

New species of *Blepharicera* Macquart (Diptera: Blephariceridae) from eastern North America, with a discussion of the phylogenetic relationships and biogeography of all Nearctic species

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Abstract. The eastern Nearctic fauna of *Blepharicera* Macquart (Diptera: Blephariceridae) is revised to include 23 species, six of which are new to science. Descriptions of the larvae, pupae and adults of *B. amnicula* sp.n., *B. conifera* sp.n., *B. crista* sp.n., *B. enoristera* sp.n., *B. hillabee* sp.n. and *B. opistera* sp.n. are presented. Keys to instar IV larvae, pupae and adults of all eastern *Blepharicera*, except *B. caudata* for which pupae and adults remain unknown, are provided. Phylogenetic studies were conducted to determine the relationships between eastern and western Nearctic *Blepharicera* and among species within these groups. Morphological data for 44 characters were used to test previous phylogenetic hypotheses on the historical relationships and biogeography of Nearctic *Blepharicera*. Maximum parsimony analyses support monophyly of the *B. tenuipes* and *B. micheneri* groups.

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 blepharicerids are known to be sensitive bioindicators (Lenat, 1993; Courtney *et al.*, 2008) and an important food for native fish (Courtney & Duffield, 2000). Habitats include cool, clear, well-oxygenated mountain streams that are flowing at velocities greater than 1 m/s. With densities sometimes greater than 1000/m², blepharicerids can be the prevailing insect in such a harsh environment (Georgian & 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).

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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, the 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, two each 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.

Adult blepharicerids 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-coloured 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 & Borkent, 1989; Courtney, 1990, 1991; Oosterbroek & Courtney, 1995), including cephalic division, six ventral suckers, cephalic apotome divided by the frontoclypeal suture, tracheal gills and stalked Malpighian tubules. Adult males have a characteristic trifold 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 behaviour 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 & Courtney, 2002). The mandibles are used to scrape periphyton off the rock surface and specialized maxillae then sweep dislodged material to the mouth opening. 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 nonfeeding 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; *Bibliocephala* Osten Sacken in western North America and Japan; *Blepharicera* 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 23 species, including six undescribed species (*B. tenuipes* group) known from eastern North America (Table S1) (Hogue, 1987; Zwick, 1990; Courtney, 2000b; Moulton & Curler, 2007; Jacobson & Courtney, 2008). Distribution of the Nearctic fauna is limited to mostly mountainous areas from southwestern Canada to southern California and Arizona and in the east from the Great Lakes to southeastern Canada and throughout the Appalachian Mountains (Hogue, 1987) (Fig. 1).

Hypothesized relationships and biogeography of the Nearctic *Blepharicera* were discussed by Hogue (1978), Zwick (1984), Hogue & 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.

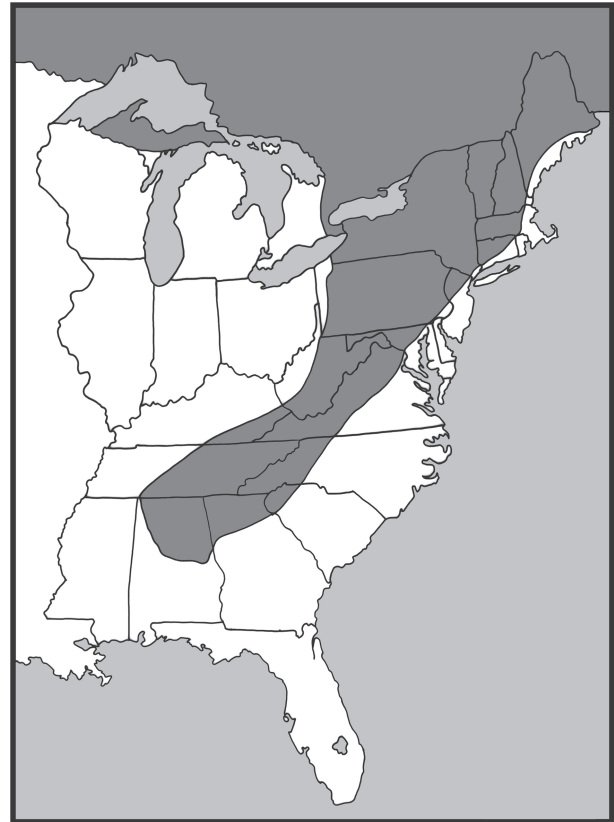


Fig. 1. Distribution of the eastern Nearctic *Blepharicera*.

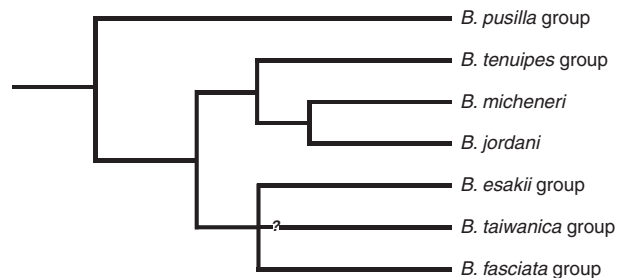


Fig. 2. Phylogenetic relationships of *Blepharicera* based on Zwick (1984).

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 (1984) constructed a phylogenetic tree that showed the *B. micheneri* group as a sister group to the *B. tenuipes* group (Fig. 2).

Hogue & Georgian (1986) concurred with Zwick (1984), concluding that the European clade is too distinct to have given rise to the eastern North American clade. They suggested that both the western and eastern Nearctic groups derived from an Asian lineage. Hogue & 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* (Fig. 2). Results of his analyses suggested a sister-group relationship between the *B. micheneri* group and the *B. tenuipes* group. Hogue & Georgian (1986) decided not to evaluate the phylogenetic relationships within the *B. tenuipes* group due to numerous similarities throughout the group.

The 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 the *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, the *B. tenuipes* group and *B. ostensackeni* arriving at different times.

The objective of the present study was to search for and describe any new species of eastern Nearctic *Blepharicera* and to hypothesize phylogenetic relationships of all Nearctic *Blepharicera*. Descriptions are given for *B. amnicula* sp.n., *B. conifera* sp.n., *B. crista* sp.n., *B. enoristera* sp.n., *B. hillabee* sp.n. and *B. opistera* sp.n. Keys are provided for all known life stages of all known species of eastern Nearctic *Blepharicera*. Phylogenetic relationships of the Nearctic *Blepharicera*, based on parsimony analyses of morphological data, are discussed.

Materials and methods

Study area

This project focused on specimens collected from the south-eastern U.S.A. Locations in Kentucky, Virginia, Tennessee, Alabama, Georgia, North Carolina and South Carolina were surveyed. If larvae and pupae were determined unique and did not fit into the current species descriptions, subsequent collections were made to obtain mature pupae for laboratory rearing.

Material

Most of the material was obtained during 2006–2010 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, IA, U.S.A.; LACM, Natural History Museum of

Los Angeles County, Los Angeles, CA, U.S.A.; USNM, National Museum of Natural History, Smithsonian Institution, Washington, DC, U.S.A. Thirty-two species of *Blepharicera* were examined in the phylogenetic portion of this study. Of these, all but one species, *B. caudata*, were available as larvae, pupae and adults (both male and female).

Specimens

Specimens were collected and preserved in 70 or 95% ethyl alcohol (EtOH). All adult specimens examined for this study were reared following the protocol described by Courtney (1998) and terminology for structures are based on Courtney (2000a). Morphological studies were based on whole-animal preparations, pupal dissections, slide mounts and scanning electron microscopy. 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%) sodium hydroxide. Specimens were examined with a Meiji Techno RZ dissecting microscope and an Olympus BH-2 compound microscope. Drawings were rendered with the aid of a drawing tube on the Olympus system. Material for scanning electron microscopy examination was sonicated briefly (5–10 s) in EtOH with a drop of dish detergent and prepared by critical point drying or hexamethyldisilazane and gold coating in a sputter coater. Material was examined with a Leo 1525 scanning electron microscope.

Measurements are given in millimetres, as a mean followed by a range in parentheses. The 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 for collection records. Abbreviations for life stages are: L = larvae; P = pupae; Pex = pupal exuviae; A = adult.

Phylogenetic analysis

Cladistic methods (sensu Hennig, 1966, as modified by Wiley, 1981; Schuh, 2000) based on synapomorphies, or shared, derived character states, were used to test the phylogenetic relationships among the Nearctic *Blepharicera*. Character polarity (i.e. the direction of character state evolution) was determined using outgroup methods (Watrous & Wheeler, 1981; Maddison *et al.*, 1984; Schuh, 2000). 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 & Courtney and the single European *Blepharicera* species, *B. fasciata* Westwood. *Blepharicera pusilla* is presumed to belong to the basal-most clade of *Blepharicera*, 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, the *B. micheneri* group and *B. ostensackeni* Kellogg (Table S1). Morphological characters were evaluated for 32 taxa, including four outgroup species. In total, 44 characters, including 12 larval, ten pupal and 22 adult characters (Table S2), were designated for use in the phylogenetic analysis. Character states were specified for each character and given a numerical code (Table S3). All characters were equally weighted and unordered, with characters coded as '0', '1', '2', '3', etc. Missing data were 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 & Maddison, 2002). In TNT, a heuristic search with the default ten random stepwise addition replicates was performed and bootstrap values were calculated for 10 000 replicates.

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 (catalogue of Diptera genera); Curran 1934: 62 (generic key); Georgian & Wallace 1983: 1237 (feeding ecology); Hogue & Georgian 1986: 1 (new species in eastern Nearctic); 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 (catalogue); 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: *Blepharicera shirakii* Alexander 1922 (synonymy by Zwick, 1990: 234).

Diagnosis. 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 six to seven 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 R₄ and R₅ asymmetrical, vein R₄₋₅ weak basally, crossvein r-m present, vein M₂ 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 nonsetate dorsally.

Male terminalia. Epandrium simple, subquadrate, moderately sclerotized and usually setose. Cerci prominent. 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 & Georgian 1986: 1 (new species in eastern Nearctic); Hogue 1987: 102 (review of Nearctic fauna); Courtney 2000: 11 (revision of eastern Nearctic fauna).

Included species. – *Blepharicera*:

annicula sp.n.

appalachiae Hogue & Georgian 1986

capitata Loew 1863

caudata Courtney 2000

chattooga Courtney 2000

cherokea Hogue 1978

conifera **sp.n.**
corniculata Courtney 2000
courtneyi Curler & Moulton 2007
coweetae Hogue & Georgian 1986
crista **sp.n.**
diminutiva Hogue 1978
enoristera **sp.n.**
gelida Courtney 2000
hillabee **sp.n.**
hispida Courtney 2000
magna Courtney 2000
opistera **sp.n.**
separata Alexander 1963
similans Johannsen 1929
tenuipes Walker 1848
tuberosa Courtney 2000
williamsae Alexander 1953

Diagnosis. 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 1.2–1.6× 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 trifold 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 (Courtney, 2000b: figs 1, 71, 73, 74); body large (>6 mm) 2
 – Lateral processes on anal division bluntly rounded apically (Courtney, 2000b: figs 2, 4, 7, 8, 10, 12, 15, 72, 77); body usually small (<6 mm) 3
 2. Anal division acutely trilobed, medial lobe elongate and tapered apically (Courtney, 2000b: figs 1, 71)
 *B. caudata* Courtney
 – Anal division bluntly trilobed, medial lobe never elongate (Courtney, 2000b: figs 73, 74) *B. magna* Courtney
 3. One or two rows of prominent dorsal tubercles (Courtney, 2000b: figs 2, 3, 15, 16, 77, 124–128, 140–142) 4

– Dorsal tubercles absent (Figs 3C, 5C, 7A–C, 9A, B, 11C, 13C, D; Courtney, 2000b: figs 4, 12, 13, 72, 78, 79, 97, 103–111, 116, 117, 132, 134) 6
 4. Dorsum with row of small, submedian tubercles on each side (Courtney, 2000b: figs 15, 16, 140, 141); tubercles consist of an area of elongate-ellipsoid sensilla (Courtney, 2000b: fig. 142); body coloration usually uniformly dark
 *B. tuberosa* Courtney
 – Dorsum with row of prominent, median tubercles, one per abdominal division (Courtney, 2000b: figs 2, 3, 77, 124–128); body coloration either piebald or uniformly pale 5
 5. Body coloration piebald (Courtney, 2000b: fig. 77); dorsal secondary sensilla sparse, mostly clavate (Courtney, 2000b: figs 126–128) *B. separata* Alexander
 – Body coloration uniformly pale (yellow), except cranial sclerites (dark brown) (Courtney, 2000b: fig. 80); dorsal secondary sensilla numerous, clavate and long setiform (Courtney, 2000b: figs 2, 3, 124, 125) *B. capitata* Loew
 6. Dorsal secondary sensilla arranged in small distinct clusters on abdominal segments (Figs 7A–C) *B. crista* **sp.n.**
 – Dorsal secondary sensilla present over larger area of abdominal segments, not arranged in small distinct clusters 7
 7. Clypeus with pair of prominent, anteriorly projected spines (Courtney, 2000b: figs 97, 98); presently known only from Chattooga River *B. corniculata* Courtney
 – Clypeal spines absent (Figs 3A, 5A, 11A, 13A) 8
 8. Dorsal secondary sensilla mostly setiform (Courtney, 2000b: figs 12, 13, 104, 105) 9
 – Dorsal secondary sensilla mostly clavate (Courtney, 2000b: figs 10, 11, 110–112) to globose (Courtney, 2000b: figs 132–135, 137) 10
 9. Dorsal secondary sensilla short (shorter than first antennal segment) (Courtney, 2000b: fig. 104)
 *B. appalachiae* Hogue & Georgian
 – Dorsal secondary sensilla elongate (longer than first antennal segment) (Courtney, 2000b: figs 12, 13) *B. gelida* Courtney
 10. Dorsal secondary sensilla mostly globose (Courtney, 2000b: figs 132–135) *B. similans* Johannsen
 – Dorsal secondary sensilla mostly clavate or digitiform (Courtney, 2000b: figs 10, 11, 110–112) 11
 11. Body and cranial sclerites uniformly coloured (light to dark brown) 12
 – Body and cranial sclerites usually with contrasting colour patterns (Courtney, 2000b: figs 72, 78, 79) 19
 12. Substernal sensilla (adjacent to first suctorial disc) dark (Courtney, 2000b: fig. 75); dorsal sensilla minute, about as long as broad (Courtney, 2000b: figs 8, 9, 108); body small (instar IV <5 mm) *B. diminutiva* Hogue
 – Substernal sensilla (adjacent to first suctorial disc) light, pale to light brown (Courtney, 2000b: fig. 76); dorsal sensilla well developed, usually dense; body large (instar IV >5 mm) . . 13
 13. Dorsal sensilla arranged in transverse anterior and posterior clusters (Figs 9A, B; Courtney, 2000b: fig. 109) 14
 – Dorsal sensilla densely distributed over segments, not arranged in distinct transverse clusters (Courtney, 2000b: figs 10, 11, 110–112) 16

14. Dorsal sensilla arranged in two transverse clusters (Courtney, 2000b: fig. 109) *B. tenuipes* (Walker)
– Dorsal sensilla arranged in four transverse clusters (Figs 9A, B)..... 15
15. Anal division slightly concave; ecdysial stem line long (Moulton & Curler, 2007: fig. 1)
..... *B. courtneyi* Curler & Moulton
– Anal division rounded; ecdysial stem line short (Fig. 9D)..
..... *B. enoristera* **sp.n.**
16. Anal division broadly rounded (Figs 9D, 13B)..... 17
– Anal division truncate to slightly concave (Figs 3B, 5B)....
..... 18
17. Dorsal sensilla elongate-fustiform; setiforms mostly absent on abdominal segments and lateral lobes (Courtney, 2000b: figs 10, 11, 110–112)..... *B. hispida* Courtney
– Dorsal sensilla fustiform; setiforms mostly absent medially, numerous on lateral lobes (Fig. 11B, C) *B. hillabee* **sp.n.**
18. Dorsal sensilla elongate-coniform and digitiform; presently known only from Cloudland Canyon State Park in northwest Georgia (Fig. 5C)..... *B. conifera* **sp.n.**
– Dorsal sensilla digitiform; presently known only from Little River Canyon National Preserve in northeast Alabama (Fig. 3C)..... *B. amnicula* **sp.n.**
19. Ecdysial stem line short, frontoclypeal apotome reaching or almost reaching posterior margin of head capsule (Courtney, 2000b: fig. 4); head capsule mottled or uniformly coloured, 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 (Courtney, 2000b: fig. 133); head capsule mottled or uniformly coloured, with frontoclypeal apotome typically darker than rest of head capsule; membranous region of antenna approximately the same length as apical sclerotized region (Courtney, 2000b: fig. 2) 21
20. Ecdysial line with little to no stem line, frontoclypeal apotome reaching posterior margin of head capsule; body with chevron-like or banded colour pattern; anal division truncate to slightly concave (Courtney, 2000b: figs 4, 72).....
..... *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. 13A, B)..... *B. opistera* **sp.n.**
21. 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 (Courtney, 2000b: figs 2, 106) *B. cherokea* Hogue
– Cephalothorax, trunk, and prolegs mostly dark but with light highlights; cranial sclerites either uniformly dark or dark with light highlights (Courtney, 2000b: figs 78, 79); antennae short, total length less than length of frontoclypeal apotome 22

22. 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 (Courtney, 2000b: fig. 79); cranial sclerites usually with contrasting colour 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 (Courtney, 2000b: fig. 109).....
..... *B. coweetae* Hogue & 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 (Courtney, 2000b: fig. 78); if cranial sclerites with contrasting colour 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

Pupae

Pupae of *B. caudata* are unknown.

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. Branchial sclerite papillose (Courtney, 2000b: fig. 160); cephalic sclerite with pair of ridges extended dorsoventrally for nearly half its height (Courtney, 2000b: fig. 161).....
..... *B. diminutiva* Hogue
– Branchial sclerite glabrous (Courtney, 2000b: fig. 136); cephalic sclerite without submedian ridges 3
3. Integument of abdominal tergites with minute, dark papillae bearing microscopic spinules apically (Courtney, 2000b: figs 93, 138, 139, 154–156).....
..... *B. chattooga* Courtney and *B. similans* Johannsen
– Integument of abdominal tergites without distinct papillae, but with minute, circular patches of spinules (Courtney, 2000b: figs 152, 153); these patches may give the appearance of light-coloured papillae or papillae surrounded by light-coloured halo (Courtney, 2000b: fig. 88)..... 4
4. Integument of abdominal tergites with micropunctures between circular patches, surface luster somewhat dull (less so than in *B. williamsae*) (Courtney, 2000b: figs 143, 144); 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 (Courtney, 2000b: figs 152, 153); abdominal tergites without submedian tubercles.....
..... *B. cherokea* Hogue
5. Integument of abdominal tergites with fine, reticulate (areolate) pattern; surface luster of tergites and branchial sclerite very dull (Courtney, 2000b: figs 169–171).....
..... *B. williamsae* Alexander

– Integument of abdominal tergites homogenous or otherwise sculpted, no reticulate pattern; surface luster shiny on at least branchial sclerite (Courtney, 2000b: figs 89, 131, 146, 149, 154, 166) 6

6. Integument of abdominal tergites without papillae, but with minute, circular patches of spinules (Courtney, 2000b: figs 152, 153); patches may give appearance of light-coloured papillae or papillae surrounded by light-coloured halo (Courtney, 2000b: fig. 89) *B. corniculata* Courtney

– Integument of abdominal tergites with minute, dark or white papillae (Courtney, 2000b: figs 90–96) 7

7. Abdominal papillae appearing as white dots against a darker background (Courtney, 2000b: figs 86, 87, 95) 8

– Abdominal papillae darker than background coloration (Courtney, 2000b: figs 90–94, 96) 9

8. Abdominal papillae large ($\geq 10 \mu\text{m}$), adjacent papillae separated by distance that approximates papilla width (Courtney, 2000b: figs 87, 157–159); anterior lamella of respiratory organ thick, broadly rounded apically (Courtney, 2000b: fig. 14); large ($> 6 \text{ mm}$) *B. gelida* Courtney

– Abdominal papillae small ($\leq 10 \mu\text{m}$), adjacent papillae separated by distance that typically exceeds papilla width (Courtney, 2000b: figs 95, 129–131); anterior lamella of respiratory organ broadly pointed apically (Courtney, 2000b: fig. 17); relatively small ($< 6 \text{ mm}$) *B. capitata* Loew and *B. separata* Alexander

9. Abdominal papillae in clusters of two, three or more, arrangement most apparent lateral to muscle scars (Courtney, 2000b: figs 96, 146, 147) *B. appalachiae* Hogue & Georgian

– Abdominal papillae more or less evenly spaced, rarely forming clusters lateral to muscle scars (Courtney, 2000b: figs 90–92, 94, 114, 121, 150, 167) 10

10. Abdominal papillae large ($\geq 10 \mu\text{m}$), adjacent papillae separated by distance that approximates papilla width (Courtney, 2000b: figs 120–122); large ($> 6 \text{ mm}$) *B. magna* Courtney

– Abdominal papillae small ($\leq 10 \mu\text{m}$), adjacent papillae separated by distance that typically exceeds papilla width (Courtney, 2000b: figs 91, 92, 113, 114, 149, 150); relatively small ($< 6 \text{ mm}$) 11

11. Integument of abdominal tergites with micropunctures or other pattern between papillae, surface luster somewhat dull (less so than in *B. williamsae*) (Figs 7D, E, 9E–G; Courtney, 2000b: figs 91, 92, 113–115, 149–151) 12

– Integument of abdominal tergites mostly glabrous between papillae, surface luster shiny (Courtney, 2000b: figs 90, 166, 167) 14

12. Integument of abdominal tergites with faint reticulation; anal tergite smooth, unwrinkled (Fig. 7D, E) *B. crista* sp.n.

– Integument of abdominal tergites with micropunctures between papillae; anal tergite wrinkled (Fig. 9E–G; Courtney, 2000b: figs 149–151) 13

13. Body outline roughly ellipsoid (as in Courtney, 2000b: 85) *B. enoristera* sp.n.

– Body outline ovoid (as in Courtney, 2000b: 83) *B. coweetae* Hogue & Georgian and *B. hispida* Courtney

14. Anal tergite smooth, unwrinkled *B. courtneyi* Curler & Moulton

– Anal tergite wrinkled 15

15. Lamellae of respiratory organs broadly pointed apically 16

– Lamellae of respiratory organs broadly rounded apically 17

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

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

17. Endemic to Great Lakes Region and northern Appalachians, north of Tennessee *B. tenuipes* (Walker)

– Endemic to southern Appalachians, south of Tennessee *B. confifera* sp.n. and *B. amnicula* sp.n.

Adult males

Adult males of *B. caudata* are unknown.

1. Dorsal and ventral eye division subequal in size (Courtney, 2000b: figs 29, 39) 2

– Dorsal division of eye much smaller than ventral division (Figs 4A, 6A, 8A, 10A, 12A, 14A; Courtney, 2000b: figs 44, 49) 7

2. Inner margin of cercus convex or expanded (Courtney, 2000b: figs 31, 36, 58) 3

– Inner margin of cercus straight, neither convex nor expanded (Courtney, 2000b: figs 26, 41, 55) 5

3. Inner margin of cercus greatly expanded near base, reaching to or beyond midline (Courtney, 2000b: fig. 58); southern Appalachians *B. williamsae* Alexander

– Inner margin of cercus convex or expanded broadly near apex (Courtney, 2000b: figs 31, 36); mostly central or northern Appalachians 4

4. Posterior margin of cercus with small process near inner edge and prominent, triangular lobe near outer margin (Courtney, 2000b: fig. 31); 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 (Courtney, 2000b: fig. 36); cercus and epandrium set with numerous, elongate setae *B. hispida* Courtney

5. Cercus with sparse setae, posterior margin relatively straight (Courtney, 2000b: fig. 26); dorsal paramere opaque (Courtney, 2000b: fig. 27) *B. corniculata* Courtney

– Cercus densely set with long setae, posterior margin with pronounced lobe near inner edge (Courtney, 2000b: figs 41, 55); dorsal paramere with pigmented outer margin (Courtney, 2000b: figs 42, 61) 6

6. Posterior margin of cercus with elongate, medially directed hook near inner edge (Courtney, 2000b: fig. 41); dorsal

- paramere without medial dorsal carina (Courtney, 2000b: fig. 42)..... *B. magna* Courtney
- Posterior margin of cercus with blunt, ventrally projected lobe near inner edge (Courtney, 2000b: fig. 55); dorsal paramere with medial dorsal carina (Courtney, 2000b: fig. 61)..... *B. coweetae* Hogue & Georgian
7. Inner margin of cercus expanded (Courtney, 2000b: figs 65, 67)..... 8
- Inner margin of cercus straight, not expanded (Figs 4B, 6B, 8B, 10B, 12B, 14B; Courtney, 2000b: figs 21, 46, 51, 53, 54, 56, 57)..... 9
8. Inner margin of cercus expanded evenly and broadly, apical margin slightly convex, making outer margin somewhat lobulate (Courtney, 2000b: fig. 65); median aedeagal filament of approximately same length as lateral filaments (Courtney, 2000b: fig. 66); 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 (Courtney, 2000b: fig. 67); median aedeagal filament distinctly longer than lateral filaments (Courtney, 2000b: fig. 68); apex of ventral parameres incurved, asymmetrically bifurcate.... *B. similans* Johannsen
9. Cercal lobes narrow, elongate, and generally pointed apically (Courtney, 2000b: figs 46, 51)..... 10
- Cercal lobes relatively broad and generally truncated apically (Figs 4B, 6B, 8B, 10B, 12B, 14B; Courtney, 2000b: figs 21, 53, 54, 56, 57)..... 11
10. Apical margin of cercal lobe with acutely pointed lobe medially (Courtney, 2000b: fig. 51); apex of dorsal paramere deeply incised on either side of pronounced medial dorsal carina (Courtney, 2000b: fig. 52)..... *B. tuberosa* Courtney
- Apical margin of cercal lobe with broadly rounded inner corner (Courtney, 2000b: fig. 46); apex of dorsal paramere entire on either side of weak, medial dorsal carina (Courtney, 2000b: fig. 47)..... *B. separata* Alexander
11. 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 (Courtney, 2000b: figs 59, 63) or expanded inner wall (Figs 4C, 6C, 8C, 12C); medium sized (wing length \approx 5–6 mm)..... 12
- Apex of dorsal paramere weakly incised or entire on either side of medial dorsal carina..... 18
12. Posterior margin of cercus with indistinct lobe near inner margin, giving appearance of small posteromedian notch (Courtney, 2000b: fig. 53)..... *B. appalachiae* Hogue & Georgian
- Posterior margin of cercus with pronounced, acute lobe apically..... 13
13. Medial margin of cercus sinuous (Figs 4B, 8B)..... 14
- Medial margin of cercus straight (Figs 6B, 12B; Moulton & Curler, 2007: fig. 8; Courtney, 2000b: fig. 57)..... 15
14. Lateral margin of cercus straight; aedeagal rods subequal in length (Fig. 8B, C)..... *B. crista* sp.n.
- Lateral margin of cercus concave arcuate; aedeagal rods equal in length (Fig. 4B, C)..... *B. amnicula* sp.n.
15. Ejaculatory apodeme short, extended approximately one-half distance to anterior margin of lateral parameral lobes (Figs 10C, 12C)..... 16
- Ejaculatory apodeme elongate, extended approximately two-thirds distance to anterior margin of lateral parameral lobes (Figs 4C, 6C)..... 17
16. Posterior margin of cercus with prominent medial hook, posterolateral margin extended into rounded lobe; body coloration brown to dark brown (Moulton & Curler, 2007: fig. 8)..... *B. courtneyi* Curler & Moulton
- Posterior margin of cercus largely undeveloped with median hook-like lobe, posterolateral margin undeveloped; body coloration light brown (Fig. 12B)..... *B. hillabee* sp.n.
17. Dorsal eye division smaller (0.30 \times) 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 (<0.25 \times) than ventral, with ten rows of ommatidia along mid-meridian; presently known only from Cloudland Canyon State Park in northwest Georgia (Fig. 6A)..... *B. conifera* sp.n.
18. Posterior margin of cercus without conspicuous lobes or processes (Courtney, 2000b: fig. 56)..... *B. diminutiva* Hogue
- Posterior margin of cercus with conspicuous lobes or processes (Figs 10B, 14B; Courtney, 2000b: figs 21, 54)..... 19
19. Posterior margin of cercus distinctly trilobate, posterolateral and posteromedial lobes developed, medial lobe hook-like (Figs 10B, 14B)..... 20
- Posterior margin of cercus not trilobate, conspicuous lobe triangular (Courtney, 2000b: figs 21, 54)..... 21
20. Dorsal eye division smaller (0.33 \times) than ventral; lateral and medial cercal margins convex; ejaculatory apodeme extended approximately one-half distance to anterior margin of lateral parameral lobes (Fig. 10A–C)..... *B. enoristera* sp.n.
- Dorsal eye division smaller (0.25 \times) than ventral; lateral and medial cercal margins subparallel; ejaculatory apodeme subequal in length to lateral parameral lobes (Fig. 14A–C)..... *B. opistera* sp.n.
21. Posterior cercal lobe near medial margin (Courtney, 2000b: fig. 54)..... *B. cherokea* Hogue
- Posterior cercal lobe equidistant between medial and outer margin (Courtney, 2000b: fig. 21)..... *B. chattooga* Courtney

Adult females

Adult females of *B. caudata* are unknown.

1. Two spermathecae, medial spermatheca vestigial (Courtney, 2000b: fig. 70)..... *B. similans* Johannsen
- Three spermathecae (Figs 4D, 6D, 8D, 10E, 12D, 14E) .. 2
2. Ducts of lateral spermathecae sclerotized and pigmented near genital aperture (Courtney, 2000b: fig. 69)..... *B. capitata* Loew

- Ducts of spermathecae membranous and unpigmented for entire length (Courtney, 2000b: figs 20, 25, 30, 35, 40, 45, 50) 3
- 3. Dorsal and ventral eye divisions contiguous laterally, without callis oculi (Courtney, 2000b: figs 28, 38) 4
- Dorsal and ventral eye divisions separated by callis oculi (Figs 10D, 14D; Courtney, 2000b: figs 23, 43, 48) 6
- 4. 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 (Courtney, 2000b: figs 28, 38); number of parietal setae usually five or fewer; scutellum distinctly lighter than scutum; scutellum with lateral sensilla arrangement diffuse 5
- 5. 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. 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 two to three dorsal ommatidia) 17
- 7. Distal palpomere long (>3× length of second palpomere). 8
- Distal palpomere short (<3× length of second palpomere) 14
- 8. Number of parietal sensilla >20 9
- Number of parietal sensilla <20 10
- 9. Spermathecae ovoid; accessory gland dilated anteriorly; number of parietal sensilla >20 *B. coweetae* Hogue & Georgian
- Spermathecae pyriform; accessory gland not wider than corpora of spermathecae; number of parietal sensilla 25–30 (Fig. 4D) *B. ammicula* sp.n.
- 10. Hypogynial valves subquadrate; number of parietal sensilla 12 (Moulton & Curler, 2007: figs 4, 6) *B. courtneyi* Curler & Moulton
- Hypogynial valves rounded; number of parietal sensilla variable (Figs 6D, 8D, 10E, 14E) 11
- 11. Number of parietal sensilla 3; clypeal sensilla ≈10 (Fig. 14 D) *B. opistera* sp.n.
- Number of parietal sensilla ≥10; clypeal sensilla ≥15 .. 12
- 12. Spermathecae spherical; number of parietal sensilla 15–17; antennal flagellomeres barrel-shaped (Fig. 6D) *B. confifera* sp.n.
- Spermathecae pyriform; number of parietal sensilla ≥10; antennal flagellomeres cylindrical in shape (Figs 8D, 10D, E) 13
- 13. Hypogynial valves rounded apicolaterally, pointed apicomeres, inner margin parallel and outer margin slightly convex, apices straight, not convergent or divergent, intervalvular area narrowly V-shaped (Fig. 10E) *B. enoristera* sp.n.

- Hypogynial valves rounded at apex, inner margin strongly convex and outer margin convex, apices slightly convergent, intervalvular area broadly U-shaped (Fig. 8D) *B. crista* sp.n.
- 14. Number of parietal sensilla 20–25 (Courtney, 2000b: fig. 18) *B. chattooga* Courtney
- Number of parietal sensilla <20 (Courtney, 2000b: figs 33, 43) 15
- 15. Hypogynial valves rounded at apex; dorsal eye division with 11 rows of ommatidia along mid-meridian; number of parietal sensilla 12–18 (Fig. 12D) *B. hillabee* sp.n.
- Hypogynial valves quadrate to subquadrate (Courtney, 2000b: fig. 35, 45); dorsal eye division with 15–20 rows of ommatidia along mid-meridian; number of parietal sensilla ≈10 16
- 16. Hypogynial valves quadrate (Courtney, 2000b: fig. 45); dorsal eye division with 15 rows of ommatidia along mid-meridian *B. separata* Alexander
- Hypogynial valves subquadrate (Courtney, 2000b: fig. 35); dorsal eye division with 18–20 rows of ommatidia along mid-meridian *B. hispida* Courtney
- 17. Distal palpomere short (<3× length of second palpomere) (Courtney, 2000b: fig. 23) 18
- Distal palpomere long (>3× length of second palpomere) 19
- 18. Number of parietal sensilla <10 *B. appalachiae* Hogue & Georgian
- Number of parietal sensilla >10 *B. tenuipes* (Walker)
- 19. Number of parietal sensilla <15 (Courtney, 2000b: figs 23, 48) 20
- Number of parietal sensilla >20 21
- 20. Hypogynial valves subrectangular, inner margin parallel and outer margin slightly divergent (Courtney, 2000b: fig. 25); number of parietal sensilla 10–15 ... *B. corniculata* Courtney
- Hypogynial valves subpentagonal, truncate apically, inner margin parallel basally and divergent apically, outer margin angulate (Courtney, 2000b: fig. 50); number of parietal sensilla <10 *B. tuberosa* Courtney
- 21. Posterolateral margin of sternite VIII with approximately ten setiform sensilla, medial depression U-shaped; medium-sized species (wing length usually >5 mm) *B. cherokee* 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

Diagnoses and descriptions of new species

Blepharicera ammicula Jacobson & Moulton, sp.n.

(Figs 3A–F, 4A–D)

Diagnosis. A medium-sized *Blepharicera*. *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;

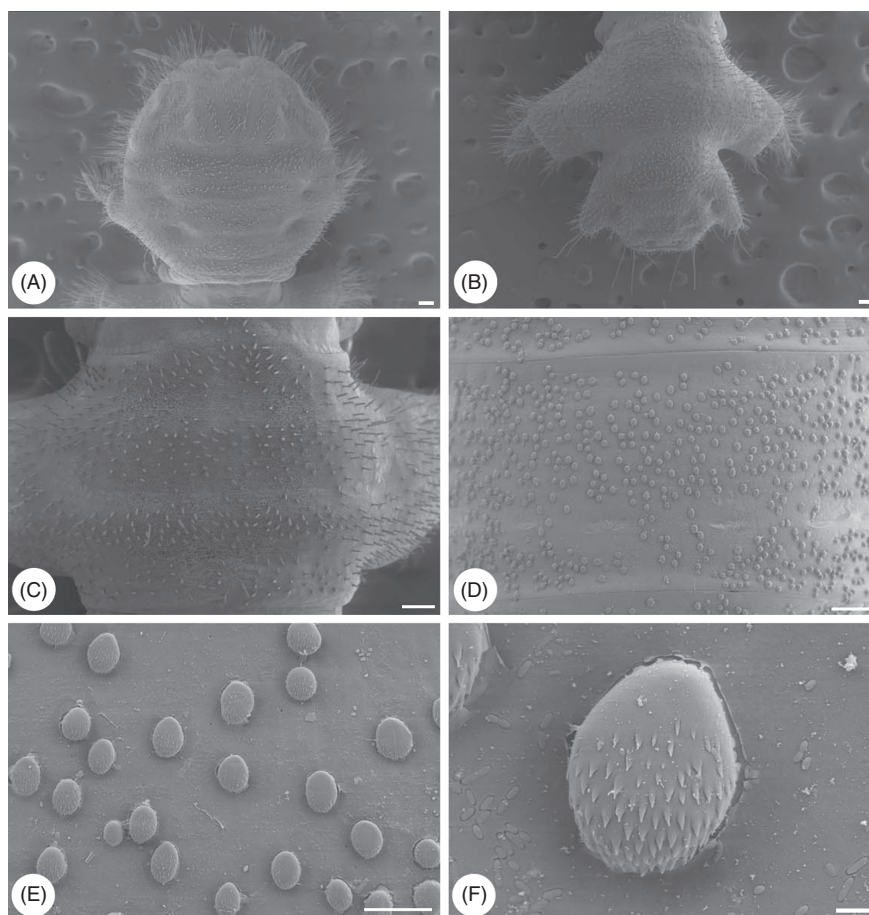


Fig. 3. Scanning electron micrographs of larval and pupal *Blepharicera ammicula*. (A) Cephalic division, dorsal view; (B) anal division, abdominal segments VI–X, dorsal view; (C) larval dorsal sensilla; (D, E) pupal abdominal tergite microsculpture; (F) pupal abdominal papilla. Scale bars = 3 μ m (F), 30 μ m (E), 100 μ m (A–D).

cuticle glabrous; anal tergite wrinkled. *Adult male*. Dorsal eye division smaller ($0.25\times$) 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 (Fig. 3A–C). Measurements, instar II ($n = 3$) total length 2.64 mm (2.33–2.86), cranial width 0.27 mm, antennal segments 0.11, 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, approximately 45 in number; dorsal secondary sensilla numerous, digitiform; anal division with six to seven prominent setiforms marginally.

Pupa (Fig. 3D–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.

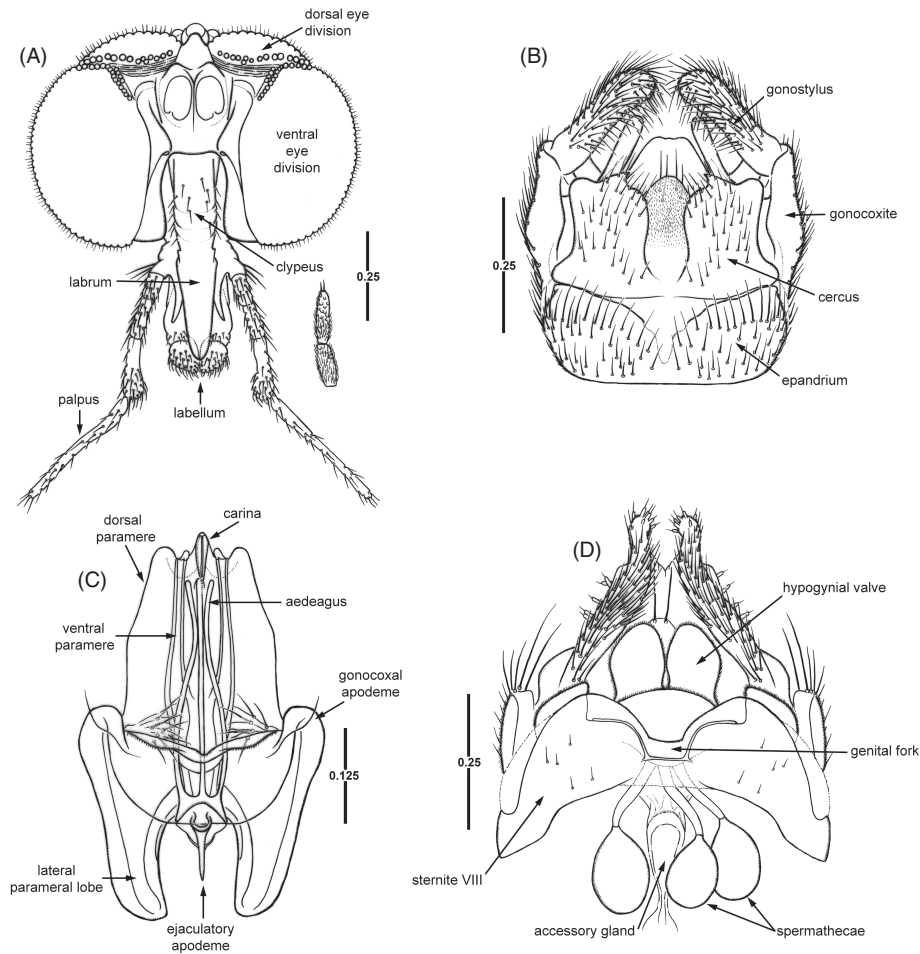


Fig. 4. Adults of *Blepharicera amnicula*. (A) Male head and antennal apex, frontal view; (B) male terminalia, dorsal view; (C) male terminalia (phallic structures), dorsal view; (D) female terminalia, ventral view.

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).

Head (Fig. 4A): Structure: eyes semidichoptic, interocular ridge present, interocular distance 0.11 mm; eye divided; callis oculi absent; dorsal division contiguous with ventral, smaller ($0.25\times$) than ventral; dorsal ommatidia larger in diameter; dorsal division with 12 rows of ommatidia along mid-meridian. Clypeus length/width = 2.0. Proboscis about $0.40\times$ head width; mandibles absent; palpi with five palpomeres, distal four segment proportions 1.0, 1.2, 1.6, 4.0. Antennal flagellomeres cylindrical; ultimate flagellomere $1.34\times$ length of penultimate flagellomere; scape light brown and pedicel brown, both with prominent setiforms; flagellomere one 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 (Table S4). Chaetotaxy: Thorax

glabrous except for few sparse setae; scutellum with numerous setae grouped at posterolateral corner ($n \approx 20$); 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 (Fig. 4B, 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; approximately 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 $\approx 1.5\times$ 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.

Adult female. 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).

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 0.46× head width; palpi with five palpomeres, distal four segment proportions 1.0, 1.2, 1.4, 3.5. Antennal flagellomeres cylindrical in shape; ultimate flagellomere 1.60× length of penultimate flagellomere; scape light brown and pedicel brown with prominent setiforms; flagellomere one 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 (Table S4). Chaetotaxy: Thorax glabrous except for sparse setae; scutellum with numerous setae grouped at posterolateral corner ($n \approx 25$); 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. 4D): 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 six to eight setiforms laterally; hypogynial plate with numerous small setae; epiproct with two prominent setiforms apically.

Type material. Holotype (adult male, reared): U.S.A.: 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 [two instar IV L (slides), two male and two female P (EtOH), one male and one female Pex (slides), two male and one female A (reared, pinned, head and genitalia slide mounted), one male and two female A (reared, pinned, genitalia in glycerin microvial)]; 12 March 2007 [nine 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' (*ammicola*) plus the diminutive suffix (*-ula*) in reference to the type locality, Little River, AL.

Distribution. *Blepharicera amnicola* 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 *B. amnicola* is probably 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 available only from Canyon Mouth Park because habitats above the falls consisted mostly of large boulders and bedrock.

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

Blepharicera confifera Jacobson, sp.n.

(Figs 5A–F, 6A–D)

Blepharicera tenuipes [in part] Hogue 1978: 23 (as 'Atypical adults').

Blepharicera sp. Courtney 2000b: 59 (as 'Unplaced Specimens').

Diagnosis. A medium-sized *Blepharicera*. *Larva.* Dorsal secondary sensilla numerous, elongate-coniform and digitiform; anal division truncate to slightly concave, lateral processes rounded. *Pupa.* Body outline ovoid; papillae brown with minute spinules; cuticle between papillae glabrous with a few micropunctures, brown; anal tergite wrinkled. *Adult male.* Dorsal eye division smaller ($< 0.25\times$) 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. *Adult female.* Eyes divided; callis oculi present; hypogynial valves short, lateral margins convex and medial margins slightly convex, inner

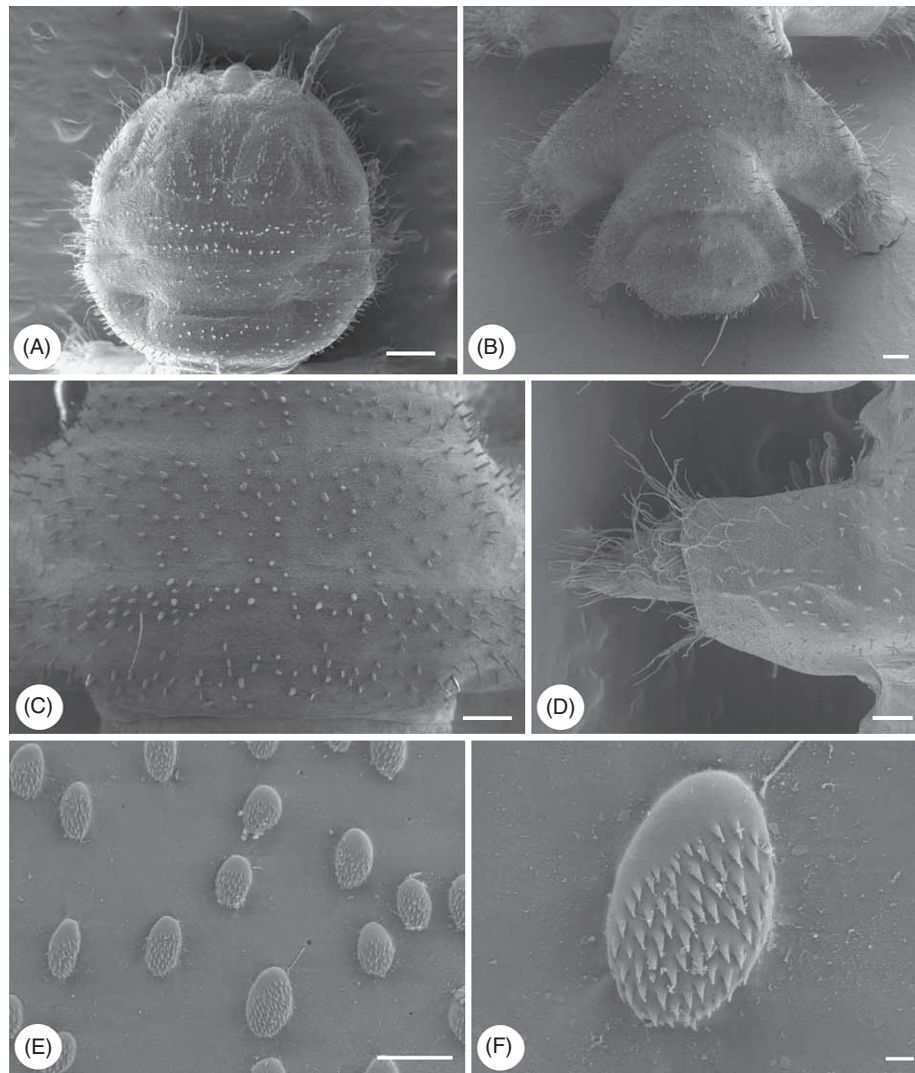


Fig. 5. Scanning electron micrographs of larval and pupal *Blepharicera confiera*. (A) Cephalic division, dorsal view; (B) anal division, abdominal segments VI–X, dorsal view; (C) larval dorsal sensilla; (D) proleg on abdominal segment III, dorsal view on left side; (E) pupal abdominal tergite microsculpture; (F) pupal abdominal papilla. Scale bars = 2 μm (F), 20 μm (E), 100 μm (B–D), 200 μm (A).

margin narrowly separated, apices rounded; intervalvular area narrowly V-shaped; three spherical spermathecae.

Description. *Larva* (Fig. 5A–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 six to eight prominent setiforms marginally.

Pupa (Fig. 5E, 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,

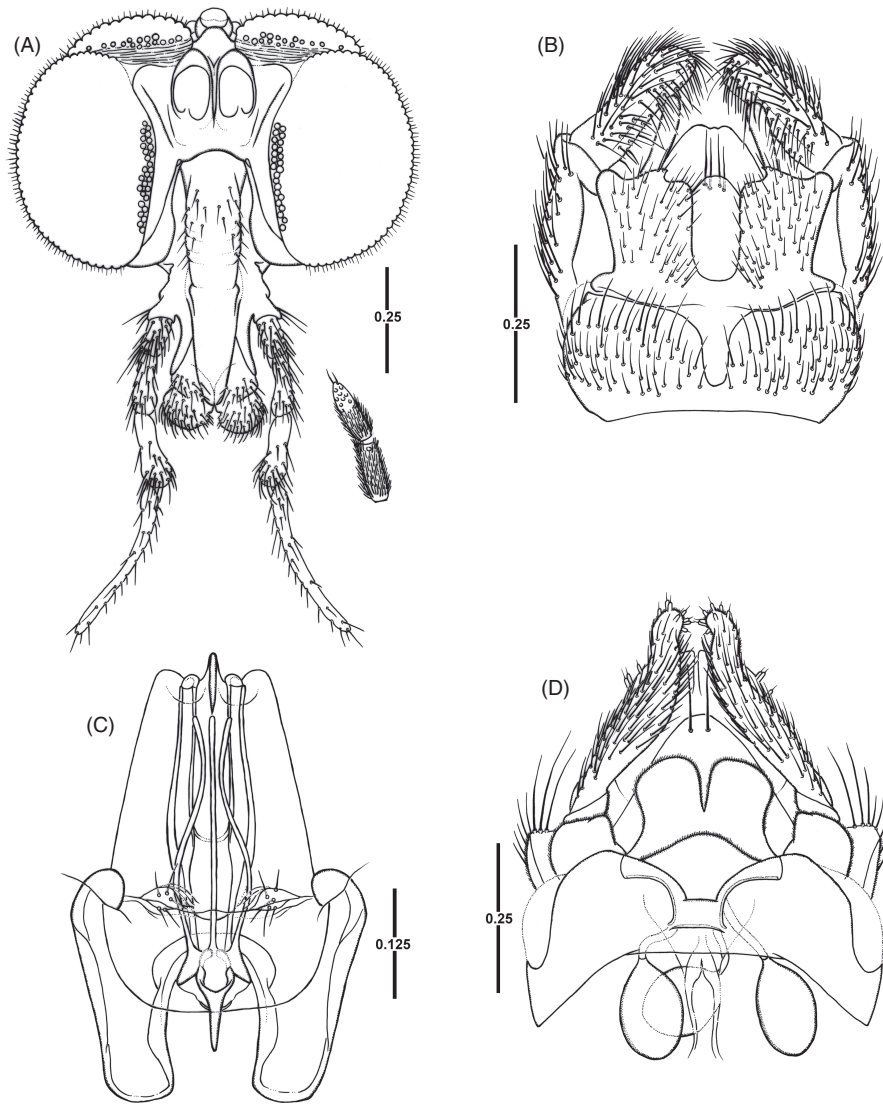


Fig. 6. Adults of *Blepharicera confifera*. (A) Male head and antennal apex, frontal view; (B) male terminalia, dorsal view; (C) male terminalia (phallic structures), dorsal view; (D) female terminalia, ventral view.

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).

Head (Fig. 6A): Structure: eyes semidichoptic, interocular distance 0.09 mm; eye divided; callis oculi absent; dorsal division contiguous with ventral, smaller ($<0.25\times$) than ventral; dorsal ommatidia larger in diameter; dorsal division with ten rows of ommatidia along mid-meridian. Clypeus length/width = 2.0. Proboscis about $0.43\times$ head width; mandibles absent; palpi with five palpomeres, distal four segment proportions 1.0, 1.3, 1.5, 3.8. Antennal flagellomeres

barrel-shaped; ultimate flagellomere $1.2\times$ length of penultimate flagellomere; scape pale and pedicel brown, both with prominent setiforms; flagellomere one 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 (Table S5). Chaetotaxy: Thorax glabrous except for few sparse setae; scutellum with numerous setae grouped at posterolateral corner ($n \approx 30$); coxae with prominent setae. Coloration: Frons, clypeus and face light brown, pruinose. Thorax light brown, pruinose. Fore-coxae light brown, other coxae pale. Abdominal tergites light

brown, sternites pale. Terminalia (Fig. 6B, 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 $\approx 1.5 \times$ 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.

Adult female. 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).

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 $0.46 \times$ head width; palpi with five palpomeres, distal four segment proportions 1.0, 1.3, 1.5, 3.8. Antennal flagellomeres barrel-shaped; ultimate flagellomere $1.65 \times$ length of penultimate flagellomere; scape light brown and pedicel brown with prominent setiforms; flagellomere one 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 (Table S5). Chaetotaxy: Thorax glabrous except for sparse setae; scutellum with numerous setae grouped at posterolateral corner ($n \approx 20$); 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. 6D): 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. Chaetotaxy: Sternite VIII with two to six setiforms laterally; hypogynial plate with numerous small setae; epiproct with two prominent setiforms apically.

Type material. Holotype (adult male, reared): U.S.A.: Georgia: Dade Co: Cloudland Canyon State Park, Daniel Creek

below upper falls, $34^{\circ}50'N$ $85^{\circ}28'W$, 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 [one male and one female Pex (slides), two male A (reared, pinned, head and genitalia slide mounted), two male and one female A (reared, pinned, head and genitalia in glycerin microvial)]; 12 March 2007 [two instar IV L (slides)], coll. A.J. Jacobson, G.R. Curler and J.K. Moulton; 25 April 2007 [nine instar IV L (EtOH), three male and three 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. *Blepharicera conifera* 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 this species was from 8 May 1952, coll. G.S. Walley (Courtney, 2000b). Additional material has recently been collected, including the unknown larval and pupal stages. Due to its unique, isolated habitat, *B. conifera* is probably an endemic, univoltine, spring species. As with some collection localities for other *Blepharicera* species, the substrate in Daniel Creek near the upper falls is mostly bedrock, making it difficult to find pupae on small rocks that can be carried out of the canyon for laboratory rearing.

Taxonomic comparison. 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 similar to those of other species in the group. Adult males of *B. conifera* are most similar to those of *B. amnicula* and *B. tenuipes*, but can be distinguished by differences in the shape of the cerci as well as differences in the aedeagus. *Blepharicera conifera* and *B. amnicula* are also much smaller in size when compared with *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*. *Blepharicera conifera* is the only species known to occur in Daniel Creek within Cloudland Canyon State Park.

Blepharicera crista Jacobson & Curler, sp.n.

(Figs 7A–F, 8A–D)

Diagnosis. A medium-sized *Blepharicera*. Larva. Dorsal secondary sensilla intermediate between digitiform and

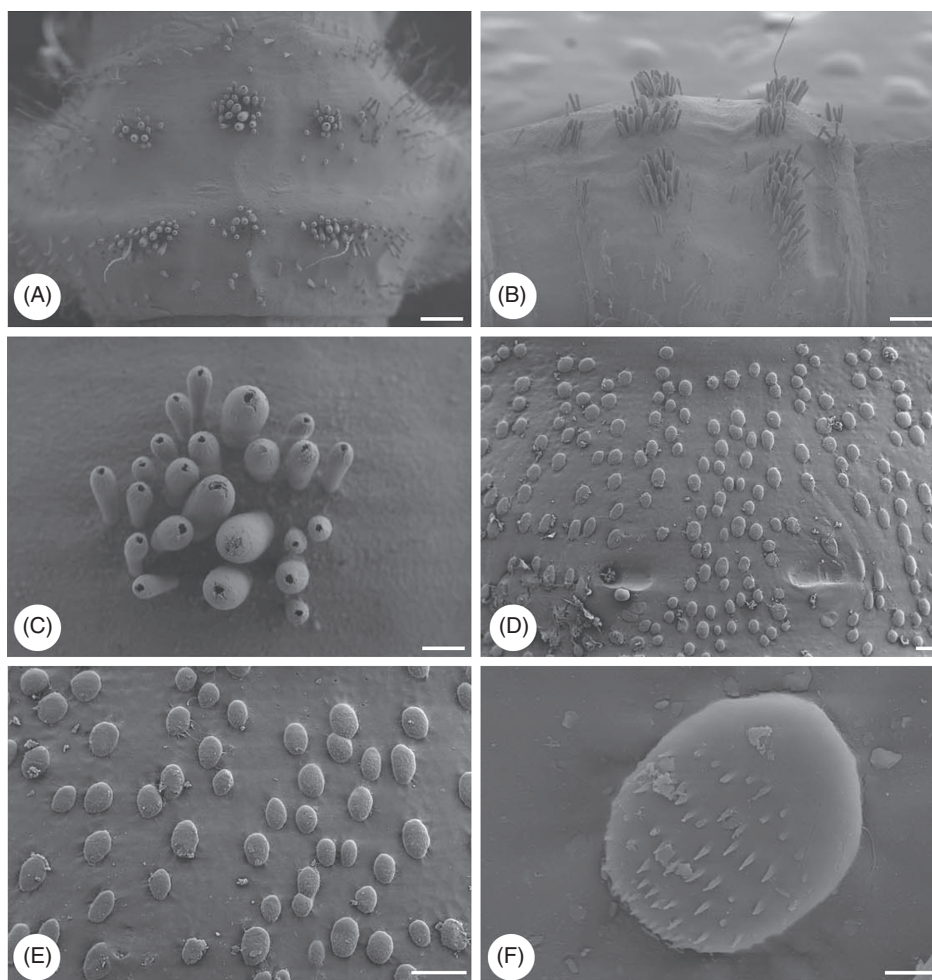


Fig. 7. Scanning electron micrographs of larval and pupal *Blepharicera crista*. (A) Larval dorsal sensilla, dorsal view; (B) larval dorsal sensilla, lateral view; (C) larval dorsal sensilla cluster; (D–E) pupal abdominal tergite microsculpture; (F) pupal abdominal papilla. Scale bars = 3 μm (F), 20 μm (C, D), 30 μm (E), 100 μm (A, B).

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 ($<0.25\times$) 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 (Fig. 7A–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, approximately 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 four to six prominent setiforms marginally.

Pupa (Fig. 7D–F). Measurements, male ($n = 10$) length 4.42 mm (4.25–4.65), width 2.67 mm (2.55–2.85); female

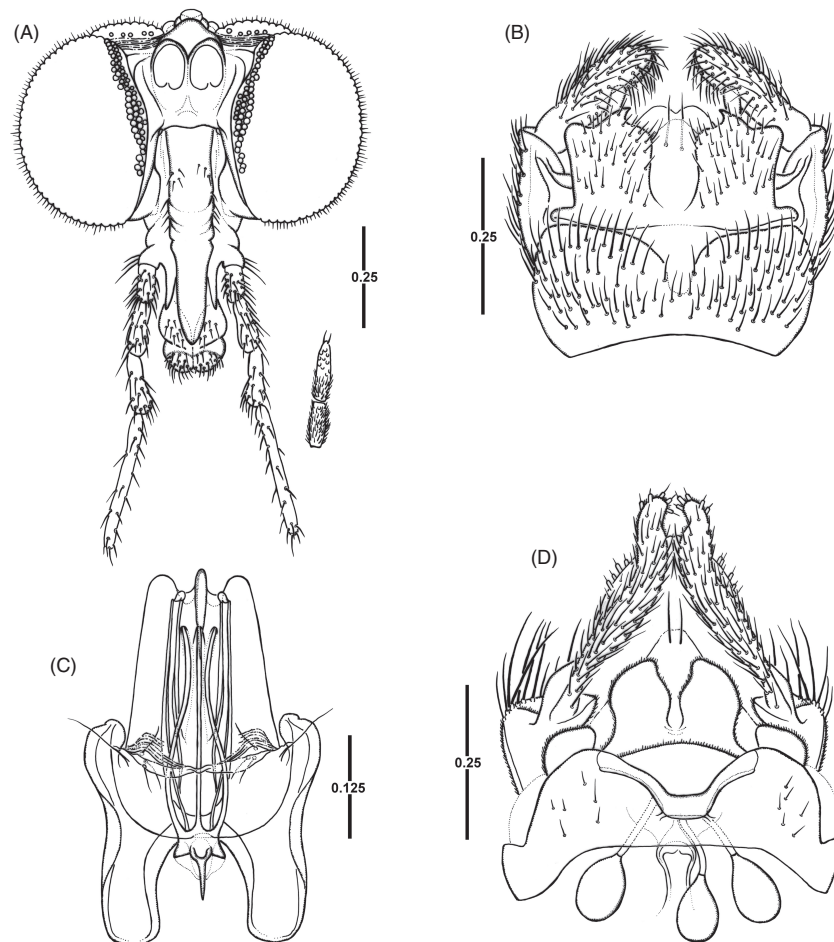


Fig. 8. Adults of *Blepharicera crista*. (A) Male head and antennal apex, frontal view; (B) male terminalia, dorsal view; (C) male terminalia (phallic structures), dorsal view; (D) female terminalia, ventral view.

($n = 10$) 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).

Head (Fig. 8A): Structure: eyes semidichoptic, interocular distance 0.12 mm; eye divided; callis oculi absent; dorsal division mostly contiguous with ventral, smaller ($<0.25\times$) than ventral; dorsal ommatidia larger in diameter; dorsal division with nine rows of ommatidia along mid-meridian. Clypeus length/width = 2.2. Proboscis about $0.39\times$ head width; mandibles absent; palpi with five palpomeres, distal four

segment proportions 1.0, 1.4, 1.6, 4.2. Antennal flagellomeres cylindrical; ultimate flagellomere $1.33\times$ length of penultimate flagellomere; scape and pedicel light brown with prominent setiforms; flagellomere one 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 (Table S6). Chaetotaxy: Thorax glabrous except for few sparse setae; scutellum with numerous setae grouped at posterolateral corner ($n \approx 15$); 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 (Fig. 8B, 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 $1.25\times$ 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.

Adult female. 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).

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 $0.45\times$ head width; palpi with five palpomeres, distal four segment proportions 1.0, 1.3, 1.4, 3.2. Antennal flagellomeres cylindrical in shape; ultimate flagellomere $1.67\times$ length of penultimate flagellomere; scape and pedicel light brown with prominent setiforms; flagellomere one 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 (Table S6). Chaetotaxy: Thorax glabrous except for sparse setae; scutellum with numerous setae grouped at posterolateral corner ($n \approx 15$); 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. 8D): 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 six to eight setiforms laterally; hypogynial plate with numerous small setae; epiproct with two prominent setiforms apically.

Type material. Holotype (adult male, reared): U.S.A.: Virginia: Giles Co: Little Walker Creek @ Hwy 100, $37^{\circ}12'N$ $80^{\circ}44'W$, 1844 ft, 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 [nine instar IV L (EtOH), three instar IV L (slides), three male and three female P (EtOH), one male and one female Pex (slides), one male and one female A (reared, pinned, head and genitalia slide mounted), two male and two female A (reared, pinned, head and genitalia in glycerin microvial)]. Paratypes deposited in CNC, LACM and USNM.

Other material examined. U.S.A.: Virginia: Giles Co: Little Walker Creek @ Hwy 100, $37^{\circ}12'N$ $80^{\circ}44'W$, 1844 ft, 27 April 2009 [LP]; Walker Creek nr confl with Little Walker Creek, $37^{\circ}12'N$ $80^{\circ}44'W$, 1796 ft, 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. *Blepharicera crista* has been collected from only two locations in Giles County, VA. The locations are within 500 m of each other.

Bionomics. Collection records indicate *B. crista* is probably 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. *Blepharicera 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 similar to those of other species in the group except for the respiratory lamellae, which are not convergent, as in many *Blepharicera*, and the dorsal cuticle, which has a faintly reticulate pattern. Adult males of *B. crista* can be distinguished by features of the genitalia and by the 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, sp.n.

(Figs 9A–H, 10A–E)

Diagnosis. A medium-sized *Blepharicera*. *Larva.* Dorsal secondary sensilla numerous, digitiform; ecdysial line with short stem line. *Pupa.* Body outline roughly ellipsoid; papillae dark brown with minute spinules; cuticle with micropunctures; anal tergite wrinkled; distance between lamellae at base $\approx 2\times$ basal width of individual lamella. *Adult male.* Dorsal eye division smaller ($0.33\times$) 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. *Adult female.* Eyes divided; callis oculi present; hypogynial valves short, rounded apicolaterally,

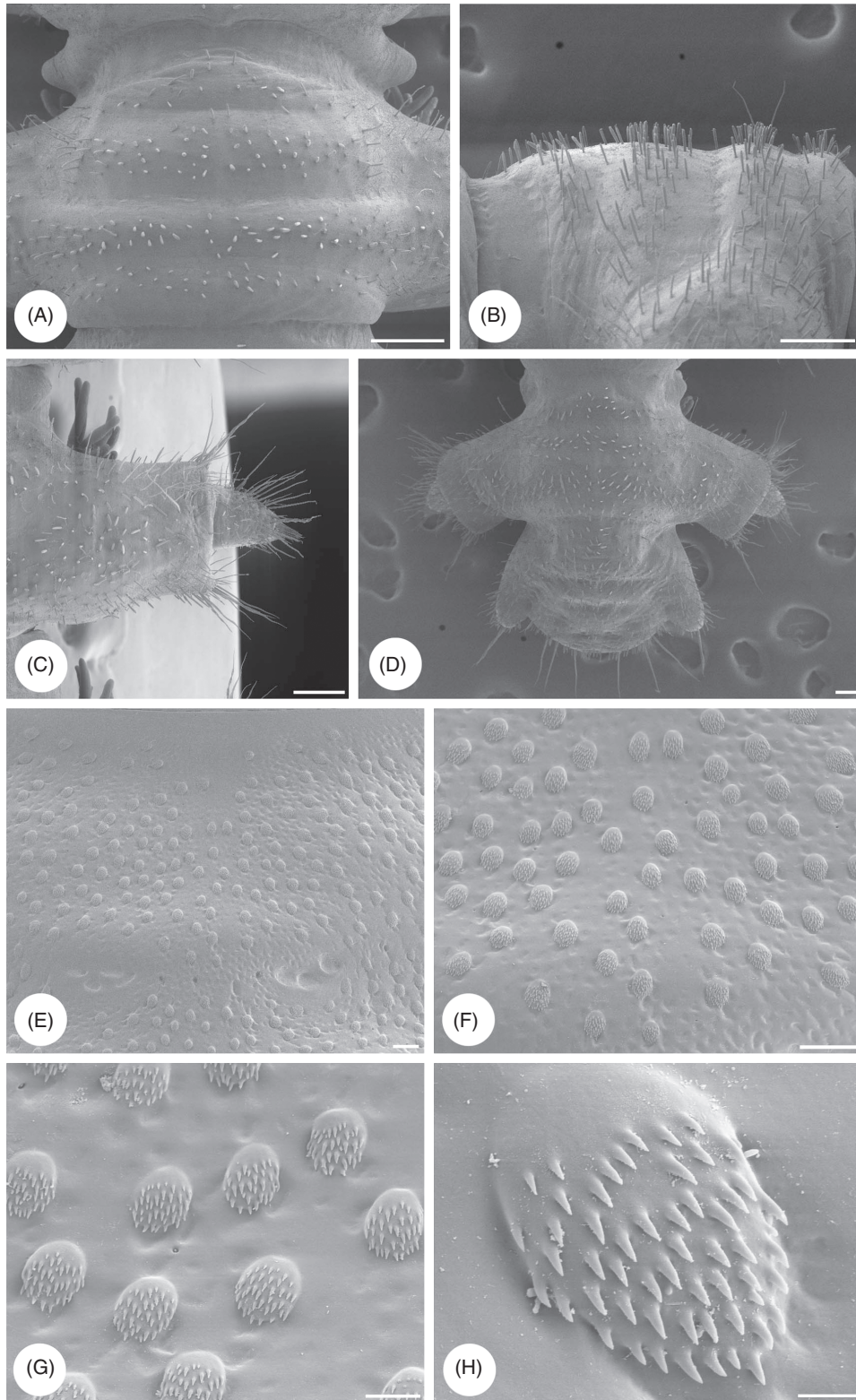


Fig. 9. Scanning electron micrographs of larval and pupal *Blepharicera enoristera*. (A) Larval dorsal sensilla, dorsal view; (B) larval dorsal sensilla, lateral view; (C) proleg on abdominal segment III, dorsal view on right side; (D) anal division, abdominal segments VI–X, dorsal view; (E–G) pupal 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).

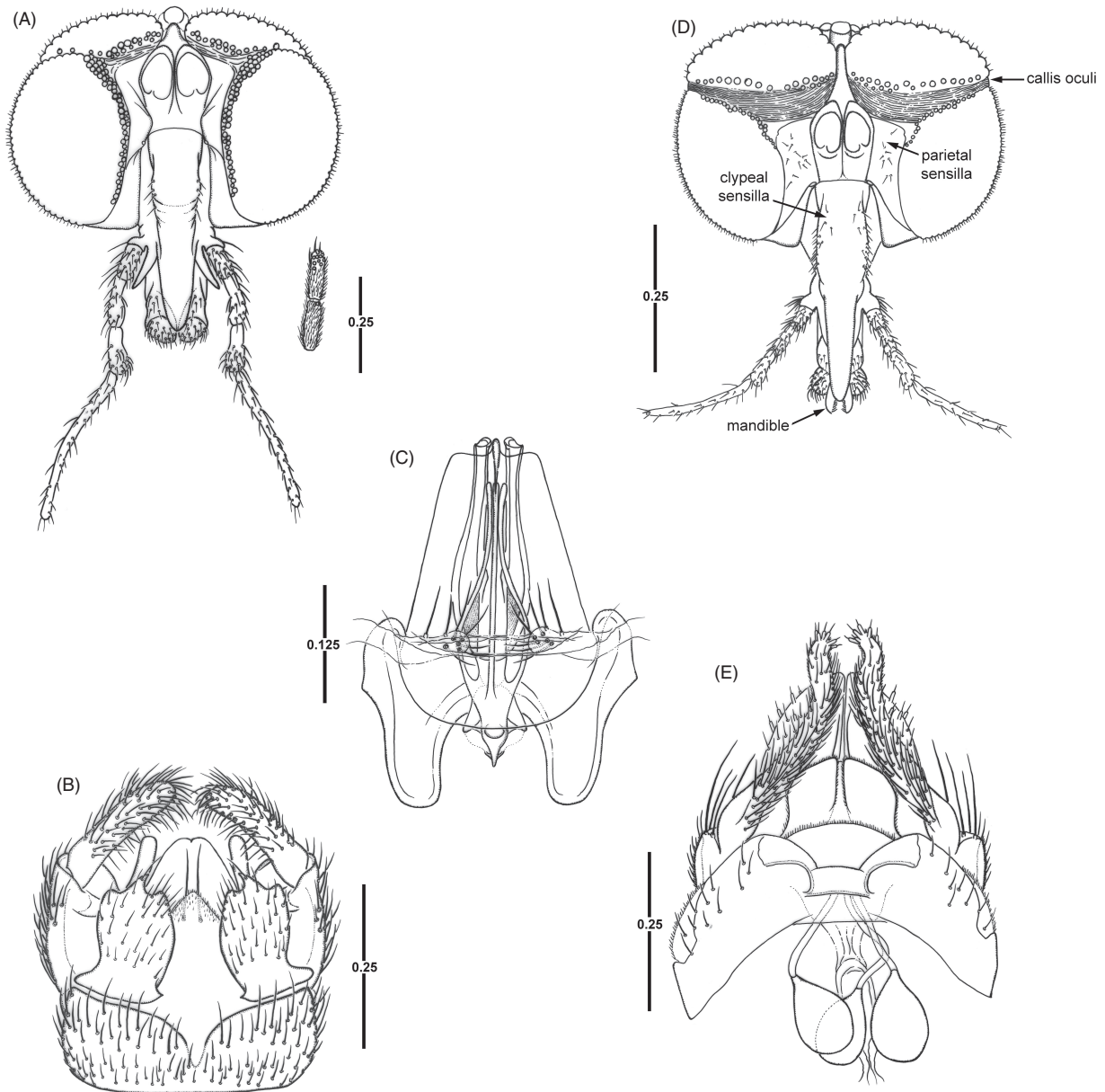


Fig. 10. Adults of *Blepharicera enoristera*. (A) Male head and antennal apex, frontal view; (B) male terminalia, dorsal view; (C) male terminalia (phallic structures), dorsal view; (D) female head, frontal view; (E) female terminalia, ventral view.

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. *Larva* (Fig. 9A–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 four to six prominent setiforms marginally.

Pupa (Fig. 9E–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 $\approx 2 \times$ 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).

Head (Fig. 10A): Structure: eyes semidichoptic, interocular ridge present, interocular distance 0.05 mm; eye divided; callis oculi absent; dorsal division contiguous with ventral, smaller ($0.33 \times$) than ventral; dorsal ommatidia larger in diameter; dorsal division with 13 rows of ommatidia along mid-meridian. Clypeus length/width = 2.2. Proboscis about $0.37 \times$ head width; mandibles absent; palpi with five palpomeres, distal four segment proportions 1.0, 1.3, 1.4, 4.5. Antennal flagellomeres cylindrical; ultimate flagellomere $1.2 \times$ length of penultimate flagellomere; scape and pedicel brown with prominent setiforms; flagellomere one 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 (≈ 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 (Table S7). Chaetotaxy: Thorax glabrous except for few sparse setae; scutellum with numerous setae grouped at posterolateral corner ($n \approx 15$); 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 (Fig. 10B, 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.

Adult female. 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).

Head (Fig. 10D): 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 $0.52 \times$ head width; palpi with five palpomeres, distal four segment proportions 1.0, 1.2, 1.4, 3.7. Antennal flagellomeres cylindrical in shape; ultimate flagellomere $1.53 \times$ length of penultimate flagellomere; scape and pedicel light brown with prominent setiforms; flagellomere one 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 (Table S7). Chaetotaxy: Thorax glabrous except for sparse setae; scutellum with numerous setae grouped at posterolateral corner ($n \approx 15$); 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. 10E): 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 apicomeres, 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 five to 15 setiforms laterally and 15 setiforms medially; hypogynial plate with numerous small setae; epiproct with two prominent setiforms apically.

Type material. Holotype (adult male, reared): U.S.A.: Georgia: Murray Co: Jacks River @ Cottonwood Patch campsites, $34^{\circ}59'N$ $84^{\circ}38'W$, 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 [four instar IV L (EtOH), three male and four female P (EtOH), one male and one female A (reared, pinned, head and genitalia slide mounted), two male A (reared, pinned, genitalia in glycerin microvial)]; 13 March 2007 [five instar IV L (EtOH), two 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. U.S.A.: 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 with the emergence dates of *B. opistera*, another new species from Jacks River.

Distribution. *Blepharicera enoristera* has been collected from Jacks River and Conasauga River where they flow through the Cherokee National Forest of southeastern Tennessee and the Cohutta Wilderness of north central Georgia. Additional larval and pupal collections were made in Mill Creek (35°52'N 84°43'W), Rock Creek (34°44'N 84°40'W) and Holly Creek (34°48'N 84°39'W) in 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 26 March 2009 yielded only males and females of *B. enoristera*. This suggests an approximately 4 week divergence in emergence times for *B. enoristera* in comparison with the emergence times of *B. appalachiae* and *B. opistera*, which were present in a collection from 29 April 2007.

Taxonomic comparison. *Blepharicera enoristera* larvae have a short ecdysial stem line and are much darker 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 apicomeresally. The hypogynial valves of *B. appalachiae* and *B. opistera* are rounded apically.

***Blepharicera hillabee* Jacobson & Courtney, sp.n.**

(Figs 11A–F, 12A–D)

Blepharicera sp. Courtney 2000b: 59 (as 'Unplaced Specimens').

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 (0.25×) 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* (Fig. 11A–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, approximately 30 in number; dorsal secondary sensilla numerous, fustiform; setiforms mostly absent medially, numerous on lateral lobes; anal division with six to seven prominent setiforms marginally.

Pupa (Fig. 11D–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).

Head (Fig. 12A): Structure: eyes semidichoptic, interocular distance 0.13 mm; eye divided; callis oculi absent; dorsal division contiguous with ventral, smaller (0.25×) than ventral; dorsal ommatidia larger in diameter; dorsal division with ten rows of ommatidia along mid-meridian. Clypeus length/width = 2.2. Proboscis about 0.37× head width; mandibles absent; palpi with five palpomeres, distal four segment proportions 1.0, 1.1, 1.3, 3.5. Antennal flagellomeres cylindrical; ultimate flagellomere 1.5× length of penultimate flagellomere; scape pale and pedicel brown, both with prominent setiforms; flagellomere one 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,

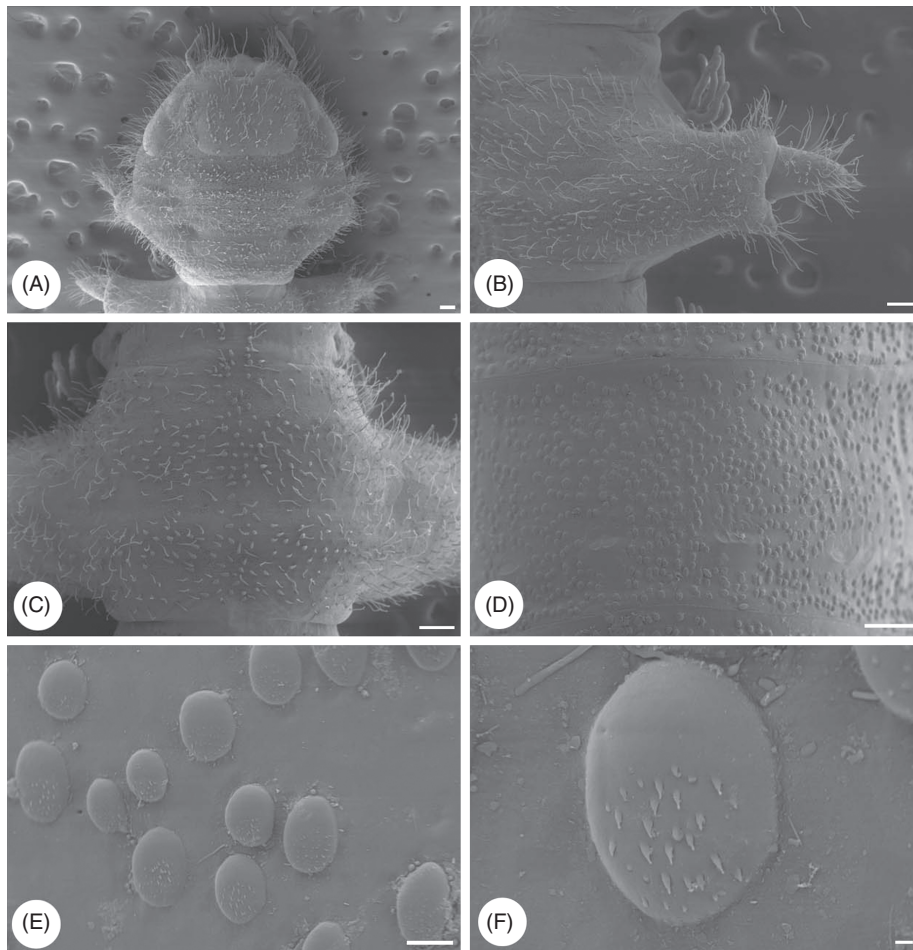


Fig. 11. Scanning electron micrographs of larval and pupal *Blepharicera hillabee*. (A) Cephalic division, dorsal view; (B) proleg on abdominal segment III, dorsal view on right side; (C) larval dorsal sensilla; (D, E) pupal abdominal tergite microsculpture; (F) pupal abdominal papilla. Scale bars = 1 μm (F), 10 μm (E), 100 μm (A–D).

hindleg 39:35:14:5:3:2:2 (Table S8). Chaetotaxy: Thorax glabrous except for few sparse setae; scutellum with numerous setae grouped at posterolateral corner ($n \approx 15$); 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 (Fig. 12B, 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.

Adult female. 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).

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 $0.47\times$ head width; palpi with five palpomeres, distal four segment proportions 1.0, 1.1, 1.3, 2.8. Antennal flagellomeres cylindrical in shape; ultimate flagellomere $1.97\times$ length of penultimate flagellomere; scape and pedicel light brown with prominent setiforms; flagellomere one 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).

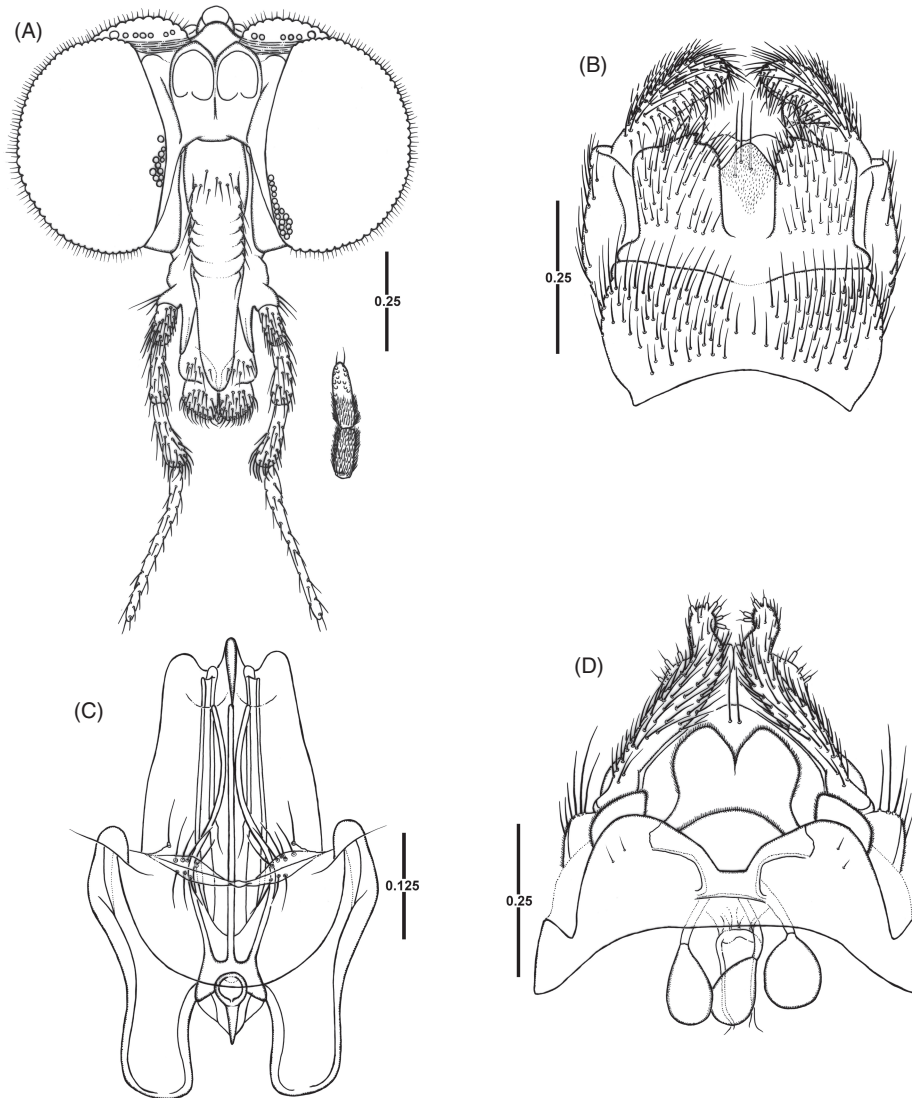


Fig. 12. Adults of *Blepharicera hillabee*. (A) Male head and antennal apex, frontal view; (B) male terminalia, dorsal view; (C) male terminalia (phallic structures), dorsal view; (D) female terminalia, ventral view.

Leg segment 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 (Table S8). Chaetotaxy: Thorax glabrous except for sparse setae; scutellum with numerous setae grouped at posterolateral corner ($n \approx 25$); coxae with prominent setae. Coloration: Frons and clypeus light brown, pruinose. Thorax light brown, pruinose. Fore-coxae light brown, other coxae pale. Abdominal tergites light brown, sternites pale. Terminalia (Fig. 12D): 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 two to four setiforms laterally; hypogynial plate with numerous small setae; epiproct with two prominent setiforms apically.

Type material. Holotype (adult male, reared): U.S.A.: 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 [two instar IV L (slides), one male and one female Pex (slides), two male and two female A (reared, pinned, head and genitalia slide mounted), two male and two female A (reared, pinned, genitalia in glycerin microvial)]; 25 April 2007 [nine instar IV L (EtOH), three male and three 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 derived from the Muskogee (Creek) word 'helype', 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 larval and pupal material and reared adults.

Bionomics. Collection records indicate *B. hillabee* is probably 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. Although 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 similar to those of 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, whereas the gland in *B. hillabee* is narrow, $\approx 3\times$ longer than wide.

Blepharicera opistera Jacobson, sp.n.

(Figs 13A–H, 14A–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 $>1.5\times$ basal width of individual lamella. *Adult male.* Dorsal eye division smaller ($0.25\times$) than ventral; cerci quadrate; posterior margin trilobate; lateral and medial margins subparallel; 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* (Fig. 13A–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 four to six prominent setiforms marginally.

Pupa (Fig. 13F–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 $>1.5\times$ 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).

Head (Fig. 14A): Structure: eyes semidichoptic, interocular ridge present, interocular distance 0.06 mm; eye divided; callis oculi absent; dorsal division contiguous with ventral, smaller ($0.25\times$) than ventral; dorsal ommatidia larger in diameter; dorsal division with 11 rows of ommatidia along mid-meridian. Clypeus length/width = 2.2. Proboscis about $0.36\times$ head width; mandibles absent; palpi with five palpomeres, distal four segment proportions 1.0, 1.2, 1.2, 3.9. Antennal flagellomeres cylindrical; ultimate flagellomere $1.1\times$ length of penultimate flagellomere; scape and pedicel brown with prominent setiforms; flagellomere one brown and glabrous in basal half, setose apically; remaining flagellomeres setose and brown. Chaetotaxy: Setiform groups as follows (number per

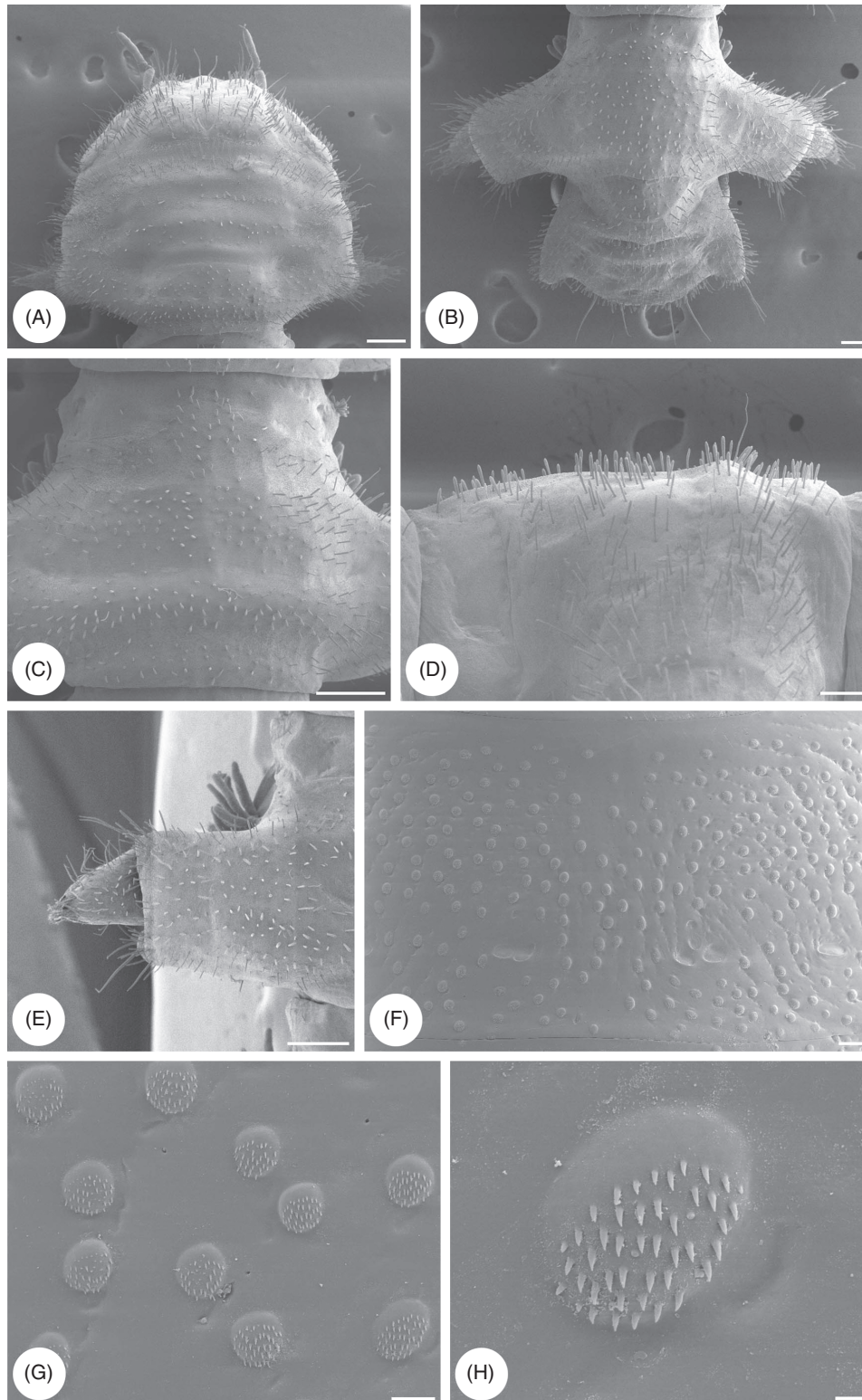


Fig. 13. Scanning electron micrographs of larval and pupal *Blepharicera opistera*. (A) Cephalic division, dorsal view; (B) anal division, abdominal segments VI–X, dorsal view; (C) larval dorsal sensilla, dorsal view; (D) larval dorsal sensilla, lateral view; (E) proleg on abdominal segment III, dorsal 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).

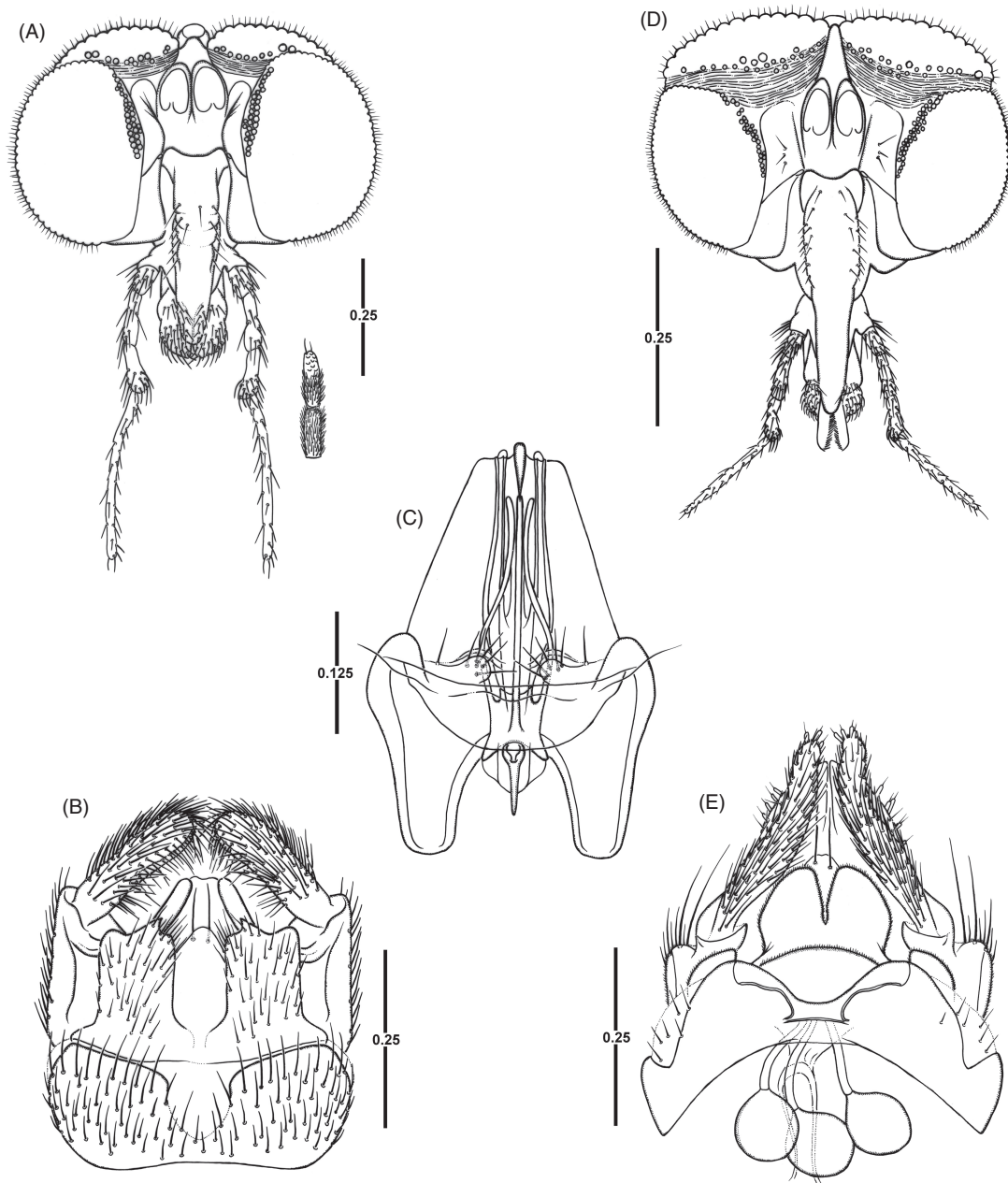


Fig. 14. Adults of *Blepharicera opistera*. (A) Male head and antennal apex, frontal view; (B) male terminalia, dorsal view; (C) male terminalia (phallic structures), dorsal view; (D) female head, frontal view; (E) female terminalia, ventral view.

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 (Table S9). Chaetotaxy: Thorax glabrous except for few sparse setae; scutellum with numerous setae grouped at posterolateral corner ($n \approx 15$); 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 (Fig. 14B, 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, intercal 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).

Head (Fig. 14D): Structure: eyes subholoptic, interocular ridge present, interocular distance 0.03 mm; eye divided; calli 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 $0.45\times$ head width; palpi with five palpomeres, distal four segment proportions 1.0, 1.3, 1.3, 3.6. Antennal flagellomeres cylindrical in shape; ultimate flagellomere $1.67\times$ length of penultimate flagellomere; scape light brown and pedicel brown with prominent setiforms; flagellomere one 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 (Table S9). Chaetotaxy: Thorax glabrous except for sparse setae; scutellum with numerous setae grouped at posterolateral corner ($n \approx 15$); 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. 14E): 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, 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 five setiforms laterally; hypogynial plate with numerous small setae; epiproct with two prominent setiforms apically.

Type material. Holotype (adult male, reared): U.S.A.: Georgia: Murray Co: Jacks River @ Cottonwood Patch campsites, $34^{\circ}59'N$ $84^{\circ}38'W$, 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 [one female A (reared, pinned, head and genitalia slide mounted), three male and one female A (reared, pinned, head and genitalia in glycerin microvial), three male A (reared, pinned, genitalia in glycerin microvial)]; 13 March 2007 [six instar IV L (EtOH), two instar IV L (slides)], coll. A.J. Jacobson, G.R. Curler and J.K. Moulton; 26 March 2009 [six instar IV L (EtOH), two male and two female P (EtOH)], coll. A.J. Jacobson

and G.R. Curler. Paratypes deposited in CNC, LACM and USNM.

Other material examined. U.S.A.: Tennessee: Polk Co: Conasauga River @ TN/GA State Line, $34^{\circ}59'N$ $84^{\circ}38'W$, 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 with the emergence dates of *B. enoristera*, another new species from Jacks River.

Distribution. *Blepharicera opistera* has been collected from Jacks River and Conasauga River in 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 yielded males and females of both *B. opistera* and *B. appalachiae*. This overlap suggests an approximately 4 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 those of *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, whereas the sensilla of *B. opistera* larvae are digitiform and randomly arranged. 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* is significantly larger than the upper eye division of *B. appalachiae*, but only slightly smaller than that 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, whereas the hypogynial valves of *B. enoristera* are straight along the entire length.

Morphological phylogenetic analysis

Results

Strict parsimony analysis of 44 characters in PAUP* 4.0b10 resulted in 180 equally parsimonious trees of 211 steps, whereas 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. 15). Character state transformations are plotted on the consensus tree (Fig. 16).

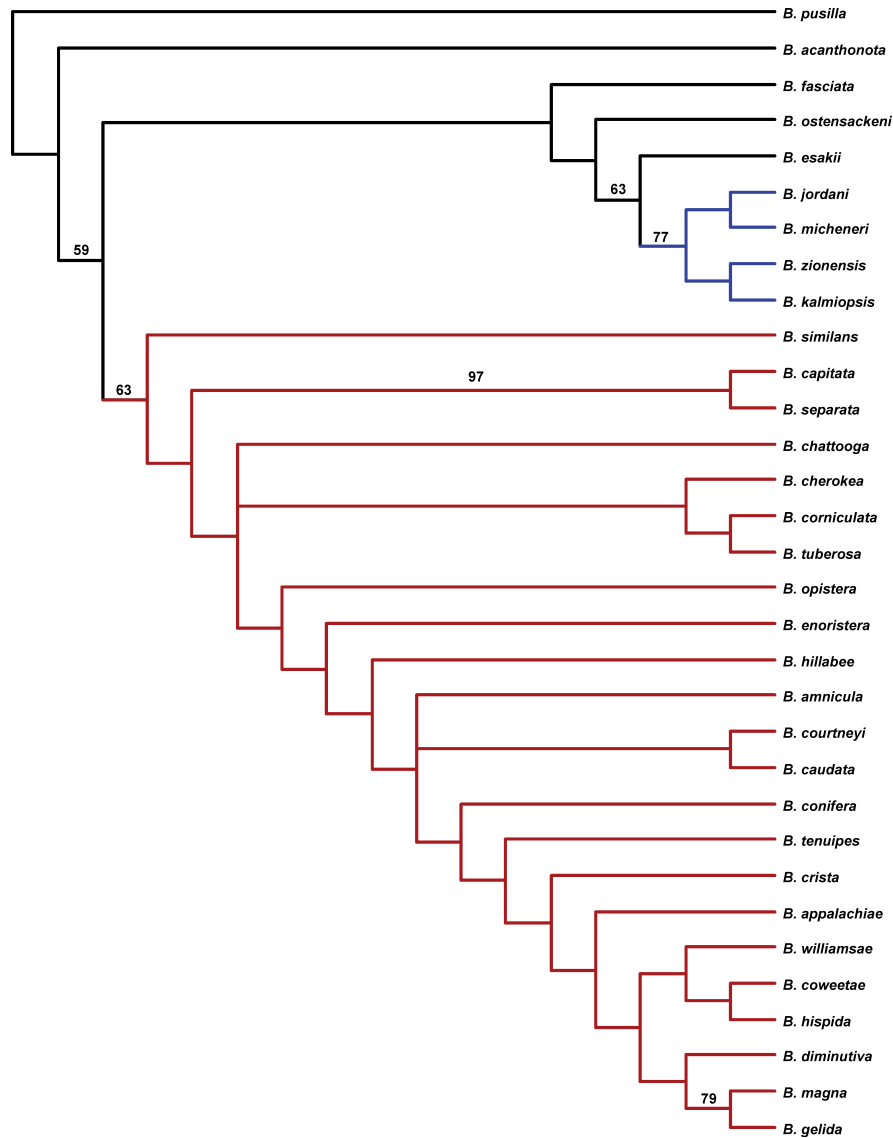


Fig. 15. Hypothesized phylogenetic relationships of Nearctic *Blepharicera* based on maximum parsimony analysis of morphological data. Values above branches denote bootstrap support. Black branches = outgroup taxa; red branches = *B. tenuipes* group; blue branches = *B. micheneri* group.

Discussion

Phylogenetic analyses were 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 from representatives of all described Nearctic species and supported the monophyly of both the *B. micheneri* and the *B. tenuipes* groups. The monophyly of the *B. micheneri* group was supported by the following synapomorphies: larval prolegs obscured in dorsal view, adult male ultimate antennal flagellomere $>2\times$ length of penultimate, adult male dorsal paramere rectangular, female ultimate antennal flagellomere $>2\times$ length of penultimate, and female spermathecal duct long extending approximately half 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 with aedeagal rods) is subequal and male cercus is triangular. The 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, probably due to conflicting characters and high levels of homoplasy.

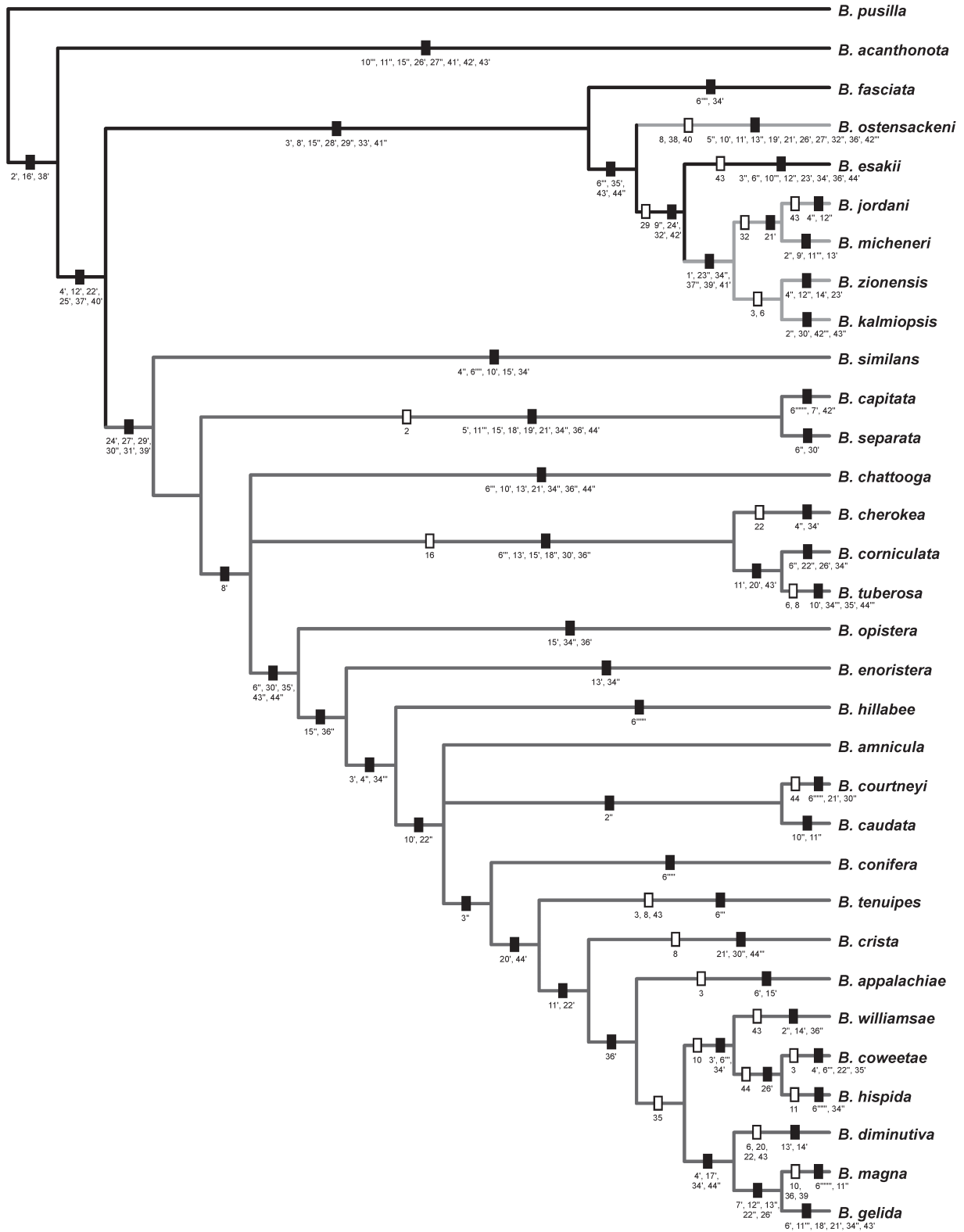


Fig. 16. Character state distribution within hypothesized phylogenetic relationships of Nearctic *Blepharicera* based on maximum parsimony analysis of morphological data. Black boxes indicate derived character states and white boxes indicate character reversals. Numbers below boxes denote characters and accents denote character states: ' = character state 1; '' = character state 2, etc. Black branches = outgroup taxa; dark grey branches = *B. tenuipes* group; light grey branches = *B. micheneri* group and *B. ostensackeni*.

These analyses provided a test of the hypotheses of Hogue (1978), Zwick (1984), Hogue & Georgian (1986) and Jacobson (2006). The results concurred with those of Jacobson (2006), revealing that the Nearctic fauna is probably 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, the *B. tenuipes* group and *B. ostensackeni* arriving at different times.

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, although 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. cherokeea*; 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*. Like the earlier morphological phylogeny, bootstrap values provide little support for resolution within the tree and many nodes collapse in the consensus. This is probably 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* is either nested within the western clade (*B. micheneri* group and *B. ostensackeni*) or forms a sister group (with *B. ostensackeni*) to the *B. micheneri* group.

Our analyses represent the most up-to-date phylogenetic study of Nearctic *Blepharicera*. However, some life stages of *B. caudata* are unknown and thus could not be included in this analysis. 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 areas of study should emphasize the discovery and description of these life stages. Additional collections in the eastern U.S.A. 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. The inclusion of more Asian *Blepharicera* species is necessary to better elucidate the origins of the Nearctic fauna. Ideally, a genus-level study would help to clarify the relationships.

General discussion

Although the *B. tenuipes* group has been extensively studied over the past 20 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 (1987) 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 (2000b), the new species discovered by Moulton & Curler (2007) and the six new species described here.

The *B. tenuipes* group is unique in its pattern of diversity. In aquatic Diptera, the generic diversity, and in some groups species diversity, is greater in the western Nearctic than the eastern Nearctic (Courtney, personal communication). However, eastern Nearctic *Blepharicera*, in comparison with the western Nearctic *Blepharicera*, exhibits a higher level of diversity, with 23 and five described species, respectively.

Extreme morphological similarities abound, particularly in immature stages, thus encumbering studies on the Nearctic fauna (Georgian & 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 many species. Most females in this study were associated through the ontogenetic method of dissecting pharate adults from pupae, but difficulties with associating females were encountered for some sympatric species.

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. *Blepharicera conifera* and *B. ammicula* 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. *Blepharicera 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*. *Blepharicera enoristera* and *B. opistera* have been discovered in several locations in the Cherokee National Forest and Cohutta Wilderness in southeastern Tennessee and north central Georgia. *Blepharicera 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 in western Virginia appeared initially to be ideal blepharicerid habitats, both incorporating drainages that are in valleys, separated from other sources. Upon further inspection of the sites, it was unclear whether blepharicerids would be present due to the high impact of agriculture in the area. Highly impacted areas typically do not yield blepharicerid midges. Surprisingly, there were up to four sympatric species at these locations, which indicates a higher level of tolerance to environmental impacts.

Unplaced specimens

Two representatives from the Locust Fork of the Warrior River in north central Alabama were included in a molecular analysis. Initially it was believed that these specimens represented a new species. Preliminary molecular results indicate they are distinct, with high levels of support, but morphologically they are nearly indistinguishable from *B. hillabee* (Jacobson & Moulton, unpublished). 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.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/j.1365-3113.2011.00595.x

Table S1. Location and species names for Nearctic *Blepharicera*.

Table S2. Characters and states used in the phylogenetic analysis of Nearctic *Blepharicera*.

Table S3. Matrix of characters and states used in the phylogenetic analysis of Nearctic *Blepharicera*.

Table S4. Leg segment measurements for adult males and females of *Blepharicera amnicula* Jacobson & Moulton **sp.n.**

Table S5. Leg segment measurements for adult males and females of *Blepharicera confifera* Jacobson **sp.n.**

Table S6. Leg segment measurements for adult males and females of *Blepharicera crista* Jacobson & Curler **sp.n.**

Table S7. Leg segment measurements for adult males and females of *Blepharicera enoristera* Jacobson **sp.n.**

Table S8. Leg segment measurements for adult males and females of *Blepharicera hillabee* Jacobson & Courtney **sp.n.**

Table S9. Leg segment measurements for adult males and females of *Blepharicera opistera* Jacobson **sp.n.**

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References

- Agassiz, L. (1846a) Nomina systematica generum dipterorum. *Nomenclator Zoologicus*, Fasc. 9/10 (ed. by L. Agassiz), pp. vi+42. Jent and Gassmann, Solothurn.
- Agassiz, L. (1846b) *Nomenclatoris Zoologici Index Universalis*. Jent and Gassmann, Solothurn.
- Aldrich, J.M. (1905) A catalog of North American Diptera. *Smithsonian Miscellaneous Collections*, **46**, 1–680.
- Alexander, C.P. (1922) An undescribed net-winged midge from Japan. *Insector Inscitiae Menstruus*, **10**, 21–23.
- Alexander, C.P. (1953) Undescribed species of nematoceros Diptera. Part II. *Bulletin of the Brooklyn Entomological Society*, **48**, 41–49.
- Alexander, C.P. (1963) Family Blepharoceridae. In Guide to the insects of Connecticut. Part VI. The Diptera or true flies of Connecticut. Eighth fascicle. *Bulletin of the Connecticut State Geology and Natural History Survey*, **93**, 39–71.
- Alverson, A.J. & Courtney, G.W. (2002) Temporal patterns of diatom ingestion by larval net-winged midges (Diptera: Blephariceridae: *Blepharicera*). *Freshwater Biology*, **47**, 2087–2097.
- Alverson, A.J., Courtney, G.W. & Luttenton, M.R. (2001) Niche overlap of sympatric *Blepharicera* larvae (Diptera: Blephariceridae) from the southern Appalachian Mountains. *Journal of the North American Benthological Society*, **20**, 564–581.
- Anderson, N.H. (1992) Influence of disturbance on insect communities in Pacific Northwest streams. *Hydrobiologia*, **248**, 79–82.
- Courtney, G.W. (1990) Cuticular morphology of larval mountain midges (Diptera: Deuterophlebiidae): implications for the phylogenetic relationships of Nematocera. *Canadian Journal of Zoology*, **68**, 556–578.
- Courtney, G.W. (1991) Life history patterns of Nearctic mountain midges (Diptera: Deuterophlebiidae). *Journal of the North American Benthological Society*, **10**, 177–197.
- Courtney, G.W. (1998) A method for rearing pupae of net-winged midges (Diptera: Blephariceridae) and other torrenticolous flies. *Proceedings of the Entomological Society of Washington*, **100**, 742–745.
- Courtney, G.W. (2000a) A.1. Family Blephariceridae. *Contributions to a Manual of Palaearctic Diptera. Appendix* (ed. by L. Papp and B. Darvas), pp. 7–30. Science Herald, Budapest.
- Courtney, G.W. (2000b) Revision of the net-winged midges of the genus *Blepharicera* Macquart (Diptera: Blephariceridae) of eastern North America. *Memoirs of the Entomological Society of Washington*, **23**, 1–99.
- Courtney, G.W. & Duffield, R.M. (2000) Net-winged midges (Diptera: Blephariceridae): a food resource for Brook Trout in montane streams. *Pan-Pacific Entomologist*, **76**, 87–94.

- Courtney, G.W., Merritt, R.W., Cummins, K.W., Berg, M.B., Webb, D.W. & Foote, B.A. (2008) Ecological and distributional data for larval aquatic Diptera. *An Introduction to the Aquatic Insects of North America*, 4th edn (ed. by R. W. Merritt, K. W. Cummins and M. B. Berg), pp. 747–771. Kendall/Hunt Publishing, Dubuque, IA.
- Curran, C.H. (1923) Studies in Canadian Diptera II. The genera of the family Blepharoceridae. *Canadian Entomologist*, **55**, 266–269.
- Curran, C.H. (1934) *The Families and Genera of North American Diptera*. The Ballou Press, New York, NY.
- Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.
- Frutiger, A. (1998) Walking on suckers – new insights into the locomotory behavior of larval net-winged midges (Diptera: Blephariceridae). *Journal of the North American Benthological Society*, **17**, 104–120.
- Frutiger, A. (2002) The function of the suckers of larval net-winged midges (Diptera: Blephariceridae). *Freshwater Biology*, **47**, 293–302.
- Georgian, T. & Wallace, J.B. (1983) Seasonal production dynamics in a guild of periphyton-grazing insects in a southern Appalachian stream. *Ecology*, **64**, 1236–1248.
- Goloboff, P.A., Farris, J.S. & Nixon, K.C. (2008) TNT, a free program for phylogenetic analysis. *Cladistics*, **24**, 774–786.
- Hennig, W. (1966) *Phylogenetic Systematics*. University of Illinois Press, Urbana, IL.
- Hogue, C.L. (1978) The net-winged midges of eastern North America, with notes on new taxonomic characters in the family Blephariceridae (Diptera). *Contributions in Science, Natural History Museum of Los Angeles County*, **291**, 1–41.
- Hogue, C.L. (1987) Blephariceridae. *Flies of the Nearctic Region*, Vol. 2 (ed. by G. C. D. Griffiths), pp. 1–172. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.
- Hogue, C.L. & Georgian, T. (1986) Recent discoveries in the *Blepharicera tenuipes* group, including description of two new species from Appalachia (Diptera: Blephariceridae). *Contributions in Science, Natural History Museum of Los Angeles County*, **377**, 1–20.
- Jacobson, A.J. (2006) *Phylogenetic analysis of Nearctic Blepharicera Macquart (Diptera: Blephariceridae)*. MSc Thesis, Iowa State University, Ames, IA.
- Jacobson, A.J. & Courtney, G.W. (2008) A new species of *Blepharicera* Macquart (Diptera: Blephariceridae) from western North America. *Proceedings of the Entomological Society of Washington*, **110**, 978–987.
- Johannsen, O.A. (1934) Aquatic Diptera. Part I. Nematocera, exclusive of Chironomidae and Ceratopogonidae. *Memoirs of the Cornell University Agricultural Experimental Station*, **164**, 1–71.
- Johns, J.A. (1996) *The net-winged midges (Diptera: Blephariceridae) of the southeastern United States: phenology and ecology*. MSc Thesis, Clemson University, Clemson, SC.
- Kellogg, V.L. (1903) The net-winged midges (Blepharoceridae) of North America. *Proceedings of the California Academy of Sciences (Series 3)*, **3**, 187–233.
- Kitakami, S. (1931) The Blepharoceridae of Japan. *Memoirs of the College of Science, Kyoto Imperial University, Series B*, **6**, 53–108.
- Lenat, D.R. (1993) A biotic index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water quality ratings. *Journal of the North American Benthological Society*, **12**, 279–290.
- Loew, H. (1844) Beschreibung einiger neuen Gattungen der europäischen Dipterenfauna. *Stettiner Entomologische Zeitung*, **5**, 118–123.
- Loew, H. (1858) Ueber einige neue fliegengattungen. *Berliner Entomologische Zeitschrift*, **2**, 101–122.
- Loew, H. (1863) Diptera Americae septentrionalis indigena. Centuria quarta. *Berliner Entomologische Zeitschrift*, **7**, 275–326.
- Loew, H. (1869) La famiglia dei Blepharoceridi (Blepharoceridae). *Bollettino della Società Entomologica Italiana*, **1**, 85–98.
- Loew, H. (1877) Revision der Blepharoceridae. *Zeitschrift für Entomologie, Breslau*, **6**, 54–98.
- Macquart, J.M. (1843) Description d'un nouveau genre d'insectes Diptères. *Annales de la Société Entomologique de France*, **1**, 59–63.
- Maddison, D.R. & Maddison, W.P. (2002) *MacClade 4: Analysis of Phylogeny and Character Evolution, Version 4.05*. Sinauer Associates, Sunderland, MA.
- Maddison, W.P., Donoghue, M.J. & Maddison, D.R. (1984) Outgroup analysis and parsimony. *Systematic Zoology*, **33**, 83–103.
- Moulton, J.K. & Curler, G.R. (2007) A new species of net-winged midge of the genus *Blepharicera* macquart (Diptera: Blephariceridae) from the Cumberland plateau of Tennessee. *Proceedings of the Entomological Society of Washington*, **109**, 920–929.
- Osten Sacken, C.R. (1895) Contribution to the study of the Liponeuridae Loew (Blepharoceridae Loew olim). *Berliner Entomologische Zeitschrift*, **40**, 148–169.
- Oosterbroek, P. & Courtney, G.W. (1995) Phylogeny of the nematoceros families of Diptera (Insecta). *Zoological Journal of the Linnean Society*, **115**, 267–311.
- Schuh, R.T. (2000) *Biological Systematics. Principles and Applications*. Cornell University Press, Ithaca, NY.
- Swofford, D.L. (2003) *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0b10*. Sinauer Associates, Sunderland, MA.
- Watrous, L.E. & Wheeler, Q.D. (1981) The out-group comparison method of character analysis. *Systematic Zoology*, **30**, 1–11.
- Westwood, J.O. (1842) Description de l' *Asthenia fasciata*. *Guérin's Magazine of Zoology*, **2**, 94.
- Wiley, E.O. (1981) *Phylogenetics. The Theory and Practice of Phylogenetic Systematics*. John Wiley and Sons, New York, NY.
- Wood, D.M. & Borkent, A. (1989) 114. Phylogeny and classification of the Nematocera. *Manual of Nearctic Diptera, Agriculture Canada Monograph*, **32**, Vol. 3 (ed. by J. F. McAlpine, B. V. Peterson, G. E. Shewell, H. J. Teskey, J. R. Vockeroth and D. M. Wood), pp. 1333–1370. Research Branch, Agriculture Canada, Ottawa.
- Zwick, P. (1977) Australian Blephariceridae (Diptera). *Australian Journal of Zoology Supplementary Series*, **46**, 1–121.
- Zwick, P. (1984) *Phylogenetic System and Distribution of the Genus Blepharicera (Diptera: Blephariceridae)*. International Congress of Entomology, Hamburg.
- Zwick, P. (1990) Systematic notes on Holarctic Blephariceridae (Diptera). *Bonner Zoologische Beiträge*, **41**, 231–257.

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