Early Nymphal Development in *Ephoron leukon* (Ephemeroptera: Polymitarcyidae) with Particular Emphasis on Mouthparts and Abdominal Gills

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ABSTRACT The early stages of nymphal development of *Ephoron leukon* Williamson (Ephemeroptera: Polymitarcyidae) are described with special attention paid to the appearance and differentiation of the mouthparts and abdominal gills. Dramatic changes in the mouthparts of *E. leukon* include outgrowth of the mandibular tusks from the mandible proper, differentiation of the galealacinia and palps of the maxillae, and splitting of the labium into multiple lobes plus the adornment of various regions of the mouthparts with dense brushes of setae functioning in filter feeding. The abdominal gills of *E. leukon* originate as short threads, and then they progressively elongate and develop a fringe of long thin extensions around their margins, branch basally to form two separate lamellae and ultimately acquire a suite of structural adornments. In *E. leukon*, nymphs develop mature mouthparts and abdominal gills during early nymphal ontogeny, a developmental pattern that may facilitate key habitat transitions.

KEY WORDS *Ephoron leukon*, nymphal development, abdominal gills, mandibular tusks, mayfly ontogeny

Early nymphal development in mayflies (Ephemeroptera) is relatively understudied because only incomplete descriptions in a handful of species provide details of the morphological changes that occur over the course of nymphal ontogeny. Partial descriptions have been reported for the earliest stages of nymphal development of *Stenacron interpunctatum* (Say) [as *Stenonema* (Needham et al. 1935, also as *Stenonema canadense* (Walker) (Ide 1935c)], *Stenonema femoratum* (Say) [as *Stenonema tripunctatum* (Banks) (Ide 1935c)], *Maccaffertium vicarium* (Walker) (as *Stenonema fuscum* Clemens [Ide 1935c]), *Epeorus vitreus* (Walker) [as *E. humeralis* (Morgan) (Ide 1935c) and *Iron humeralis* (Morgan) (Needham et al. 1935)], *Epeorus pleuralis* (Banks) (as *Iron* [Ide 1935c]), *Leucrocuta hebe* (McDunnough) (as *Heptagenia* [Needham et al. 1935]), *Heptagenia pulla* (Clemens) [Ide 1935c], *Ecdyonurus forcipula* (Pictet) [Gros 1923] [Heptageniidae]; *Ephemera simulans* Walker (Ide 1935c), *Hexagenia bilineata* (Say) (Wiebe 1926), [Ephemeridae]; *Palingenia longicauda* (Olivier) (Landolt et al. 1997) [Palingeniidae]; *Isonychia bicolor* Walker (Ide 1935c) [Isonychiidae]; *Ephemerella subvaria* McDunnough (Ide 1935c) [Ephemerellidae]; *Paraleptophlebia debilis* (Walker) (as *Leptophlebia* [Ide 1935c]), *Leptophlebia cupida* (Say) (Clifford et al. 1979) [Leptophlebiidae]; *Siphlonurus typicus* (Eaton) (Kosnicki and Burian 2003) [Siphlonuridae]; and *Cloeon cognatum* Stephens (as *Chloeon dimidiatum* Curtis [Lubbock 1864]) [Baetidae]. Descriptions also exist for three polymitarycid species: Britt (1962) detailed the first six instars of *Ephoron album* (Say), Tsui and Peters (1974) described the embryos and first two instars of *Tortopus incertus* (Traver) (Polymitarcyidae), and Ide (1935a) figured the first two instars for *Ephoron leukon* Williamson.

Dramatic changes in the morphology of abdominal gills and mouthparts have been reported during early nymphal development (Ide 1935b). For example, the abdominal gills of newly hatched mayfly nymphs are strikingly different from that of black wing pad stage nymphs (e.g., see Ide 1935b for data from 11 species). Likewise, most species of burrowing mayflies possess large and spiny mandibular tusks absent from early instars but conspicuous in mature nymphs (Ide 1935b). In *Ephoron* spp., mandibular tusks are used in the creation of burrows in fine silt and gravel in rivers and lakes (Ide 1935a, McCafferty 1975).

Ephoron leukon (Polymitarcyidae) is a common burrowing mayfly found in medium- to large-sized rivers in the eastern and midwestern United States (McCafferty 1975). Synchronized emergence of nymphs into dense mating swarms of adults is characteristic of the genus *Ephoron* (Edmunds et al. 1956).

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After mating, females oviposit immediately and the eggs become attached to sand grains and other materials to form large clumps of egg masses [e.g., in *Ephoron shigae* (Takahashi) (Watanabe and Ohkita 2000)]. Eggs spend the winter in an obligate diapause, which is well documented in several species of *Ephoron* (Edmunds et al. 1956, Britt 1962, Giberson and Galloway 1985, Watanabe and Takao 1991, Greve et al. 1999). The eggs of *E. leukon* hatch in the spring, beginning in mid-May as reported for a populations in Virginia (Snyder et al. 1991). Nymphal development in *E. leukon* proceeds until emergence in mid- to late summer (Ide 1935a, Snyder et al. 1991).

Herein, I present a detailed description of the early nymphal stages of development in *E. leukon,* with special emphasis on the development of the mouthparts and abdominal gills. Previously, the early instars of *Ephoron album* (Say), *E. leukon*'s sister species (McCafferty 1975), were described in a series of light micrographs (Britt 1962), but the sole account of early nymphal development for *E. leukon* is a brief descriptions of the first two instars (Ide 1935a). A detailed understanding of nymphal development for a wider range of mayfly species is desirable to broaden the knowledge of mayfly developmental biology and understand the details of how ecologically important features develop.

Materials and Methods

E. leukon was cultured in the laboratory by first collecting eggs from fertilized females in the field using the approach developed for *Ephoron virgo* (Olivier) (Greve et al. 1999). A population of *E. leukon* from the Housatonic River (near Cornwall, CT) was sampled for the laboratory culture. Sampling efforts targeted hatches occurring in late July to mid-August, dependent upon collection year (2002–2006). Eggs were brought back to the laboratory and maintained at room temperature in glass culture dishes to complete prediapausal development in ≈6 wk. No aeration of the cultures was necessary because Britt (1962) demonstrated that *Ephoron* eggs are capable of surviving low oxygen conditions. Eggs were transitioned to cold storage at 4°C to undergo obligate diapause once development ceased and eyespots were clearly visible through the chorion.

To induce hatching, *E. leukon* eggs were removed from cold storage after ≈12 wk and placed in shallow glass culture dishes with ambient temperature water (20–23°C); hatching commenced in 5–7 d. Early instar nymphs were maintained on either an algal and diatom diet consisting of *Pediastrum tetras* (Ehrenberg) and *Navicula* (Bory) sp. or the ambient algal community that arose naturally in the culture dishes and was collected along with the eggs. Cultures were periodically refreshed with refrigerated filtered river water (3.0-μm then 0.45-μm filter size) (Millipore, Billerica, MA).

Black wing pad stage nymphs were collected from near the river's banks at dusk with a kicknet. Nymphs were placed in 80% ethanol upon collection for use in scanning electron microscopy (SEM) imaging. Both laboratory-reared and field-caught nymphs were then immersed in fixative containing 6% formaldehyde in phosphate-buffered saline + 0.1% Tween (PBTw) and subsequently heptanes were added to make a 2:1 ratio of buffer to heptanes. Specimens were shaken vigorously in fixative for 25 min at room temperature. The fixative was removed and the specimens were rinsed twice in PBTw. Specimens were dehydrated in an increasing ethanol series and left overnight in absolute ethanol. The following day, specimens were transferred into hexamethyldisilazane (HMDS) and washed three to four times for 20–25 min, each time in fresh HMDS. After the final wash, excess HMDS was removed and the specimens were air-dried for at least 10 min. Alternatively, the HMDS step was omitted, and specimens were dehydrated in an ethanol series, culminating in an overnight soak in absolute ethanol. All specimens were mounted on aluminum stubs by using silver paint or two-sided tape, sputter-coated with gold/palladium for at most 3 min to produce a 270-Å (or 27–30-nm-thick) coating, left overnight in a desiccator, and examined using a DSM982 Gemini Zeiss field electron microscope (Carl Zeiss, Oberkochen, Germany) at the University of Connecticut Biosciences Imaging Facility (Storrs, CT).

Results and Discussion

Development of Early Stage Nymphs of *E. leukon*: Mouthparts. *E. leukon* emerges from its egg case with fully functional mouthparts. The mandibles of hatchlings are quite large relative to the head capsule size and have the distal-most tips modified into 4–5 small teeth (Fig. 1A). The teeth correspond to the canine region of the mandible, whereas the molar region is located at <90° from the canines (obscured by the maxillae and not visible in the preparations). The canine and molar regions of the mandibles in mayflies are thought to function to strain organic material out of the water and/or grind tough plant material before ingestion (McShaffrey 1988). At the hatchling stage, the maxillae are composed of a fused galealacinia and short, unsegmented palps. The distal-most regions of the maxillae bear toothed structures highly similar to those on the distal tips of the mandibles that lie very close to the tips of the mandibles (Fig. 1A), suggesting that these teeth may act in concert during feeding in very young nymphs. The labium of the hatchling is composed of a medially split lingual region with small palps flanking it on either side (Fig. 1A). The wrinkled structure of the maxillary palps and most of the labium proper at this stage are not artifacts of specimen preparation, but instead reflect weak sclerotization, because adjacent mouthparts (and the majority of the body surface) are not wrinkled.

By the second instar, the mandibles enlarge (Fig. 1B) and the maxillae become elongated and bifid at their apices, composed of a toothed structure at the outer edge, and an inner brushlike structure protruding from a socket (Fig. 1B, inset). In combination, the toothed structures of the canine region of the man-
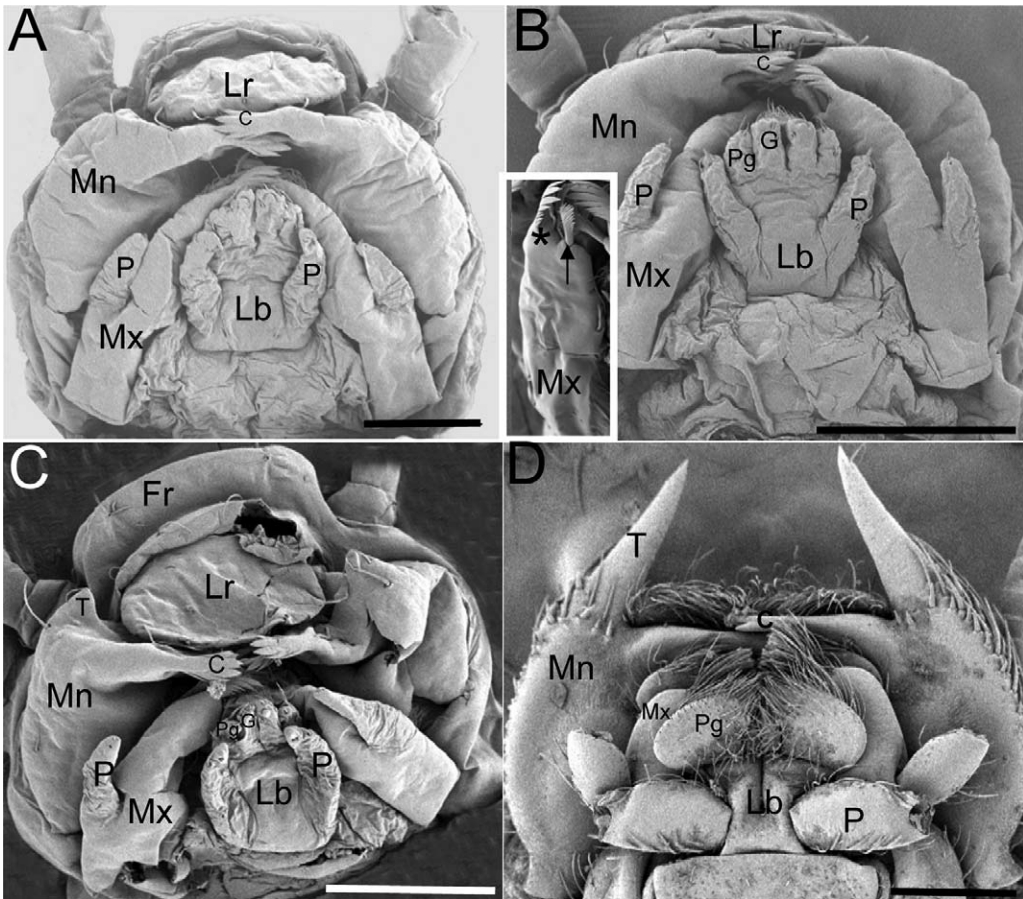
Fig. 1. Development of nymphal mouthparts in *E. leukon*. (A) First instar hatchling. (B) Second or third instar showing the enlargement of the mandibles and maxillae and the further splitting of the labium into two paraglossal and two glossal lobes. Note the canine region of the developing mandible (arrowhead) with its toothed apex. Inset shows the bifid apex of the maxilla: 1) a toothed comb on the outer region (* marks the base of the comb) that looks very similar to the canine region of the mandibles and 2) a brushlike structure on the inner margin (marked with an arrow). (C) Undetermined instar showing early outgrowth of tissue from the mandible that will form the tusk. (D) Undetermined instar showing small mandibular tusks. The maxillae are positioned dorsal to and consequently behind the labium in this prep. The third and most distal segment of the labial palps was broken off in preparation and the labrum is not visible from this perspective. Scale bar = 50 μm (A–C) and 200 μm (D). Ventral view in all specimens, anterior is up. Lr, Labrum; Mn, Mandible; Mx, Maxilla; Lb, Labium; P, Palp; T, tusk; Fr, frontal process; Pg, paraglossae; G, glossa; C, canine region.

dibles and maxillae form a set of teethlike structures situated ventral to the mouth. The maxillary palps have elongated but are not sclerotized, as is obvious from their wrinkled surfaces (Fig. 1B). The labium has split further across its lingual region into four lobes corresponding to two inner glossae and two outer paraglossae (Fig. 1B).

In contrast to the ventral mouthparts (mandibles, maxillae, and labium), the mandibular tusks extend anteriorly from the head and are initially evident as small growths off of the anterior-most margin of the mandible in hatchlings (Fig. 1C and D). The frontal process lies directly between the tusks and is the result of the fusion of the labrum and a portion of the anterior clypeus (Figs. 1C, 2B). The nascent tusks are short triangular stubs and are positioned at ≈90 from the canine region (Fig. 1C and D). As outgrowth

continues, the tusks become more pointed and progressively adorned with spines along their dorsal and lateral surfaces in addition to setae (Fig. 1D). Each tusk has at least 30 spines at black wing pad stage, a characteristic that distinguishes *E. leukon* from its sister species, *E. album* (McCafferty 1975). Strikingly, a related species, *T. incertus* hatches from the egg with small tusks in the first instar (Tsui and Peters 1974), illustrating variation in developmental modes even between species in two closely related genera.

Dramatic changes in the mouthparts of *E. leukon* occur over the early nymphal stages. Mature nymphal mouthparts in *E. leukon* are hypognathous with the mandible proper, maxillae, and labium positioned ventrally and mandibular tusks directed anteriorly (Fig. 2A) as well as a prominent frontal process (Fig. 2B) and dense brushes of setae scattered about the head and mouthparts (Fig.
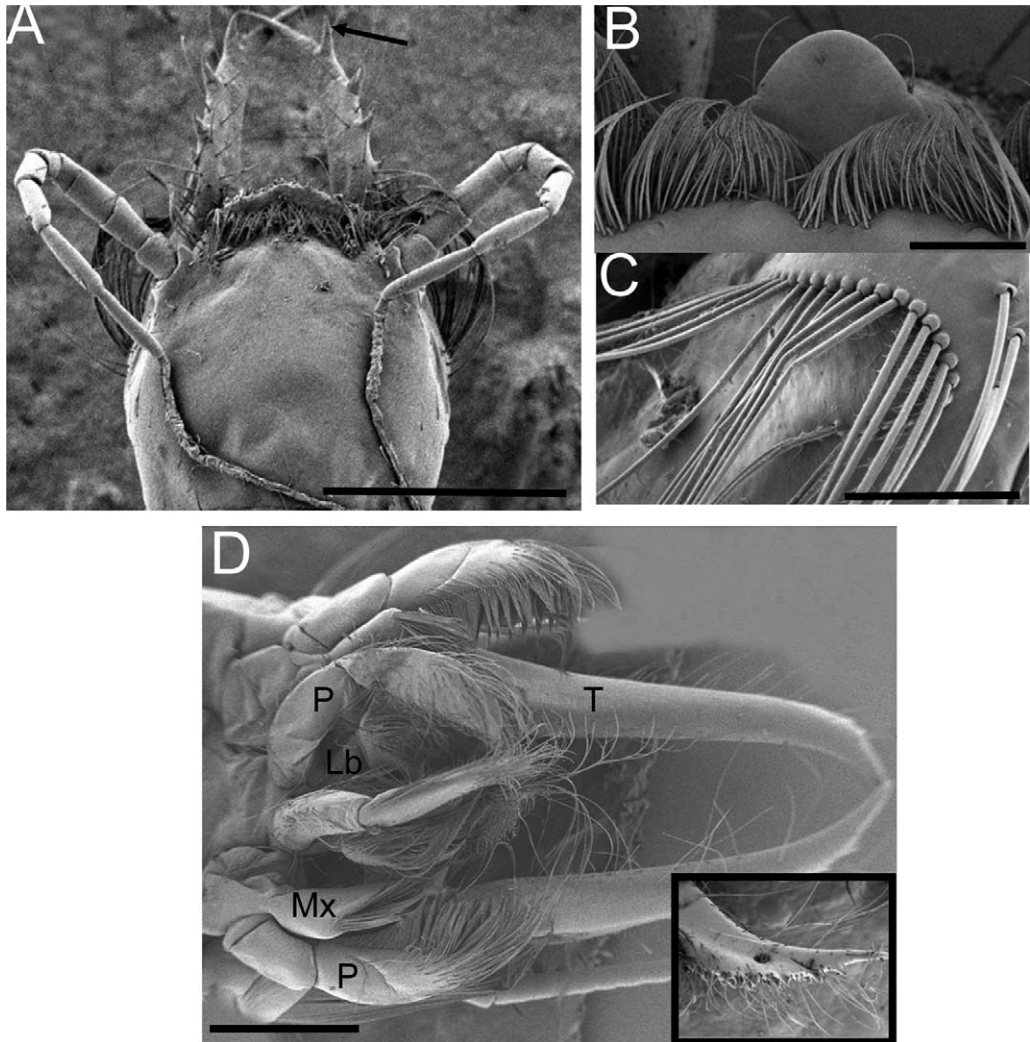
Fig. 2. Head and mouthpart morphology of submature and mature *E. leukon* nymphs. (A) Dorsal view of the head of a submature nymph showing elongate antennae, spines on the tusks (arrow), and regions of setation on the head. A close view of the lateral edge of the head is shown in C (marked with *). (B) Dorsal view of the rounded frontal process of a submature nymph with fringes of dense setae. (C) Close view of the setae adorning the anterolateral head of a mature nymph showing the rows of setae. (D) Ventral view of the mouthparts of a mature nymph, including the prominent mandibular tusks and the three-segmented maxillary and labial palps. Note that the base of the mandibles, including the canine and molar regions are completely obscured, and the labial base is recessed behind the labial palps and that the maxillary palps are much larger relative to the galealacinia region of the maxillae. Inset is a light micrograph of a dorsal view of a mature tusk showing the spines and setae lining its dorsal surface. Scale bar = 200 μm (A and B), 40 μm (C), and 1 mm (D). C, canine region; Mx, maxilla; Lb, labium; P, palp; T, tusk.

2B and C, D) thought to play a role in filter feeding (Bae and McCafferty 1995, McShaffrey 1988). As in other ephemeroid species (Ide 1935b), tusks in *E. leukon* arise from the body of the mandibles as opposed to the canine/incisor region as in leptophlebiid species (Edmunds and McCafferty 1996). In *E. album*, tusks are very prominent by the fifth or sixth instar (Britt 1962). The maxillae of mature *E. leukon* nymphs have three-segmented palps attached to the maxilla proper (or galealacinia) (Fig. 2D). The apices of the galealacinia of the maxillae have long, dense brushes of setae and shorter, more sparsely distributed setae laterally, whereas the tips

of maxillary palps are adorned with dense brushes of setae laterally and apically (Fig. 2D). The fusion of the galea and lacinia occurs late in embryogenesis in *E. leukon* (B.C.O'D., unpublished data) as is reported to occur embryonically in *Baetis* (Murphy 1922). The labial palps are composed of three segments, and the paraglossae of the labium are highly setaceous, distinctly rounded, and large enough to obscure the glossae (Fig. 2D).

Development of Early Stage Nymphs of *E. leukon*: Abdominal Gills. Mature *E. leukon* nymphs have one pair of short stubby gills (on abdominal segment 1) followed by six pairs of large, bilamellate, and feathery
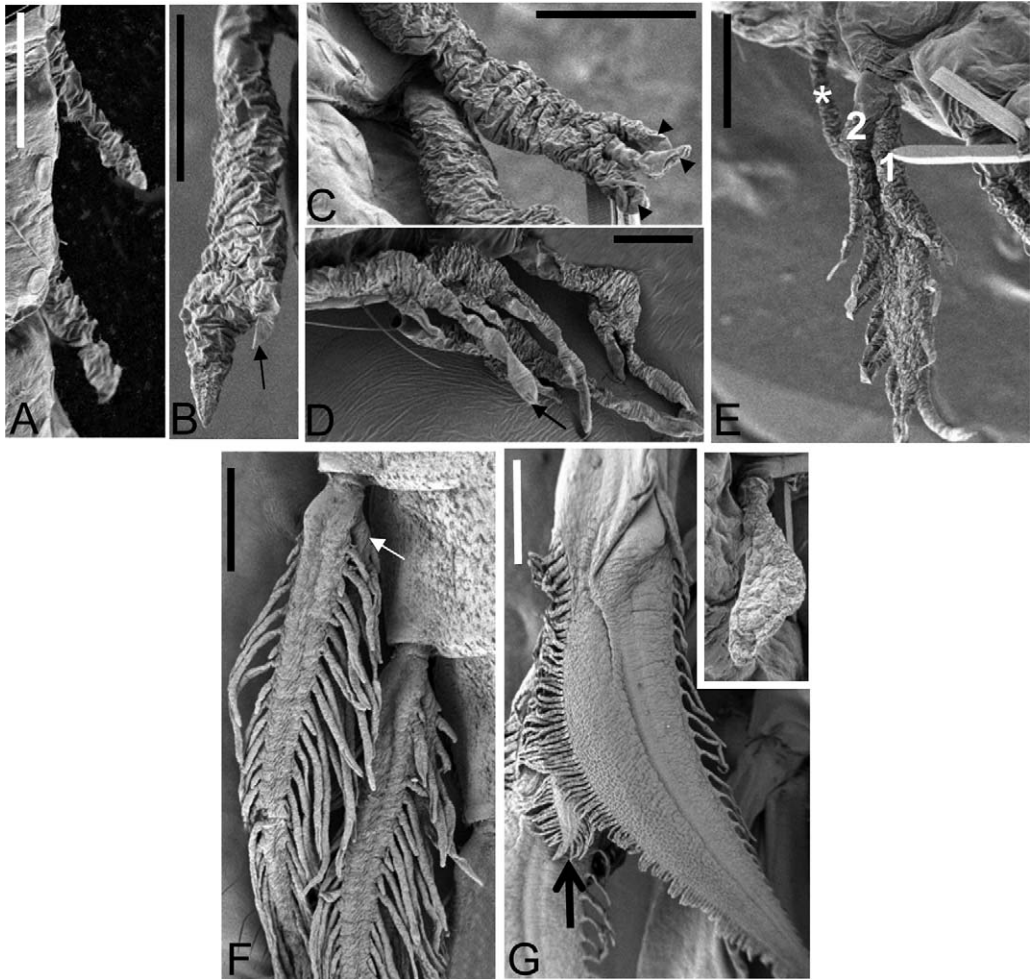
Fig. 3. Gill development during early nymphal development in *E. leukon*. (A) Gills are absent in hatchlings and then occur in the second instar as short and undifferentiated stubs. (B) A small apical split is evident near the tip of the gill (arrow), and the gill has elongated to about twice its original length. (C) As the main gill axis elongates, further splits along the apex occur to eventually form three distinct extensions (arrowheads). (D) The entire gill axis has elongated considerably and the first small extension of the lateral fringe (arrow) has elongated as well. Note that at this stage, gills are unilamellate and highly ramified along their margins. (E) Two distinct axes are now visible: a dorsal lamella (1) that is highly developed relative to the small ventral lamella (2). The gill on abdominal segment 1 (*) has not differentiated in form past its initial morphology. (F) The gills elongate further; note that the lamella of the gill is narrow and the ventral lamella is located directly beneath the dorsal gill branch (arrow denotes the base of the ventral lamella). (G) Dorsal lamella of the mature nymph is large and broad with a fringe of projections along its entire margin. Note that the second lamella is directly beneath this gill, denoted with the arrow. Inset shows the final morphology of gill 1 (≈75 μm in length) from the same individual; gill 1 has flattened considerably from its initial rounded shape. Scale bar = 40 μm (A–E), 100 μm (F), and 500 μm (G).

gills (on abdominal segments 2–7); however, gills occur in the second instar as six pairs of short, tubular rods attached to the posterolateral edges of abdominal segments 2 through 7 (Fig. 3A). Gills originate as uniramous structures ≈40 μm in length (Fig. 3A). These structures will develop into the dorsal lamellae of the mature gills, with the ventral lamella developing later. Across mayflies, Needham et al. (1935) described variation in the morphology of young gills as either short buds or long, thin threads. In *E. leukon*, the initial form of the gill is a short bud (compared with the thread form, for example, in *L. hebe*), and a similar morphology is described for gills of the second instar of *E. album* (Britt 1962). The simultaneous appearance of six gill pairs at the second instar also has been reported in *H. bilineata* (Wiebe 1926) and *E. simulans* (Ide 1935b). *H. bilineata* has gill buds that look highly similar to those in *E. leukon*. The simultaneous appearance of multiple gill pairs is in contrast to the sequential addition of gill pairs as reported in several species of heptageniids (Needham et al. 1935) as well as in *Cloeon cognatum* (Lubbock 1864), *Ecdyonurus forcipula* (Gros 1923), and *L. cupida* (Clifford et al. 1979).

Following the second instar, the gills of *E. leukon* elongate along their main axis while still highly wrinkled (Fig. 3B). The main gill axis then becomes split at the distal tip (Fig. 3B); this split constitutes the first of many that will dissect the entire margin of the gill into a fringe of long thin extensions (Fig. 3C and D). Early dissection of the tip of the dorsal lamella results in marginal fringing in *E. leukon*, as it does in another burrowing mayfly, *H. bilineata* (Wiebe 1926). *H. bilineata* gills become dissected along the dorsal margins (termed "distal crenulation") starting in the third instar (Wiebe 1926) before the split of the gill into two distinct lamella. The same is true for *E. album* as dissections (or "protuberances") on the gill surface become apparent in the third instar (Britt 1962). By the fourth instar, most of the gills of *E. album* are flattened and fringed (Britt 1962).

The next major morphological transition is the formation of the secondary axis near the base of the primary gill axis that will develop into the ventral lamella of the mature gill (Fig. 3E). Similarly, in *H. bilineata*, the secondary axis arises from near the basis of the primary gill axis after the distal dissection (Wiebe 1926). *E. album* buds the secondary gill lamellae off the main gill axis during the fourth instar (Britt 1962). The origination of the secondary gill axis from the gill base seems to be widespread in mayflies: Clifford et al. (1979) document the appearance of the secondary gill axis at the base of the dorsal lamellae in *L. cupida*, and the tufts of filaments comprising the ventral branch of the gills arise basally in several species of heptageniids, among others (Ide 1935b). The gill base in *E. leukon* is smooth compared with the wrinkled lamella (Fig. 3E). Over successive molts, both gill lamellae lengthen and continue to become dissected over their margins (Fig. 3F). Ultimately, the lamellae broaden to become roughly 50 times their original width (Fig. 3G) and a mid-line furrow is evident in the mature gill (Fig. 3G). The first gill pair elongates and flattens to form the mature morphology (Fig. 3G, inset) and is strikingly different (unilamellate, small, flat with no marginal extensions) relative to the remaining gill pairs (Fig. 3G).

Several elements of mature gill ultrastructure in *E. leukon* (Fig. 4A) are similar to structures that function in oxygen uptake, osmoregulatory balance and sense of the local environment (Komnick and Stockem 1973, Wichard et al. 1973, Wichard 1975, Filshie and Campbell 1984, Keil 1998). The gill base (Fig. 4B) serves as the point of attachment of the gill lamellae to the abdomen, each of which is articulated and independently movable. Several mayfly species [e.g., *Leptophlebia marginata* (L.)] move their gills in conditions of oxygen deficiency to bring highly oxygenated water close to the body and replace the anoxic boundary layer that develops around insects, a behavior commonly observed in mayfly species that construct burrows (Eastham 1934, 1936a, 1936b, 1939). In addition, gill beating has been suggested to reduce the thickness of the viscous sublayer surrounding the insect; reduction of this sublayer promotes more efficient transfer of oxygen via diffusion (Vogel 1996). Reinforcement

of the bases of the gill lamellae is suggested by the ridged margin and smooth surface of gill bases (Fig. 4B) compared with the distal, more wrinkled gill surface. Wiebe (1926) reported that third-instar nymphs of *H. bilineata* have freely moveable gills. Similarly, *E. leukon* hatchlings beat their small gills actively in the still waters of the rearing dishes (B.C.O'D., unpublished data).

Rows of elongate structures extend vertically from the gill lamellae, near the base of the marginal fringe (Fig. 4C and D). These structures are similar to the clavate sensilla found on the caudal filaments of the cricket *Gryllus bimaculatus* De Geer (Keil 1998); thus, they may perform some sensory role. In *G. bimaculatus*, these structures are mechanoreceptors, and the clavate morph in particular has been linked to gravity perception. For a small insect in an aqueous environment the function of rows of clavate sensilla may be to perceive changes in the nymph's microenvironment and avoid predation. Gaino and Rebora (1999) report the existence of mechanosensory structures on the antennae of nymphal mayflies, presumably used for perception of small scale turbulence. Larger versions of clavate sensilla highly similar to those found on the gills are located on the second antennal segments in *E. leukon* nymphs (data not shown).

The gills of *E. leukon* have numerous raised disclike structures on their surfaces (Fig. 4E). Cuticular discs are scattered across the gill lamellae of *E. leukon* with denser mats near the basal region of the gill and clustered at the bases of the marginal fringe (Fig. 4A and E); these discs are also found on the dorsal region of the abdomen (data not shown). The raised rims and recessed center of these discs in *E. leukon* (Fig. 4E, inset) are similar to structures described from gills in several baetid species (Komnick and Stockem 1973) as well as in *Coloburiscoides* Lestage (Oligoneuriidae) (Filshie and Campbell 1984). The basal location and structure of the discs on the gills of *E. leukon* are also similar to structures described in stonefly gills (Mill 1998).

Tightly packed thin extensions arise from the perimeter of the gill lamellae (Fig. 4F). The tracheal system extends into the marginal fringes (Fig. 4F, inset). The gill surface is highly wrinkled on either side of the mid-line furrow and gills removed from live specimens also show high levels of wrinkling across their surfaces when examined by light microscopy (data not shown).

In sum, the course of gill development in *E. leukon* involves the appearance of small gill buds at the second instar, progressive elongation of the gill bud into a thin thread, division of the gill margin into numerous extensions, basal bifurcation of the main gill axis into a dorsal and ventral lamella, flattening and widening of the gill lamellae, and finally adornment of the gill surface with a suite of secondary structures.

Implications of Early Nymphal Development for *E. leukon* Biology. Comparison of early instars in *E. leukon* relative to mature nymphs coupled with close inspection of successive stages of early nymphal development suggests that changes in the morphology of
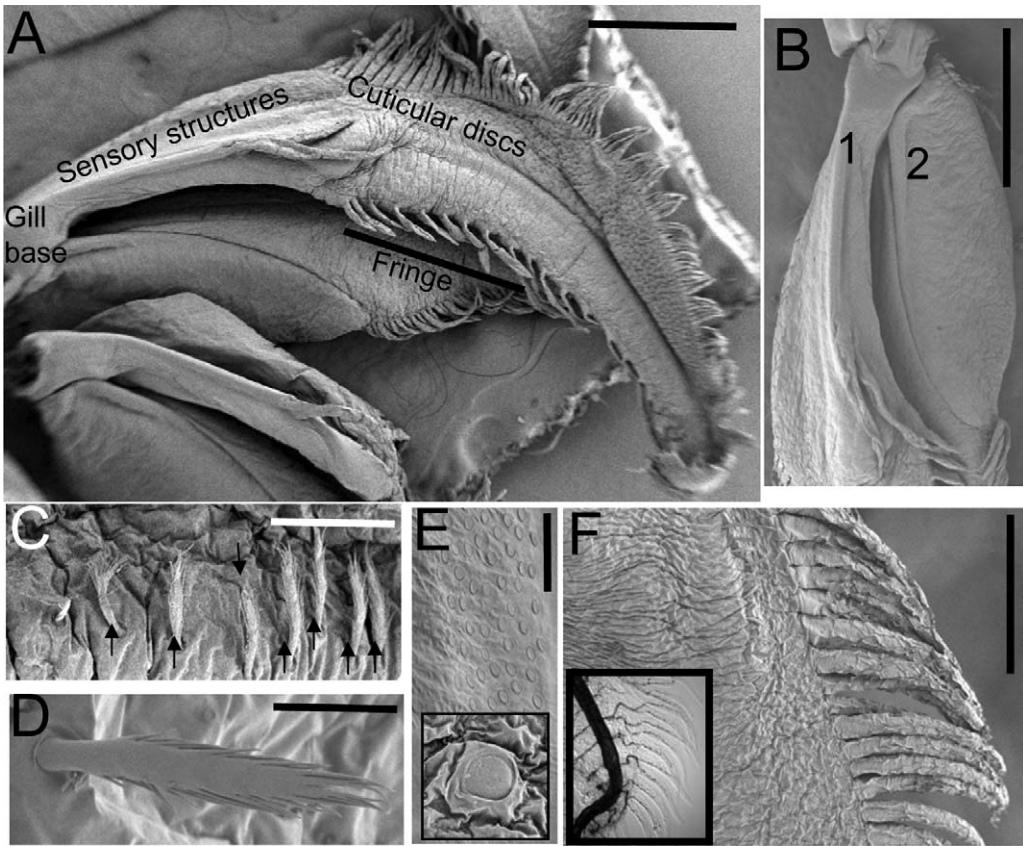
Fig. 4. Ultrastructure of the mature gills of *E. leukon.* (A) View of entire lamella with regions of interest labeled. Detailed pictures of each aspect of gill ultrastructure are shown in B–F. (B) Gill base showing origin of two separate lamellae (1, dorsal; 2, ventral) from the abdomen of the nymph. (C) Sensory structures near gill margin are arranged in rows along this edge (arrows). (D) One of the sensory structures in isolation from C. (E) Cuticular discs found near the base of the gill proper; each disc has a raised lip and a recessed center. These discs are usually clustered and distributed over the surface of the gill with denser collections of them near the gill base and at the bases of the marginal fringe. Inset shows a close view of a single disc, measuring 5 μm in diameter. (F) Gill margin with fringe. Inset shows a light micrograph of the gill margin in a mature nymph showing that the tracheal system extends directly into each individual gill extension. Scale bar = 500 μm (A and B), 50 μm (C), 30 μm (D), 50 μm (E), and 200 μm (F).

mouthparts and abdominal gills of *E. leukon* occur relatively rapidly over the early instars (e.g., see Figs. 1 and 3). Both gills and mouthparts require a small number of molts to achieve their final form, and mature morphology is attained at a relatively small total body size (relative to black wing pad stage). Ide (1935b) estimated that mature nymphal form is reached in as little as eight to 10 molts, while nymphs are still quite small. The number of nymphal molts in mayflies varies from ≈10 to >40 (Waltz and Burian 2008), and growth rates are influenced by both biotic and abiotic factors (for a study in *E. leukon,* see Snyder et al. 1991). Britt (1962) reported that black wing pad stage *E. album* nymphs from Lake Erie measured between 17.4–20.5 mm; in this study, SEM-imaged *E. leukon* nymphs with well-formed mandibular tusks and abdominal gills and highly similar to their mature form were typically around 1 mm in body length (excluding cerci and antennae). Britt (1962) estimated that *E. leukon* undergoes ≈13 molts; this cou-

pled with the current study would have the mouthpart and abdominal gill groundplan of *E. leukon* nymphs in place relatively early in nymphal development. Likewise, Clifford (1970) documented nonisometric growth in certain nymphal structures (relating specifically to reproductive tissues) during early development in *L. cupida,* describing a critical period between small "immature" and "mature" nymphs occurring at a size range of ≈7–9-mm body length. The mature form of the mouthparts and gills in *E. leukon* is in place early in nymphal ontogeny, at a small relative body size (≈1 mm in this study). Development of the mouthparts and gills are likely to be critical in facilitating key habitat transitions during the life cycle of *E. leukon.*

Acknowledgments

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