

## Microstructure of Attachment Mechanisms of Newly Hatched Larvae of Four Cyprinid Species with Comments on Terminology

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### ABSTRACT

An adhesive organ is a prominent, protruding mucus secreting gland that is used by newly hatched tadpoles and larvae of some fishes to attach to aquatic vegetation. The objective of this research is to test the hypothesis that newly hatched cyprinid larvae of *Hybognathus hankinsoni*, *Notemigonus crysoleucas*, *Cyprinus carpio* and *Gila atraria* contain cephalic adhesive organs. Newly hatched larvae of *Semotilus atromaculatus*, which do not attach to submerged aquatic vegetation, were used as the control. SEM examination of newly hatched larvae indicate there were no adhesive organs on the control species (*S. atromaculatus*) or test species (*H. hankinsoni*, *N. crysoleucas*, *C. carpio* and *G. atraria*). Rather, newly hatched larvae of test species contain a localized highly modified epidermis (i.e., primarily on the ventral cephalic and anteroventral yolk sac surfaces of *H. hankinsoni*, *N. crysoleucas*, *C. carpio*, and *G. atraria*, and sometimes on dorsal cephalic epidermal cells of *H. hankinsoni*, *C. carpio*, and *G. atraria*). This modified epidermis is composed of epidermal cells with unculi-like projections, elevated microridges at peripheries of epidermal cells, and mucus from apical pores of goblet cells that probably are responsible for attachment of test species to substrates. We hypothesize that the unculi-like projections at centers of epidermal cells in newly hatched larval test cyprinids are true unculi. There is a need to define and clarify the meanings of words and phrases (i.e., cement gland, cement gland apparatus, cement gland-like structure, casquette, temporary adhesive glands, adhesive apparatus, adhesive gland, adhesive organ, attachment organ, and glue secretion and adhesion) for structures used by newly hatched larvae to attach to substrates. Definitions should be based on homologies, crucial in phylogenetic reconstructions of species relationships and in identifying developmental homologues of cells, tissues, glands, and organs that have been described as mechanisms for attachment by newly hatched larvae of various species to substrates. We proposed the phrase “attachment mechanism” as a broad definition for the ways in which newly hatched larvae attach and adhere to substrates during early development. This broad definition, however, should be modified to define specific methods of attachment (e.g. attachment mechanism of unculi, elevated epidermal microridges, and mucus) to assist in defining homologies.

Keywords: newly hatched cyprinid larvae, attachment mechanism, SEM

## INTRODUCTION

Various terms (e.g. cement gland, adhesive organ) have been used to describe the attachment mechanism of newly hatched larvae of some frogs and fishes to substrates. Snyder et al. (2016), following Auer et al. (1982) and Wallus et al. (2008), presented a broad definition of cement gland: “discrete or diffuse structures which permit a larva to adhere to a substrate.” Other researchers, however, have proffered a strict definition of cement gland/adhesive organ in which an adhesive organ is a single prominent, protruding gland composed of an aggregation of elongated, tubular secretory cells surrounding a pyriform acinus (Rétaux and Pottin, 2011; Bennemann and Pietzsch-Rohrschneider, 1978), which contain adhesive secretions. Adhesive organs (described as cement glands by Schäperclaus, 1962 and Blaxter, 1969) are used by newly hatched tadpoles and larvae of some fishes to attach to aquatic vegetation and other substrates during early development (Rétaux and Pottin, 2011). Adhesive organs have been reported in frogs (e.g. *Xenopus laevis* (Boothby and Roberts, 1992) and a variety of fishes [*Lepisosteus osseus* (Lepisosteidae), *Amia calva* (Amiidae), *Astyanax mexicanus* (Characidae), *Cichlasoma dimerus* (Cichlidae), *Oreochromis niloticus* (Cichlidae), *Pterophyllum scalare* (Cichlidae), and *Tilapia mariae* (Cichlidae)] (Eycleshymer and Wilson, 1908; Peters, 1965; Morrison et al., 1978; Boothby and Roberts, 1992; Britz et al., 2000; Meijide and Guerrero, 2000; Morrison et al., 2001; GropPELLI et al., 2003; Pottin et al., 2010). In presenting a chordate phylogenetic tree highlighting the presence of adhesive organs in one sarcopterygian (Dipnoi) and seven species of actinopterygians (Polypteriformes, Acipenseriformes, Lepisosteiformes, Amiiformes, Osteoglossiformes, Characiformes, and Perciformes), Rétaux and Pottin (2011) hypothesized that the adhesive organ in *A. mexicanus* was homologous to that in *X. laevis* based on characteristics common to both: adhesive gland secretory cell morphology, mucus production, innervation by the trigeminal nerve, and the development of the adhesive organ by the same gene regulatory network, including *Bmp4* and *Pitx1/2*.

Blaxter (1969) and Balon (1975) hypothesized that the newly hatched larvae of some phytophilous species of Cyprinidae (Ostariophysi: Cypriniformes) attach themselves to aquatic vegetation with adhesive organs/cement glands. Likewise, Loos and Fuiman (1978) and Loos et al. (1979) hypothesized that newly hatched larvae of *Notemigonus crysoleucas* and *Cyprinus carpio* contained cephalic cement glands that allowed them to attach to aquatic vegetation during early development. To date, there have been no studies to test the adhesive organ/cement gland hypothesis in these cyprinids. The objective of this research was to test the hypothesis that cyprinid newly hatched larvae contain cephalic adhesive organs/cement glands described by Pottin et al. (2011). Independent variables are the New World newly hatched larval cyprinids, *Hybognathus hankinsoni*, *N. crysoleucas*, and *Gila atraria* and the Old World cyprinid, *C. carpio* (test group). *Hybognathus hankinsoni* and *G. atraria* were included as they are reported to be phytophilous spawners by Snyder et al. (2016). Newly hatched larvae of *Semotilus atromaculatus*, which do not adhere to substrates, were used as the control group. Dependent variables were the presence of an adhesive organ/cement gland and variations in epidermal characteristics (e.g. apical pores of goblet cells, epidermal uncili-like projections, mucus, and the peripheral microridge) of newly hatched larvae in control and test groups.

## MATERIALS AND METHODS

Newly hatched (1 day old) larval specimens of control and test species, preserved in 10 % buffered formaldehyde, were acquired from Colorado State University, University of Richmond, and Academy of Natural Sciences of Drexel University. Collection data are available upon request.

**Scanning electron microscopy (SEM):** Three specimens of each species were dehydrated by the following procedure: one quick distilled water wash, two 10-minute distilled water washes; incremental 30, 50, 70, 90, and 100% ETOH (ethanol) 10-minute soaks, followed by two additional 100% ETOH 10-minute soaks. After dehydration, specimens were vacuum dried in a Samdri-795 critical point dryer. Critical point dried specimens were mounted on SEM stubs, and coated with two 6-micron palladium coatings in a Denton-4 sputter coater. The dorsal, anterior, ventral, and lateral areas of the head, the anteriorventral surface of the yolk sac, and the lateral body trunk of each early hatched larval specimen were examined with SEM to identify the presence/absence of adhesive organs, apical ends of goblet cells, mucus, and epidermal projections. Three newly hatched larvae of *S. atromaculatus* were used as controls.

## RESULTS

SEM indicated there are no adhesive organs/cement glands as described by Rétaux and Pottin (2011) and Bennemann and Pietzsch-Rohrschneider (1978) on any cephalic areas of newly hatched larvae of cyprinid control species (*S. atromaculatus*) and all cyprinid test species (*H. hankinsoni*, *N. crysoleucas*, *C. carpio*, and *G. atraria*) (Table 1; Fig. 1). In all control and test species, surfaces of hexagonal (rarely pentagonal) epidermal cells contain micro-ridges (MR) *albeit* with varying lengths and patterns, and have well demarcated cell borders formed by peripheral micro-ridges of adjoining cells. Descriptions of the epidermis of all species is presented by region.

**Dorsal Cephalic Region** – In *S. atromaculatus*, the most striking feature of the dorsal epidermis is the abundance of goblet cell pores (Figs. 1A and 2A). Each epidermal cell is bordered by one to two surface pores of goblet cells (Figs. 1A and 2A). Micro-ridges at cell peripheries are elevated at adjoining cells and where they contact the elevated pores of goblet cells (Fig. 2A). No unculi-like projections were present.

In *H. hankinsoni*, the dorsal epidermis has few pores but a preponderance of unculi-like projections that emanate primarily from the central regions of epidermal cells (Figs. 2B). Ventral and lateral margins of the eyes contain numerous unculi-like projections (Fig. 2B). The dorsal surface of one specimen of *H. hankinsoni* had a thick mucus embedded with debris (Fig. 2F).

As in *H. hankinsoni*, the dorsal epidermis of *N. crysoleucas* has few pores. However, dorsal epidermal cells of *N. crysoleucas* do not have unculi-like projections (Fig. 2C).

The micro-ridge pattern of dorsal epidermal cells in *C. carpio* is not as pronounced as those in other species (Fig. 2D). The dorsal epidermis of *C. carpio* has numerous pores, and the

centers of epidermal cells have nubby uncili-like projections. Lateral cephalic epidermal cells have longer uncili-like projections (Figs. 2C).

In *G. atraria* the dorsal epidermis contains numerous pores. Its epidermal cells have uncili-like projections and short MR (Fig. 2E). Location of the uncili-like projections is variable. Some are present in the center of cells whereas others are located at or near the periphery of the cells (Fig. 2E). Some epidermal cells have more than one uncili-like projections (Fig. 2E). The dorsal cephalic surface of one *G. atraria* specimen has a thick mucus embedded with debris (Fig 2G).

**Ventral Cephalic Region** – In *S. atromaculatus*, the ventral cephalic epidermis is much like that on the dorsum (Fig. 3A). There is clear definition of the micro-ridges in the hexagonal cells which do not have uncili-like projections (Fig. 3A). Numerous pores punctuate the epidermis where mucus spheres are present in some pores (Fig. 3A).

In *H. hankinsoni*, the ventral epidermis has an abundance of both apical pores of goblet cells and small to large uncili-like projections (Fig. 3B). The uncili-like projections are distributed in the centers of some cells, and at or near the periphery of other cells (Fig. 3B).

In *N. crysoleucas*, small to large uncili-like projections were present in ventral and anteroventral cephalic epidermal cells (Fig. 3C). Few apical pores of goblet cells were apparent in the ventral epidermis (Fig. 3C). Anteriolateral cephalic epidermal cells have long uncili-like projections (Fig. 3F).

The ventral epidermis of *C. carpio* has uncili-like projections and pores (Figs. 3D and 3G). Epidermal cells containing uncili-like projections appeared to be segregated from apical pores of goblet cells (Figs. 3D). Mucus and debris often obscure the epidermal uncili-like projections and pores like that imaged on the anteroventral cephalic region in one specimen (Fig. 3G).

In *G. atraria*, pores are prevalent (Figs 1E and 3E), and the uncili-like processes are relatively short (Figs. 3E and 3H). Some uncili-like projections appear to arise from the periphery of cells adjacent to apical pores of goblet cells (Fig. 3H).

**Anteroventral Yolk Sac Region** – The epidermis on the anteroventral regions of the yolk sac of *S. atromaculatus* has numerous apical pores of goblet cells which rise above the level of the hexagonal epidermal cells (Fig. 4A). No epidermal cells have uncili-like projections.

In *H. hankinsoni*, anteroventral and lateral epidermal cells of the yolk sac contain small to large uncili-like projections (Fig. 4B). Few pores are apparent. The anteroventral epidermal cells on the yolk sac of *N. crysoleucas* contain central, small to large uncili-like projections (Figs. 4C and 4F). Few pores are apparent. In *C. carpio*, epidermal cells of the anteroventral and ventrolateral areas of the yolk sac contain uncili-like projections and pores (Figs. 4D and 4G). Anteroventral yolk sac epidermal cells of *G. atraria* contain both uncili-like projections and pores (Fig. 4E).

**Lateral-Ventral Trunk Region** – In *S. atromaculatus*, the epidermal cells of the lateral trunk have well defined micro-ridge patterns (Fig. 5A) which and rise towards the cell periphery where they meet those of other cells (Fig. 5A). Numerous apical pores of goblet cells rise above the surface of the centers of the other epidermal cells (Fig. 5A). No unculti-like projections are present.

Lateral, lateroventral, and ventral trunk areas of *H. hankinsoni* contain primarily hexagonal epidermal cells with unculti-like projections that increase in numbers toward the ventral finfold (Fig. 5B). Apical pores of goblet cells are common but not prevalent.

In *N. crysoleucas*, epidermal cells on the lateral trunk do not have unculti-like projections but have short, pectinate MR (Fig 5C and 5F). Few apical pores of goblet cells were present on the lateral trunk (Fig. 5C).

The lateral trunk epidermis of *C. carpio* has apical pores of goblet cells (Fig. 5D). The frequency and size of unculti-like projections increases towards the ventral finfold (Fig. 5D).

In *G. atraria*, lateroventral and ventral epidermal cells have goblet cell pores and well-defined unculti-like projections (Figs. 5E).

## DISCUSSION

The newly hatched larvae of test species (*H. hankinsoni*, *N. crysoleucas*, *C. carpio*, and *G. atraria*) in the present study do not possess cement glands/adhesive organs (i.e., a single prominent, protruding gland composed of an aggregation of elongated, tubular cells surrounding a pyriform acinus) as described for *X. laevis* (Amphibia) and *A. mexicanus* (Characidae) by Rétaux and Pottin (2011), *P. scalare* (Cichlidae) by Bennemann and Pietzsch-Rohrschneider (1978) and Gropelini et al. (2003), *Oreochromis niloticus* (Cichlidae) by Morrison et al. (2001), *Hypsophrys nicaraguensis* (Cichlidae) by Arias (2011), and *Amphilophus xiloaensis* (Cichlidae) by Kratochwil et al. (2015), *A. calva* (Amiidae) by Eycleshymer and Wilson (1908), *L. osseus* (Lepisosteidae) by Eycleshymer (1903) and Long and Ballard (2001), *Cichlasoma dimerus* (Cichlidae) by Meijide and Guerrero (2000), and *Octolasmis angulate* (Crustacean: Poecilasmatidae) by Yap et al. (2017). In contrast to these prominent protruding glands, a localized highly modified epidermis (i.e., primarily on the ventral cephalic and anteroventral yolk sac surfaces) occurs in newly hatched larval *H. hankinsoni*, *N. crysoleucas*, *C. carpio*, and *G. atraria*, and sometimes on dorsal cephalic epidermal cells of *H. hankinsoni*, *C. carpio*, and *G. atraria*. This modified epidermis is composed of epidermal cells with unculti-like projections, elevated microridges at peripheries of epidermal cells, and mucus from apical pores of goblet cells that in combination probably are responsible for attachment of test species to substrates. Unculti-like projections have been reported previously in newly hatched larval ostariophysans, *C. carpio* (Britz et al., 2000; Appelbaum and Riehl, 1997), and *Rhodeus uyeekii*, described as minute tubercles by Suzuki et al. (1985). Britz et al. (2000) reported the presence of projections in epidermal cells in specific cephalic regions in the gymnotiforms, *Apteronotus albifrons* and *Apteronotus leptorhynchus* (Britz et al., 2000), and proposed they aid in the adhesion of the newly hatched larvae to substrates during early development. Appelbaum and Riehl (1997) reported that centers of surface epidermal cells of the yolk sac of newly hatched larval *C. carpio*

contained protrusions, and that epidermal cells of the mouth region were comprised of keratinizing cells, but did not relate either to attachment. The reported presence of projections (Britz et al., 2000), protrusions (Appelbaum and Riehl, 1997), and minute tubercles (Suzuki, et al., 1985) is consistent with the presence and locations of the unculti-like projections on anteroventral yolk sac and ventral cephalic epidermal areas of newly hatched larval *H. hankinsoni*, *N. crysoleucas*, *C. carpio*, and *G. atraria* in our study.

We hypothesize that the unculti-like projections at centers of epidermal cells in newly hatched larval test cyprinids in our study, those reported for newly hatched larval gymnotiforms by Britz et al. (2000), and the minute tubercles primarily on the head and yolk sac of newly hatched larval *R. uyekii* by Suzuki et al. (1985) are true unculti (horny keratinized projections arising from single epidermal cells) as described by Roberts (1982). We plan to test this hypothesis with special keratin stains (i.e., Dane Herman method, ayoub-shklar method, Alcian blue-periodic acid Schiff's stain or hemotoxylin and eosin stains) described in Rao et al. (2014). Our hypothesis is based on the report by Li et al. (2011) who demonstrated the production of keratinized projections in epidermal cells of larval *Danio rerio* after treatment with morpholino-mediated knockdown of the *anca12* and *snap29* genes, and the fact that the unculti-like projections on epidermal cells of newly hatched larvae in this study, as well as those of the gymnotiforms, *A. albifrons* and *A. leptorhynchus* (Britz et al., 2000), *R. uyekii* (Suzuki et al., 1985), and *C. carpio* (Appelbaum and Riehl, 1997) are strikingly similar in appearance to unculti in SEM micrographs of adult epidermis reported in Roberts (1982). We do not find the presence of unculti in newly hatched larvae to be unexpected. Unculti are unique to ostariophysan fishes and have been found on adults of numerous species (Roberts, 1982). They also are part of the attachment mechanism in adult cyprinid hill stream fishes *Garra gotyla*, *Garra lissorhynchus*, *Garra pectinopterus*, and *Garra sulcatus* (Gaur et al., 2013; Massar, 2015) and adult mountain-stream catfish, *Pseudocheneis sulcatus* (Joshi et al., 2012), which use a combination of unculti and mucus to adhere to substrates in swift water currents.

Loos and Fuiman (1978) and Loos et al. (1979) stated that newly hatched larval *N. crysoleucas* and *C. carpio* contain cephalic adhesive organs/cement glands. With SEM, we did not find adhesive organs (cement glands) on any cephalic areas or anteroventral epidermal yolk sacs of Old World and New World cyprinid newly hatched larvae examined in the present investigation. Loos and Fuiman (1978) and Loos et al. (1979) provided no evidence for the presence of cephalic adhesive organs/cement glands in newly hatched larval *N. crysoleucas* and *C. carpio*. Rather, their hypotheses were based on observations of these newly hatched larvae attaching themselves to aquatic vegetation as well as to the sides of aquaria, a plausible assumption at the time considering published descriptions of adhesive organs as means of attachment to submerged aquatic vegetation by newly hatched larvae of other species (e.g. adhesive glands described on larval *Amia calva* by Eycleshymer and Wilson, 1908, and those on larval *L. osseus* by Eycleshymer, 1903).

We could not corroborate the presence of an adhesive organ on the anterior end of the head of newly hatched larvae of *C. carpio* reported by Appelbaum and Riehl (1997). Appelbaum and Riehl (1997) simply labeled a sunken area on their specimen as an adhesive organ, not a protruding one as described by Rétaux and Pottin (2011). Our observations of the absence of an adhesive organ on newly hatched larval *C. carpio* are corroborated by the studies of Maitland

and Campbell (1992), Steffens (1980), and Sola et al. (1983) who state that newly hatched larval *C. carpio* do not possess cephalic adhesive organs but attach themselves to substrates with their mouths. Similarly, we did not find evidence of the presence of bilateral cement glands and pores anterior to the developing mouths of our test species that Fletcher and Wilkins (1999) reported for newly hatched larval *Pteronotropis hypselopterus*. Fletcher and Wilkins (1999) indicate that newly hatched larval *P. hypselopterus* do not have fibrils or specialized attachment appendages that were prevalent on ventral epidermal cells and those on the anteroventral surface of yolk sacs of test species in the current study.

The bilateral cement glands and pores anterior to the developing mouths in *P. hypselopterus* and the attachment mechanism described for our test species indicate there are at least two significantly different attachment mechanisms for newly hatched larval cyprinids (i.e., a combination of uncili-like projections, MR pattern, and mucus present in newly hatched larvae in the current study; and the bilateral cement glands without fibrils or specialized attachment appendages of *P. hypselopterus* reported by Fletcher and Wilkins (1999).

**Mode of spawning and larval development relationship** – We propose that the uncili-like projections at the centers of epidermal cells on the ventral cephalic regions and anterior ventral regions of all test species, mucus from the apical pores of goblet cells, and the elevated peripheral ridge of epidermal cells account for the attachment of the newly hatched larvae of test species to substrates (i.e., aquatic vegetation, roots, and rocks) during early development. Most likely, the micro-ridge pattern of epidermal cells helps to distribute mucus. Mucus from goblet cells contain mucopolysaccharides and serves as an adhesive (Long et al., 2013), and also facilitates gas exchange (Horng et al., 2009). Physical attachments of newly hatched larval *N. crysoleucas* and *C. carpio* to substrates during their early development have previously been confirmed by Loos et al. (1979). Developmental attachment dispositions of newly hatched larval *H. hankinsoni* and *G. atraria*, however, are unknown. Based on our results and life history aspects of these two species, we hypothesize that their newly hatched larvae attach and adhere to substrates during their early life history using a combination of the uncili-like projections, mucus, and the MR pattern of their epidermal cells that are present on their cephalic and anteroventral region of their yolk sacs. *Hybognathus hankinsoni* is an open-substrate phytophilous (non-obligatory plant spawner) that broadcast small, demersal, adhesive eggs over vegetation when available (Snyder et al., 2016). *Gila atraria* is an open-substrate phytophilous breeder, spawning adhesive eggs in shallow littoral shoals, usually over vegetation from April through August (Snyder et al., 2016). Graham (1955) reported that newly hatched fry were scattered through emergent vegetation and present in bank depressions in well protected pockets created by driftwood. In contrast, fertilized non-adhesive eggs are spawned by adult *S. atromaculatus* (control) in gravel nests where they hatch and develop progressively through protolarval, mesolarval, and metalarval stages before exiting the nest through its interstices (Maurakis et al., 1990, Maurakis et al., 1993). The cephalic epidermis of newly hatched larvae of the control (*S. atromaculatus*) do not contain uncili-like projections and is similar to the newly hatched larval epidermis of *D. rerio*, which has no uncili-like projections (Li et al., 2011). These results are comparable to those of Rétaux and Pottin (2011) who stated that the genome model cyprinid, *Danio rerio* (Zebrafish), does not possess a cement gland-like adhesive organ. *Danio rerio*, considered a group spawner and egg scatterer, spawns non-adhesive eggs (Spence et al., 2008), but unlike species of *Semotilus*, does not prepare a pebble nest.

**Clarification of Terms** – There is a need to define and clarify the meanings of words and phrases used to describe structure(s) “used to attach the larvae to the substrate” as stated in the Dictionary of Ichthyology (2017). Definitions of the various attachment mechanisms that have been described in the literature should be based on homologies, which are crucial in phylogenetic reconstructions of species relationships and in identifying developmental homologues of cells, tissues, glands, and organs that have been used to describe attachment of various species to substrates. We identified 10 terms (i.e., cement gland, cement gland apparatus, cement gland-like structure, casquette, temporary adhesive glands, adhesive apparatus, adhesive gland, adhesive organ, attachment organ, and glue secretion and adhesion) that have been used by others to describe how larval and adult fishes, frogs, and barnacles attach themselves to various substrates (Table 1). For example, “adhesive organ” has been used synonymously in four different ways to describe non-homologous attachment mechanisms. The paired anteriodorsal *adhesive organs* (sucking discs) on head of newly hatched larval *A. calva* are endodermal in origin (Eycleshymer and Wilson, 1908). In contrast, the single anteroventral *adhesive organ* of newly hatched larval *L. osseus* is considered as ectodermal in origin (Eycleshymer, 1903; Eycleshymer and Wilson, 1908; Long and Ballard, 2001). Additionally, Meijide and Guerrero (2000) used *adhesive organ* to denote the three to six pairs of glands in newly hatched larval *Cichlasoma dimerus* (Cichlidae) whereas Gomes et al. (2007) described *adhesive organ* as a secretory prismatic epithelial cell in *Hoplias malabaricus* (characiform). More research like those of Retaux and Pottin (2011) and Pottin et al. (2010) who identified the posterior dorsal cephalic cement gland-like structure (casquette) in *A. mexicanus* as homologous to the cement gland in *X. laevis* are warranted to clarify and define the various terms (Table 1) that have been used to describe structures associated with adhesion ability. In this context, “cement” is explicitly used and correctly applied by Retaux and Pottin (2011) and Pottin et al. (2010) to denote a cement-like substance that adheres a newly hatched larva to a substrate, consistent with the definition of “cement” as a noun (i.e., a binding element or agency, such as a substance to make objects adhere to each other) and transitive verb (i.e., to unite or make firm by or as if by cement) in Webster (2016). As such, we disagree with the use of “cement gland” by Snyder et al. (2016), Auer (1982), and Wallus and Simon (2008). We propose “attachment mechanism” is more appropriate for a broad definition of attachment: “discrete or diffuse structures and/or substances which permit a larva to attach and adhere to a substrate.” For the test species in our study, attachment mechanism of uncini and mucus is appropriate.

This study has generated new questions and research areas. For example, newly hatched larvae of other ostariophysan fishes, particularly phytophilous spawners, should be examined using SEM to characterize epidermal structures relative to their roles in attachment mechanisms. For example, newly hatched larvae of *Notropis bifrenatus* (Cyprinidae) have been reported to adhere vertically (head up) on their ventral sides to aquarium glass (Harrington, 1947), but no studies have been published on their cephalic and trunk epidermal microstructures that could be associated with adherence. Secondly, is the production of mucus in cephalic epidermal cells of newly hatched larvae controlled by the same or different genes involved in mucus production in trunk epidermis during later stages of larval development?



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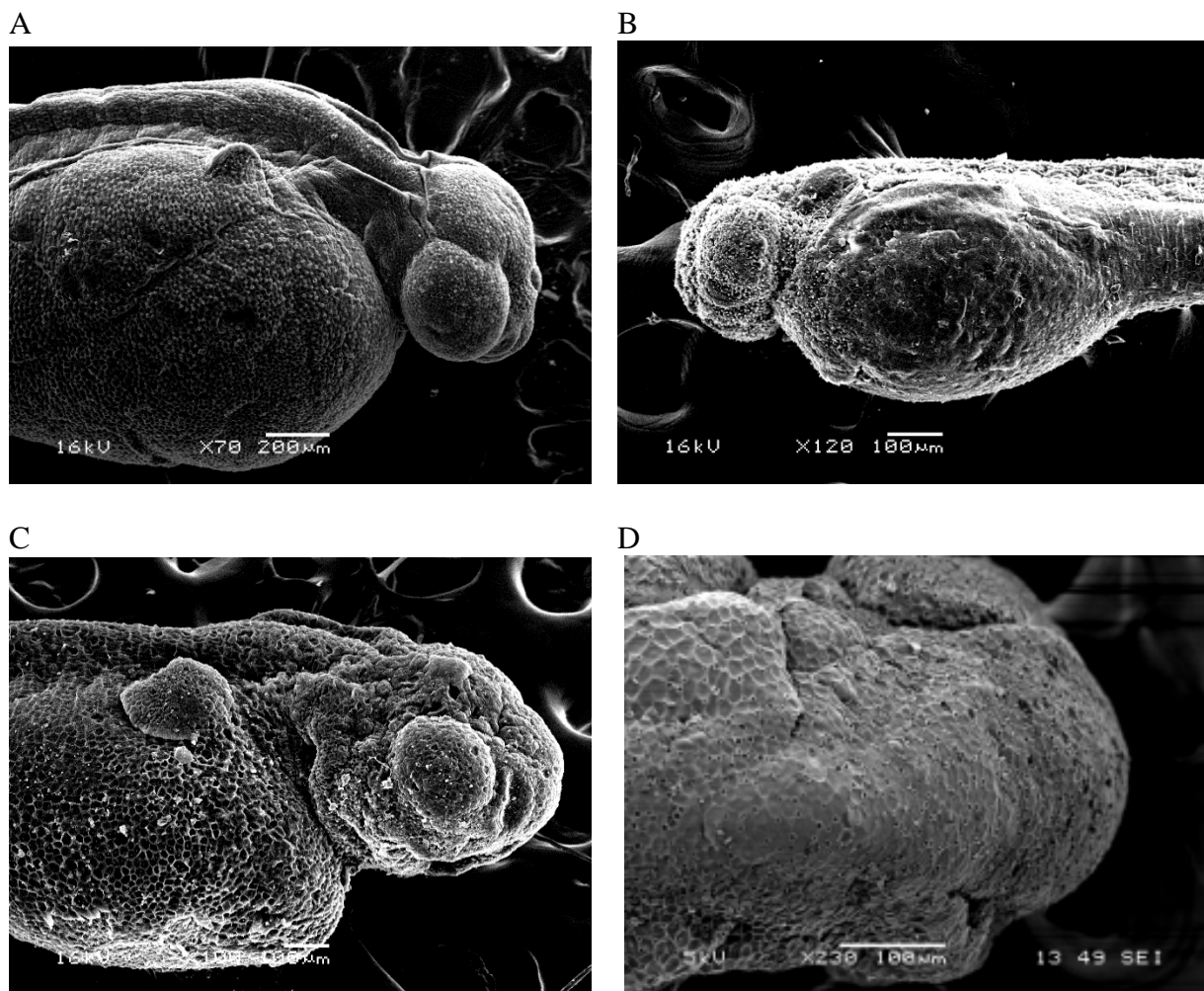
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Table 1. Diversity of terminology used to describe the attachment mechanisms of larval and adult organisms to substrates.

<b>Name</b>	<b>Species</b>	<b>Stage</b>	<b>Family</b>	<b>Group</b>	<b>Reference</b>
cement gland	<i>Xenopus laevis</i>	larvae	Pipidae	Amphibia	Sive & Bradley, 1996
"	<i>Pterophyllum scalare</i>	larvae	Cichlidae	Actinopterygii	Bennemann & Pietzsch-Rohrschneider, 1978
"	<i>Octolasmis angulata</i>	larvae	Poecilasmataidae	Crustacea	Yap et al., 2017
cement gland apparatus	<i>Pterophyllum scalare</i>	larvae	Cichlidae	Actinopterygii	Groppelli et al., 2003
cement gland-like structure	<i>Astyanax mexicanus</i>	larvae	Characidae	Actinopterygii	Pottin et al., 2010
casquette	<i>Astyanax mexicanus</i>	larvae	Characidae	Actinopterygii	Pottin et al., 2010
temporary adhesive gland	<i>Octolasmis angulata</i>	larvae	Poecilasmataidae	Crustacea	Yap et al. 2017
adhesive apparatus	<i>Pseudocheneis sulcatus</i>	adult	Sisoridae	Actinopterygii	Joshiet et al., 2011
"	<i>Glyptothorax pectinopterus</i>	adult	Sisoridae	Actinopterygii	Joshiet et al., 2011
"	<i>Garra lissorhynchus</i>	adult	Cyprinidae	Actinopterygii	Massar, 2015;
adhesive gland	<i>Cichlasoma dimerus</i>	larvae	Cichlidae	Actinopterygii	Meijide & Guerrero, 2000
"	<i>Tilapia marie</i>	larvae	Cichlidae	Actinopterygii	Retaux & Pottin, 2011
"	<i>Cyprinus carpio</i>	larvae	Cyprinidae	Actinopterygii	Applebaum & Riehl, 1997
adhesive organ	<i>Amia calva</i>	larvae	Amiidae	Actinopterygii	Eycleshymer & Wilson, 1908
"	<i>Lepisosteus osseus</i>	larvae	Lepisosteidae	Actinopterygii	Long & Ballard, 2001; Retaux & Pottin, 2011
"	<i>Hoplerythrinus unitaeniatus</i>	larvae	Erythrinidae	Actinopterygii	Gomes et al., 2007
"	<i>Hoplias lacerdae</i>	larvae	Erythrinidae	Actinopterygii	Gomes et al., 2007
"	<i>Hoplias malabaricus</i>	larvae	Erythrinidae	Actinopterygii	Gomes et al., 2007
"	<i>Brycon orthotaenia</i>	larvae	Characidae	Actinopterygii	Gomes et al., 2011
attachment organ	<i>Apteronotus albifrons</i>	larvae	Gymnotidae	Actinopterygii	Britz et al., 2000
"	<i>Apteronotus leptorhynchus</i>	larvae	Gymnotidae	Actinopterygii	Britz et al., 2000
glue secretion & adhesion	<i>Pteronotropis hypselopterus</i>	larvae	Cyprinidae	Actinopterygii	Fletcher & Wilkins, 1999

Figure 1. Epidermis of lateral views of newly hatched larvae of A. *Semotilus atromaculatus* (note abundance of apical pores of goblet cells), B. *Hybognathis hankinsoni* (note abundance of white dots on dorsal, lateral, and ventral cephalic epidermal areas and anteroventral epidermis of yolk sac identified as unculti-like projections under higher magnification), C. *Notemigonus crysoleucas* (note absence of white dots on dorsum but abundance of white dots on lateral and ventral cephalic areas and anteroventral yolk sac epidermis identified as unculti-like projections under higher magnification), D. dorsal cephalic view of newly hatched larva of *Cyprinus carpio* (note nubby unculti-like projections on dorsum and longer ones on lateral cephalic areas), and E. ventral cephalic view of *Gila atraria* (note white dots on ventral cephalic area and on anteroventral yolk sac epidermal areas identified as unculti-like projections under higher magnification).



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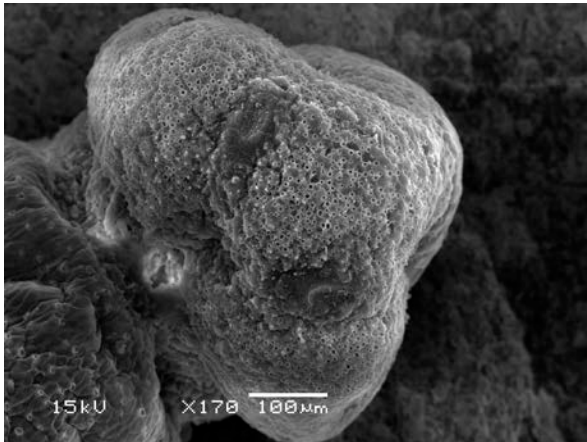
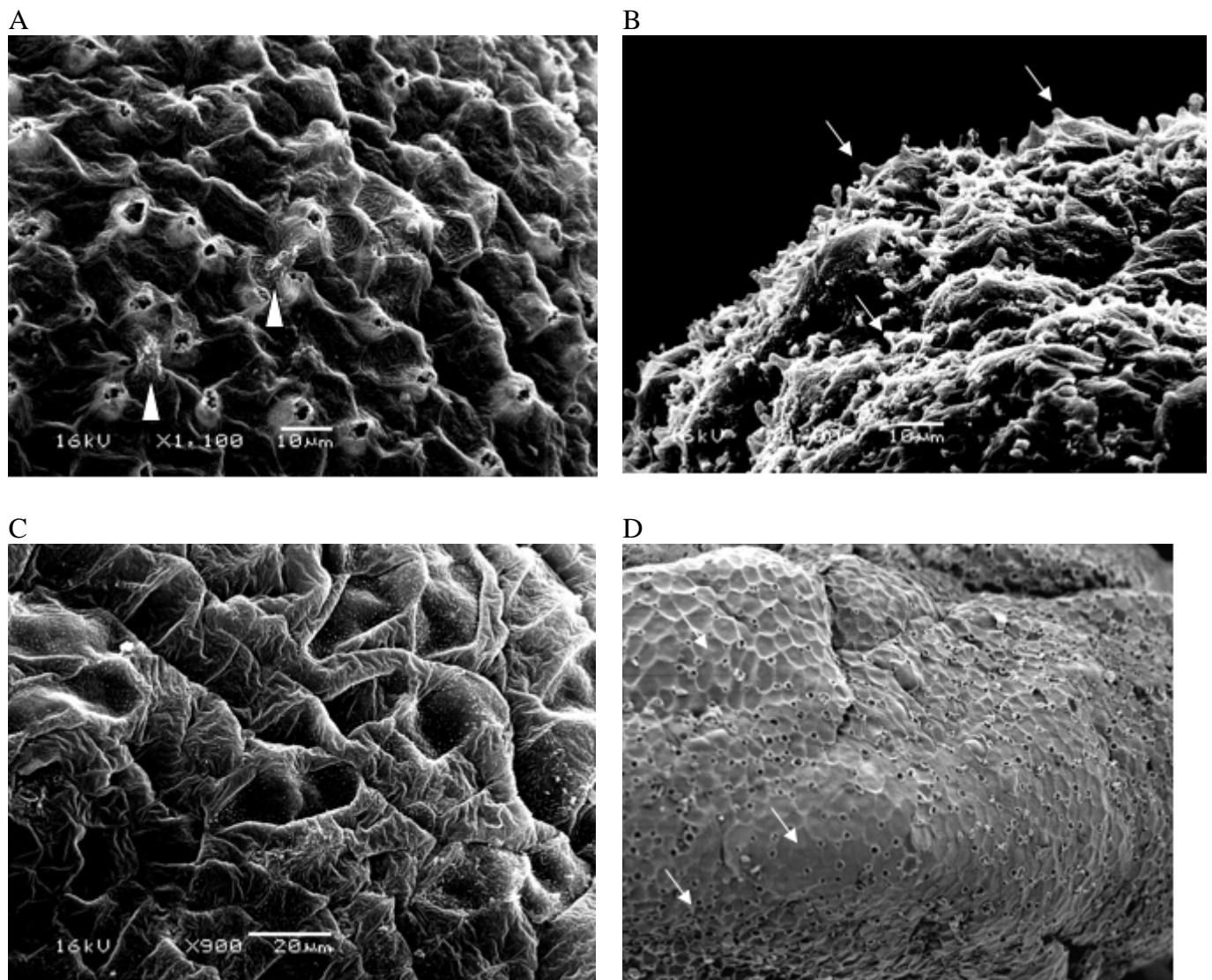
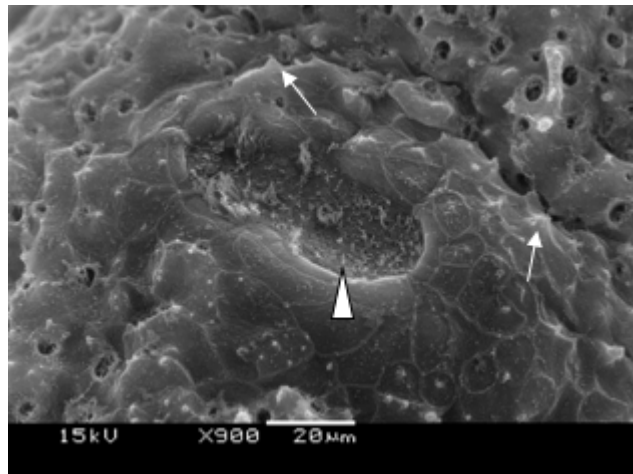


Figure 2. Dorsal cephalic epidermal areas of newly hatched larvae. A. *Semotilus atromaculatus* (numerous pores surrounded by hexagonal epidermal cells without uncili-like projections, well-defined microridge (MR) pattern, and free neuromasts (triangle), B. *Hybognathis hankinsoni* with well-defined MR and widespread uncili-like projections, C. *Notemigonus crysoleucas* hexagonal cells with well-defined MR and no uncili-like projections, D. *Cyprinus carpio* with widespread nubby uncili-like projections on dorsum and well defined uncili-like projections on lateral cephalic area, E. *Gila atraria* with short MR, widespread well-defined uncili-like projections on dorsum, and developing olfactory organ (triangle), F. *H. hankinsoni* (thick mucus embedded with debris; note uncili-like projections on lateral cephalic margin), and G. *atraria* (heavy mucus coating embedded with debris on dorsal epidermis).

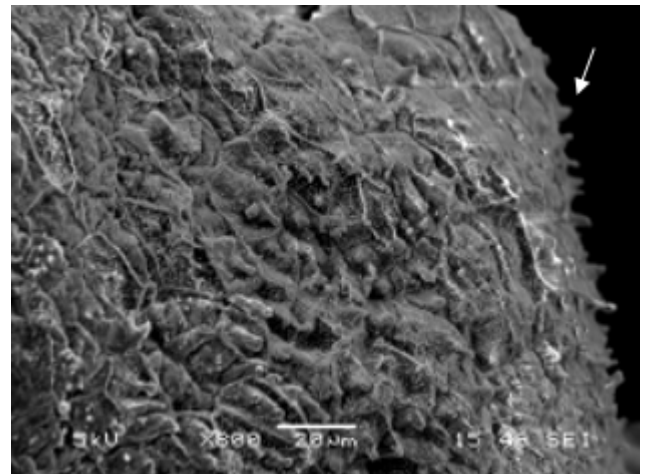




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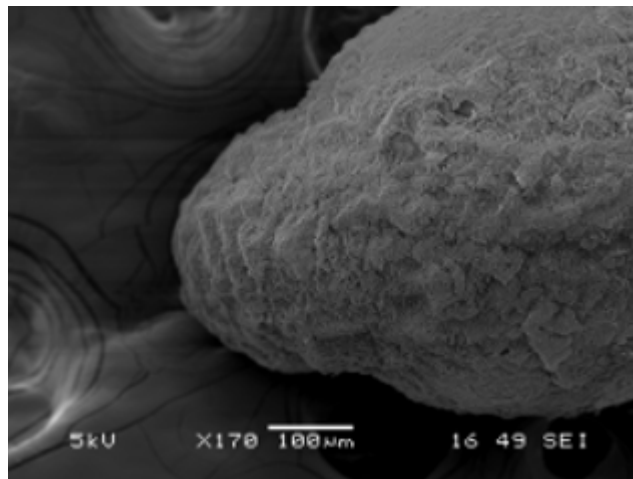
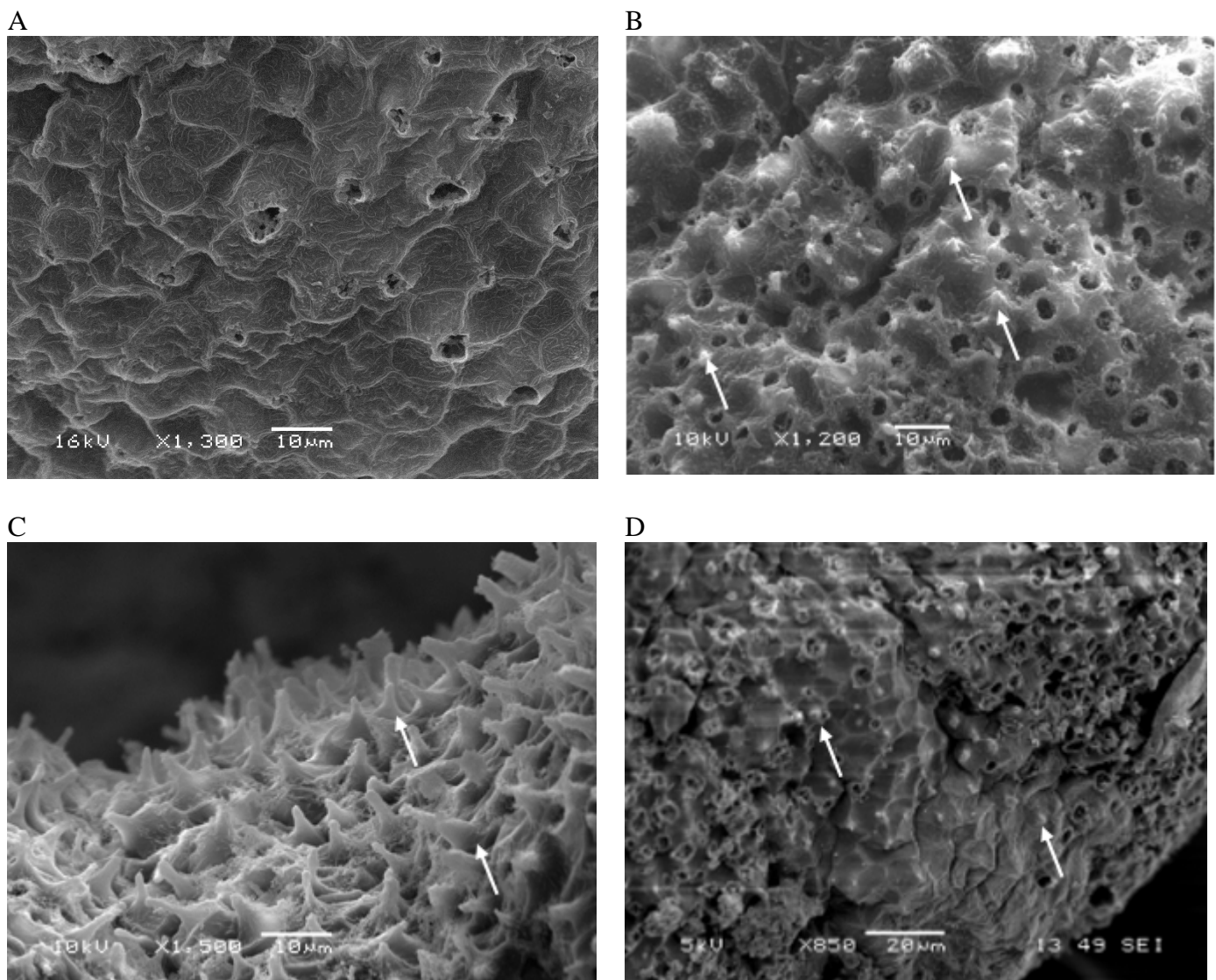
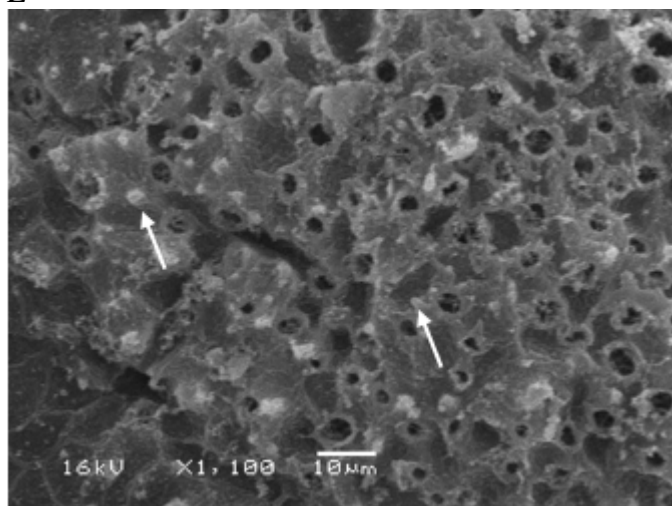


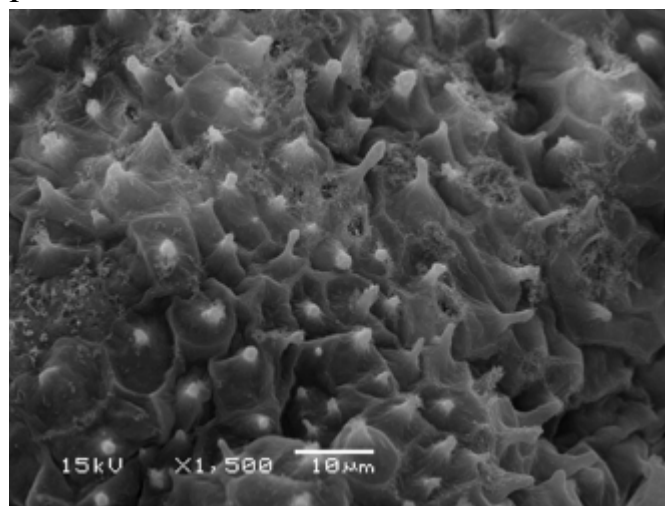
Figure 3. Ventral cephalic epidermal areas of newly hatched larvae. A. *Semotilus atromaculatus* (epidermal cells and apical pores of goblet cells and no uncili-like projections, B. *Hybognathis hankinsoni* (abundant uncili-like projections emanating primarily from cell centers, and apical pores of goblet cells, C. *Notemigonus crysoleucas* (abundant and long uncili-like projections), D. *Cyprinus carpio* (uncili-like projections and apical pores of goblet cells abundant displaying some segregation of cell types), E. *Gila atraria* (abundant uncili-like projections emanating from cell centers and at or near the cell peripheries, and abundant apical pores of goblet cells), F. *N. crysoleucas* (anteriolateral cephalic epidermis contains long uncili-like projections), G. *C. carpio* (mucus covering epidermal uncili-like projections on anterioventral portion of head), and H. *G. atraria* (uncili-like projections emanate from central and at or near cell peripheries).



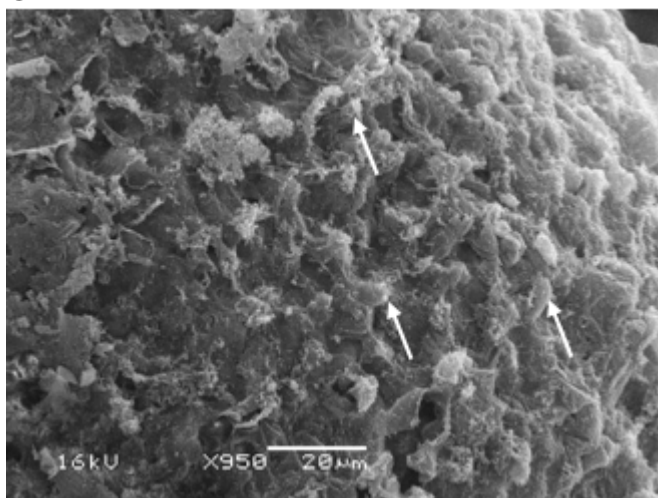
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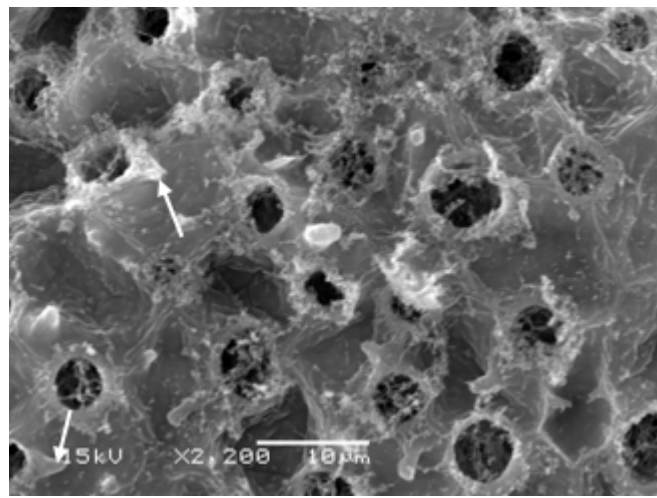
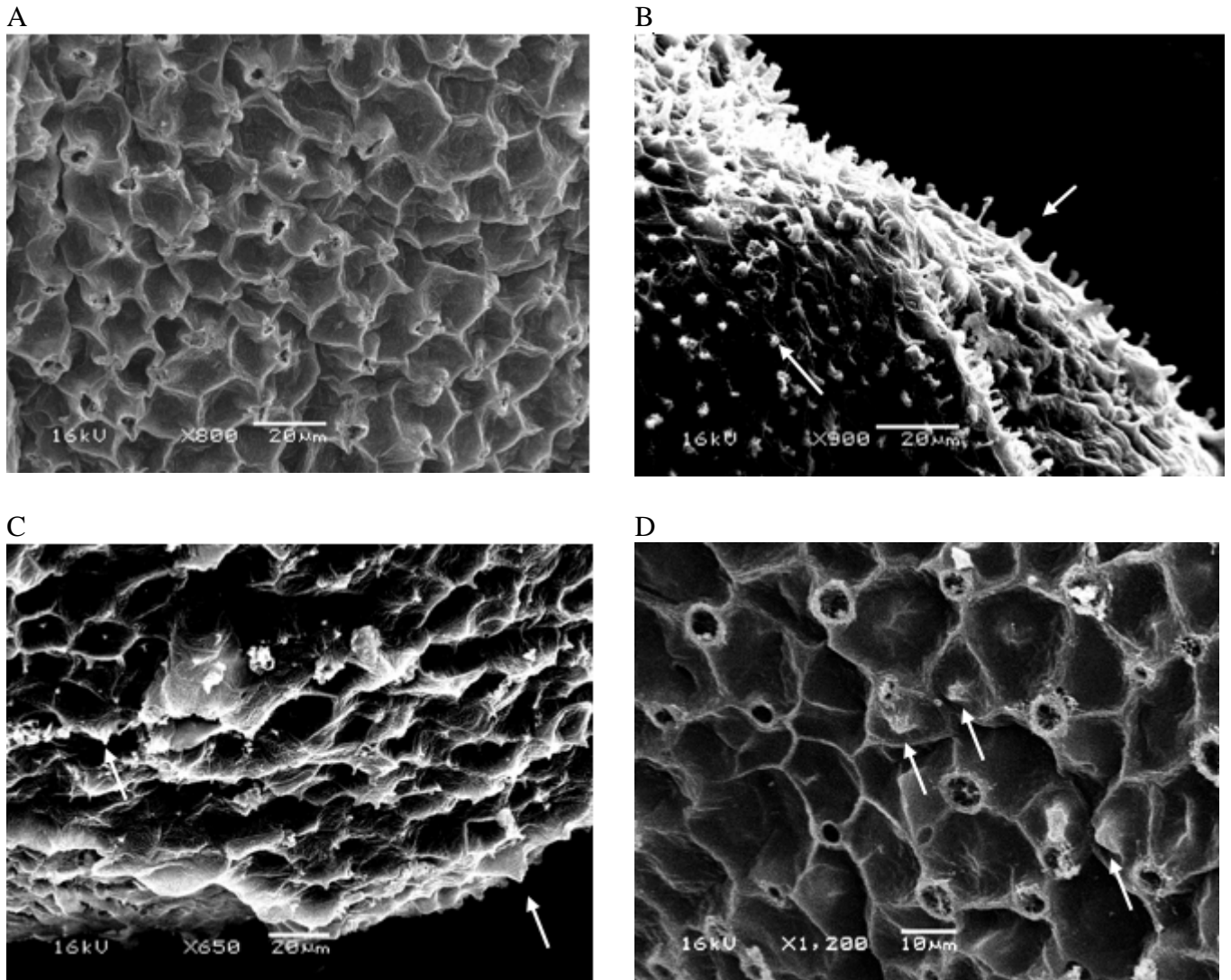
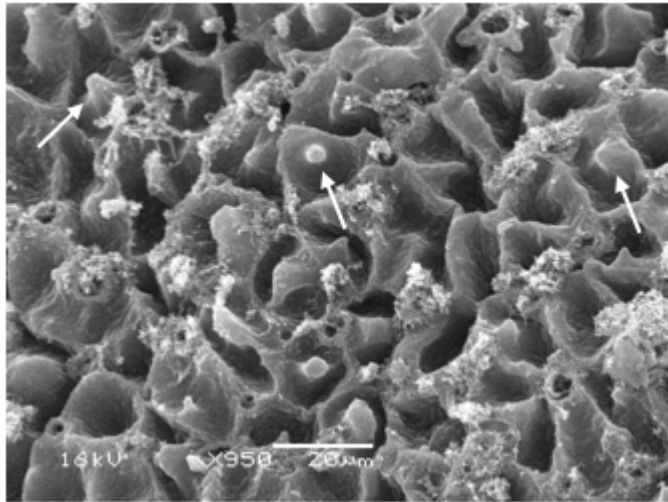


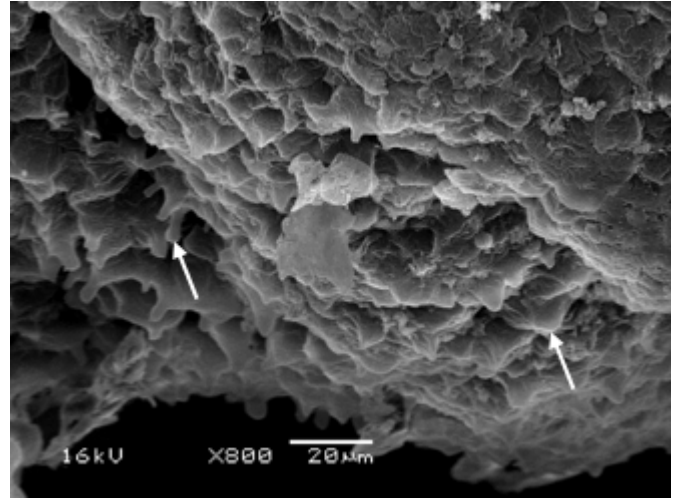
Figure 4. Anterioventral yolk sac epidermal areas of newly hatched larvae. A. *Semotilus atromaculatus* (apical pores and no unculti-like projections), B. *Hybognathis hankinsoni* (long unculti-like projections), C. *Notemigonus crysoleucas* (well developed to nubby unculti-like projections present), D. *Cyprinus carpio* (unculti-like projections present), E. *Gila atraria* (well-developed unculti-like projections and numerous pores present), F. *N. crysoleucas* (well-developed unculti-like projections), and G. *C. carpio* (unculti-like projection magnified at 3,700x).



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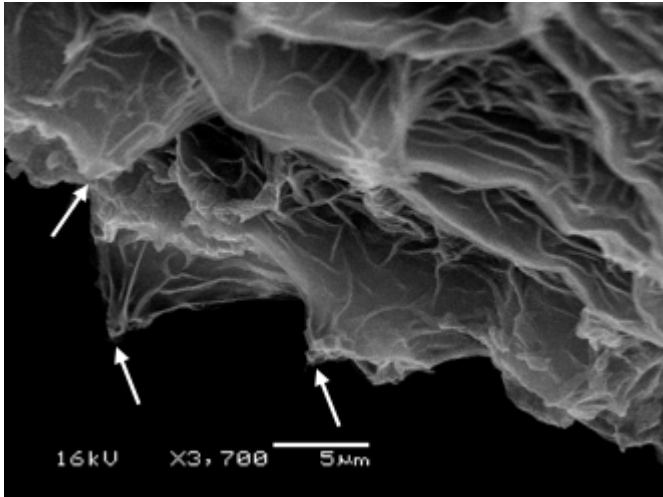
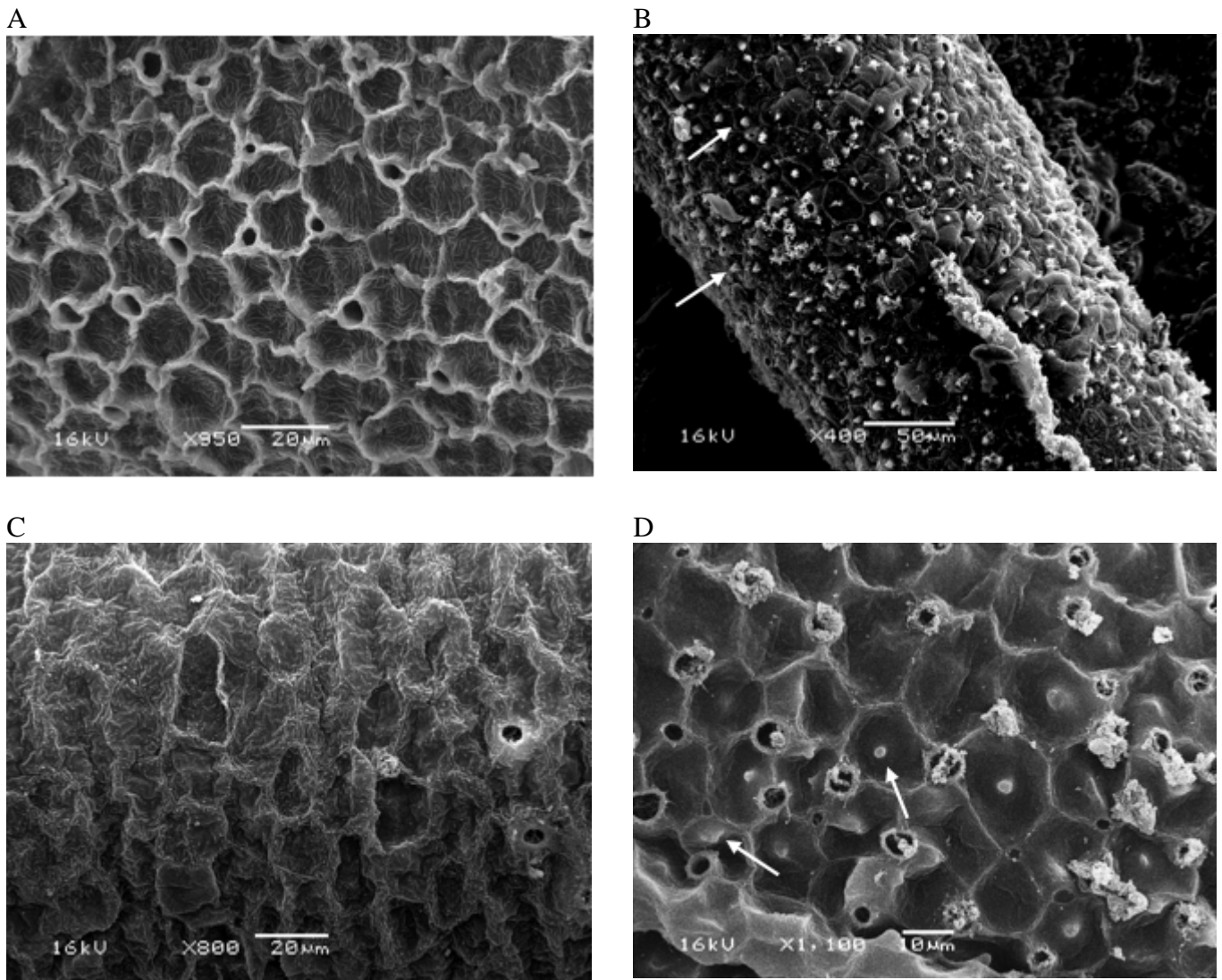
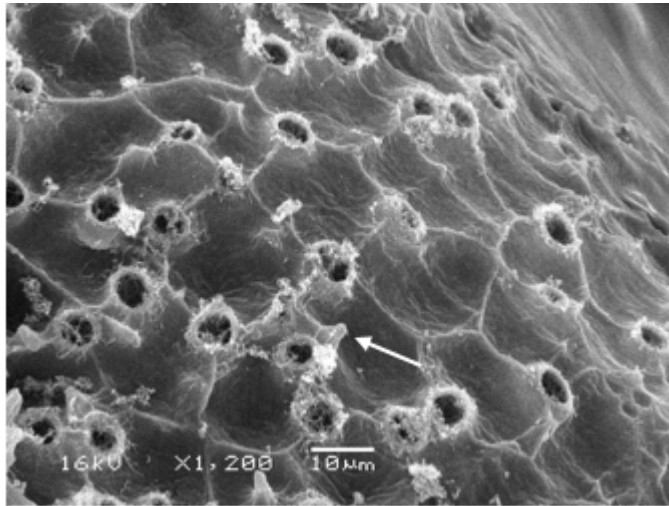


Figure 5. Ventrolateral trunk epidermal areas of newly hatched larvae. A. *Semotilus atromaculatus* (unculi-like projections absent), B. *Hybognathis hankinsoni* (unculi-like projections present), C. *Notemigonus crysoleucas* (unculi-like projections absent on lateral trunk), D. *Cyprinus carpio* (unculi-like projections increase towards ventral finfold), E. *Gila atraria* (few unculi-like projections present on ventrolateral and ventral epidermal cells), and F. *N. crysoleucas* (unculi-like projections present on ventral trunk).





E



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