



## SYMPOSIUM

# The Role of Developmental Integration and Historical Contingency in the Origin and Evolution of Cypriniform Trophic Novelty

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**Synopsis** While functional morphologists have long studied the evolution of anatomical structures, the origin of morphological novelties has received less attention. When such novelties first originate they must become incorporated into an integrated system to be rendered fully functional. Thus, developmental integration is key at the origin of morphological novelties. However, given enough evolutionary time such integration may be broken, allowing for a division of labor that is facilitated by subsequent decoupling of structures. Cypriniformes represent a diverse group of freshwater fishes characterized by several trophic novelties that include: kinethmoid-mediated premaxillary protrusion, a muscular palatal and post-lingual organ, hypertrophied lower pharyngeal jaws that masticate against the base of the neurocranium, novel pharyngeal musculature controlling movement of the hypertrophied lower pharyngeal jaws, and in a few species an incredibly complex epibranchial organ used to aggregate filtered phytoplankton. Here, we use the wealth of such trophic novelties in different cypriniform fishes to present case studies in which developmental integration allowed for the origin of morphological innovations. As proposed in case studies 1 and 2 trophic innovations may be associated with both morphological and lineage diversification. Alternatively, case studies 3 and 4 represent a situation where ecological niche was expanded but with no concomitant increase in species diversity.

“The history of life is not necessarily progressive; it is certainly not predictable. The earth’s creatures have evolved through a series of contingent and fortuitous events.”

—SJ Gould, 1994

## Introduction

Historical contingency has played an important role in the origin and evolution of morphological novelties. Morphological novelties are often defined as unique structures that arise in a clade, not homologous to any feature in the ancestral lineage, that may allow an organism to perform a new function or behavior (Mayr 1960, 1963; Hall 2005). Novelties represent the wholesale origin of a structure, as opposed to a change in an existing structure

(Brigandt 2010). Morphological novelties offer the potential of opening new regions of the adaptive landscape within which diversification can occur. Such innovations may lead to increased lineage diversification (evidenced by bursts of speciation) and/or morphological diversification. Galis (2001) proposed that innovations *potentially* allow for species divergence, rather than cause it, making diversification dependent on the new functional interaction with the environment created by the innovation. Liem (1989, 1990) also noted that key innovations may trigger lineage diversification when ecological conditions offer the proper environment for such alternate designs to prosper.

There are times when morphological novelties do not lead to species diversity but rather allow for increased morphological diversification and

concomitant expanded trophic niche by a few species. Lineage diversification is generally the measure of “evolutionary success” but need not be accompanied by great ecological diversity. Alternatively, morphological diversification may lead to substantial increase in ecological niche without a concomitant lineage diversification. Such morphological specializations have been referred to as evolutionary dead ends (Day et al. 2016; evolutionary one-offs of Blount et al. 2018), however the significant advantage that is provided to the small number of species possessing the specialization may still allow them to exploit resources not available to most (Cohen and Hernandez 2018a). When such species find themselves in an area with no natural predators, as is seen with invasive species, such specializations may allow them to outcompete native species potentially destroying natural food webs. Regardless of whether the novelty is associated with lineage diversification or not these novelties are often parts of complex systems. Increased structural complexity is often associated with the genesis of morphological innovations (Galis 2001), and such morphogenesis is often tied to developmental integration.

Developmental integration presupposes that morphological building blocks will interact during their development being driven either by an extrinsic factor such as selection or intrinsic factors driven by historical contingency. Regardless of underlying cause developmental integration may then lead to functional integration within an individual (Cheverud 1996). Thus, developmental integration may provide the historical contingency that plays a significant role in the origin of morphological novelty. In addition to determining which modules survive or are lost (Eble 2004), developmental integration may also exist in the form of well-integrated “scaffolds” into which evolutionary novelties can be incorporated. Broader developmental integration within trophic systems may have allowed for the origin of some cypriniform evolutionary novelties.

Cypriniformes represent a large monophyletic group (Saitoh et al. 2006) of teleosts containing at least 4250 species and 400 genera (Fricke et al. 2019) and making up 25% of the world’s freshwater fishes. They are the sister group to all remaining taxa within Otophysi (Fink and Fink 1981, 1996; Dimmick and Larson 1996; Briggs 2005). Fishes within this order have a number of trophic synapomorphies that include: (1) a palatal organ, a thick muscular cushion on the roof of the mouth, that helps process food within the buccal chamber (Sibbing and Uribe 1985; Callan and Sanderson

2003; Finger 2008); (2) a post-lingual organ on the base of the pharynx located between posterior branchial arches; (3) a kinethmoid, a novel sesamoid ossification that effects a unique type of premaxillary protrusion (Harrington 1955); and (4) novel pharyngeal muscles inserting on a greatly hypertrophied ceratobranchial. (5) Some species also possess the most complex epibranchial organ (EBO) yet described that serves to aggregate small particles collected during filter feeding in silver carp (Cohen and Hernandez 2018a). Britz and Conway (2016) pointed out that eight of nine unreversed synapomorphies uniting Cypriniformes were trophic in nature. These authors have also detailed the origin of morphological novelties within miniaturized danionins (Conway and Britz 2007; Britz and Conway 2009, 2016; Britz et al. 2009). Increased ecological diversity associated with these novelties has characterized the history of this group (de Silva et al. 1980; de Graaf et al. 2000; Sibbing and Nagelkerke 2001).

Here we will present four case studies illustrating the roles of both developmental integration and historical contingency in the origin and evolution of a subset of trophic novelties within Cypriniformes. The first case study illustrates the primary role of developmental integration as a permissive factor in the evolutionary origin of a novelty that is associated with both morphological and lineage diversification. The second case study outlines the role of historical contingency in the origin and evolution of several muscular trophic novelties within Cypriniformes. The third case study describes the importance of functional and historical contingency in the evolution of complex systems by presenting the multifunctional role of respiratory muscles to a specific type of prey processing used by cypriniform filter feeders. This novelty is not associated with lineage diversification; instead it has allowed the few species possessing it to outcompete native filter feeders. The final case study illustrates how once independent modules can be secondarily functionally (and developmentally) coupled to form an incredibly complex trophic system, a filtering apparatus that epitomizes a multi-structural function.

### Case Study No. 1. Developmental integration and historical contingency allowed for the origin of a novel means of premaxillary protrusion

Premaxillary protrusion has evolved at least five times during actinopterygian evolution (Hernandez and Staab 2015; Wainwright et al. 2015). Increased premaxillary protrusion is associated with a

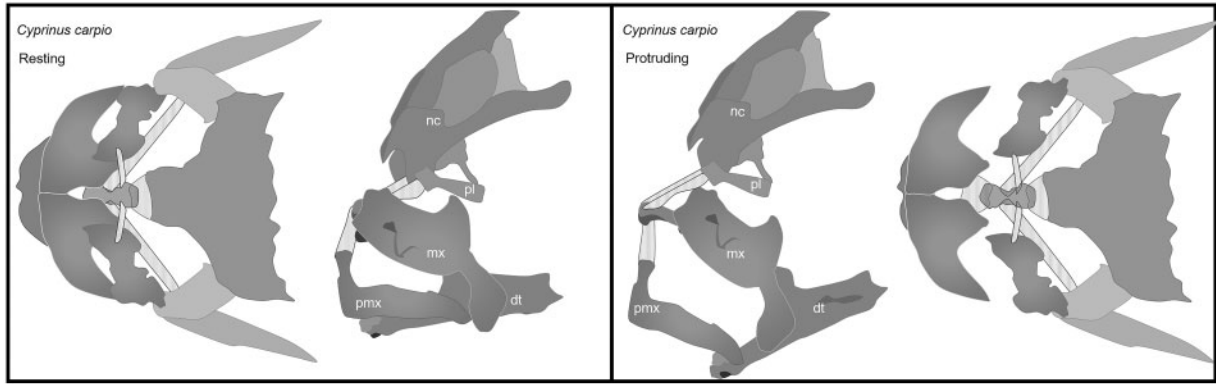
lengthening of the ascending process, a trait that has evolved several times within many groups of acanthomorphs (Cooper et al. 2017). In Cichlidae increases in the length of the ascending process of the premaxilla, and thus maximum jaw protrusion, have evolved several times (Hulsey et al. 2010), while in Haemulidae elongation of the ascending process has been correlated with an increase in benthic feeding (Tavera et al. 2018). Within acanthopterygians, depression of the lower jaw causes premaxillary protrusion; a taut ligament tying the premaxilla to the lower jaw pulls the premaxilla forward as the jaw is lowered. Kinethmoid-mediated premaxillary protrusion requires secondary ligamentous attachments and has evolved only once at the base of Cypriniformes. Mayrinck et al. (2015) identified a kinethmoid in the basal otophysan fossil *Chanoides macropoma* (suggesting that an earlier lineage had a kinethmoid), although Patterson (1984) concluded that the structure was not homologous to the cypriniform kinethmoid.

Cypriniform premaxillary protrusion is decidedly more complex, relying on the evolution of the kinethmoid, a novel median ossification in the rostral skeleton of these fishes. Located between the ascending processes of the premaxillae and the neurocranium (Fig. 1), this bone is suspended via ligamentous attachments to the palatines and maxillae. Upon mouth opening (or ventral translation of the maxilla) tension produced at the connections to the neurocranium and ascending processes of the premaxillae effects a 90–180° rotation of the kinethmoid thus pushing the premaxilla forward (Gidmark et al. 2012). While there is significant morphological diversification in the size and shape of cypriniform kinethmoids (Hernandez et al. 2007), the ligamentous attachments are more conserved. The complex ligamentous system that predated Cypriniformes (Alexander 1967) was a requisite historical contingency for the evolution of a novel means of premaxillary protrusion, and may have resulted from strong selective forces for increased cranial kinesis. In addition to allowing for significant protrusion (Staab et al. 2012; Hernandez and Staab 2015), this type of jaw protrusion facilitates closed mouth protrusion, an important part of buccal processing that allows species to winnow detritus and other nutritious particles from sediment (Gidmark et al. 2012). We agree with Sibbing et al. (1986) that the kinethmoid was involved in opening up unique modes of benthic feeding among cypriniforms. The ecomorphological importance of this novel ossification is likely the reason it develops so early.

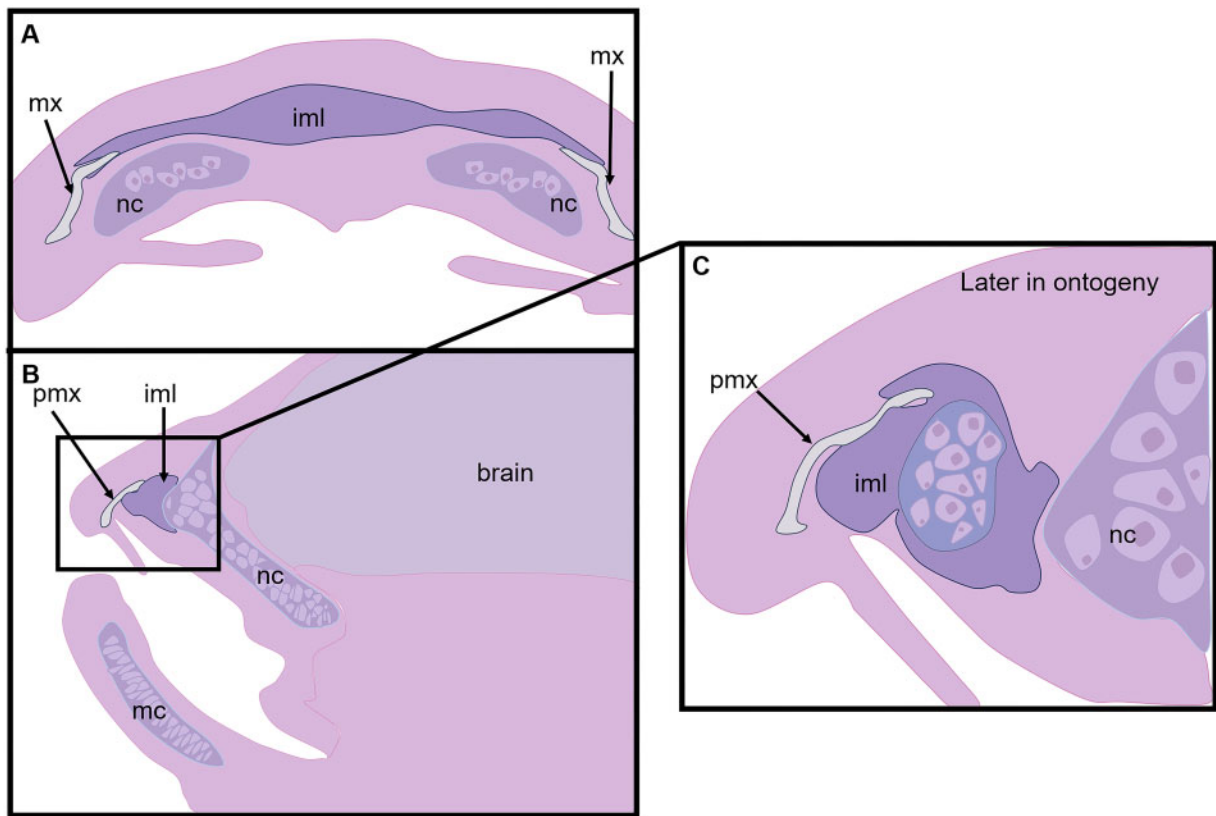
A cartilaginous kinethmoid can be identified as early as 5.8 mm SL within zebrafish. This small cartilaginous condensation forms within the intermaxillary ligament, located between the contralateral hooks on the medial maxilla (Fig. 2). This cartilage starts to ossify during early metamorphosis (at around 6.6 mm SL), with points of ligamentous attachment showing the earliest signs of ossification (Danos and Staab 2010; Staab and Hernandez 2010). This cypriniform sesamoid appears most like a hybrid category between embedded sesamoids (contained entirely within ligaments) and interosseous sesamoids (found adjacent to ligaments; *sensu* Jerez et al. 2010). While the initial cartilaginous kinethmoid is completely contained within the intermaxillary ligament, later during growth it is only partially encased within the ligament. Danos and Staab (2010) as well as Staab and Hernandez (2010) suggested that this intermaxillary ligament was transient, however there is no indication that this ligament disappears during development. This ligament likely played a pivotal role in the evolutionary origin of this sesamoid bone. Likely attesting to its functional importance, in only some extremely miniaturized (and specialized) cyprinids has the kinethmoid been lost (Roberts 1986; Britz and Conway 2016).

Sesamoids, differentiating from mesenchymal tissue within ligaments and tendons (Fabrezi et al. 2017), are commonly found within fishes (Patterson 1977; Arratia 1990; Summers and Koob 2002). The capacity for ligaments and tendons to ossify under mechanical forces appears to be a vertebrate synapomorphy. A phylogenetically broad assessment of sesamoid development has consistently shown that the development of this kind of bony element appears to be homoplastic and often associated with areas experiencing mechanical stress (Nussbaum 1982; Mikic et al. 2000; Scott 2005; Fabrezi 2006; Vickaryous and Olson 2007; Jerez et al. 2010; Montero et al. 2017; Amador et al. 2018). Several teleostean bones are derived from ossified ligaments including the “entopterygoid” of both Nematogenys and Diplomystes (Arratia 1990), as well as the urohyal in most fishes (Wainwright et al. 2006). These sesamoids tend to fully ossify only late in development again implicating mechanical forces in the proper ossification of these elements.

To better understand how such a novel ossification may have originated during the early evolutionary history of this clade it is necessary to determine the basal condition of the rostral skeleton within this group. Otocephala (Fig. 3) is comprised of



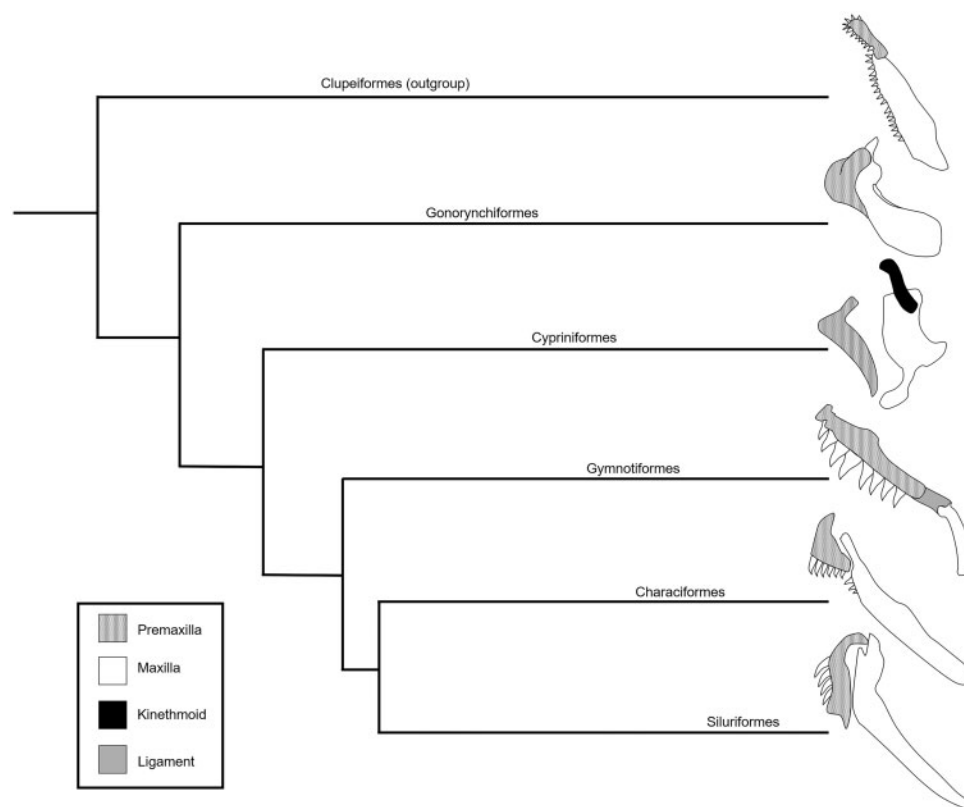
**Fig. 1** *Cyprinus carpio* showing resting and protruded state of the kinethmoid and premaxilla. Mouth opening (or depression of the maxilla) elicits premaxillary protrusion via rotation of the kinethmoid. dt, dentary; mx, maxilla; nc, neurocranium; pl, palatine; pmx, premaxilla.



**Fig. 2** Initial chondrogenesis of the kinethmoid in larval zebrafish. (A) An intermaxillary ligament connects the two maxillae. (B) At approximately 5.8 mm SL a small condensation of cartilaginous cells appears completely encased within the intermaxillary ligament. (C) Close-up of B showing cartilage later in ontogeny. iml, intermaxillary ligament; mc, Meckel's cartilage; mx, maxilla; nc, neurocranium; pmx, premaxilla. Modified from [Staab and Hernandez 2010](#).

Clupeiformes, Gonorynchiformes, and the four orders within Otophysi (Cypriniformes, Characiformes, Siluriformes, and Gymnotiformes), with Cypriniformes at the base of Otophysi ([Arratia 2018](#)). Clupeiformes (herrings and their relatives) and Gonorynchiformes (sister to Otophysi) have strongly linked maxillae and premaxillae with

little movement between them, thus cypriniforms represent one of the earliest evolutionary experiments with such decoupled, and increasingly kinetic, upper jaws ([Gosline 1961](#)). A constructional (and developmental) constraint present at the base of Otocephala was that the premaxilla and maxilla were strongly tied together. Decoupling the



**Fig. 3** Tree showing basal condition of maxilla and premaxilla (diagrammed from lateral aspect) in different orders within Otocephala. Cypriniforms represent one of the earliest experiments with decoupled maxilla and premaxilla. Species were chosen to best represent the basal condition for each clade. Clupeiformes: *Denticeps clupeoides*. Gonorynchiformes: *Chanos chanos*. Cypriniformes: *Cyprinus carpio*, diagrammed from the medial aspect. Gymnotiformes: *Gymnotus carapo*. Characiformes: *Bryconops affinis*. Siluriformes: *Diplomystes camposensis*.

premaxilla and maxilla likely allowed for increased cranial kinesis profoundly impacting the force regime experienced by adjacent cranial features.

In actinopterygians the evolution of a kinetic skull and the concomitant change in cranial forces affected both skeletal and ligamentous structure (Schaeffer and Rosen 1961). Tensile forces can sculpt ligamentous architecture early in development (Schaeffer and Rosen 1961; Vogel and Koob 1989) while compressive forces can lead to the ossification of sesamoids (Summers and Koob 2002). Such epigenetic changes in ligamentous connections (likely driven by strong selective forces; Schaeffer and Rosen 1961) were not necessarily concomitant with protrusion, but by loosening up the skull they may have facilitated evolution of protrusile mechanisms. We hypothesize that such changes may have also characterized the lineage leading to cypriniforms. Breaking such developmental constraints may have also allowed for the origin of novel features (Brigandt 2010), but it is essential to consider how a system could have initially integrated the novelty (Müller and Newman 2005) without rendering the entire system nonfunctional.

While selection can easily mold a structure once it originates, the forces molding its origin remain less straightforward. The side effect hypothesis (Müller 1990), which emphasizes constructional architecture, suggests that selective forces may act on areas adjacent to where novelties will originate. For example, a change in the size or architecture of cranial elements (acted on by selection) may bring about a change in the force regime experienced within adjacent cranial structures. Such epigenetic forces may then indirectly impact the genesis of morphological novelties. Such a hypothesis is appealing in that it explains how natural selection may indirectly lead to generation of a novelty. While such epigenetic hypotheses have been largely ignored until recently they provide a reasonable mechanism by which novelties can originate and rapidly spread throughout a population (Müller 1990; West-Eberhard 2003, 2005).

Much like our own knee caps, a sesamoid evolutionary novelty created by mechanical stress would be fully integrated upon initial development. The increased structural complexity afforded by the ligamentous scaffolding supporting the increasingly

kinetic skull allowed for the origin of a sesamoid bone that could be seamlessly functionally integrated into the system. Thus, the intermaxillary ligament may represent a key ligamentous attachment vital to the proper initiation of premaxillary protrusion. Indeed, functional analyses have shown that movement of this “maxillary bridge” (i.e., the taut intermaxillary ligament uniting the contralateral maxillae) predicts kinethmoid-mediated premaxillary protrusion better than any other variable (Gidmark et al. 2012). Moreover, functional studies of zebrafish larvae have shown that there is an immediate improvement in premaxillary protrusion once the kinethmoid first appears (Hernandez 2000; Hernandez et al 2002; Staab and Hernandez 2010). Unlike the independent origins of elongated ascending arms of the premaxilla which liberally pepper the acanthopterygian tree, the addition of a novel medial ossification effecting jaw protrusion happened only once, vividly illustrating that the great morphological and lineage diversification that characterizes Cypriniformes “evolved through a series of contingent and fortuitous events.” Both the loosening of the skull and the myriad episodes of elongation of the ascending process of the premaxillae can readily be explained by strong selection for increased kinesis and protrusion. Alternatively, the single origin of a protrusion-facilitating sesamoid strongly supports one contingent and fortuitous episode.

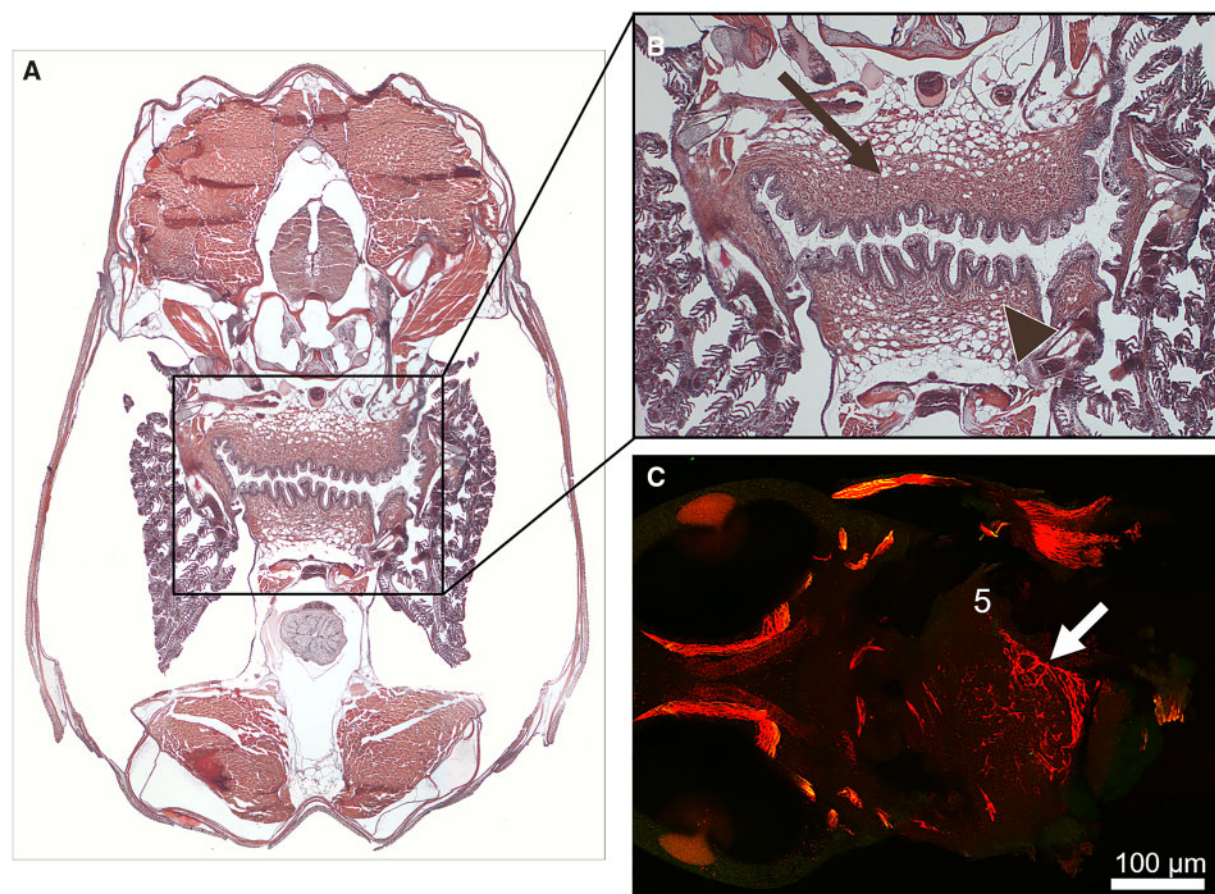
The decoupling of the maxilla and premaxilla and selective forces acting on increased kinesis may have set the stage for the origin of the kinethmoid. Selection may have been acting on a different set of elements allowing a permissive developmental milieu for the origin of the kinethmoid. Strong selection for increased cranial kinesis, even divorced from jaw protrusion, would have a pronounced effect on suction performance. Such selective forces would drive the loosening up of the skull and increased kinesis of feeding elements. Once a median ossification was fully formed selection could then act directly to mold the shape and architecture of the kinethmoid (Hernandez et al. 2007). Once cypriniforms possessed this novel protrusile mechanism evolution likely acted on the rostral skeleton (and its requisite muscular drivers) which was associated with profound morphological and lineage diversification. Changes in shape (Hernandez et al. 2007) and length of the kinethmoid (Staab et al. 2012), mouth position (Hernandez and Staab 2015), as well as histological properties of associated ligaments have significantly affected feeding performance (Gidmark et al. 2012; Staab et al. 2012). But importantly all this concomitant trophic diversity was

likely initially spurred by the early developmental integration that allowed for the origin of the kinethmoid.

## Case Study No. 2. Developmental contingency leads to several muscular trophic innovations within the posterior pharynx

Early in the evolution of cypriniforms a preponderance of muscle progenitors in the posterior pharynx was likely another developmental contingency that allowed for the origin of a variety of muscular trophic novelties. Cypriniforms are characterized by several muscular trophic innovations including: (1) a palatal organ located on the roof of the mouth, (2) a ventral post-lingual organ located between the posterior pharyngeal arches, and (3) several pharyngeal muscles that insert on a greatly hypertrophied ceratobranchial 5 (itself a novel lower pharyngeal jaw). Here we will focus on the palatal organ given the profound effect that this structure has had on trophic evolution within cypriniforms.

Both the palatal and post-lingual organ seemingly evolved at the base of Cypriniformes, as they have not been identified in any outgroup taxa. The palatal organ is a heavily muscularized pad of tissue located on the pharyngeal roof of cypriniform species (Fig. 4; Matthes 1963; Morita and Finger 1985; Sibbing 1988; Callan and Sanderson 2003; Finger 2008). It is composed of a complex mesh of differently sized skeletal muscle fibers of various lengths and diameters that are covered by an epithelium studded with mucus cells and taste buds (arrow in Fig. 4B; Evans 1931; Bhimachar 1935; Miller and Evans 1965; Morita and Finger 1985; Sibbing 1988; Gomahr et al. 1992; Lamb and Kiyohara 2005; Finger 2008). In goldfish and carp this structure aids in food selection and transport by forming local protuberances that protrude ventrally to trap larger particles against the base of the pharyngeal cavity (Morita and Finger 1985; Sibbing 1988; Callan and Sanderson 2003; Finger 2008). The large surface area of the palatal organ enables a great amount of food sorting to take place thus increasing feeding efficiency (Sibbing 1982, 1988; Finger 2008). The ventral postlingual organ (Sibbing and Nagelkerke 2001) is a median pad of small skeletal fibers similar in anatomy to those of the palatal organ (arrowhead in Fig. 4B). Sibbing et al. (1986) showed that the palatal organ and postlingual organ together generate peristalsis-like waves that transport food to the pharyngeal jaws. Preliminary data (S. Keer and L. P. Hernandez, unpublished data) suggest that both the



**Fig. 4** Palatal and postlingual organs of zebrafish. **(A)** Histological cross-section through adult zebrafish. **(B)** Close-up showing dorsal palatal organ (arrow) and ventral postlingual organ (arrowhead). **(C)** 60 h post-fertilization embryo showing the first evidence of skeletal muscle fibers of the palatal organ moving anteriorly; 5 represents the position of the 5th ceratobranchial.

palatal organ and postlingual organ are derived from embryonic muscle precursors moving rostrally from an area adjacent to the esophagus (Fig. 4C).

The palatal organ has been shown to assist in the selective retention of food particles during benthic feeding (Sibbing 1982, 1988). Taste buds on the palatal organ and throughout the pharynx enable fish to distinguish edible from non-edible particles (Sibbing and Uribe 1985; Lamb and Finger 1995; Finger 2008). Callan and Sanderson (2003) hypothesized that the palatal organ protrusions also provide localized chemosensory information on the palatability of the particles. Indeed, Lamb and Finger (1995) observed that when goldfish were fed food pellets covered with quinine, caffeine, and other bitter compounds particles were rejected. A hypertrophied vagal lobe in goldfish and common carp controls sensorimotor function of the palatal organ. Consistent with the muscular architecture of the palatal organ muscular hydrostats are often characterized by seemingly disorganized muscle fibers that run in many different directions (Kier and Smith

1985). Such a muscular hydrostat could serve a variety of functions within the pharyngeal cavity of cypriniforms and we believe that we have only started to scratch the surface of the functional potential of this cypriniform novelty.

Pharyngognath, and the requisite modifications that provided a stronger pharyngeal bite, is known to have evolved independently several times within acanthopterygian fishes (Wainwright et al. 2012). Similarly, it is widely held that many of the pharyngeal muscles of cyprinids evolved independently from those of Acanthopterygii (Takahasi 1925; Holstvoogd 1965; Vandewalle 1975). Many of these pharyngeal muscles are hypothesized to be derived from the sphincter esophagi, a muscle that develops early during larval development. It grows around the esophagus with fibers ultimately spreading forward to form both discrete obliqui and retractor muscles (Millard 1966; Nelson 1967a). Outgrowth from the embryonic sphincter esophagi may have also led to the origin of the muscular palatal organ.

While zebrafish have long served as excellent biomedical models of developmental processes, less attention has been paid to the development of structures not found in humans. Schilling and Kimmel (1997) published an excellent summary of the development of craniofacial muscles in the zebrafish. Unfortunately, they did not address the specific muscles associated with the pharyngeal jaws. However, one figure (Fig. 8H; Schilling and Kimmel 1997) showed expression of *myoD* (an early marker of muscle precursors) at 65 h post fertilization in the area where both pharyngeal muscles and the incipient palatal organ would be forming (S. Keer and L. P. Hernandez, unpublished data). Using immunohistochemistry we have shown that the first fibers that will comprise the palatal organ arise at 60–62 h post-fertilization in the posterior pharynx adjacent to the esophagus (Fig. 4C, arrow) and stretch laterally to the pharyngeal jaws (Fig. 4C, #5). This incredibly early development of the palatal organ may also explain the loss of upper pharyngeal jaws in cypriniforms.

Developmental mechanisms underlying large macroevolutionary changes have often been elusive. In an elegant exception Brylski and Hall (1988) showed that the seemingly major switch from internal to external cheek pouches in rodents could be explained by a relatively simple heterotopic shift early in development. First characterized by an early evagination of the buccal epithelium, a slight anterior shift in this evagination instantaneously shifted this pocket from internal to external. In a similar manner, we hypothesize that a slight heterotopic and/or heterochronic shift in the development of the sphincter esophagi could have led to the origin of the palatal organ as well as the overgrowth of the pharyngobranchials (which made up the upper pharyngeal jaws within outgroup taxa).

### Case Study No. 3. Historical contingency allows for the function of a unique type of epibranchial organ

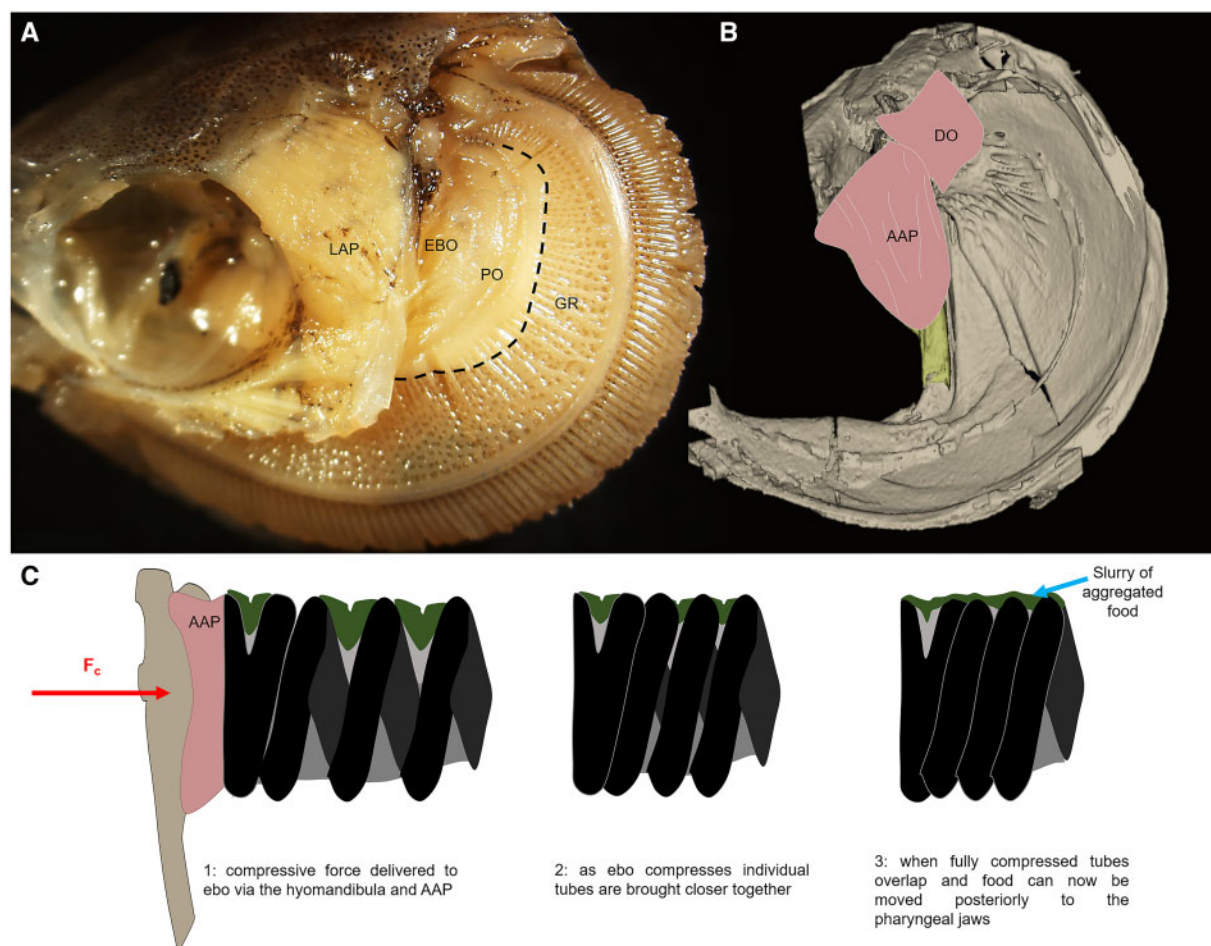
While Clupeiformes contain a large proportion of filter feeders with aggregating EBOs (Nelson 1967a), such organs are not as common within members of Otophysi. Most Gonorynchiformes (Pasleau et al. 2009), and some characiforms (Bertmar et al. 1969) possess these aggregating structures. EBOs are paired epithelial diverticula surrounded by muscle located in the posterodorsal pharynx supported by branchial arches 4 and 5 (Nelson 1967b). They can be relatively small invaginations or may become more complex, possessing

multiple coils. These epithelial invaginations are surrounded by skeletal muscle which serves to move particulate matter collected within the EBO into the esophagus. As opposed to this relatively simple architecture the EBO of silver (and bighead) carp is incredibly complex.

The EBO of silver carp is the most complex yet described (Figs. 5 and 6; Cohen and Hernandez 2018a). These aggregating organs span the entirety of the pharyngeal chamber. The paired organs are dorsal to the branchial arches and composed of four discrete epithelial (invaginating) tubes that are surrounded and supported by cartilaginous pharyngobranchials. Each tube spirals forming a snail-like structure into which the corresponding modified epibranchial associated with each tube penetrates (Figs. 5C, 6B). Each tube expands at its terminus forming a sac lined with abundant goblet cells. The most posterior tube of the EBO is formed by the involution of the fifth set of gill rakers that extend from the modified fifth ceratobranchial. Importantly each of the epithelial tubes is surrounded by circumferential muscles used to squeeze food out of the tubes and toward the pharyngeal jaws. The paired EBOs are surrounded by a greatly hypertrophied palatal organ (Fig. 6A, D).

Detailed analyses of the EBO and surrounding tissues have resulted in an anatomically-driven functional model for this complex organ (Cohen and Hernandez 2018a). Collected phytoplankton in both the terminal sac of each tube as well as food adhering to the food collecting groove of the modified epibranchials is squeezed out via circumferential muscle surrounding all epithelial tubes. Indeed, the thickest layer of circumferential muscle surrounds the final two continuous tubes that open on to the triturating pharyngeal jaws (Fig. 6B, see 4/5).

Silver carp are unusual among filter feeders in being pump filter feeders, thus the same muscles that are used for buccal pumping while respiring are used to move water into the mouth and over the large modified filtering plates. The operculum is thick, well-ossified, and tied to the hyomandibula via strong ligaments. The muscles used in buccal pumping also attach to the cartilaginous anterior cap of the EBO. The greatly hypertrophied levator arcus palatini (LAP) inserts along the lateral face of the hyomandibula (Fig. 5A), and the adductor arcus palatini (AAP) connects the hyomandibula to the EBO (Fig. 5B, C). The strong muscular connection between the EBO and the hyomandibula suggests that the force generated by these muscles during pumping is transferred to the EBO thus compressing and expanding this organ. The partially interlocking



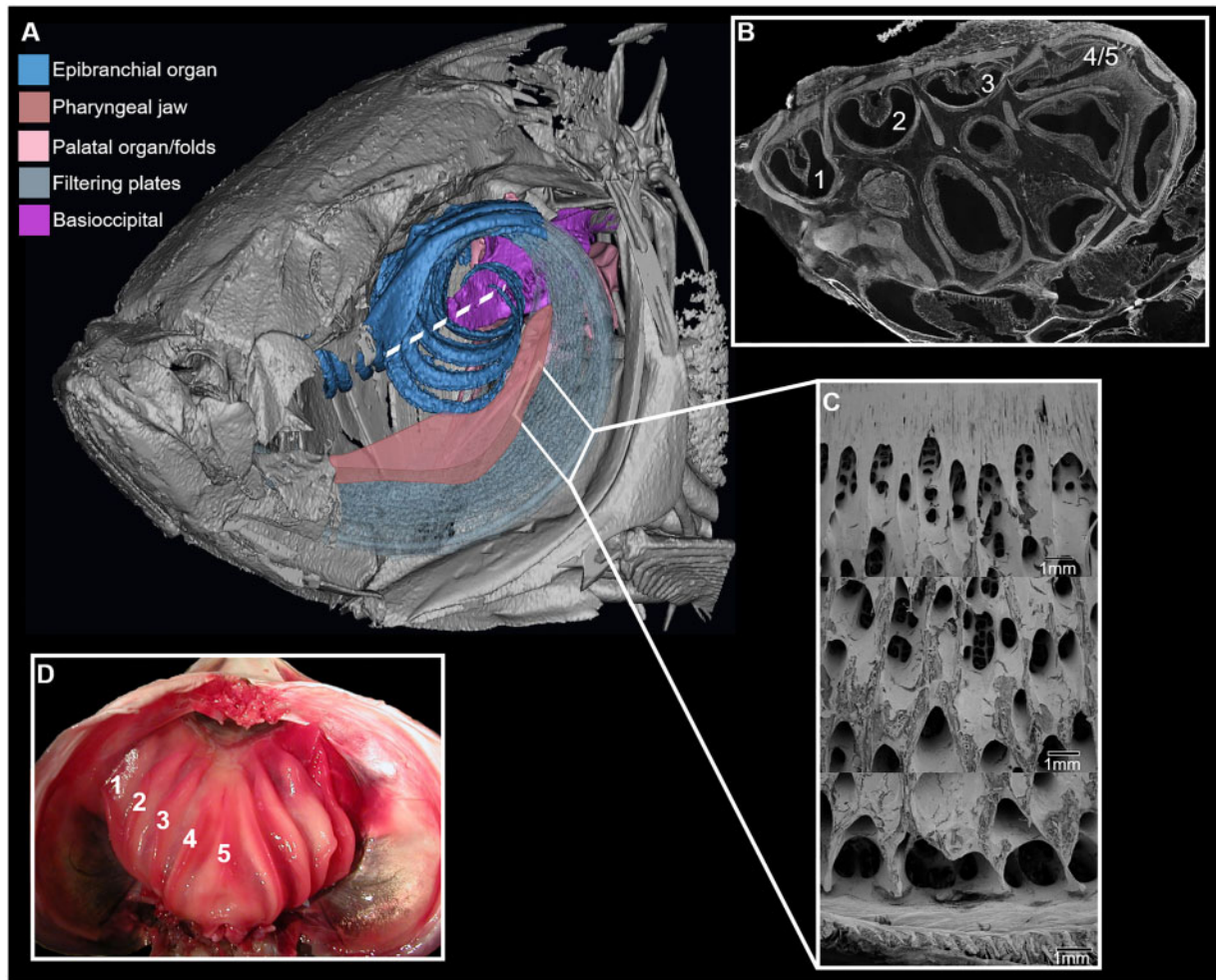
**Fig. 5** Greatly hypertrophied muscles attach to hyomandibula and the EBO of *Hypophthalmichthys molitrix*. (A) Lateral dissection shows a greatly hypertrophied LAP. Also note fold of palatal organ (PO) tucked into the two filtering plates that extend from each ceratobranchial. (B) Medial aspect of the operculum and hyomandibula showing hypertrophied AAP. (C) AAP connects the medial side of the hyomandibula to the rostral end of the EBO. The individual coils of the branchial arches can be compressed moving food medioposteriorly to the pharyngeal jaws. Modified from [Cohen and Hernandez 2018a](#).

cartilaginous plates that surround the individual tubes of the EBO can collapse against each other (much like an accordion) allowing the tubes to be compressed moving the aggregated slurry of phytoplankton and mucus back into the entrance of each tube (Fig. 5). The thick layer of well-innervated circumferential muscles (Fig. 6B) surrounding the epithelial tubes (and modified gill rakers holding food) serves to move accumulated particles on to the pharyngeal jaws.

The historical (and functional) contingency is that cypriniform filter feeders (bighead and silver carp) rely on buccal pumping. The hypertrophied muscles that perform opercular pumping during respiration have been coopted to move phytoplankton through the complex EBO. Without the hypertrophied muscles that move the operculum during buccal pumping being available to move particles of food through the EBO, it is highly unlikely that such a

structure could have evolved. The lack of such a complex, anteriorly expanded EBO in fishes that use ram filtration lends support to this hypothesis. Thus, co-option of respiratory muscles for the purposes of food aggregation may have led to the complex structure of the EBO in silver and bighead carp.

In the first case study we saw developmental integration providing a permissive scaffold (literally and figuratively) for the origin of the kinethmoid, while in the second case study we hypothesized about the role that errant muscle progenitors may have played in the construction of muscular trophic novelties. Here we see that structures that were once functionally modular (EBO and respiratory muscles) have become developmentally and functionally integrated; systems that should be modular work in an integrated fashion, thus breaking functional modularity. Moreover, while the majority of filter feeders are ram feeders it is actually the silver carp, using



**Fig. 6** Complex filtering apparatus of the silver carp, *Hypophthalmichthys molitrix*. (A) CT scan of the main skeletal components of the trophic apparatus showing the relative position of the palatal folds. (B) Cross-section of the EBO showing the three (1, 2, 3) individual anterior tubes associated with branchial arches 1–3, as well as the continuous tube (4/5) associated with branchial arches 4 and 5. (C) Three discrete sections of the epithelium covering the filtering plate. (D) Greatly modified palatal organ is comprised of muscular folds (1–5) that interdigitate into the corresponding filtering plates as shown in Figs. 5A and 6A.

buccal pumping, that is able to consume particles smaller than nearly any vertebrate filter feeder (Cremer and Smitherman 1980; Smith 1989; Zhou et al. 2009; Battonyai et al. 2015). The cypriniform historical contingency of having to use buccal pumping to filter feed is associated with the morphogenesis of the most complex EBO yet described. Moreover, the type of EBO found in the very destructive filter feeding silver carp is unlikely to have evolved anywhere but within Cypriniformes, where it was surrounded by a developing cushion of muscle progenitors. Next, we will examine the entire complex of filtering and aggregating structures that characterize silver and bighead carp suggesting how this multi-structural filtering function may have evolved.

#### Case Study No. 4. Role of integration in building a complex filtering structure

Complex systems are generally defined as systems with multiple parts that may have intricate levels of hierarchical organization (Gregory 2008). Such complex systems are built using multiple cell types and tissues that develop in complex ways, certainly when compared with the basal condition that preceded the evolution of the complex phenotype. They epitomize situations in which several structures have become tied together to achieve one function (multi-structural functions). Filter-feeding fishes generally use the same morphological structure for both filtering and aggregating particles. Alternatively, silver and bighead carp have two different specialized features to carry out this division of labor. In silver carp four

structures (gill rakers, EBO, palatal organ, and respiratory muscles) are combined to produce one of the most complex vertebrate filter feeding structures yet identified.

While the aggregating EBO has already been described, the filtering plates are greatly modified from the basal state and have an incredibly complex ontogenetic trajectory. During early ontogeny silver carp have two rows of comb-like rakers like those seen in most teleosts (Cohen and Hernandez 2018b). Shortly thereafter these individual gill rakers elongate and start to grow together to form two fused plates protruding from each branchial arch at an acute angle to each other. These filtering plates (formed from greatly modified gill rakers; Figs. 5A and 6A) provide a scaffold for the overlying topographically complex epithelium. This epithelium forms a series of canals dotted with pores of various sizes (Fig. 6C) making up a complex filtering surface (Cohen and Hernandez 2018b). As fluid moves over these topographically complex plates vortices form that move tiny particles into the canals (Cohen et al. 2018) where a mucus-secreting epithelium helps to collect particles. Particles are then moved from the filtering side of the complex plate to the inner side of the plate adjacent to elongated, fleshy palatal folds (Fig. 6A, D). These palatal folds are thought to play a role in moving particles into the food collecting groove and/or clearing the filtering plates to prevent clogging. An earlier hypothesis (Pichler-Semmelrock 1988) proposed that the palatal organ moved like a piston to bring water through the filtering plates, with the filtering plates serving as both mechanical and hydrosol filters (Adamek and Spittler 1984). The same modified gill rakers that make up the filtering plates also penetrate the epithelial tubes of the EBO. Indeed, it is along the food collecting groove (at the base of the V-shaped branchial arch) that food collected at the filtering plates is passed along and into the EBO (Fig. 6A, B).

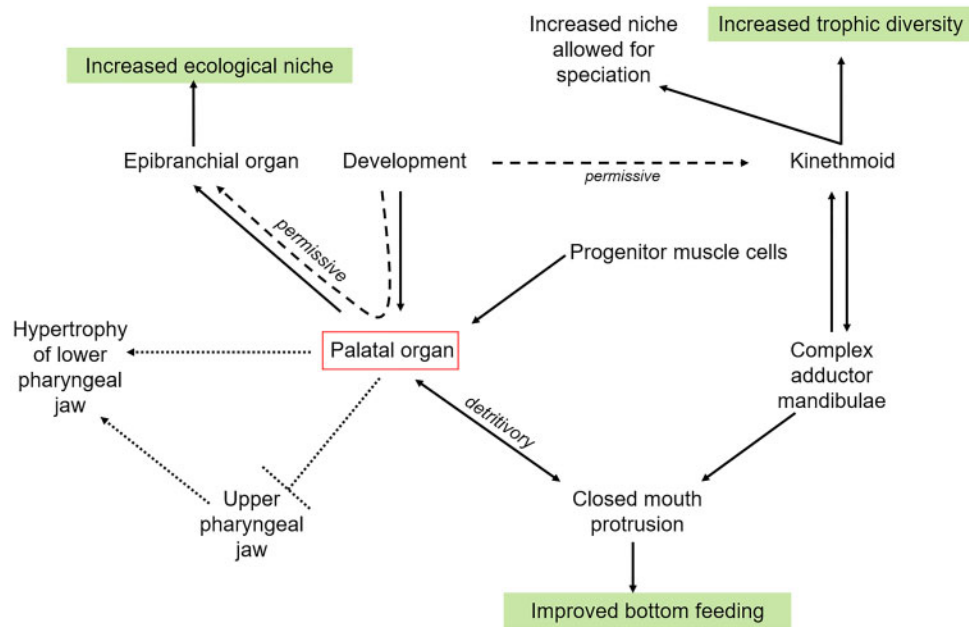
To build such a complex filtering and aggregating apparatus, structures that had not previously been associated, had to become integrated (or potentially re-integrated, see below). While outgroup taxa build EBOs from only the most posterior gill arches, in silver carp all the branchial arches have become incorporated. Moreover, the muscles controlling the respiratory buccal pump now work in the service of aggregating small particles. Previous work has suggested that at the base of the cyprinid tree the palatal organ and gill arches may have been functionally integrated (Eastman 1977), but such integration likely broke down during cyprinid evolution as the palatal organ was deemphasized. Here, the

developing palatal organ and filtering plates have become developmentally integrated anew. Palatal folds develop early in ontogeny and interdigitate into the filtering plates before the system has even become functional (unpublished data). This complex structure may have evolved piecemeal, but it is hard to imagine a similarly complex structure evolving within any group other than Cypriniformes. Catostomids, now considered to be near the base of the cypriniform tree (Conway 2011; Stout et al. 2016), have epibranchial bones that are completely subsumed by the muscular palatal organ (Grey and Mabee 2012). This sort of historical contingency may have facilitated the origin of the complex EBO of bighead and silver carp. While these species represent a miniscule proportion of extant cypriniforms they represent a compelling case study for illustrating the importance of developmental and historical contingency in the evolution of complex morphological novelties.

### Hypothesized role of trophic novelties on cypriniform ecology and evolution

Although almost certainly derived from bottom feeders (Hernandez and Staab 2015) cypriniforms have invaded nearly every freshwater trophic niche (Howes 1991) becoming master filter feeders (Cohen et al. 2018), piscivores (Sibbing and Nagelkerke 2001), herbivores (Vincent and Sibbing 1992), zooplankton pickers, algae scrapers, and secondary consumers (Sibbing 1991). In instances during which they might suffer performance deficits given their muscular palatal organ this fleshy structure has decreased in size (Doosey and Bart 2011). Using the palatal organ in combination with modified branchial arches even common carp can combine cross-flow filtration on small particles with palatal protrusions to catch larger items (Callan and Sanderson 2003), thus showing tremendous functional flexibility. Yashpal et al. (2009) suggested that in some species epithelial projections from the palatal organ beat synchronously to help move food particles into the esophagus. The enormous capacity for the palatal organ to become modified to fit different feeding modes is illustrated in silver carp where the palatal organ has become greatly hypertrophied yet seamlessly incorporated into the complex means of filter feeding which characterizes this species (Fig. 6).

We hypothesize that the trophic novelties outlined here have greatly affected cypriniform ecology and evolution (Fig. 7). The origin of the kinethmoid coupled with subsequent morphological evolution of the adductor mandibulae complex allowed for increased



**Fig. 7** Hypothesized role of morphological novelties in cypriniform trophic diversity. Development of the kinethmoid led to increased trophic diversity and expansion of trophic niche. Concomitant changes in the morphology of the adductor mandibulae led to improved bottom feeding. The palatal organ also played an important role in improved bottom feeding, as well as allowing for the origin of a novel type of EBO. Finally, we hypothesize that initial growth of the palatal organ may have led to the loss of the upper pharyngeal jaws thus indirectly affecting the hypertrophy of the pharyngeal jaw.

trophic diversity (Hernandez et al. 2007; Staab et al. 2012). The closed mouth protrusions that are greatly facilitated by the kinethmoid required novel reorientation of the adductor mandibulae complex (Gidmark et al. 2012) and allowed for bottom feeding as well as detritivory; detritivory also being facilitated by the function of the palatal organ. The palatal organ may have played a permissive role in the development of the complex EBO that increased the ecological niche of Asian carp. Finally, we hypothesize that early development of the palatal organ may have led to loss of the upper pharyngeal jaws, and concomitant hypertrophy of the lower pharyngeal jaws. Subsequent evolution of the lower pharyngeal jaws, palatal and postlingual organs, and pharyngeal pad on the basioccipital may have allowed for subsequent exploitation of novel trophic niches.

## Conclusion

Cypriniform novelties undergo substantial ontogenetic changes in integration and modularity, changes that profoundly affect function and performance. Early patterns of developmental integration may later become hidden by increasing modularity or integration with different structures (Hallgrímsson et al. 2007; Andjelković et al. 2017). Since early patterns of integration will undergo ontogenetic change complex phenotypes need to be investigated from a

developmental perspective (Damian et al. 2017). Much of this developmental work has informed our hypotheses regarding the role of morphological integration in the cypriniform skull. With better phylogenetic trees now available we can rigorously test specific hypotheses regarding evolutionary patterns of morphological integration within this speciose clade. These complex phenotypes need to be investigated within a developmental and functional context to clearly detail the effect on organismal performance, as well as morphological and lineage diversification. Within the evolutionary history of Cypriniformes we see developmental integration serve as the basis for morphological novelties (Case Studies Nos. 1 and 2), but we also see previously independent modules coming together to form newly integrated, complex functional solutions (Case Studies Nos. 3 and 4). The number of evolutionary novelties that characterize this group as well as the degree to which their development and evolution is integrated provides rich raw material for the great trophic diversity found within Cypriniformes. Indeed, the evolutionary history of Cypriniformes is a seemingly endless Russian doll of novelties comprised of hierarchical degrees of integration and modulation.

## Author contributions

K.E.C. and L.P.H. conceived the study and analyzed the data. L.P.H. wrote the initial draft while K.E.C.

produced all figures. K.E.C. and L.P.H. were both involved in revision and final approval of the manuscript and all figures.

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