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Morphology of a picky eater: A novel mechanism underlies premaxillary protrusion and retraction within cyprinodontiforms

L. Patricia Hernandez[a],*, Lara A. Ferry-Graham[b], Alice C. Gibb[c]

[a]*Department of Biological Sciences, The George Washington University, 2023 G. Street NW, Washington, DC 20052, USA*
[b]*Moss Landing Marine Labs, California State Universities, 8272 Moss Landing Road, Moss Landing, CA 95039, USA*
[c]*Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640, USA*

Abstract

Upper jaw protrusion is hypothesized to improve feeding performance in teleost fishes by enhancing suction production and stealth of the feeding event. However, many cyprinodontiform fishes (mid-water feeders, such as mosquitofish, killifish, swordtails, mollies and pupfish) use upper jaw protrusion for "picking" prey out of the water column or off the substrate; this feeding mode may require improved jaw dexterity, but does not necessarily require increased stealth and/or suction production. We describe functional aspects of the bones, muscles and ligaments of the anterior jaws in three cyprinodontiform genera: *Fundulus* (Fundulidae), *Gambusia* and *Poecilia* (Poeciliidae). All three genera possess a premaxillomandibular ligament that connects the premaxilla of the upper jaw to the mandible. The architecture of this ligament is markedly different from the upper–lower jaw connections previously described for basal atherinomorphs or other teleosts, and this loose ligamentous connection allows for more pronounced premaxillary protrusion in this group relative to closely related outgroup taxa. Within poeciliids, a novel insertion of the second division of the adductor mandibulae (A2) onto the premaxilla has also evolved, which allows this jaw adductor to actively retract the premaxilla during mouth closing. This movement is in contrast with most other teleosts, where the upper jaw is retracted passively via pressure applied by the adduction of the lower jaw. We postulate that this mechanism of premaxillary protrusion mediates the cyprinodontiforms' ability to selectively pick specific food items from the water column, surface or bottom, as a picking-based feeding mechanism requires controlled and coordinated "forceps-like" movements of the upper and lower jaws. This mechanism is further refined in some poeciliids, where direct muscular control of the premaxillae may facilitate picking and/or scraping material from the substrate.
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Introduction

Teleosts have demonstrated an enormous capacity to evolve new feeding morphologies, mechanisms and behaviors. Trophic strategies in this group include: (1) biting tough, encrusting algae (e.g. robust-jawed scarids; Bellwood and Choat, 1990); (2) sucking miniscule crustaceans from the water column (e.g. gracile-jawed syngnathids; Bergert and Wainwright, 1997); (3) scraping epiphytic algae from the substrate (e.g. loricarids; Schaefer and Lauder, 1986); (4) suspension feeding on zooplankton and phytoplankton from the water column

*Corresponding author. Tel.: +1 202 994 6930;
fax: +1 202 994 6100.
E-mail address: phernand@gwu.edu (L.P. Hernandez).

(e.g. tilapia; Goodrich et al., 2000); (5) ambush attacks on mobile prey (e.g. *Luciocephalus*; Lauder and Liem, 1981); (6) rotational feeding to tear chunks from larger prey (e.g. eels; Helfman and Clark, 1986); and (7) suction feeding on highly elusive prey (e.g. largemouth bass; Sanford and Wainwright, 2002). Although these feeding mechanisms are diverse, they can generally be divided into one of two broad categories: biting or suction feeding. While there is a continuum between these two end points, there is also a functional trade-off associated with specialization for one category versus the other (Bouton et al., 1998). Effective suction-feeding performance is associated with a relatively small mouth aperture, well-developed hyoid musculature and associated skeletal elements, lateral occlusion of the gape during a feeding event, and rapid movements of cranial elements. In contrast, effective biting is associated with a large mouth aperture to maximize contact with the prey, well-developed adductor muscles to generate a forceful bite, as well as robust cranial bones to withstand forces generated by the jaws.

Another example of trophic diversity in teleosts is demonstrated by "picking"-based prey capture; this is a subcategory of biting where individual prey items are grasped from the water column and/or substrate (Motta, 1982, 1985; Ferry-Graham et al., 2008). In contrast to most forms of biting, however, fish with this capture mechanism may procure individual nutritive prey items from the substrate or water column, while leaving non-nutritive items behind (Horn and Ferry-Graham, 2006). Because fish first select and then grasp specific individual food items, fine control of the jaw apparatus is key. Thus, a picking-based feeding mechanism should be most effective when performed using precisely controlled and coordinated "forceps-like" movements of the upper and lower jaws (Motta, 1988).

Anyone who has used a pair of fine forceps knows that brute force is not the preferred mode of operation; rather, more delicate control is key. Moreover, forceps/tweezers are often curved to concentrate forces at the anterior tips. This analogy, coupled with the functional demands of picking, suggests that pickers possess a suite of morphological features distinct from those seen in either suction feeders or strong biters. Effective pickers should possess: (1) fine control of the oral jaws, (2) jaws in which force application has been shifted anteriorly and (3) a biomechanical coupling that enables synchronized movements of the upper and lower jaws.

Many species in the teleost order Cyprinodontiformes are characterized by a picking-based feeding behavior that employs a beak-like, open mouth to grasp prey from the substrate, water column or surface (Alexander, 1967b; Ferry-Graham et al., 2008). This order, which includes mosquitofish, killifish, swordtails, mollies and pupfish, is often considered a large group of picking, mid-water and substrate feeders (Weisberg, 1986;

Taylor, 1992; Mansfield and McArdle, 1998; Hargrave, 2006). However, while some basal members are omnivorous mid-water feeders (e.g., Hargrave, 2006), more derived members of Poeciliidae appear to be specialized for herbivory (e.g., Fares Alkahem et al., 2007). To date, most research on feeding behavior within this group has focused on the role of the premaxilla and maxilla (upper jaw bones) in effecting upper jaw protrusion (Eaton, 1935; Alexander, 1967a, b). In contrast, the muscles and ligaments that produce and coordinate movement of the anterior cranial elements have been given less attention, but may greatly inform our understanding of the feeding mechanism and behavior.

Here, we assess the underlying mechanism of premaxillary protrusion and retraction within Cyprinodontiformes in two ways: (1) through a morphological description of the musculoskeletal and ligamentous architecture of the upper jaws and (2) with simple manipulative tests of competing models of jaw protrusion. To this end, we describe an unusual jaw ligament (a greatly modified premaxillomandibular ligament) within three cyprinodontiform genera, and evaluate its potential role in upper jaw protrusion. A ligament tying together the premaxilla and dentary is commonly found within teleosts, including within the sister group of cyprinodontiforms, the atherinomorphs; however, we propose that the distinct architecture of the premaxillomandibular ligament in cyprinodontiforms mediates a heretofore unrecognized mechanism of premaxillary protrusion. We also describe the unusual insertion of a key jaw-closing muscle (A2, a division of the adductor mandibulae, the primary jaw adductor) within Poeciliidae. We discuss the relationship between these anatomical features and previously proposed mechanisms of premaxillary protrusion for cyprinodontiform fishes, and use simple manipulations on one species to assess the validity of these proposed mechanisms. Moreover, we hypothesize that this suite of morphological modifications constitutes an evolutionary innovation that enables cyprinodontiform fishes to use fine-scale manipulations of the anterior jaws in a forceps-like manner to procure specific prey items via carefully controlled jaw opening and closing movements. Finally, we note that within more derived poeciliids these morphological features have undergone secondary changes that result in a jaw apparatus well suited for algal scraping and that other unrelated teleostean families show ecomorphological convergence in these features.

Material and methods

Morphology of the oral jaws

We initially hypothesized that members of the Poeciliidae are highly specialized for a picking habit,

relative to other Cyprinidontiformes. The following live adult specimens of three target species were obtained from commercial suppliers and sacrificed according to the Northern Arizona University IACUC approved protocols (NAU IACUC ♯ 04-007): *Fundulus rubrifrons* ($n = 10$, 26–44 mm snout length (SL)), *Gambusia affinis* ($n = 5$, 20–28 mm SL) and *Poecilia sphenops* ($n = 5$, 27–43 mm SL). Additional specimens preserved in 10% formalin and stored in 70% ethanol were obtained from teaching collections from the University of South Florida; these specimens were used for assessment of intra-clade variation in feeding morphology. These additional species were *Fundulus similis*, *Fundulus chrysotus*, *Fundulus grandis*, *Gambusia affinis*, *G. holbrooki*, *Poecilia latipinna* and *P. sphenops*. Fresh and preserved specimens were dissected and stained with iodine to facilitate identification of muscle fiber orientation. These specimens were used to examine basic features associated with feeding – features likely to affect both jaw protrusion and retraction. Between five and ten specimens of each species were dissected to determine musculoskeletal and ligamentous architecture, as well as to assess inter-individual variation in feeding morphology for a given species.

Additional specimens of the above species were cleared and stained using a protocol presented in Dingerkus and Uhler (1977) with modifications by Potthoff (1984). Cleared and stained specimens were used to assess the osteology of the anterior jaws. Line-drawn illustrations of specimens were made from photographs taken using an Olympus DP12 digital camera attached to an Olympus SZX12 dissecting microscope, or using an Olympus SZX-DA camera lucida attached to the same dissecting microscope. Digital illustrations of skeletal and muscle morphology were constructed by creating vector drawings from the camera lucida drawings using a technical illustration program (Canvas X and/or Adobe Illustrator CS3). Images captured using an Olympus DP12 digital camera attached to an Olympus SZX12 dissecting microscope were imported into Adobe Photoshop (CS3) and brightness, contrast and color of the images were adjusted as necessary to facilitate the visualization of fine detail.

Mechanism of premaxillary protrusion

As multiple hypotheses have been proposed regarding how the premaxilla is protruded within certain members of Cyprinodontiformes (Eaton, 1935; Alexander, 1967a; Gosline, 1981; Motta, 1984), we used ligament ablation and subsequent manipulations of fresh specimens of *Fundulus* to evaluate the validity of each hypothesis and to determine how our morphological data might influence these models. We also performed simple manipulations of cleared and stained *Gambusia* and *Poecilia*. After first describing the more conventional mechanism of premaxillary protrusion within teleosts, a brief overview of the various hypotheses is given to provide the rationale behind the specific ligament ablations performed on *Fundulus*.

Within many acanthopterygians, depression of the lower jaw enables premaxillary protrusion; lower jaw depression is generated via the opercular linkage, which connects the neurocranium to the lower jaw (Fig. 1A). A taut ligament tying the premaxilla to the lower jaw pulls the premaxilla forward as the jaw is lowered. During jaw protrusion the maxilla also swings dorsally and anteriorly to partially occlude the lateral gape (Fig. 1B).

Within cyprinodontiforms, two different mechanisms of premaxillary protrusion have been suggested. Gosline (1981) proposed that the "lip membrane" that links the paired premaxillae to the dentary bones of the lower jaw is the primary agent that effects protrusion in this group
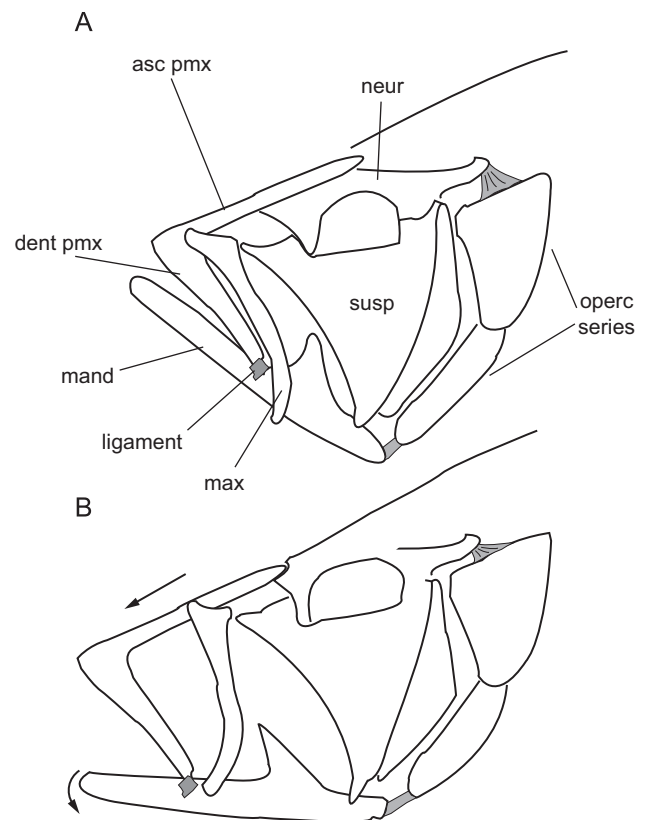

Fig. 1. Premaxillary protrusion mediated by abduction of the mandible. (A) Resting state, (B) protruded state. The jaw is lowered and via tension placed on the ligament tying the premaxilla to the mandible the premaxilla is pushed forward. An elongated ascending process of the premaxilla slides along the neurocranium. *Monocirrhus* sp. modified from Liem (1970). Abbrev.: asc pmx, ascending process of the premaxilla; dent pmx, dentigerous process of the premaxilla; mand, mandible; max, maxilla; neur, neurocranium; operc, opercular; susp, suspensorium.

(Fig. 2A). In this model, ventral rotation of the lower jaw distends the lip membrane and places tension on the premaxillae, which are then pulled anteriorly (Fig. 2B). Alexander (1967b) and Eaton (1935) also describe the protrusion exhibited by cyprinodontiform fishes and consider this pattern of protrusion as functionally distinct from the mechanisms characterizing other acanthopterygians. However, Eaton (1935) and Alexander (1967b) both suggest that lateral twisting of the paired maxillae along their dorsoventral axes, brought about by contraction of the first branch of the adductor mandibulae (AM) (A1, which inserts on the ventral aspect of the maxilla), causes movement of the paired premaxillae. In this scenario, the ascending processes of the premaxillae are affected by movements of the maxillae such that they are either forced or pulled anteriorly, which creates upper jaw protrusion (Fig. 2C–F). While Eaton (1935) suggests that the ascending processes are "squeezed", Alexander (1967a)

suggests that this twisting pulls the ascending processes anteriorly via a thick rostral cartilage.

These different mechanisms have been suggested based on morphology; however, ablations of individual ligaments to test these hypotheses have not been performed. We performed a number of experiments in which key ligaments were severed and the subsequent degree of protrusion was examined following the application of an input force. Thus, we performed the following sequential ablations on fresh specimens of *F. rubifrons* and *F. stellifer*, as all previous workers in this area also used this genus as a model: (1) removal of the lip membrane, followed by (2) decoupling the premaxilla from the lower jaw by severing the ligamentous attachment (described in detail below) connecting the descending process of the premaxilla to the lower jaw, but leaving the maxilla and premaxilla attached to one another at their ventral ends; (3) decoupling the maxilla and premaxilla by severing the ligament that connects the maxilla to the premaxilla, but leaving the premaxilla attached to the lower jaw; (4) freeing the premaxilla and maxilla from one another and the lower jaw; (5) on an additional set of specimens, freeing the descending process of the premaxilla and maxilla (as in step 4), while leaving the lip membrane intact. After each ablation, the lower jaw of the specimen was manually rotated to approximately 30° (previously determined to represent a typical feeding event; Gibb and Ferry-Graham 2005), and resultant movements of the upper jaw were recorded.
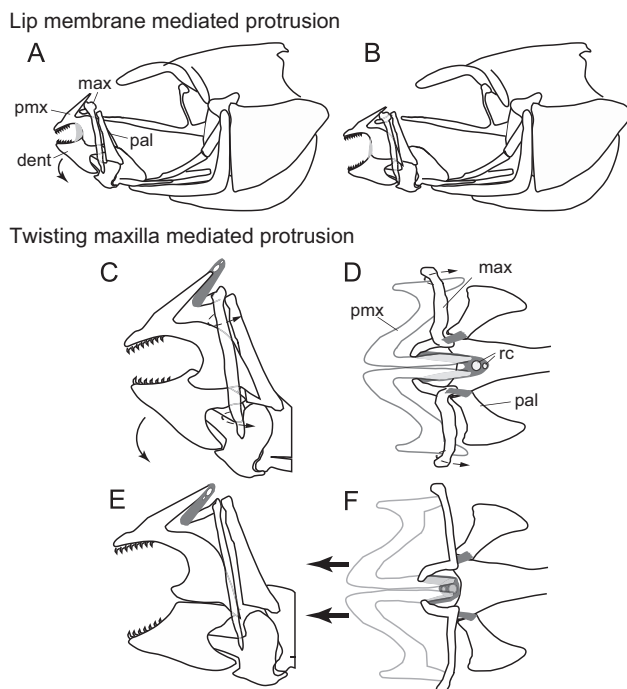

Fig. 2. Two hypotheses explaining protrusion within *Fundulus*, lip membrane- and twisting maxilla-mediated premaxillary protrusion. (A, C, D) Resting state; (B, E, F) protruded state. In lip membrane-mediated protrusion the jaw is lowered (A), which produces tension on the lip membrane resulting in premaxillary protrusion (B). In twisting maxilla-mediated premaxillary protrusion, lowering of the jaw coupled with contraction of the A1 division of the adductor mandibulae (C, D) causes the maxilla to rotate laterally along its dorsoventral axis. This rotation causes the medial hooks of the maxilla to rotate outward, which pulls on the ligament tying together the maxilla and premaxilla. This rotation causes premaxillary protrusion (E, F). Abbrev.: dent, dentary; max, maxilla; pal, palatine; pmx, premaxilla.

Results

Morphology

Musculoskeletal architecture of *Fundulus* spp.

Within *F. rubifrons*, a species chosen to represent the more primitive cyprinodontiform condition that is present in the Fundulidae, the bony elements comprising the anterior jaws are similar to those described for many other teleosts. The descending (or alveolar) arms of the paired premaxillae have a strongly recurved shape (Fig. 3A, B). The ascending arms of the premaxillae are relatively long and diverge from one another at their postero-dorsal tips (Fig. 3B). The space between the dorsal ends of the ascending processes is filled with thick connective tissue within which two rostral cartilages are located (Fig. 3B). This connective tissue ties the ascending processes of the premaxillae to the medial arms/hooks of the paired maxillae, such that anterior movement of the medial hooks of the maxillae pulls on this ligament, which pulls the premaxillae forward. The ventral end of each maxilla is relatively thin. However, the dorsal end of each maxilla is more
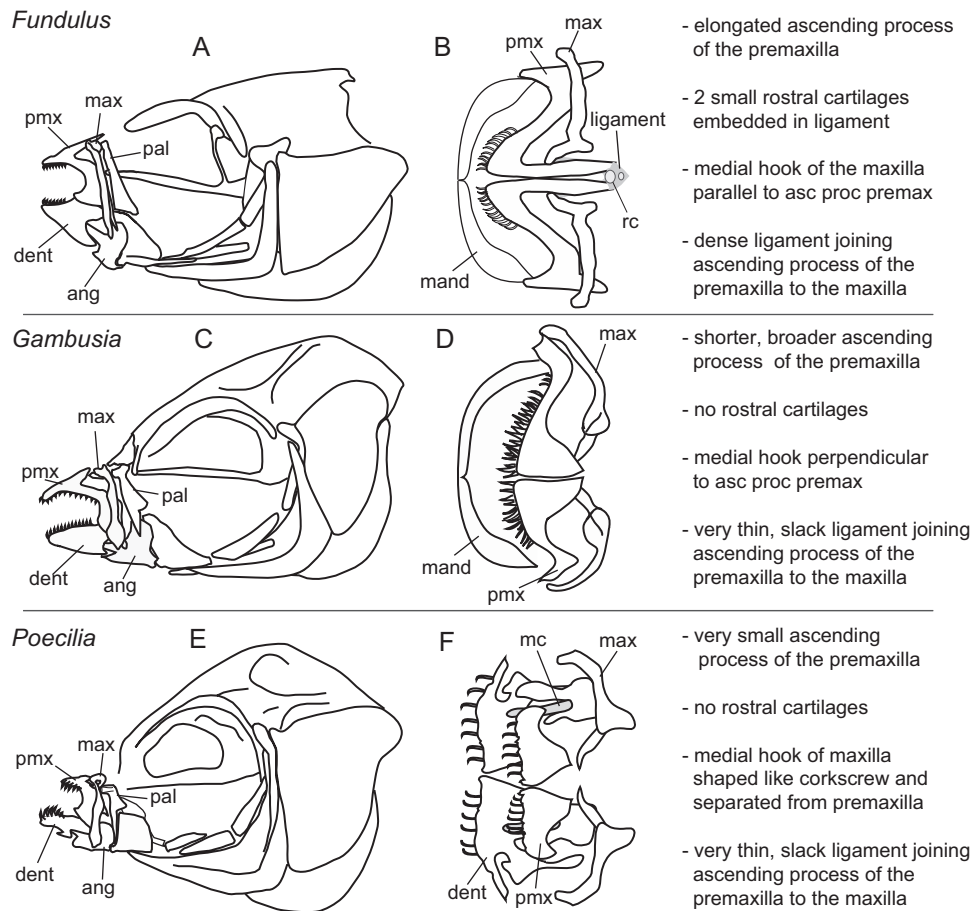
Fig. 3. Gross skeletal anatomy of the head of *Fundulus heteroclitus* (A, B), *Gambusia affinis* (C, D), *Poecilia sphenops* (E, F) from a lateral and dorsal aspect. Drawn from cleared and stained specimens. For clarity, aspects of the orbital series and suspensorium have been removed. Abbrev.: ang, anguloarticular; dent, dentary; mand, mandible; max, maxilla; mc, Meckel's cartilage; pal, palatine; pmx, premaxilla; rc, rostral cartilages.

complex, with a relatively small dorsal knob, but a large, twisted medial hook (Fig. 3B). Prominent ventral and dorsal processes are present on each dentary where they articulate with the anguloarticular. The process on the ventral aspect of the anguloarticular is quite long and inserts into the "V" formed by the two processes of the dentary; as a result, the anguloarticular and dentary are tightly articulated, relative to the other species examined here.

Within *F. rubrifrons*, the musculature that controls movements of the anterior jaws is similar to that seen in many generalized teleosts, thus the nomenclature used here is taken from Winterbottom (1974). There are two discrete branches of the first division of the AM (A1): A1α and A1β (Fig. 4A). A1α is substantially larger than A1β, and the fibers of A1α proceed horizontally from their origin on the hyomandibula and preopercle to their insertion on the ventral third of the maxillae (Fig. 4A). A1β originates from the ventral edge of the suspensorium and proceeds anterodorsally to insert on the ventral third of the maxilla. Each of the species examined here

also has a very small branch of A1 going to the ventral edge of the lacrimal (as has been previously noted by Parenti, 2005; data not shown here).

The fibers of the second and third divisions of the AM (A2 and A3) are medial to A1 and closely associated, although they insert on discrete regions of the lower jaw or mandible (Fig. 4B). Most of the fibers of A3 are "deep" (medial) to A2, and insert on the medial face of the anguloarticular via a discrete tendon. A2, largely combined with A3, originates from the hyomandibula and symplectic (medial to A1) and inserts muscularly (i.e., without a distinct tendon) on the dorsocaudal edge of the dentary and anguloarticular. As in most teleosts, the Aω originates from the medial surface of the ventralmost portion of the quadrate and inserts on the medial side of the dentary, filling most of the Meckelian fossa (Winterbottom, 1974). We noted a lack of variation in Aω among all three genera examined here, thus this division will not be discussed further. Comparisons among *Fundulus* spp. revealed that interspecific variation within this genus consists predominantly of
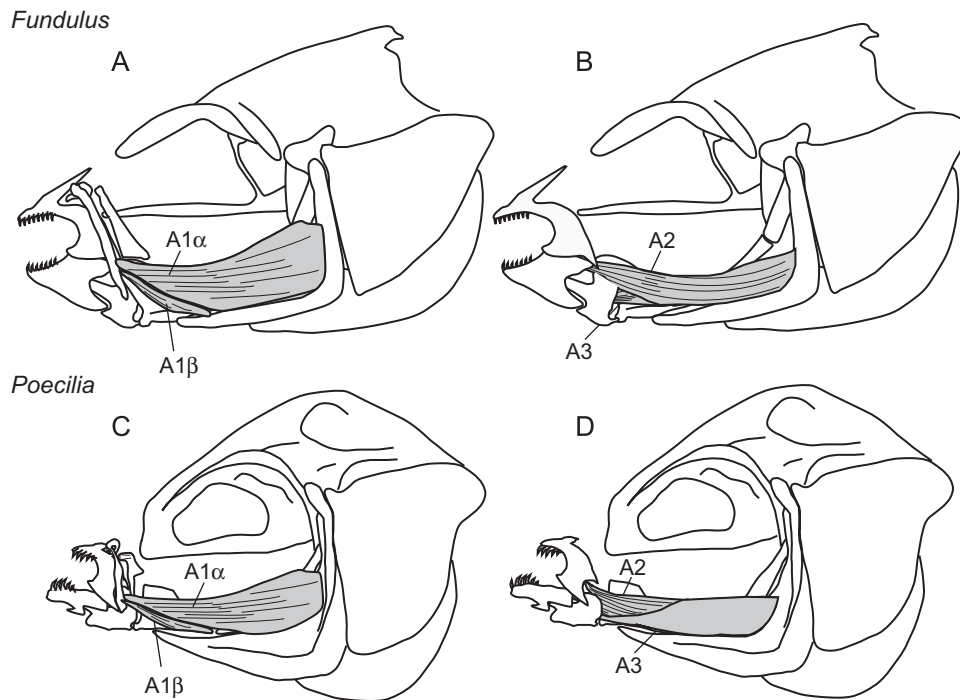
Fig. 4. Adductor mandibulae complex of *Fundulus heteroclitus* (A, B) and *Poecilia latipinna* (C, D) from a superficial (A, C) and deep aspect after removal of branches of adductor mandibulae A1 (B, D). Drawn from fresh and preserved specimens.

subtle changes in the size of distinct divisions of the AM complex, but not in origin or insertion site.

Musculoskeletal architecture of *Gambusia* spp.

Within *G. affinis*, a basal poeciliid, the bony elements comprising the anterior jaws are generally similar to those described for *Fundulus* (Fig. 3C, D). The premaxilla has the same characteristic shape, with the descending arm strongly recurved, although less so than in *Fundulus* (Fig. 3C). The ascending arm of the premaxilla is relatively long, though not as long as seen in *Fundulus*. Moreover, from a dorsal aspect, the ascending processes of the paired premaxillae of *Gambusia* are more triangular in shape and quite broad where they connect with the descending arms (Fig. 3D). The maxilla in *Gambusia* is more robust than that of *Fundulus*. In contrast to *Fundulus*, where the dorsal end of the maxilla had a large twisted medial hook with a simple dorsal knob, in *Gambusia* the maxilla has an elaborate dorsolateral process, in addition to the medial hook. This process consists of a large dorsal knob and a strongly curved, flattened medial hook. In a non-protruded state, the medial hooks of the maxilla were more medially directed, whereas those of *Fundulus* were more anteriorly directed (Fig. 3D vs. B). More importantly, the ligament connecting the ascending arms of the premaxilla to the medial hooks of the maxillae has been greatly reduced and is composed of very slack connective tissue. Rostral cartilages have been lost. In addition, the ventral and dorsal processes of

each dentary are not as long in *Gambusia* as they are in *Fundulus* (Fig. 3C).

In *G. affinis*, the musculature controlling movements of the anterior jaws is similar to that of *Fundulus*, thus is not figured here. There are two branches of A1 (A1α and A1β); again, as in *Fundulus*, A1α is significantly larger than A1β. The fibers of A1α are directed horizontally from their origin on the hyomandibula and preopercle to their insertion on the ventral third of the maxilla. A1β originates from the ventral edge of the suspensorium and its fibers proceed anterodorsally to insert on the maxilla. The maxilla, slightly wider in *Gambusia* than in *Fundulus* (Fig. 3A, B), allows for a greater separation between the insertion of A1α and A1β. As in *Fundulus*, A2 and A3 are somewhat interconnected; however, the bulk of A2 originates from the suspensorium (deep to A1) and inserts directly on the dorsocaudal edge of the dentary with few fibers inserting on the anguloarticular. A very small dorsal portion of A2 appears to insert on ligamentous tissue that invests the premaxilla. The bulk of A3 is found deep to A2 and inserts on the medial face of the anguloarticular via a discrete tendon. Examination of *G. holbrooki* suggests that, as in *Fundulus*, variation within the group consists predominantly of changes in the size of distinct divisions of the AM complex, but not in origination or insertion sites.

Musculoskeletal architecture of *Poecilia* spp.

Within *P. sphenops*, a more derived poeciliid (Ghedotti, 2000; Parenti, 2005), the general cyprinodontiform

osteology of the anterior jaws is retained; however, we see several substantial morphological changes with likely functional implications (Fig. 3E). As in *Gambusia*, the strongly recurved descending arm of the premaxilla is obvious, although the relative length of the ascending arm is reduced. As in *Gambusia*, the ascending processes of the paired premaxillae have broad bases where they connect with the wide descending arms (Fig. 3F); this condition is more pronounced in *Poecilia* than in *Gambusia*. The posterodorsal portion of the maxilla, which articulates with the palatine bone, has become more elaborate in *Poecilia* (characterized by a long medial hook of the maxilla), relative to the condition seen in *Gambusia*. More importantly, the thick ligament connecting the ascending arms of the premaxilla to the medial hooks of the maxillae has been lost. Although the maxilla is thinner in *Fundulus*, within *Poecilia* this element is somewhat wider (Fig. 3E). The medial hooks of the paired maxillae, already complex in *Gambusia*, are significantly elongated and even more complex in shape in *Poecilia* (given the twisted appearance of the bones, they resemble corkscrews). Moreover, the complex medial hook of each maxilla has become slightly more mediolaterally directed (Fig. 3F). In *Poecilia*, as in *Gambusia*, there is little overlap between the medial hook of the maxilla and the ascending process of the premaxilla in the resting state (Fig. 3F). In addition, the retroarticular process on the ventral aspect of each dentary is considerably foreshortened. The coronoid process of the dentary is widely separated from the coronoid process on the corresponding anguloarticular, potentially allowing for some intramandibular bending.

In *P. sphenops*, as in *Fundulus* and *Gambusia*, A1 is split into two subdivisions, both of which insert on the ventral third of the maxillae (Fig. 4C). However, the morphology of A2 and A3 is significantly different from that seen in either *Fundulus* or *Gambusia*. A2 and A3 originate largely from the preopercle and symplectic, forming a cylindrical mass found deep to A1. As in all species examined, A3 inserts on the medial face of the anguloarticular. However, the insertion and origin of A2 is markedly different from that described previously: A2 constitutes a fairly thin, cylindrical muscle that origi-nates both from the suspensorium and from part of the muscular belly of A3, then bifurcates to insert on both the lower jaw (dentary) and, notably, the premaxilla. Insertions of A2 on the premaxilla and dentary are equally robust (Fig. 4D). However, the insertion of A2 on the lower jaw is via a relatively thick, discrete tendon to the caudal edge of the dentary, the insertion of A2 on the upper jaw is via a more diffuse tendinous connection to the anterior aspect of the premaxilla. Given the foreshortened nature of the lower jaw elements, the insertion of A2 on the dentary is more anterior than that of other species examined here. Examination of *P. latipinna* suggests that variation within this genus also

consists predominantly of changes in the size of distinct divisions of the AM complex, but not in insertion site.

Ligaments of the anterior jaws

For all three genera examined (*Fundulus*, *Gambusia* and *Poecilia*), a premaxillomandibular ligament ties the lateral face of the premaxilla to the medial edge of the dentary or anguloarticular (Fig. 5). Although the homology of this ligament is uncertain, we refer to it
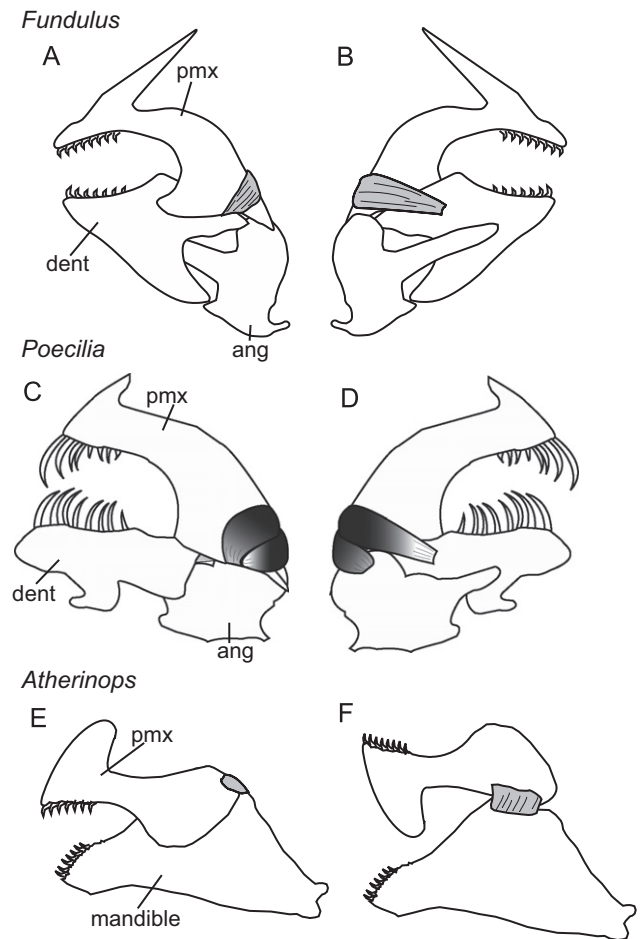

Fig. 5. Premaxillomandibular ligament linking the premaxilla to the lower jaw in *Fundulus rubrifrons* (A, lateral; B, medial) and *Poecilia latipinna* (C, lateral; D, medial). Within *Fundulus* a simple ligament ties the ventrolateral aspect of the premaxilla (A) to the medial aspect of the dentary (B). Within *Poecilia* a larger and more complex pair of ligaments ties the ventro-lateral aspect of the premaxilla (C) to the medial aspect of the dentary and anguloarticular (D). In (C–D) ligaments are drawn such that the lighter portion represents typical ligamentous tissue, while the darker portion represents the more cellular portion of this ligament (hyaline cell cartilage, sensu Benjamin, 1989). (E, F) *Atherinops californiensis* show-ing the presumed ancestral ligament tying together the premaxilla and mandible, in resting lateral view (E), as well as with the premaxilla folded over dorsally to show the extent of taut ligamentous attachment (F). Abbrev.: ang, anguloarti-cular; dent, dentary; pmx, premaxilla.

here as the premaxillomandibular ligament, because it ties the premaxilla to the mandible. Whereas others have noted generalized connective tissue uniting the premaxilla to the dentary in this group, the complex morphology outlined below has not been previously described. For example, Alexander (1967a) recognizes what he terms "tracts of thickened dermis" that attach the premaxilla to the lower jaw; however, this description does not capture the complex biomechanical architecture of this coupling.

Within *Fundulus*, this ligament originates from the ventral aspect of the lateral descending arm of the premaxilla (Fig. 5A). It wraps around the posterior edge of the premaxilla and turns anteriorly to insert on the medial side of the dentary or anguloarticular. This ventral attachment is often adjacent to the insertion of A2 on the dentary (Fig. 5B). Importantly, this ligament is not attached to the posterior margin of the premaxilla, but rather slides freely across this region of the bone when the jaws are manipulated.

There is some variation in the architecture of this ligament among the cyprinodontiform species examined here. In both *Fundulus* and *Gambusia*, the connective tissue across the lateral face of the premaxilla constitutes a small and discrete ligament, which is localized on the caudal face of the premaxilla (Fig. 5A; data not shown). After wrapping around the posterior aspect of the premaxilla, it then attaches to the medial aspect of the dentary (Fig. 5B). In *Gambusia*, a bit of tissue inserts on the anguloarticular as well. Within *Poecilia*, this ligament has become increasingly complex, and is split into two discrete elements: the ligament bifurcates distally and attaches to both the coronoid process of the dentary (adjacent to the insertion to the A2) and to the anguloarticular (Fig. 5C, D). Moreover, this "ligament" has an unusual morphology in that the points of attachment are composed of what appears to be typical ligamentous, fibrous tissue, but the freely moving, central portion of this connective tissue appears to be composed of more cellular, and less fibrous, material (see Fig. 5C, D).

In comparison, *Atherinops californiensis* (Atheriniformes; sister group to Cyprinodontiformes + Beloniformes) has a small, taut ligament that ties the medial edge of the descending arm of the premaxillae to the lateral surface of the anguloarticular (Fig. 5E, F). Within *Atherinops*, the bulk of this ligament is found between these two bones and serves to connect the premaxilla firmly to the lower jaw. Unlike in the cyprinodontiforms, in *Atherinops* this taut connection does not appear to allow significant premaxillary protrusion.

Thus, in an evolutionary cross-section that encompasses basal atherinomorphs and derived poeciliids, we see increasing complexity of this premaxillomandibular ligament. Within *Atherinops* it is a small and very taut

ligament, made of typical collagenous material. Within cyprinodontiforms, it has become a much looser and longer ligament. Finally, within derived poeciliids it has increased in size, complexity and cellular structure (see Fig. 5C, D).

Basal members of Cyprinodontiformes also have a taut ligament that ties the lateral face of the maxilla to the lacrimal (data not shown). This ligament ties each lacrimal to a discrete lateral portion of the corresponding maxilla halfway along its shaft (Parenti, 2005). Alexander (1967b) briefly mentions a ligament uniting the maxilla and lacrimal and notes its importance for the twisting maxilla model. This ligament is quite thick in *Fundulus*, is greatly reduced in *Gambusia* and is present in *Poecilia*.

Mechanism

Manipulation of both fresh, intact specimens and cleared and stained specimens of *Fundulus* confirmed that depression of the lower jaw consistently generates anteriorly directed protrusion of the premaxillae. Rotation or twisting of the paired maxillae (along their dorsoventral axes) was also observed when viewed from the dorsal aspect, both when the lower jaw was depressed and when the paired premaxillae were grasped and pulled anteriorly. Rotation or twisting was such that the medial hooks on the dorsal ends of the maxillae rotated outward, away from both the midline and one another. The maxillae rotated along their long axes nearly $90°$ in response to lower jaw depression of 30–$40°$. Induced rotation of the medial hooks of the maxillae pulls on the thick connective tissue that connects the medial hooks to the ascending processes of the paired premaxillae; this action appears to pull the ascending processes forward (Fig. 2C–F).

When the lip membrane was removed in *Fundulus* spp., protrusion of the premaxilla occurred with no observable differences in protrusion direction or distance (relative to intact specimens); this result suggests that Gosline's (1981) hypothesis is incorrect (Fig. 2A, B). It also appeared that the lower jaw could rotate to a larger degree (i.e., greater jaw depression) when this membrane was removed, which suggests that the lip membrane may constrain the degree of jaw opening. When each maxilla was detached from the corresponding premaxilla, but each premaxilla left connected to the lower jaw, lower jaw depression resulted in anteriorly directed protrusion of the paired premaxillae, and it again appeared that the lower jaw could rotate to a larger degree in the absence of the attachments to the maxillae. When the descending process of each premaxilla was subsequently freed from its attachment to the lower jaw (but remained attached to the maxilla, which is lateral to the premaxilla at the ventral tip), it

tended to slide under (i.e., medial to) the maxilla. Depression of the lower jaw, however, still generated anteriorly directed protrusion of the paired premaxillae, presumably due to the action of the lower jaw through the remaining connection between the maxillae and the premaxillae. It is noteworthy, however, that the protrusion distance observed under these circumstances was approximately half of that observed when the connections between the premaxillae and the lower jaw remained intact. When both maxillae and premaxillae were detached from one another *and* from the lower jaw, then no protrusion occurred. If these ligaments were transected, but the lip membrane kept intact, protrusion only occurred when the lower jaw was depressed to extreme and biologically unrealistic angles ($>180°$); even in this scenario, protrusion was minimal.

Discussion

Based on our morphological investigations, we propose that cyprinodontiform fishes employ a novel mechanism of premaxillary protrusion, which we suggest is largely mediated by a previously undescribed conformation of the premaxillomandibular ligament that connects the premaxilla to the lower jaw. We posit that, within Cyprinidontiformes, this ligament may increase premaxillary protrusion distance (relative to outgroup taxa), and enhance the use of the anterior jaws as forceps for picking individual prey items by enabling the upper and lower jaws to move in a highly coordinated fashion (see also Ferry-Graham et al., 2008). We also note that the novel insertion of AM A2 division on the premaxillae (Fig. 4) – an unusual condition in teleosts – may allow for fine control of the closing of the anterior tips of the jaws when prey are individually "picked" and fine control of retraction of the upper jaws once prey are caught. Relative to the anterior jaws of teleosts with more "typical" mechanisms of jaw protrusion (Motta, 1984), these two mechanisms – an unusual ligament and a novel insertion of a major jaw adductor – appear to have transformed the anterior jaws of derived poeciliids into a mechanism that is extremely well suited for picking- and scraping-based feeding behaviors.

Refining previous models of premaxillary protrusion

In our manipulations, protrusion of the paired premaxillae could be initiated via depression of the lower jaw, without transfer of force through the maxillae. Thus, a "Type A" protrusion mechanism (sensu Motta, 1984), in which force is transferred directly from the lower jaw to the premaxillae, exists in this group (Fig. 6C, D). We posit that this mechanism
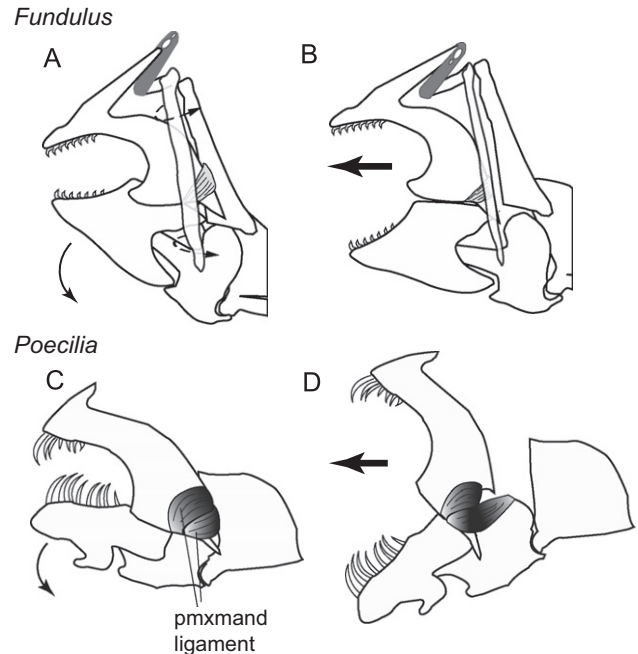

Fig. 6. New models of premaxillary protrusion for *Fundulus* and *Poecilia*. (A, B) Schematic indicating dual mechanisms of premaxillary protrusion in *Fundulus*. The twisting maxilla indirectly transfers force to the premaxilla from the lower jaw via the thick ligament connecting the premaxilla and maxilla, while the premaxillomandibular ligament directly transfers force from the lower jaw to the premaxilla. Within *Poecilia* (C, D), taut ligamentous attachments between the premaxilla and maxilla have been lost, thus premaxillary protrusion is largely caused by the lowering of the jaw. Since the maxilla would obscure these ligaments, it has been removed from C and D. Abbrev.: pmxmand, premaxillomandibular.

is mediated in cyprinodontiform fishes by the aforementioned premaxillomandibular ligament that connects each premaxilla to the lower jaw, and *not* by the lip membrane (as postulated by Gosline, 1981). However, we also note that premaxillary protrusion still occurred when the ligament connecting the lower jaw to the premaxilla was severed (bilaterally), although it was much less pronounced. This suggests that the connection between the maxilla and the premaxilla also plays a role, albeit a smaller one, in jaw protrusion. Apparently, none of the previous models of jaw protrusion for cyprinodontiform fishes is entirely correct, as none accounts for the role of the premaxillomandibular ligament in force transfer. We suggest that members of this group demonstrate a distinct mechanism of premaxillary protrusion that, at least within *Fundulus*, works in concert with the twisting maxilla model outlined by Alexander (1967b) and Eaton (1935).

This mechanism is distinct in that the premaxillomandibular ligament is structurally different from any ligaments previously proposed to be of importance during premaxillary protrusion. Because this ligament

wraps around the posterior edge of the premaxilla, it will allow for a much wider gape within species with such a ligament. Given the architecture of this ligamentous attachment, the only thing constraining maximum gape production is the degree of jaw depression.

During jaw opening in *Fundulus* spp., the medial hook of each maxilla rotates outward, pulling the thick ligament (associated with the rostral cartilages) anteriorly (Fig. 6A). As suggested by Alexander (1967b), the thick ligament attaching each maxilla to the lacrimal bone is important for stabilizing the maxilla along its posterior margin, such that the bone can pivot outward along its long axis, rotating the medial hook by as much as 90°. Eaton (1935) proposed that the medial hooks of the paired maxillae "pinched" the ascending processes of the premaxillae, forcing them to slide anteriorly. However, we hypothesize that the ascending processes of the premaxillae are pulled forward by the rotational force imparted by movement of the medial hooks of the maxillae. The medial hooks are attached to the ascending processes of the paired premaxillae via a thick, Y-shaped ligament, which Alexander (1967a) described as the "rostral cartilage". Two ligamentous arms of the Y attach to the medial hooks of the maxillae, whereas a single ligament attaches to both tips of the ascending processes of the premaxillae. This Y-shaped structure appears to be a thickened ligament in which two rostral cartilages are embedded. Twisting/rotation of the maxillae causes the premaxillae to pivot forward through a rotational motion (Fig. 2).

In this sense, this constitutes a "Type B" mechanism (sensu Motta, 1984), in which twisting of the paired maxillae effects anterior movement of the premaxillae. However, given that rotation of the lower jaw is also likely required to generate such twisting of the maxilla, *Fundulus* may show a combination of Types A and B mechanisms. Interestingly, the ligaments as well as the ascending processes of the premaxilla are all less well developed in more derived species (i.e., *Gambusia* and *Poecilia*). Indeed, rostral cartilages have been lost in these more derived species (Fig. 7). We suggest that derived cyprinodontiform species rely more heavily on transfer of force through the premaxillomandibular ligament (Fig. 6C, D). The fact that this ligament is much more complex within derived species supports this contention.

Alexander (1967b) refers to the ligament that ties each premaxilla to the lower jaw in *Fundulus* as a "thickening", but does not assign it further functional significance. However, our manipulations confirm that this ligament mediates jaw protrusion by transferring force directly to the paired premaxillae from the lower jaw. Interestingly, this ligament also limits the anterior excursion of the descending process of the premaxillae during mouth opening; in other teleosts, the descending arms of the premaxillae rotate anteriorly and laterally occlude the gape. This restriction of the ventral ends of the premaxillae gives the cyprinodontiforms their characteristic "beak-like" appearance during mouth opening. The lip membrane creates some lateral occlusion,
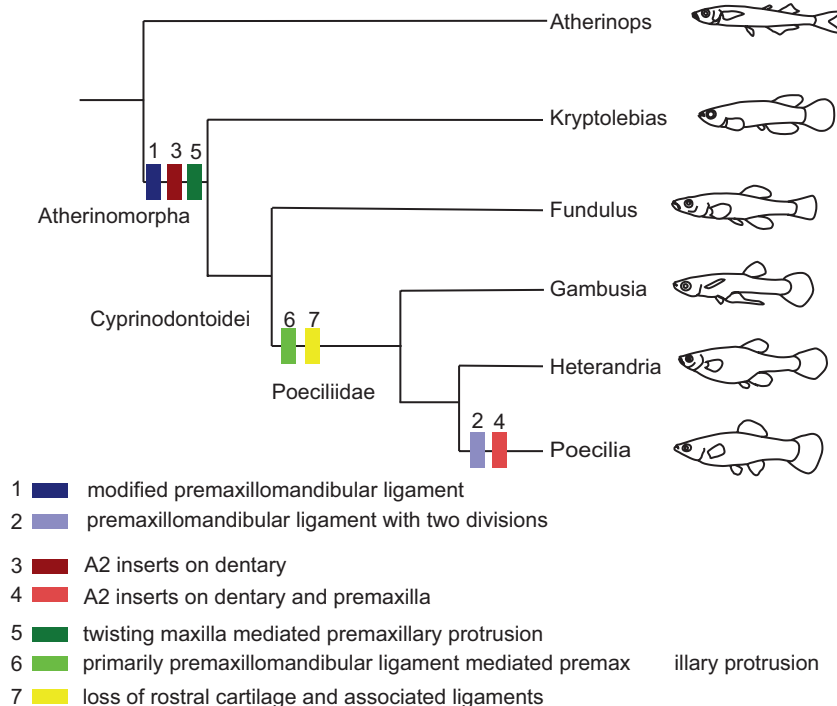

Fig. 7. Phylogenetic position of species examined in this study. Character states are as noted on the figure. Atheriniform and cyprinodontiform relationships are after Costa (1998), Ghedotti (2000) and Parenti (1981, 2005).

although occlusion is much less pronounced than that seen in other teleosts where the descending processes of the premaxillae laterally occlude gape.

The distinct architecture of the premaxillomandibular ligament (Fig. 5A–D) may be a synapomorphy of Cyprinodontiformes, as this conformation is not present in Atheriniformes. As previously mentioned, within *A. californiensis* there is a ligament that attaches the premaxilla to the dentary; however, it is a short, taut, fibrous ligament that attaches the medial edge of the descending arm of each premaxilla to the lateral surface of the corresponding anguloarticular (Fig. 5E, F). It does not curve around the caudal edge of the dentigerous arm of the premaxilla. Curiously, the poeciliid premaxillomandibular ligament also differs histologically from typical ligaments within teleostean heads. Benjamin (1989) described tissue surrounding the jaws of *Poecilia sphenops* as hyaline cell cartilage, which is characterized by closely packed cells within an extracellular matrix. Based on Benjamin's histological sections (Fig. 1 in Benjamin, 1989), it is clear that the premaxillomandibular ligament in *Poecilia* is at least partially composed of hyaline cell cartilage.

While the architecture of the cyprinodontiform ligament that connects the premaxilla to the lower jaw is certainly distinct as compared to basal atherinomorphs, the presence of a ligament connecting the premaxilla to the lower jaw is not. Indeed, it is likely that many teleosts have such a ligament. A "premaxillomandibular" ligament is described in the Nandidae (Liem, 1970). However, these ligaments appear to be similar to the simple, taut ligaments that we have identified in *Atherinops californiensis* (Fig. 5E, F); thus important functional aspects of these ligaments either are not present in these other species or have gone unnoted. Until a more thorough phylogenetic comparison can be performed, we cannot determine if the ligament described herein is homologous with the premaxillomandibular ligament previously described within other fish groups (e.g., Nandidae), or represents an evolutionary novelty.

Functional significance of the insertion of the adductor mandibulae on the premaxilla

In the vast majority of teleosts, the lower jaw simply pushes the premaxilla back into place as the mandible is adducted; thus, no direct, muscle-mediated retraction of the premaxilla occurs. However, there are a few teleostean taxa outside of the cyprinodontiforms in which a division of the AM inserts onto the premaxilla. Within the Siluriformes, two lineages have evolved a connection between the AM and the upper jaw. One group, the loricariids (comprised of approximately 1,100 species), are characterized by a discrete insertion of A2

unto the premaxilla (Schaefer and Lauder, 1986, 1996). Indeed, Schaefer and Lauder (1986) document a gradual transformation of the anterior jaws from minimal premaxillary protrusion within primitive loricariids to a derived condition in which the paired premaxillae are controlled independent of the lower jaw via the AM complex. This morphological transformation appears to be associated with functional specialization for algal scraping. Independent control of the premaxillae via direct insertion of the AM may enable the increased dexterity necessary for scraping algae from uneven surfaces. In addition, recent work has established that members of another siluriform group, the Synodontidae, also have a distinct insertion of the AM on the premaxilla (Celine Ide and Dominique Adriaens, pers. comm.). Interestingly, synodontids also use algal scraping as their predominant feeding mode.

Although discrete branches of A2 insert on both the premaxilla and dentary within derived poeciliids (i.e., *Poecilia*), the overall size of the AM A2/3 is relatively small, which suggests that forceful biting is not being produced. Although the thin, largely muscular connection of the A2 to the premaxilla is unlikely to generate large forces during biting, it may allow small amounts of force to be applied directly to the anterior upper jaw – and thus to the prey items. This small addition of force to the upper jaw may prevent small, but elusive prey items from escaping, or allow a "nipping" behavior to be produced. Given the insertion of A2 on both the upper and lower jaws, contraction of A2 will allow fine control of captured items as the jaws are retracted, as both the upper and lower jaws can hold the prey item. Moreover, as is seen within loricariids, some poeciliids have secondarily adopted a grazing habit (Fares Alkahem et al., 2007) – a trophic niche for which this feeding mechanism is well suited.

A small, mobile and independently controlled premaxilla, in association with the intramandibular joint that characterizes *Poecilia*, should facilitate a shift to a grazing habit. Such a mechanism appears to allow for both a much wider gape, due to the architecture of the premaxillomandibular ligament, as well as the ability to generate force at the anterior ends of the upper and lower jaws; both of these functional traits are likely to be important during scraping. During cyprinodontiform oral jaw evolution, we also see an anterior shift in the insertion of A2 on the dentary; the insertion of A2 on the premaxilla probably serves to increase and direct force to the tips of the jaws. In addition, synchronized abduction of the upper and lower jaws, via the premaxillomandibular ligament described herein, coupled with controlled adduction of both the lower *and* upper jaws, via the novel insertion of A2 on the premaxilla, apparently allows poeciliids to perform precise occlusal movements with their jaws, much as is possible with forceps.

Schaefer and Lauder (1986) suggest that an increase in the complexity of the AM complex in the Loricarioidea generates the refined jaw movements necessary to scrape material from irregular surfaces. Correspondingly, we suggest that the evolutionary changes in jaw mechanism outlined here for cyprinodontiform fishes increase jaw dexterity during feeding events (Fig. 7). During the early evolution of cyprinodontiforms (Fig. 7), increased dexterity and precision granted by the ligament that directly connects the upper jaw to the lower jaw may have enhanced the ability of basal species to select individual prey items from the substrate or water column using picking-based prey capture behavior. During the later evolution of this clade, the direct control of upper jaw movements during retraction granted by the insertion of A2 on the upper jaw enhanced the ability of derived species to remove encrusting material using a nipping or scraping-based feeding behavior.

Acknowledgments

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