

# Historical and biomechanical analysis of integration and dissociation in molluscan feeding, with special emphasis on the true limpets (Patellogastropoda: Gastropoda)

by

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## ABSTRACT

Modifications of the molluscan feeding apparatus have long been recognized as a crucial feature in molluscan diversification, related to the important process of gathering energy from the environment. An ecologically and evolutionarily significant dichotomy in molluscan feeding kinematics is whether radular teeth flex laterally (flexoglossate) or do not (stereoglossate). In this study, we use a combination of phylogenetic inference and biomechanical modeling to understand the transformational and causal basis for flexure or lack thereof. We also determine whether structural subsystems making up the feeding system are structurally, functionally, and evolutionary integrated or dissociated.

Regarding evolutionary dissociation, statistical analysis of state changes revealed by the phylogenetic analysis shows that radular and cartilage subsystems evolved independently. Regarding kinematics, the phylogenetic analysis shows that flexure arose at the base of the Mollusca and lack of flexure is a derived condition in one gastropod clade, the Patellogastropoda. Significantly, radular morphology shows no change at the node where kinematics become stereoglossate. However, acquisition of stereoglossy in the Patellogastropoda is correlated with the structural dissociation of the subradular membrane and underlying cartilages. Correlation is not causality, so we present a biomechanical model explaining the structural conditions necessary for the plesiomorphic kinematic state (flexoglossy). Our model suggests that plesiomorphically the radular teeth must flex laterally as they pass over the bending plane as a result of the mechanical restrictions in the flexible but inelastic subradular membrane and close association between subradular membrane and cartilages. Relating this model to the specific character states of the clades, we conclude that lack of flexure in patellogastropods is caused by the dissociation of the subradular membrane and cartilage supports.

## INTRODUCTION

Joseph Needham ('33) used the wonderful analogy of a series of shafts and gears that may or may not engage to describe the integrated but potentially decouplable or dissociable elements of the developmental machinery. Like developmental programs, anatomical parts and their kinematics are also partially integrated and partially dissociable. Unlike developmental programs, the metaphorical engaging shafts can become reality; biological structures and kinematics sometimes very much resemble gear or pulley systems. As T. H. Huxley (1853) first pointed out, and Herrick ('06), Eigenbrodt ('41), and Branch ('81) also discussed, something close to a pulley system exists in the functioning (=kinematics sensu Lauder, '90) of the feeding system of molluscs. In this paper we link Needham's concept of integration and dissociability with Huxley's description of the kinematics of the molluscan feeding system, focusing on integration and dissociability in structure and function of the subparts that make up the feeding system in molluscs. We discuss "dissociability" in three different contexts: (1) dissociation or decoupling of structural elements, which may cause (2) dissociation of those parts during the use of structures, and (3) dissociation in evolution of subparts that make up systems (i.e., lack of coordinated evolution).

The molluscan buccal apparatus is composed of many discrete elements that operate in unison during feeding. These elements include the numerous muscles that power the buccal machinery, pairs of underlying cartilage that serve as the pulley wheel, and the radular apparatus itself, which is drawn over the cartilages during feeding (Fig. 1A,B). The buccal apparatus, in terms of its structure and function, is one of the most thoroughly studied of molluscan anatomical systems (Huxley, 1853; Geddes, 1879; Plate, 1897; Amaudrut, 1898; Woodward, '01; Herrick, '06; Crofts, '29; Carrier, '43; Starmühlner, '52; Hubendick, '56; Lemche and Wingstrand, '59; Fretter and Graham, '62; Graham, '64; Fretter, '65; Morris and Hickman, '81; Wingstrand, '85; Hickman and Morris, '85). Unfortunately, much of this work focuses on individual, or a few closely related, species.

Graham ('73) compared coordinated change of the gastropod and polyplacophoran musculature and dentition. His goal of documenting coordinated changes was based on an *a priori* belief that the feeding system of molluscs has been highly

integrated. For example, in the *introduction* to his paper he stated that "[t]he evolution of the radular pattern has a parallel evolution in the anatomy of the odontophore, and, in particular, in the cartilages which support it and the muscles by which it is manipulated" (Graham, '73: 318). Our approach is different from Graham's. Rather than looking for correlated characters, we examine the buccal apparatus using, in part, a historical approach, tracing character state transformations of structure and function and relating these transformations to one another. Thus, one outcome of our analysis is a statement of whether subparts of the molluscan feeding system have evolved congruently *or not*. More importantly, we clarify ambiguities regarding molluscan feeding function by integrating character transformation with a general biomechanical model of one of the most important aspects of the feeding stroke.

The functional shift that we focus upon is perhaps the most highly recognized aspect of the use of the radula during feeding. Radular teeth can either laterally flex outwards and then sweep inwards (the *flexoglossate* condition) or the teeth can remain fixed (the *stereoglossate* condition) during the feeding stroke (Salvini-Plawen, '88; Ponder and Lindberg, '97). Figure 2A shows an example of flexoglossy, with the teeth laterally rotating at the anterior of the buccal system. In this study, we ask the following seven questions:

- (1) Where in the evolution of the Mollusca have changes from flexoglossy to stereoglossy (or vice versa) occurred?
- (2) Do changes in the morphology of the feeding system subparts change in concert or independently through evolution?
- (3) What changes in the radula and cartilages, if any, accompany (that is, occur at the same node as) shifts from stereoglossy to flexoglossy or vice versa?
- (4) Which of these evolutionary structural changes are *directly causally related* to change in function, and which merely represent noise in the system?
- (5) Are these causal state changes related to associations or dissociations of the many interacting parts involved in the feeding stroke?
- (6) If different parts of the feeding system can change independently from one another, can similar radular morphologies be used in completely different ways?
- (7) Have dissociations led to functional flexibility or stereotypy in descendant taxa?

### MATERIALS AND METHODS

In order to document structural changes, we determine historical patterns of change for the feeding system of Polyplacophora (chitons), Monoplacophora, and basal Gastropoda with a special emphasis on the Patellogastropoda, or true limpets. In particular, we use a phylogenetic hypothesis of Patellogastropoda with exemplars from the Orthogastropoda (the clade containing all gastropods except the Patellogastropoda, as discussed in Ponder and Lindberg, 1997), Polyplacophora, and Monoplacophora serving as outgroups. Although our focus is on non-gastropod outgroups and the more basal Gastropoda, we are currently sampling the more nested gastropod clades, the Caenogastropoda and Heterobranchia, to completely resolve evolutionary patterns across the Gastropoda. Kinematic data for feeding function, largely culled from the literature, is used along with primary morphological data as a character in the analysis. Primary data were collected from dissections, histological examinations, and three-dimensional reconstructions based on the histology. Mechanical models are based on structural relationships from our primary data and information about kinematics from the literature and our own work.

#### Phylogenetic hypothesis

This phylogenetic hypothesis is based on the data-sets available in Lindberg and Hedegaard ('96), Lindberg ('98) and Sasaki ('98) and includes sampling of new cartilage and radular characters and coding for five taxa not included in those original analyses. With the exception of *Bathyacmaea*, the included taxa all form polytomies with taxa already in previous analyses. Thus our re-analysis of the existing dataset, despite additions of operational taxonomic units, has similar information content in terms of branching patterns compared to the already published Patellogastropod trees. As well, the new characters and their distribution on the tree are presented in this paper. The new taxa that have been sampled since Lindberg ('98) are *Bathyacmaea*, *Erginus*, *Paralepetopsis*, *Acmaea virginea*, and *Rhodopetala*. A total of eighteen ingroup and four outgroup taxa were analyzed. Sampling within OTUs is shown in Table 1. Re-analysis of the original dataset with new characters was performed in PAUP3.1 using tree-bisection and reconnection with ten replicates. The most parsimonious trees

were imported to MacClade3.06 to further analyze character evolution.

Although we focus on changes in major clades, our phylogenetic analysis also allows us to determine how variability within subclades does or does not affect function. Thus, state changes at all hierarchical levels in the phylogeny are examined. The radular and cartilage characters that we discuss are shown in Table 2. One functional character, whether or not the radular teeth flex laterally as the radula is pulled over the bending plane, was also included (labeled with *FS* for stereoglossy or *FF* for flexoglossy in Fig. 3B) in the analysis, although the sampling for this character is not as good as for the structural characters. This character was coded largely from the literature. Data about the flexoglossate condition have been based on the work of Ankel ('36a, b, '38), who studied *Helcion*, Eigenbrodt ('41) who worked with *Patella*, and Padilla ('85) who examined *Acmaea mitra* and the lottiid *Notoacmaea*. Ankel ('36a, b, '38), Eigenbrodt ('41), Runham ('69), Morris and Hickman ('81) and Hawkins et al. ('89) studied taxa from the other major gastropod clades, the Vetigastropoda, Neritopsina, Caenogastropoda, and Heterobranchia (see Ponder and Lindberg, '97), and all these taxa were found to show flexure, although the amount of flexure depends on the group. In the non-gastropod outgroups, Salvini-Plawen ('88) and many subsequent authors have assumed stereoglossy but have not directly studied kinematics. However, Jüch and Boekschoten ('80, see especially Fig. 10) examined the kinematics and clearly showed that in the polyplacophoran *Lepidochitona* the main lateral teeth flex during the feeding stroke. Graham ('73) also noticed that the teeth tend to flex in *Chitonina*. This is also confirmed by D. Padilla (pers. comm.) working on *Katharina tunicata*. Radular kinematics in Monoplacophora have not been directly examined.

The distribution of character states is shown on accompanying cladograms (Fig. 3A for radular and Fig. 3B for functional and cartilage characters), which display the strict consensus of the most parsimonious trees. Numbering and lettering on Figure 3 is isomorphic with numbering and lettering in Table 2 for the radular and cartilage characters. If a state is ambiguous (optimized differently under ACCTRAN and DELTRAN), a question mark follows the number as opposed to a letter.

#### Three-dimensional reconstructions

Three-dimensional reconstructions were used in determining some morphological character states and to help visualize major differences in relative

shape and position of structures. Reconstructions were generated from transverse sections for seven taxa included in the phylogenetic analysis: *Mopalia mucosa*, *Nerita polita*, *Fissurella volcano*, *Cellana transomerica*, *Bathymacraea* sp., *Collisella scabra*, and *Acmaea mitra*. Sections were captured from a digital camera, either hooked to a polarizing light microscope or a dissecting microscope, and stored on a Macintosh PowerPC. Using NIH image, the digital images of sections were converted to a stack. Due to memory limitations, the stack size was kept at or below thirty images. Thus, the spacing between each transverse section varied depending on specimen size. Although a consistent spacing between transverse sections was generally kept for each sample, we did vary this if the section located at the exact interval was inadequate.

Once a complete stack was generated, each image in the stack was hand registered to the other images. We tried to minimize the amount of overall change in position of any one morphological feature during registration; such features included the cartilage midpoints, radula, radular diverticulum, esophagus, ganglia, salivary glands, and mantle. Once registered, each image in the stack was edited so that only subradular membrane and cartilages remained—all the other morphology was erased from the images and the radula and cartilage were filled with different gray scale values.

We used the nearest-point projection function of NIH image to build a three-dimensional reconstruction, rotating around the *x*- or *y*-axis. Twenty degree increments were used for our rotations. The lower and upper thresholds were set such that strongly white and strongly black pixels are considered background, and everything between, foreground. Colorized snapshots from the 3-D reconstructions are shown in Figure 4 for six of the sampled taxa. *Collisella scabra* and *Fissurella volcano* reconstructions were built twice, once by the authors and once by an independent research team. We did this to ensure that reconstructions were consistent when done by independent researchers, since hand registration of sections may lead to biases. Independent reconstructions of the same taxon showed a high degree of consistency.

## RESULTS AND DISCUSSION

### *Character State Distribution*

#### **Kinematic character evolution**

All aspects of the kinematics of the buccal apparatus involve the muscles, radular apparatus, and odontophoral cartilages over which the radula is

drawn, not the radular teeth or muscles alone. The feeding stroke involves two separate but coordinated movements: (1) odontophoral protraction and retraction, which moves the buccal mass forward and downward as a unit towards the substrate, and (2) radular protraction and retraction, which involves movement of the radula relative to the underlying cartilages (Graham, '73). During the feeding stroke, radular protractor muscles (Fig. 1) pull the radular apparatus over and around the anterior end of the paired supporting structures; the point where the radula goes over the most anterior portion (that is, where the teeth are shifted to point down instead of up) is defined as the bending plane. Thus, when protracted the anterior-most teeth on the ribbon are located ventral to the bending plane, along the lower surface of the cartilages (Fig. 1). Since whether or not the radular teeth flex is determined only during *radular* protraction and retraction, we focus on these two events and not protraction and retraction of the odontophore.

Patellogastropods have a stereoglossate feeding stroke while all other gastropod and non-gastropod outgroups are flexoglossate. Lack of rotation can be seen in the parallel feeding traces left on the substrate (Fig. 2B). Besides lack of flexure, patellogastropod feeding shows additional differences compared to other intertidal molluscan grazers (Fretter and Graham, '62). For example, the patellogastropod radula strikes the substrate during odontophoral protraction, not retraction as in other groups (but see Hawkins et al., '89). As well, numerous tooth-rows simultaneously contact with the substrate anterior to the bending plane, unlike single-row contact seen in other groups. Finally, the buccal apparatus of patellogastropods forcefully strikes the substrate on which they feed, collecting not only plant material but rock gouged from the substrate (Hawkins et al., '89).

Although our sampling of kinematics is not as good as our sampling of structural features, when optimized along with the rest of the characters using parsimony the distribution of states is not ambiguous (Fig. 3, states *FF* and *FS*). The flexoglossate condition is primitive given our sampling and the stereoglossate feeding stroke has arisen only once as a synapomorphy for the Patellogastropoda.

#### **Structural character evolution**

In order to better clarify the distribution of radular character states (Fig. 3A), we briefly review the various elements composing the radula. The radular subsystem is composed of distinct elements:

the radular and subradular membranes and the radular teeth (see Fig. 5 for tooth morphology). The radular teeth and membrane are secreted as organic structures in the posteriorly located radular sac and are then moved progressively anteriorly towards the bending plane. While moving anteriorly during ontogeny, the teeth are often hardened by the deposition of minerals; it is important to distinguish between the bases and cusps of the teeth, which may differentially mineralize. The subradular membrane is similar in composition to the radular membrane but is produced along the sides of the pharynx, far anterior to the radular sac. The subradular membrane underlies and affixes to the radula, and also extends to either side of the radular teeth and membrane.

Radular and subradular membranes are synapomorphic for either the Mollusca or the clade containing all Mollusca besides the Aplousobranchia, dependent on the position of that group phylogenetically, which is still in doubt. These structures have also been lost in some groups (the bivalves, for example). Unlike the subradular membrane, which is generally a flat sheet of chitinous material, the radula is complex in its composition and morphology, rendering it character-rich. The radulae of polyplacophorans and patellogastropods have been considered very similar, reflected by the traditional unification of these groups as the “Docoglossa” (Fig. 5C, D for generalized views of these radulae). These taxa have few plate-like marginal teeth, the number of which has been subsequently reduced, from six in polyplacophorans to three in monoplacophorans, to even fewer in patellogastropods—the patellids have two or three, and acmaeoidaeans (Fig. 3A for definition of Acmaeoidae clade) have between zero and two. The marginals in these “docoglossan” groups are the most weakly developed teeth; on the other hand, in Vetigastropoda and Neritimorpha the marginals are numerous and highly plicate, forming brushlike structures (Fig. 5A, B).

In our character coding we distinguish between inner lateral (IL) and outer lateral (OL) tooth fields (following Lindberg, ‘88; McLean, ‘90) and count the number of cusps for those fields. Monoplacophorans, polyplacophorans, and some patellogastropods have only one IL cusp, although the state at the patellogastropod node is equivocal. Inner laterals have been lost in *Bathycypraea* and two pairs are present in the patellid and the lepetid clades (see Fig. 3A for clade names). In the Vetigastropoda and Neritimorpha, the number of IL cusps increases; these groups have three or more.

The number of OL cusps is plesiomorphically two, but varies in gastropod outgroups from one in *Nerita* to multiple in *Fissurella*. Most Patellogastropoda have two OL cusps; however, the cusps have been greatly multiplied in *Pectinodonta*, while the patellid clade usually has three to four cusps. A rachidian is plesiomorphically reduced in patellogastropods, but this median tooth is as large as the inner laterals in the Lepetid clade (although fused to the inner laterals in lepetids according to Sasaki, ‘98) and in some patellids.

Ferrous oxide minerals (e.g., goethite) are deposited in the tooth cusps and bases of several taxa. The lateral teeth of Polyplacophora and most Patellogastropoda are mineralized; exceptions among basal patellogastropods include *Eulepetopsis* (no mineralization on ILs or OLs) and Lepetidae (no OL mineralization). Deposition of iron oxides is also lacking in some Monoplacophora, most Vetigastropoda, and Neritimorpha.

Compared to the outgroups, patellogastropod subclades are apomorphic in terms of the integration of the radular apparatus. In the outgroups, the bases of the OL teeth attach at one point on the membrane, leaving the rest of the base free above the radular membrane. The OL bases in all patellogastropods except the basal *Bathycypraea* and lepetids are not free but are attached along their length to the membrane. However, the state at the base of the Patellogastropoda is equivocal. The attachment of tooth bases to the radular membrane is only one way that the patellogastropods have integrated their radular apparatus. For example, the IL and OL teeth within a row are apomorphically fused in the Acmaeoidae (Guralnick and Lindberg, 1999); plesiomorphically the outer laterals are not fused with the inners within the row but instead extend into the row behind (Guralnick and Lindberg, 1999). Teeth do not always rest directly on the membrane, and clear “plate structures” lying between teeth and membrane have arisen homoplastically. Plates can be numerous (as in *Cellana* and *Nacella*) or singular (as in the Acmaeoidae and *Bathycypraea*).

The similarity between the Patellogastropoda and non-gastropod outgroups has long been recognized, and our analysis confirms that the patellogastropod radula retains plesiomorphic states. No unequivocal radular apomorphies diagnose the Patellogastropod clade. By contrast, patellogastropods have many diagnostic cartilage apomorphies (Fig. 3B). Buccal cartilages in molluscs are more or less discrete pieces of tissue composed of what appears morphologically to be hyaline cartilage. Although buccal cartilages are

found in all Mollusca, the homologies of different cartilages have not been well established (but see Sasaki, '98). We code not only number of cartilages, as in Ponder and Lindberg ('97) and most molluscan workers, but also determine putative homologies based on position and shape. Use of shape and position suffers from one potential drawback; the position of the structures varies. In some of our specimens, the apparatus is protracted, while in others it is in a resting state. Although the position of structures does vary from one specimen to the next, the shifts are not dramatic and patterns are still discernible among and between taxa. As well, we can use these differences to document position and shape change of elements during the feeding stroke.

The presence of a medial cartilage pair is plesiomorphic for Mollusca. These cartilages are usually long rods that run just lateral to the midline of the buccal mass. Although their presence is plesiomorphic, medials have different shapes and detailed morphology in different groups. In the Patellogastropoda, they tend to become dorsoventrally elongated near the anterior of the buccal apparatus. By contrast, the medials in the other groups show no change in height from anterior to posterior (compare Fig. 6B,C to Fig. 6E). Also, the medials in patellogastropods are ventrally fused or closely approximated at their anterior ends. In the outgroups, the medials are more separated at the bending plane.

Like medial cartilages, dorsolateral cartilages are also probably plesiomorphic for Mollusca, although this again depends on the placement of Aplacophorans (which lack dorsolaterals) within the Mollusca. Sasaki ('98) considered the dorsolaterals (anterolaterals in his nomenclature) as *de novo* structures in patellogastropods and not related to the laterally placed cartilages in poly- or monoplacophorans. However, based on position, shape, and composition, we argue that chitons and monoplacophorans and patellogastropod dorsolaterals are putative homologs. In the "placophorans" the medials and dorsolaterals are attached by a connective tissue sheath, the space between constituting the hollow vesicles (Figs. 7C, 8A). The dorsolaterals are also more lateral than dorsal in the non-gastropod outgroups. This position is largely maintained in patellids, with limited dorsal shifting. In acmaeioideans this pair is further reduced, especially in its anteroposterior length, and tends to be located even more dorsally, often lying directly above the medial pair (Figs. 7D, 8C). Dorsolateral cartilages are absent in all other gastropods.

At first glance the medials appear to be the main supports of the radular apparatus. In all gastropod clades besides Patellogastropoda, the medials are very closely associated with the subradular membrane, the membrane conforming to the shape of the groove between the medials (see Figs. 7A,B, Fig. 8B and especially Fig. 4B). However, in Polyplacophora and Patellogastropoda the situation is different. In Polyplacophora a small, flattened dorsal cartilage pair lies just above the vesicle; for most of its length the radula rests on the hollow vesicles, but it is lifted slightly above the vesicles to lie on the dorsal cartilages where they occur (Figs. 7C, 8A and especially Fig. 4A). In monoplacophorans, the dorsal cartilage is not present, and the radula is associated with the vesicles.

In patellogastropods, the subradular membrane is raised far above the medial cartilages, though this has been accomplished in different ways (Fig. 6B and especially Figs. 7D and 8C). In the primitive patellogastropod condition, the anterior portion of the subradular membrane rests on a dorsal cartilage pair located above the dorsolateral pair (Figs. 7D, 8C), although in some taxa this situation has been subsequently modified. More derived conditions include those of: *Patella*, in which the dorsal pair is replaced by a connective tissue pad; *Patelloida*, in which the subradular membrane rests on the dorsolateral pair; and lottiids and *Rhodopetala*, in which the dorsal pair is absent and the subradular membrane rests instead on the hypertrophied muscles of the buccal mass. The dorsal cartilages are only found in the anterior portion of the buccal mass (Fig. 6C). Posterior to the dorsal cartilages, however, the radula still remains dissociated from the medials and rests on muscle (Acmaeioidea) or a pair of muscle rich dorsomedial cartilages (patellids).

Posterior cartilages show great variability in presence and absence throughout the Mollusca, and state assessment is equivocal at the base of the Mollusca. They are present at least in some chitons (*Kathrina* and *Mopalia*), although they are quite small and contained within the connective tissue making up the vesicle. Similarly positioned but larger cartilages are found in the patellid lineages. The posteriors are absent in Monoplacophora and acmaeioidean patellogastropods. In polyplacophoran and the patellid clade, there are two posterior cartilages; a dorsal and a ventral pair. A single posterior pair is found in other gastropod groups, potentially a fusion of the two pairs (based on its large dorsoventral extent). Although absent in

*Fissurella*, they are present in *Haliotis* (Crofts, '29); thus the character is polymorphic for the Vetigastropoda. One posterior cartilage pair is present in *Nerita*.

Some aspects of these radula and cartilage characters are best visualized in the three-dimensional reconstructions. One of the more striking aspects of patellogastropod buccal morphology is the change in slope of the radula along the dorsal surface of the odontophore. Moving from posterodorsal to anteroventral, the radula has a long region of zero slope (segment 1 in Fig. 6B, D; see also Figs. 4C,D,E) before bending sharply downwards at an angle usually greater than thirty degrees (segment 2 in Fig. 6B). This distinctive downturn occurs toward the anterior end of the radula, more than three quarters along its length from the radular sac. Importantly, the slope angle is much higher in those patellogastropod specimens where the buccal mass is in a semi-protracted state. After this downward sloping, the radula passes over the anterior portion of the medial cartilages (the bending plane) before moving back posteroventrally (segment 3 in Fig. 6B). In all other groups, the radula usually remains at zero slope from the radular sac posteriorly up to the bending plane anteriorly (segment 1 in Fig. 6D).

Given the distribution of cartilage and radular character states, we want to test whether the radular and cartilage suites show strong or weak patterns of correlated evolution. Testing this question with character suites involves determining if the number of inferred changes along branches for each suite is correlated or independent. We use the same statistical approach as Smith et al. ('95): the non-parametric Spearman's rank correlation. Here we exclude states that are equivocal depending upon optimization method. Across the twenty-four branches in our phylogenetic analysis, the  $R_s$  value is 0.015. This analysis therefore strongly supports the claim that radular morphology and cartilage morphology do not evolve in concert but are uncorrelated. Also, at the node where function changes from flexoglossate to stereoglossate, the radula shows no change while the cartilages show major changes in shape and position. This suggests the "docoglossan" radula may either be flexoglossate or stereoglossate in function.

#### ***Structural and functional model of molluscan feeding***

The evolution of characters recognized from phylogenetic analysis is an important first step toward determining how changes in structure and

function inter-relate. The next step is to decide which character state changes are directly, causally related to function. Models attempting to tie together structure and function in gastropods have been proposed since the work of Huxley (1853). Previous descriptions of morphology and function have been exhaustive and excellent, but as Morris and Hickman ('81) point out, most models have simplified the feeding action and thus do not take into account the complexity of structure *or* function. Analysis of all of the components of the buccal apparatus is daunting, particularly without robust phylogenies to detail the sequence of character state changes, and workers have tended to focus on one morphological component to the exclusion of the others. For example, the functional-morphological studies of Vera Fretter and Alastair Graham (Fretter and Graham, '62; Fretter, '65; Graham, '64, '73) attempted to explain major differences in function by inferring the actions of the numerous muscles involved in the feeding process.

Morris and Hickman's ('81) now well-established "slit-cylinder" model instead focused on the morphology of the radula. They conceptualized the radula as rolled up on both sides until the bending plane when it is pulled outwards, opening up the cylinder and forming a semicircular crease at the point where the radula contacts the substrate. This model does make explicit the importance of radular shape change during feeding. However, Morris and Hickman ('81) were primarily concerned with describing the kinematics, in one particular taxon (Trochidae), of the radula itself and only allude to the importance of the cartilage and muscles. They correctly point out that "protractions [in] species with fundamentally different radular morphologies and odontophore shapes will reveal somewhat different functional configurations" (Morris and Hickman, '81:89). It is precisely the limited structural and taxonomic focus of their model that makes it inadequate for examining broader form-function relationships in the Mollusca. Without reference to *all* the structures necessary to set the model in motion, it is difficult to interpret which structures are necessary and sufficient to explain function. As well, without knowing which states are related to the functional mode of the system, it is difficult to apply the Morris-Hickman model to other taxa with differing morphologies. We utilize a biomechanical model of function and character state transformations as reciprocally illuminating types of explanation that can be applied to all radulate molluscan groups.

### A biomechanical model of radular flexing in Mollusca

Because the flexoglossate condition is plesiomorphic given our phylogenetic hypothesis, we use this condition as our baseline in model generation. We suggest four morphological parameters that must be fulfilled in order for the radula to function in a flexoglossate manner. If each condition is met, then feeding mode will be flexoglossate. If any of these conditions is not met then the feeding mode will be stereoglossate. Although our conditions clearly relate to synapomorphies, we wait until presentation of the model to fit character state transformations into the framework of the model.

(1) *Some sturdy anterior structure must be present that will support the membrane as it is pulled over the bending plane.* Without structural support, the teeth can neither be flexed outward along the edge of the support nor can they impart forces generated by the muscles to the substrate.

(2,3) *The radula must be infolded, about a longitudinal axis, and the subradular membrane closely associated with the underlying structural supports, just prior to reaching the anterior end of the primary supports (the location of the bending plane).* If the teeth are not folded inward, they cannot flex out as they pass over the bending plane. If the subradular membrane is not closely associated with the support structure prior to the bending plane, the teeth cannot unfold along the curved edge of the supporting structure as discussed below. Folding of the radula occurs because a groove is present between the supporting structures in which the radula sits (especially clear in Fig. 7B; see also Fig. 8B). This groove must be narrower than the width of the radula, such that the entire width of the radular ribbon may not fit into the groove without folding.

(4) *The radular apparatus must be partially or fully flattened as it passes over the bending plane.* We suggest this is largely the result of a mechanical restriction imposed on the radula as it is protracted and retracted. Consider a transverse section of radula (one tooth row, for example) as it approaches the bending plane (Fig. 9A,B), assuming all other parameters are met and the anterior portion of the odontophore is approximately hemispherical.

Two different measurements of motion can be made concerning change in position of teeth at the bending plane during protraction and retraction (Fig. 9C). The first is circular: the teeth are rotated around a fixed point in the odontophore and

consequently move a certain number of degrees about this point ( $P$ ); this is the angular excursion,  $q$ . The angular excursion for any point along the tooth row, regardless of whether it lies on the midline (points  $B$  in Fig. 9A,B) or lateral margin of the radular ribbon (points  $A$  in Fig. 9A,B), will be the same. Second, the radular teeth also move some absolute distance through space, which is related to the arc described by the motion of any particular tooth around the pivot ( $P$ ). For a point located on the midline of the radular ribbon (distance from  $P =$  radius  $R_b$ ; Fig. 9C), the absolute distance traveled is given by:

$$S_b = (2\pi R_b) q$$

where  $q$  is in radians; similarly a point on the lateral margin of the ribbon (radius  $R_a$ ; Fig. 9C) will travel a distance given by:

$$S_a = (2\pi R_a) q$$

If the radula is folded prior to reaching the bending plane, teeth along the lateral margin will be further from the pivot point (as shown in Fig. 9C) than those lying along the midline (i.e.,  $R_a > R_b$ ), and hence the absolute distance traveled will also be greater for the lateral-lying teeth ( $S_a > S_b$ ). This can be clearly seen when two rows of teeth are viewed together as they pass over the bending plane (Fig. 9D). As the absolute distance traveled by lateral teeth is greater than that traveled by teeth along the midline, the lateral teeth in successive rows become spread apart from one another. For this to occur, the radular and subradular membranes must stretch. However, though these membranes are *flexible*, they are relatively *inelastic* due to tanning and deposition of calcium salts (Rinkevich, '93). Significant tensional strain is thus implausible, and distance between teeth (along the membrane) may not change during protraction and retraction: the ribbon must maintain its structural integrity. Therefore, the radula must flatten as it begins to rotate around the anterior end of the odontophore. The radular teeth, which are more or less fixed in relation to the subradular membrane, then passively change orientation by rotating laterally, or "flexing out." In this way, distances between teeth are not altered during the feeding stroke. Though the simple equations used in this analysis are applicable only to a circular rotary path, a similar argument could be applied to any surface with curvature.

Beside the flattening caused passively by protraction of the subradular membrane, two active mechanisms promote greater flattening. Pull from muscles originating lateral to the radular apparatus and attaching to the subradular membrane can exert



a lateral component of force on the radula when contracted. This has the effect of pulling the subradular membrane (and associated radula) taut as it passes over the bending plane, aiding to flatten the groove. Flattening can also occur due to dorsal divarication of supporting structures *if* the supports and subradular membrane are closely associated. Dorsal divarication at the bending plane forces the groove to be flattened by increasing its width.

### **Biomechanical model and character state transformations**

Polyplacophorans conform to all three structural parameters of the model, and despite statements to the contrary (e.g., Salvini-Plawen, '88; Ponder and Lindberg, '97), kinematic studies (Jüch and Boekschoten, '80) have shown them to be flexoglossate. In chitons a combination of cartilage, connective tissue and turgidity from vesicular fluid furnishes the supporting structure at the bending plane. These elements are highly integrated and the vesicle as a whole acts as the medials alone in the orthogastropods (see below). The space between the two vesicles anteriorly is the groove in which the radula sits as it is pulled over the bending plane. The radular apparatus is not situated deep within the groove but is found more dorsally, lying atop the space between the medial cartilages (Fig. 8A).

Vetigastropods, Neritopsines and as far as we aware all other Orthogastropoda (all gastropods excluding the Patellogastropoda as defined by Ponder and Lindberg, '97) also fulfill each of the conditions enumerated above, and they have been clearly demonstrated to be flexoglossate feeders (Ankel, '36a, b, '38; Eigenbrodt, '41; Morris and Hickman, '81; Hawkins et al., '89). The radular teeth and membrane also usually sit low in the groove between the medials deeper than in Poly- or Monoplacophorans, especially in the neritids (Figs. 4B, 7B). During the feeding stroke dorsal divarication of cartilages and lateral force exerted by slips of the radular protractor muscles have both been shown to be important in increasing flattening. Dorsal divarication was emphasized by Starmühlner ('52), who showed that ventral approximator muscles shorten the width at the ventral part of the cartilage and thus divaricate the dorsal part. Redundant systems for flattening may be a way to build flexibility in the amount of flattening, and such flexibility has been shown by Hickman and Morris ('85) in trochids. A certain basic amount of flattening occurs passively due to protraction and retraction, while greater flattening (and hence

greater flexure) involves a combination of lateral protractors and cartilage divarication.

The Patellogastropoda fulfill some but not all of the conditions described above. The robust cartilages provide ample structural support at the very anterior end of the odontophore. There is a groove between the supporting cartilages along the length of the radula that is narrower than the width of the radular ribbon. Based on the hypothesis of character evolution, patellogastropods have a relatively plesiomorphic radular configuration, and other taxa with similar radulae (i.e., polyplacophorans) certainly infold their radula prior to the bending plane so nothing inherent in radular morphology may be construed as preventing infolding. To reiterate—based on our sections it is not that chitons or Orthogastropoda have folded radulae and the Patellogastropoda do not. In fact, in our sections all are clearly folded posteriorly and anteriorly. As well, the protractor muscles of the subradular membrane include both lateral and ventral slips (a plesiomorphic condition), ensuring that some lateral force is exerted during radular protraction. Thus nothing apparent in the gross morphology of the buccal mass prevents folding of the radula prior to reaching the bending plane.

The main way in which patellogastropods differ from polyplacophorans and more derived gastropods is their lack of close association of the radular apparatus and underlying structural supports near the bending plane. This dissociation is most clear and pronounced in animals whose radulae are in the semi-protracted state suggesting that dissociation is achieved both structurally and functionally. Dissociating the subradular membrane from the supporting structure for most of the length of the buccal mass means that the radular teeth in patellogastropods are never in position to rotate around the edge of the cartilage prior to the bending plane. Further, the teeth are never forced into the groove between the cartilages and thus are also not infolded prior to the bending plane. Thus the patellogastropod buccal apparatus does not fulfill parameters two and three of our model, and we hypothesize that the structural dissociation of subradular membrane from medial cartilages is the causal change in structure involved in the shift in function.

Dissociation of radula from cartilage is structurally accomplished by dissociations in underlying support structures. The cartilages that were plesiomorphically integrated into the vesicles in the Mono- and Polyplacophora have become separated in Patellogastropoda. In the latter, the

dorsolaterals and medials remain, but the connective tissue linking the two is lost and only the medials support the radula at the bending plane, as in Orthogastropoda. Unlike the orthogastropods, the subradular membrane does *not* become associated with the medials posterior to the bending plane. Instead, the subradular membrane rests on dorsal cartilages or musculature. The dorsals and dorsal muscles (and hence radula) appear to move anteriorly and even further dorsally relative to the medials during the feeding stroke (based on semi-protracted specimens), causing even greater dissociation from the medials and increasing the dorsoventral distance the radula travels over the bending plane. Thus the structural changes cascade into function and lead to greater dissociation.

### Generation of hypotheses from the model

Because the model attempts to explain the structural parameters for radula function, we can use the model to generate explicit hypotheses concerning function based on structure for other molluscan clades. Just as importantly, we can also turn the problem on its head and hypothesize structure based on function. Hypotheses based on the model are just that; we can recognize that the model needs to be modified (at best) or discarded if our hypotheses turn out to be inaccurate. One set of hypotheses we can make relates to the inference of function in other molluscan clades. Recent analyses (Wingstrand, '85; Salvini-Plawen, '90; Lindberg and Ponder, '96) support the position of polyplacophorans and aplacophorans as basal molluscan clades and sister taxa to the Conchifera. The basal conchiferan clade is the Monoplacophora, which itself is the sister taxon to the scaphopods and bivalves on one branch and the cephalopods and gastropods on the other. Using this broader phylogenetic context, we can sample from these other major clades to determine structure and kinematics. Monoplacophorans provide one excellent test case. The monoplacophoran radula most closely resembles that of lepetid patellogastropods (Wingstrand, '85) but its supporting apparatus is like that of chitons (Wingstrand, '85; Haszprunar, '88). Both have fluid-filled vesicles and a radular apparatus resting in the groove formed by the vesicles, resulting in folding of the radula prior to the bending plane. Therefore, we predict [as have Lemche and Wingstrand ('59) and Wingstrand ('85)] that monoplacophorans use their radula in a flexoglossate manner. Literature concerning the feeding morphology or kinematics of single species in the

other extant molluscan clades (including Scaphopoda, Cephalopoda and Aplacophora) is available. Heath ('05), for example, carefully described both the structure and function of the radular apparatus in the aplacophoran caudofoveate *Limifossor talpoideus*. In *Limifossor*, "from the point of entrance into the pharynx to their extreme anterior end the two rows of teeth separate along the mid line. As the rows of teeth diverge and their long bases shift to the outer rounded surface of the supports their tips become more inclined outward" (Heath, '05: 714). Thus

*Limifossor* appears flexoglossate. As well, the radula in *Limifossor* is kept deep within the groove formed by the supporting structure anterodorsally (Heath, '05: Fig. 4). Thus feeding in *Limifossor* supports our model and also bolsters the hypothesis that flexoglossy is plesiomorphic in Mollusca given that the basal Aplacophora and Polyplacophora have flexoglossate feeding strokes.

Structural and functional information is also available in the more derived conchiferan clades Scaphopoda and Cephalopoda. The literature on cephalopod feeding morphology and kinematics is scattered. In *Loligo pealii*, Williams ('10) states that although the teeth usually point backwards, as they are pulled over the bending plane they point first upwards and then forward, suggesting rotation during feeding. In *Octopus*, Wells ('78) observed that the lateral teeth fan outward during protraction and then move back inward during retraction, again suggesting flexoglossate kinematics. Finally, Griffin (1900) thought it probable that the long lateral teeth in *Nautilus* are erected so that they no longer cover more central teeth. These different groups have different supporting structures. *Loligo* does have medial cartilages, but these appear to be replaced by muscle and connective tissue in *Nautilus* and *Octopus*. Boucaud-Camou and Boucher-Rodoni's ('83) cross-sections of *Sepia* show teeth folded anteriorly into a groove formed by a combination of muscle and cartilage, although muscle appears to predominate at the bending plane. In Scaphopoda, the radular apparatus is folded between medial cartilages, most resembling the situation in Vetigastropoda or Neritopsina (Salvini-Plawen, '88: Fig. 29), and Lacaze-Duthier (1856: Pl. 10, Fig. 2) diagrams the anterior portion of the radular apparatus flexing outward.

Although our model appears to be consistent with the data in the literature, we should state an important caveat. It is not entirely clear in the work of Wells ('78) on *Octopus* and Griffin (1900) on *Nautilus*, whether their descriptions of feeding

kinematics are based on primary observations of function or inferences based on buccal morphology or “artificial radular protraction.” More rigorous analysis and sampling of radular kinematics and structure in these clades are needed to fully test our model.

Another way to test and extend this model is to examine feeding kinematics and buccal morphology in more Patellogastropoda. Within the Patellogastropoda, McLean ('90) thought that *Eulepetopsis* showed an intermediate condition between stereoglossy and flexoglossy based on examination of partially extended radulae in fixed material. If McLean ('90) is correct and neolepetopsids do show flexure, then our model as it stands would be falsified since they would not fulfill the conditions of the model and would still be at least partially flexoglossate. As well, lepetids and *Bathymacra* have OL teeth that are neither fused to ILs nor have overlapping bases as in patellids and Acmaeoidea, and these taxa have not been sampled for kinematics. It is possible that a highly integrated, inflexible radula is also correlated with stereoglossy.

Yet another way to test this model is to instead infer structure given function. In *Haliotis*, juveniles are stereoglossate feeders (Garland et al. '85) while adults are presumed flexoglossate. We would hypothesize that differences in buccal morphology exist between juvenile and adult abalone and that these relate to the fulfillment of our model parameters. We predict that the radula in juvenile *Haliotis* is either not in the groove between the medials as it passes over the bending plane or that a groove is missing at the bending plane (or both). These predictions can be tested by examining buccal morphology in juvenile and adult animals.

Finally, we can predict based on our model that the amount of flexing relates to the amount of folding of the subradular membrane (parameter 2 of our model) and amount of spreading (parameter 3). The amount of flexing varies between taxa and we should be able to predict how much flexing occurs based purely on morphological relationships. Feeding traces clearly show that teeth farther from the pivot point flex more than closer ones, a fact consistent with the model (see Fig. 2C).

### ***Ecological and Evolutionary Implications of Feeding Patterns***

One important variable usually related to the distinction between stereoglossate and flexoglossate feeding is force production during the feeding stroke

and thus also utilization of the substrate. For example, the Patellogastropoda can penetrate and even excavate hard substrates while other taxa can only penetrate softer substrates or brush the surface of the substrate (Hawkins et al., '89). The determination of a model of force production is complex, but flexure or lack thereof is probably one important parameter. We might expect that flexing teeth exert force laterally as well as downward, and thus do not have as strong a net downward force compared to the stereoglossate true limpets. It is important, however, to clarify that lack of flexure is one of many state changes that might be responsible for higher force production. Other potential changes leading to higher force production can be determined by examining all state changes at the base of the Patellogastropoda.

Two such changes that have been discussed in the literature are hypertrophied muscles and use of the radula during protraction of the odontophore in Patellogastropoda (Fretter and Graham, '62; Graham, '64). Given our analysis, we suggest that any model of force production also include the shape of the medial cartilages and the radular apparatus. Only Patellogastropoda have medials that change dramatically in shape anteroposteriorly. At the bending plane, the cartilage is usually a small nub that gets taller dorsally toward the posterior before leveling out. Perhaps correlated with the shape change in the medial cartilages, the radular apparatus itself changes slope dramatically at the anterior portion before also leveling out (Fig. 5B), especially in a semi-protracted state. These changes may or may not affect some aspect of feeding function, like force production, but should be included in any biomechanical simulation.

Although our analysis focuses on intrinsic aspects of form and function, differences in true limpet feeding kinematics have long been linked to differential substrate use and different selective environments. The ability to penetrate the hard substrate has often been related to the ecological success of the Patellogastropoda in the rocky shore intertidal (Branch, '81). Although feeding kinematic shifts in the Patellogastropoda are possibly an adaptation given the selective environment in the rocky shore intertidal, such shifts are also potentially limiting in both a kinematic and evolutionary sense. In particular, flexoglossy appears flexible or labile in regards to diet while stereoglossy is not.

As we have shown above, all molluscan groups besides Patellogastropoda are likely flexoglossate. These groups are quite diverse in their diet. Chitons,

for example, have carnivorous (*Placiphorella*) and herbivorous (*Lepidochitonia*) members. Members of the Aplacophora either feed on foraminifera (caudofoveates) or are carnivores living on coelenterate fluids and tissues (solenogastres). Cephalopods are active carnivores or scavengers, and scaphopods are microcarnivores to microomnivores (Salvini-Plawen, '88). Members of the Orthogastropoda show a wide range of dietary habits, including carnivory, herbivory and detritivory, and these different dietary types have arisen independently numerous times at many phylogenetic levels (Ponder and Lindberg, '97). Thus, flexoglossy is found associated with many different independently derived dietary types, perhaps because this type of feeding mode allows the animal to: (1) grasp and hold items immobile while pulling food towards the midline of the esophagus, and (2) keep ingested food from moving back towards the mouth (Runham, '72). Grasping and holding onto food items either directly or after ingestion may be essential for carnivores seeking active prey, and give extra flexibility in food choice and method of obtaining food for herbivores. For example, herbivorous polyplacophorans can not only scrape the microbial film but also snip off and consume algal blades (Himmelman and Carefoot, '75). Snipping blades with the radula requires that the teeth cut the material like scissors. This is only possible for flexoglossate feeders.

The stereoglossate condition, in contrast, does not appear as labile. Animals with such a feeding stroke cannot use their radular teeth to grab, hold, cut or bite items, but only to rasp and collect the dislodged food. Based on the interconnected data we do have concerning structure, function, ecology, and evolution in the patellogastropods, we suggest the following dialectic: (1) Specific novel modifications in the buccal apparatus have led to novel functions which allow the Patellogastropoda to efficiently collect food in hard substrate habitats (Branch, '81), and (2) these same modifications of structure and function may also have disallowed the Patellogastropoda some flexibility in feeding mode and food choice, perhaps confining them more closely to a particular niche (small particle grazing on hard substrates) than other clades.

### Conclusions

We have tried to emphasize the importance of phylogenetic analysis, biomechanical models, and independent kinematic and structural data when explaining how form and function change in a

lineage. With our phylogenetic hypothesis, we show that change in function from flexoglossy to stereoglossy occurs at the base of Patellogastropoda. We also use a simple statistical test to document one kind of dissociation, the discordance of character state transformations in systems thought to be integrated. *Contra* Graham ('73), the cartilages and radula do not have to change in concert during evolution. Thus, even though the patellogastropod radula is relatively plesiomorphic, the cartilages are not, and the buccal apparatus is used in a different manner during the feeding stroke.

We then determine which of the state changes in our phylogenetic analysis directly relate to change in function. We do this by first building a biomechanical model for the plesiomorphic function and then determining which parameters must be altered to produce change in function. Our historical analysis and biomechanical model are reciprocally illuminating in that the model is built on knowledge of character evolution and changes in model parameters and in character states are both necessary in understanding pattern and mechanism of feeding system change. For example, patellogastropod radular teeth lack flexure because the radular apparatus is neither folded in the groove nor associated with main supporting structures prior to the bending plane, an assertion based on the model. The phylogeny shows the state changes that have led to these conditions not being fulfilled. In Patellogastropoda, the subradular membrane is not associated with medial cartilages like in other gastropods but instead becomes associated with plesiomorphic dorsolateral and dorsal cartilages and thus is raised far above the medials except at the point of the bending plane.

Modification of the feeding systems has been an obvious feature in molluscan diversification, related to the important process of gathering energy from the environment. Unfortunately, the feeding system has not been utilized to its fullest in analytical studies. For example, few workers have directly measured or modeled force production given the components that make up the buccal apparatus (but see Padilla, '85), and no one has tied these differences to some kind of optimality (or other) criterion to understand potential payoffs for differences in feeding mode. Also, other key components involved in kinematics of the buccal system have been unstudied, especially the nervous system control on muscle activation patterns. Although much remains to be done, the molluscan feeding system clearly has the potential to be a model system for understanding and synthesizing the

underlying phylogenetic, developmental, and ecological controls on diversity and diversification of form and function.

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## TABLES

Table 1. Sampling within operational taxonomic units.

Species name	OTU	N	Source	Procedure	Staining
<i>Cymbula compressa</i>	<i>Cymbula</i>	2	H.C.	D	N/A
<i>Cymbula safiana</i>	<i>Cymbula</i>	1	H.C.	Hi	H+E
<i>Scutellastra longicosta</i>	<i>Scutellastra</i>	1	H.C.	Hi	H+E
“ <i>Patella</i> ” <i>granularis</i>	<i>Scutellastra</i>	1	H.C.	Hi	H+E
<i>Patella vulgata</i>	<i>Patella</i>	6	UCMP 10635	Hi, D	H+E; M
<i>Nacella concinna</i>	<i>Nacella</i>	1	UCMP 10232	Hi	H+E
<i>Nacella magellanica</i>	<i>Nacella</i>	1	H.C.	Hi	H+E
<i>Cellana ardosiaea</i>	<i>Cellana</i>	1	H.C.	Hi	H+E
<i>Cellana tramoserica</i>	<i>Cellana</i>	2	H.C.	Hi, 3D	H+E;
<i>Paralepetopsis terreguvara</i>	<i>Paralepetopsis</i>	2	SMNH Uncat.	Hi, D	H+E; H+GT
<i>Eulepetopsis vitrea</i>	<i>Eulepetopsis</i>	2	USNM860497, LACM146402	Hi, D	H+E; H+GT
<i>Iothia fulva</i>	<i>Lepeta</i>	2	H.C.	Hi	WH+E, H+GT
<i>Lepeta caeca</i>	<i>Lepeta</i>	1	H.C.	Hi	WH+E
<i>Lepeta concentrica</i>	<i>Lepeta</i>	2	H.C.	Hi, D	H+E; PAS
<i>Propilidium sp.</i>	<i>Propilidium</i>	1	H.C.	Hi	H+E
<i>Bathyacmaea sp.</i>	<i>Bathyacmaea</i>	3	MNHN uncat.	Hi, D, 3D	H+E; HH+E, GT
<i>Pectinodonta aupouria</i>	<i>Pectinodonta</i>	1	USNM859397	Hi	H+E
<i>Pectinodonta sp.</i>	<i>Pectinodonta</i>	2	H.C.	Hi	H+E
<i>Acmaea mitra</i>	<i>Acmaea mitra</i>	3	H.C.	Hi, D, 3D	H+E
<i>Acmaea virginea</i>	<i>Acmaea virginea</i>	1	H.C.	Hi	H+E
<i>Rhodopetala rosea</i>	<i>Rhodopetala</i>	1	H.C.	Hi	H+E
<i>Erginus apicina</i>	<i>Erginus</i>	1	H.C.	Hi	H+E
<i>Notoacmaea scutum</i>	<i>Lottia</i>	1	H.C.	D	N/A
<i>Patelloida profunda</i>	<i>Patelloida</i>	1	H.C.	Hi	H+E
<i>Collisella scabra</i>	<i>Lottia</i>	4	H.C.	Hi, 3D	H+E; WH+VG; M
<i>Patelloida pustulata</i>	<i>Patelloida</i>	1	H.C.	Hi	H+E
<i>Nerita polita</i>	Neritopsinaa	1	CAS021197	Hi, 3D	H+E
<i>Fissurella volcano</i>	Vetigastropoda	1	CAS001023	Hi, 3D	H+E
<i>Kathrina tunicata</i>	Polyplacophora	1	H.C.	Hi	M
<i>Mopalia sp.</i>	Polyplacophora	2	H.C.	Hi, 3D	H+E
<i>Neopilina galathea</i>	Monoplacophora	1	N/A	Hi*	H+E

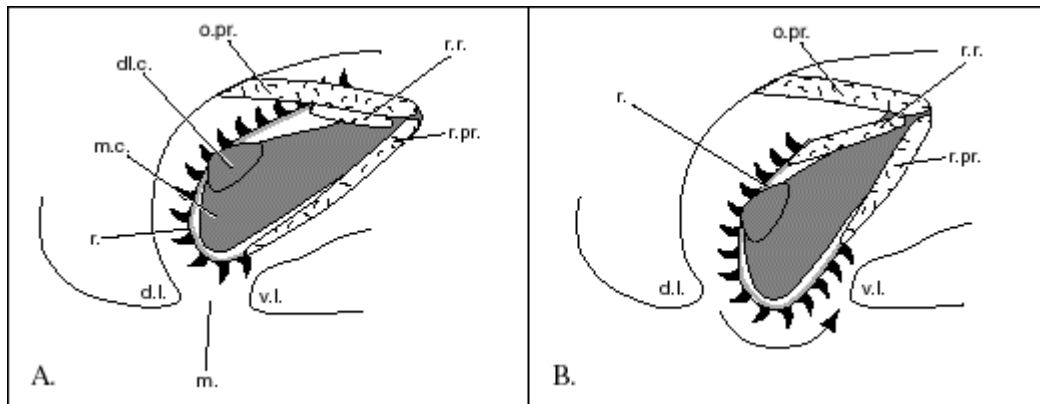
\* Histological sections made by Lemche and Wingstrand ('59) were examined.

Abbreviations for Source: H.C., hand collected; BNHM, British Natural History Museum; LACM; Los Angeles County Museum; MNHN, Muséum National d'Histoire Naturelle; SMNH; Swedish Museum of Natural History; UCMP, University of California Museum of Paleontology; USNM, United States National Museum. Procedure Abbreviations: Hi, histology; D, dissection; 3D, three-dimensional reconstruction. Staining Abbreviations: GT, Gomori's trichrome; H+E, haematoxylin and eosin, HH+E, Heidenhain's haematoxylin and eosin; M, Mallory's trichrome; PAS, periodic acid Schiff; WH+E, Weigert's haematoxylin and eosin; VG, Van Geison's.

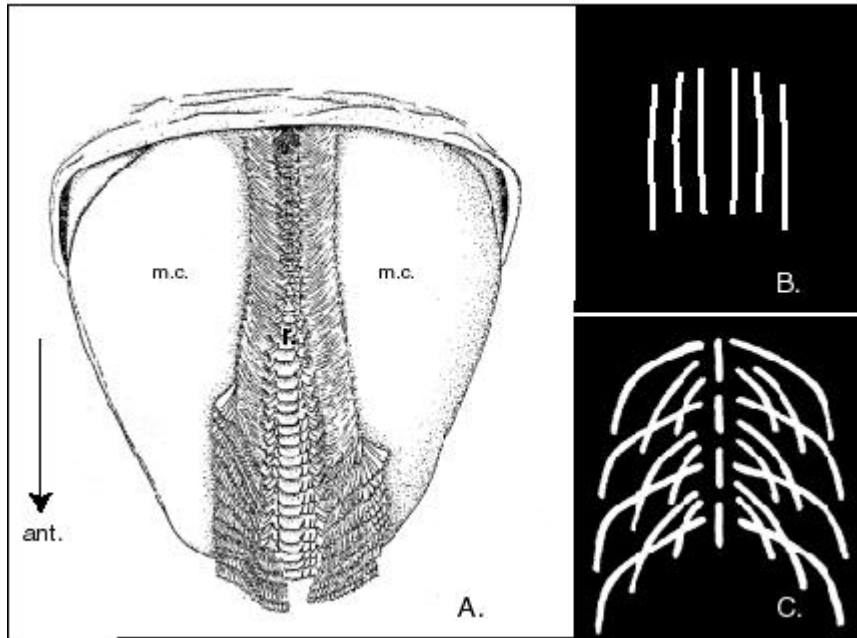


Table 2. Radular and cartilage characters sampled in this analysis. Characters are numbered and states lettered. The numbering and lettering are isomorphic with Figure 3.

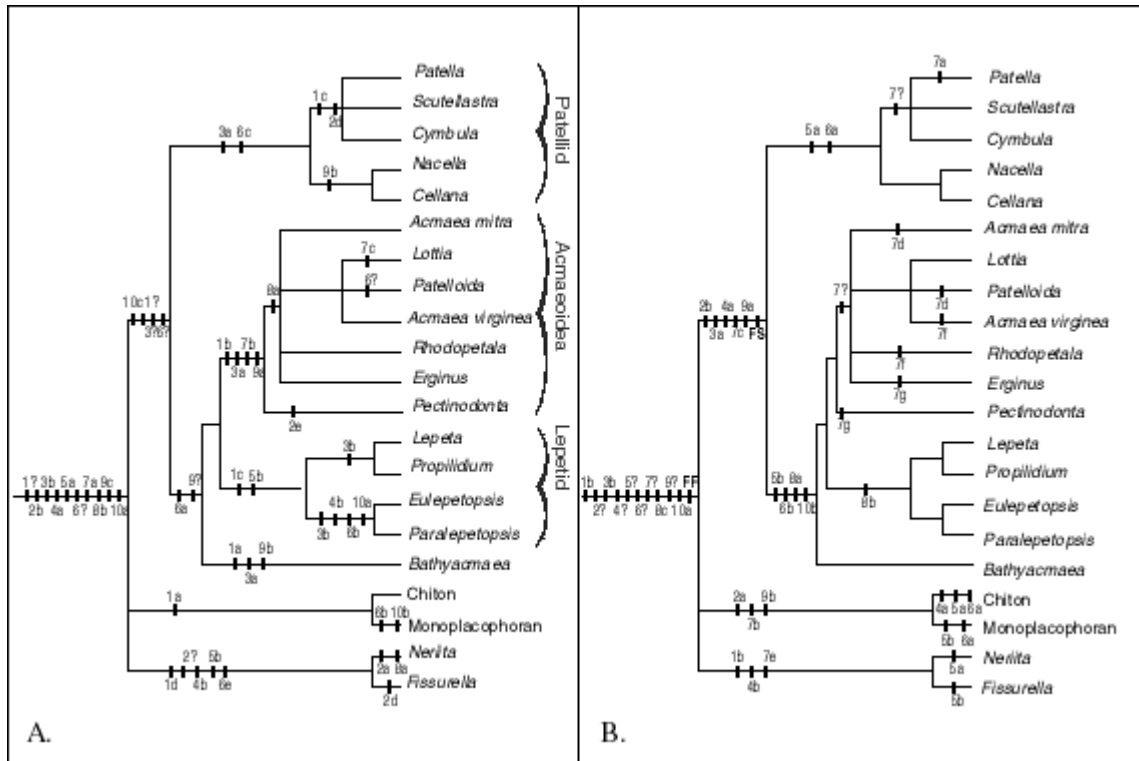
Char #	RADULAR CHARACTERS	CARTILAGE CHARACTERS
1	Number of inner lateral tooth cusps: a, zero pairs; b, one pair; c, two pairs; d, three or more pairs.	Dorsolateral cartilage: a, present; b, absent.
2	Outer lateral tooth cusps: a, one pair; b, two pairs; c, three pairs; d, four pairs e, more than six pairs (multiple).	Medials and dorsolaterals: a, linked by connective tissue to form hollow vesicles; b, not linked.
3	Outer lateral bases attached to the membrane: a, fully attached; b, free.	Shape of medials: a, elongate anteriorly; b, same height from anterior to posterior.
4	Ferrous oxide in inner laterals: a, present; b, absent.	Dorsal cartilage: a, present; b, absent.
5	Ferrous oxide in outer laterals: a, present or b, absent.	Posterior ventral cartilage: a, present; b, absent.
6	Number of marginal teeth (uncini): a, zero pairs; b, two pairs; c, three pairs; d, six pairs; e, ten or more pairs (multiple).	Posterior dorsal cartilage: a, present; b, absent.
7	Outer laterals overlap with inner laterals within a row of radular teeth: a, absent; b, partial; c, complete.	Subradular membrane support: a, connective tissue; b, hollow vesicle; c, strong dorsal cartilage; d, dorsolateral cartilage; e, medial cartilage; f, muscle; g, weak dorsal cartilage.
8	Outer lateral bases extending into the next row: a, absent; b, present.	Medials cartilages: a, fused; b, closely appositioned; c, far apart anteriorly.
9	Basal plates lying between tooth bases and radular membrane: a, present as a single large unit; b, present as many small units; c, absent.	Dorsolaterals: a, dorsal of the medials; b, flush with the medials in the dorsoventral plane.
10	Middle (rachidian) tooth: a, present; b, present but reduced; c, absent.	Dorsolaterals: a, widely separated from the medials; b, overlapping the medials in the mediolateral plane.



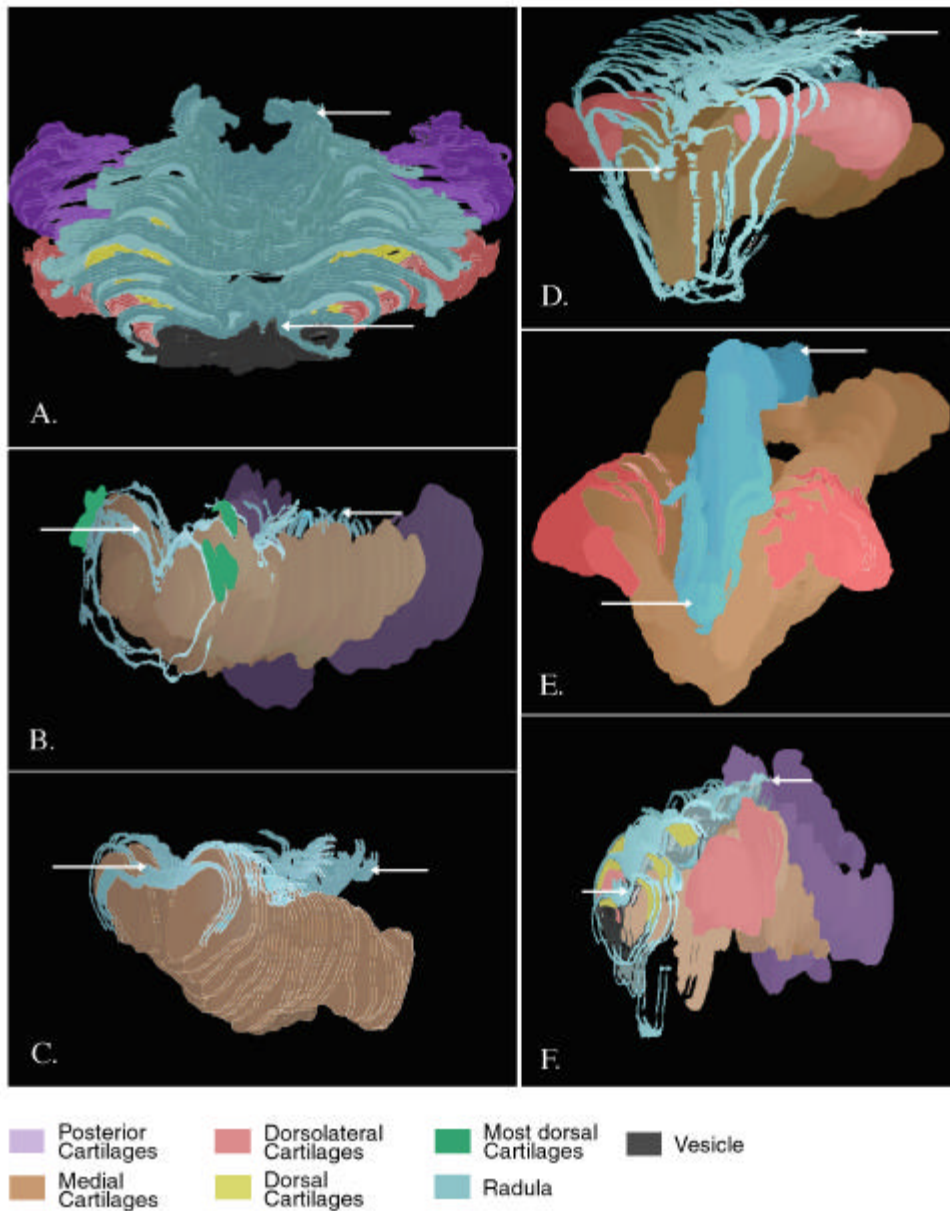
**Fig. 1.** Generalized view of the main structural components involved in the feeding stroke in the **(A)** normal, retracted position and in **(B)** protracted position. B.p., bending plane; d.l., dorsal lip; dl.c., dorsolateral cartilage; m, medial cartilage; o.pr., odontophore protractor; r., radula; r.pr., radular protractor; r.r., radular retractor; v.l., ventral lip.



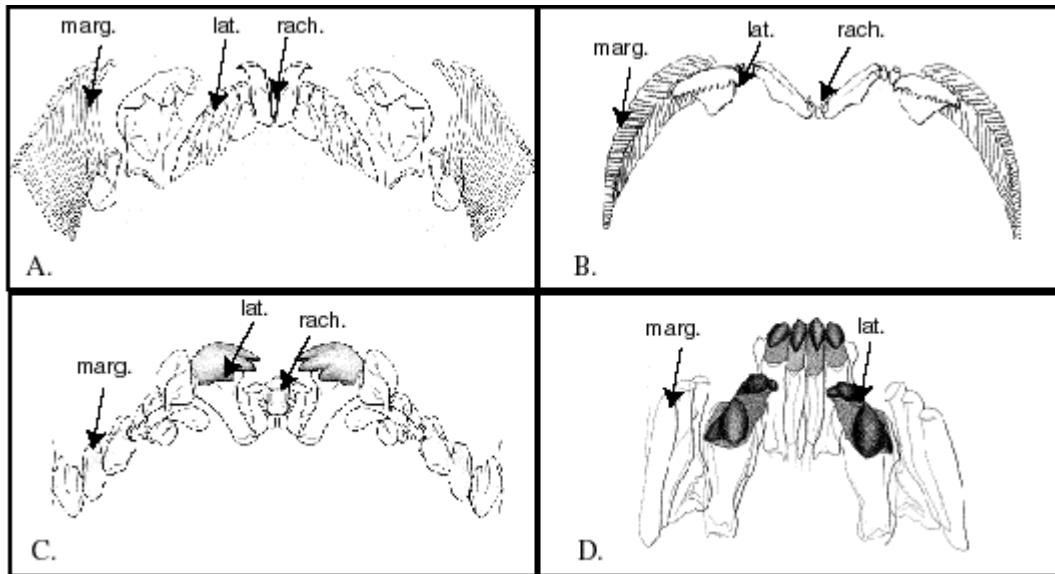
**Fig. 2.** The flexoglossate condition showing teeth rotating around the cartilage and feeding traces for flexoglossy and stereoglossy. **A:** Dorsal view of *Emarginula* (Vetigastropoda) showing teeth rotating and flexing outwards from folded to unfolded position near the bending plane. Redrawn from Eigenbrodt ('41). **B:** Feeding traces left on the substrate from a stereoglossate feeder (based on Hawkins et al., '89) **C:** Feeding traces left on the substrate from a flexoglossate feeder. Redrawn from Jüch and Boekschoten ('80). M.c., medial cartilages; r., radula.



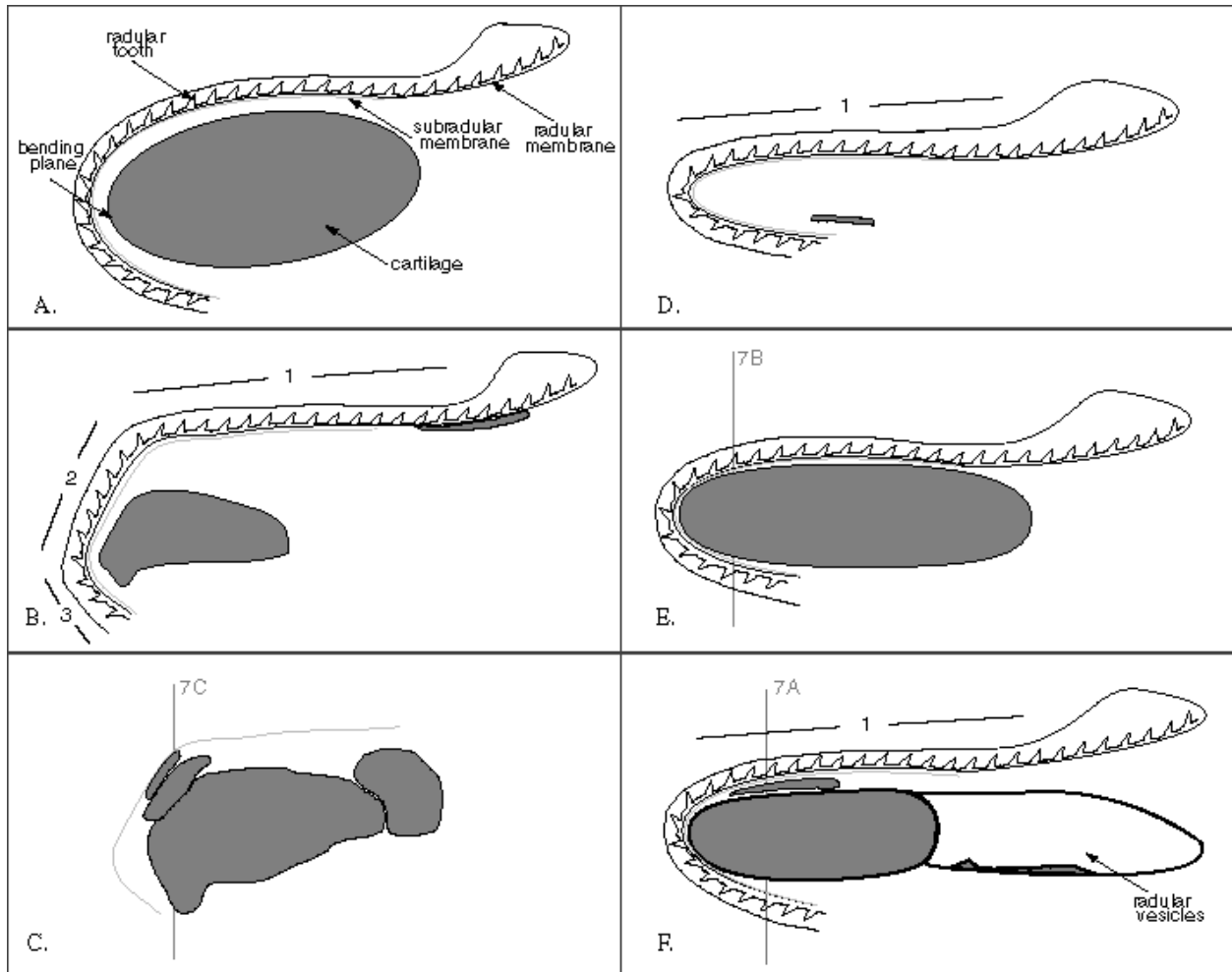
**Fig. 3.** Character state evolution based on the phylogenetic analysis. Characters are numbered, and states are lettered for the structural characters. For the one functional character, the flexoglossate state is labeled **FF** and stereoglossate is labelled **FS**. Refer to the text for a description of characters and states. If character evolution along a branch was ambiguous, we placed the character number and a question mark to reflect that ambiguity. **A:** Radula characters and clade names for Patellogastropoda subclades. **B:** Cartilage characters and functional character.



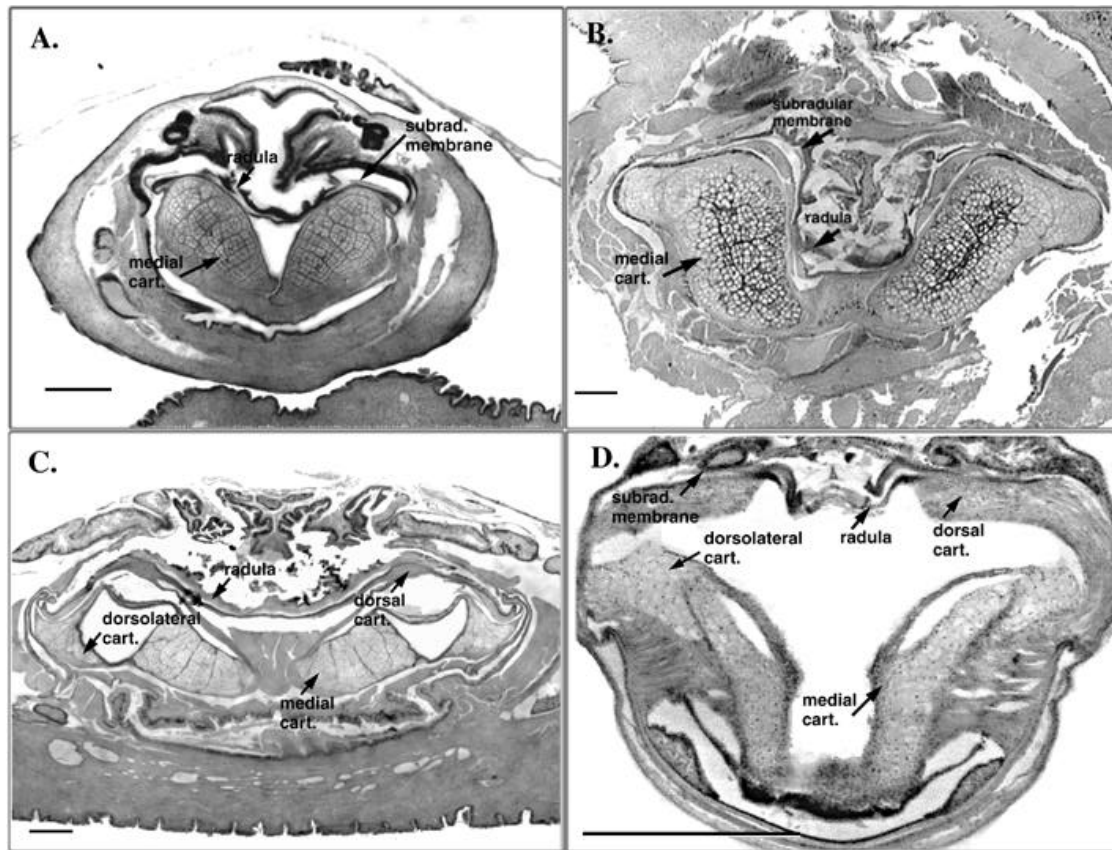
**Fig. 4.** Colorized snapshots from the animated three-dimensional reconstructions. Note labelling on image for color-scheme showing various elements. **A-C** are outgroups and **D-F** are ingroups (Patellogastropoda) in the phylogenetic analysis. **A:** Reconstruction of *Mopalia mucosa*. (Polyplacophora) rotated around the vertical axis. **B:** *Nerita polita* (Neritopsina) rotated around the horizontal axis. **C:** *Fissurella volcano* (Vetigastropoda) rotated around the horizontal axis. **D:** *Collisella scabra*, horizontal axis. **E:** *Bathyaemaea* sp., horizontal axis. **F:** *Cellana transomerica*, horizontal axis. Arrows denote the change in the position of the radula from anterior to posterior.



**Fig. 5.** Diagrams of the radula for the outgroups (excluding monoplacophorans) and ingroup flat mounted (as opposed to the position occupied in life) to show the morphology of the individual units (i.e., tooth “rows”). Marginals, laterals and rachidian are labelled for each diagram. **A:** Fissurellid. **B:** Neritid. **C:** Polyplacophoran. **D:** Patellogastropod. All redrawn from Troschel (1866-1893). Lat., lateral teeth; marg., marginal teeth; rach., rachidian teeth.

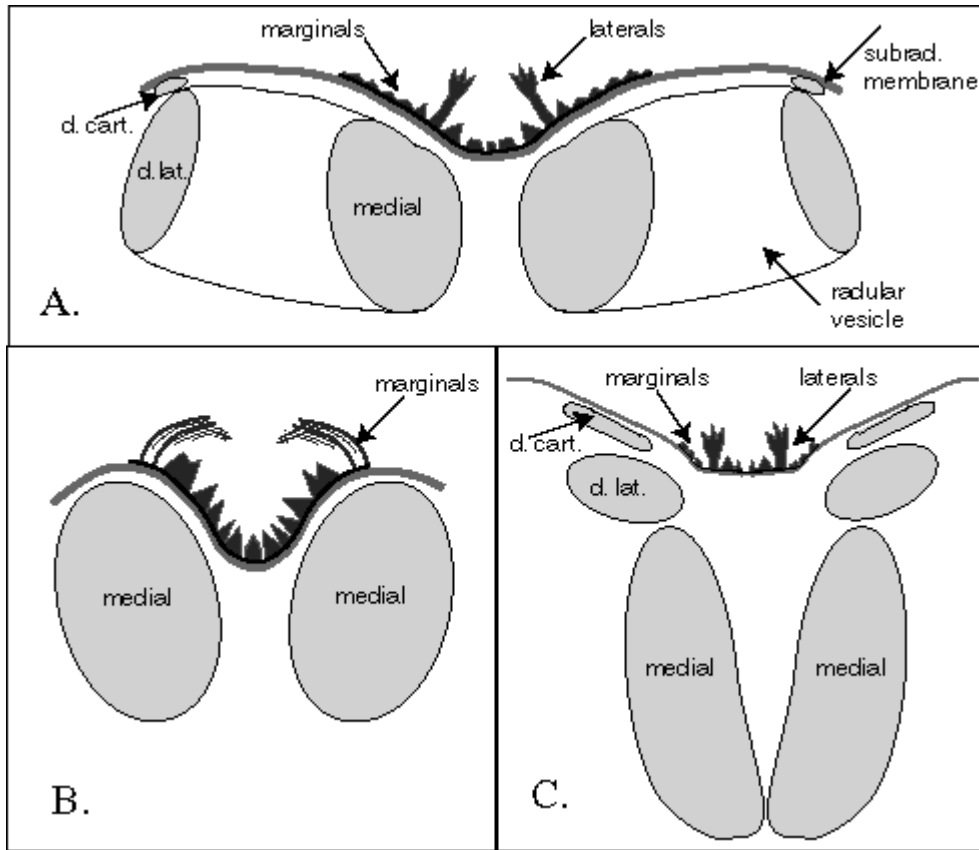


**Fig. 6.** Schematics of sagittal sections of the radula. The position of the radular sac differs in each group from that shown here but has been generalized: The main elements are labelled. **A:** Generalized “textbook” version showing the position of radula and cartilages. **B:** Just off midline cut of the patellogastropod buccal mass. Medial cartilage remains fused posteriorly and the radula is not associated with cartilage until the bending plane. Segments 1, 2, and 3 clarify areas where the slope of the radula changes along its length. **C:** More lateral slice of the patellogastropod buccal mass: The teeth are not present here, with only the subradular membrane resting on dorsal cartilages. (Vertical line shows place of transverse section for Figure 7C.) **D:** Just off midline cut of the neritid buccal mass. Segment 1 represents almost zero-slope from anterior to posterior. **E:** More lateral cut of the neritid radula. (Vertical line shows position of transverse section in Figure 7B.) **F:** Lateral cut of the polyplacophoran radula through the dorsolateral and dorsal cartilage with the vesicle extending posteriorly past the dorsolaterals. (Vertical line shows place of transverse cut shown in Figure 7A.). D.c., dorsal cartilages; dl.c, dorsal lateral cartilages; m.c., medial cartilages; p.d.c., posterior dorsal cartilage; p.v.c., posterior ventral cartilage; r, radula; r.m., radular membrane; r.ves., radular vesicle; s.r.m, sub-radular membrane.

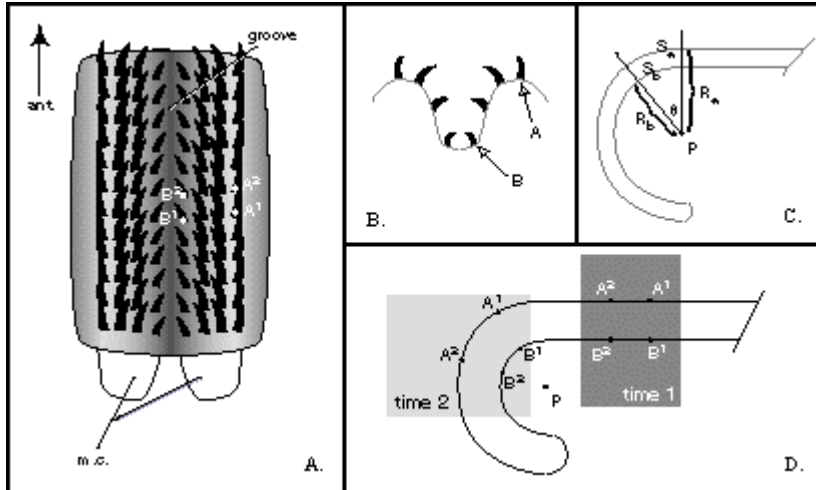


**Fig. 7.** Histological transverse sections showing the morphology of the anterior portion of the buccal mass. Radula, subradular membrane and cartilages are labelled. Note the position of the subradular membrane and cartilages in each diagram. **A:** *Fissurella volcano*. **B:** *Nerita polita*. **C:** *Mopalia mucosa*. **D:** *Erginus apicina*.





**Fig. 8.** Schematic version of Figure 6 showing the positions and shapes of the cartilage and radula systems. Important components are labelled on the figure directly. **A:** Polyplacophoran **B:** Vetigastropod and Neritopsina **C:** Patellogastropod. D. cart, dorsal cartilages; d. lat, dorsal lateral cartilages.



**Fig. 9.** A generalized model for flexure. **A:** Top view of the radula and cartilage showing many rows of teeth lying in the groove formed by the medial cartilages (or vesicle). Points A<sup>1</sup> and B<sup>1</sup> are lateral and medial points, respectively, within one row, and A<sup>2</sup> and B<sup>2</sup> the analogous points in the next most posterior row. Note groove and medial cartilages (m.c.). **B:** A cross-sectional view of one tooth row from the same radula showing the location of points A and B. **C:** Semi-sagittal view near bending plane. Pivot point (P) is located at center of the hemisphere formed by the horns of the cartilages. The distance (R) to the pivot point is greater for the more laterally placed point A than for medial point B. The absolute distance (S) travelled is also greater given that angular excursion stays the same. **D:** The position of lateral (A) and medial (B) points in two rows before the bending plane (time 1) and as the teeth cross the bending plane (time 2). The lateral teeth of successive rows (A<sup>1</sup> and A<sup>2</sup>) are spreading apart due the greater absolute distance they must travel as they are rotated around the pivot.