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Modeling a Model Organism: The Lamprey, Petromyzon marinus

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Abstract

The jawless lampreys are model organisms for evolutionary studies because their transitional features place them between the protochordates and the gnathostome, or jawed, vertebrates. However, their use as a model organism for evolutionary studies can only be valid if the model of their functional morphology is accurate, coherent, and complete. Modeling lampreys, like modeling all organisms, involves a systematic investigation into the function(s) and form(s) of their features so that their emergent faculties and biological roles can be understood and explained. Faculties are explained by auxiliary models, and these models causally amalgamate to form a holistic model that attempts to explain all the faculties of an organism, as well as the respective causal relationships among them. This investigation utilized hypothesis-driven dissection as a method to demonstrate the process of building an auxiliary model that attempts to explain the locomotive faculty of lampreys. The myomeres and myosepta were dissected in order to elucidate their structural relationships, and a functional model, the Helical Contraction Model, was constructed to explain their causal relationships. The Helical Contraction Model states that lampreys generate the sinusoidal motion observed during swimming by the alternating contraction of a left-handed and a right-handed helix *via* the contractile units of the myomeres and the belt-like tendons of the myosepta. The Helical Contraction Model provides new predictions as to the structure and function of the myomeres and myosepta, and the testing of these predictions will drive future research on lamprey locomotion.

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1. Introduction

1.1. The Lamprey as a Model Organism for Vertebrate Evolution

Lampreys (Order Petromyzontiformes) and hagfishes (Order Myxiniformes) are the only extant members of the chordate Class Agnatha. Agnathans, or jawless fishes, were once represented by over six hundred species of ostracoderms, but today only lampreys and hagfishes retain the ancestral jawless condition (Forey 1995). The once abundant jawless forms from the Devonian have all gone extinct, leaving behind only a limited sample of their fossilized skeletal structures and some ambiguous imprints of their soft tissues (Whiting 1972). Therefore, insights into the morphological changes leading from the protochordates to the origin and evolution of the gnathostomes, or jawed fishes, must be sought from extant lampreys and hagfishes, because they provide information that fossils cannot, namely a coherent understanding of physiological and developmental processes, and of an organism's functional morphology.

Lampreys are classified as the only extant jawless vertebrates because, unlike hagfishes, they possess rudimentary vertebrae called arcualia (Homberger & Walker 2004). These vertebral structures suggest a closer kinship between lampreys and gnathostomes than between hagfishes and gnathostomes (e.g., Hardisty 1981; Jefferies 1986; Mallatt 1996; Janvier 1998, 2008). Transitional characteristics between protochordates and gnathostomes, such as the absence of jaws, a single nostril, keratinized teeth, and a notochord (Hardisty 2006) make lampreys crucial model organisms for addressing fundamental questions concerning the evolution of vertebrate organs, structures, and tissues, such as the thyroid gland (e.g., Youson 1997; Kluge *et al.* 2005), cartilage (e.g., Wright *et al.* 2001; Ohtani *et al.* 2008), skeletal musculature (e.g., Kusakabe & Kuratani 2005), vertebral column (e.g., Grotmol *et al.* 2006), and the jaws (e.g., Mallatt 1996;

Horigome *et al*. 1999; Kuratani 2008). Even though the kinship between lampreys and gnathostomes has recently been challenged by molecular studies (e.g., Kuraku & Kuratani 2006; Heimberg *et al.* 2010), lampreys can still be useful as an organism for comparison to aid in understanding the origin and evolution of complex structures in gnathostomes (e.g., Mallatt 1996; Kuratani 2008; Osorio & Retaux 2008).

1.2. Rationale and Methodology for Modeling Organisms

1.2.1. Rationale

Before lampreys can be used to address fundamental questions concerning the evolutionary history of vertebrates, their functional morphology must first be understood. Understanding a lamprey's functional morphology is important because it reflects its interactions with the environment.

Like all organisms, lampreys are inherently complex. In turn this means that their population structure and dynamics will be even more complex than the individuals themselves. Furthermore, the evolution of their populations will be even more complex than the individual lampreys and their populations. This rapid increase in complexity from the individual to the population's evolutionary history demands that an adequate understanding of the individual's functional morphology precedes the explanation of the evolution of a population. Therefore, the fruitfulness of the lamprey as a model organism for vertebrate evolution depends on the validity (see section **1.3.1.** for a definition of validity) of the lamprey model itself.

Lampreys can be models and can be modeled. This suggests that there are two types of models in organismal biology: (1) Individual organisms can be modeled to understand their functional

morphology[1]; and (2) individual organisms can act as models to understand evolutionary history. In the first sense, the model is constituted of empirical data gathered from an individual organism, whereas in the second sense, the model is constituted of principles generated from the models of individuals. These principles describe potential adaptive changes in an organism's functional morphology given specific adaptive scenarios (Bock 1999; Goodwin 2009). However, the organism must be modeled before evolutionary questions can be addressed (Bock & von Wahlert 1965).

It is necessary to model organisms for many practical reasons, the first and foremost being that organisms are complex entities functioning as an aggregate of many parts. Models act as simpler representations of reality and provide a manageable grasp of organismal complexity (Homberger 1988). Models are conceptualizations of particular features of reality. In order to build valid models of organisms, their complexity must be conceptually organized.

Organismal complexity is an emergent property of morphology. It is composed of hierarchical levels of complexity ranging from the subatomic to the behavioral. These levels come together to produce a morphologically integrated organism that can interact with its environment. Although levels of complexity span a wide range of dimensions, not all are of immediate concern for biologists. Many of the lower levels of complexity, such as quantum mechanics, the properties of atoms, molecular dynamics, etc., are explained by the laws of physics and chemistry and function only as background knowledge. The lowest relevant level of complexity for modeling organisms is the biochemical level, and the highest level of importance is the behavioral level.

[1] Although the model is strict to the individual, observations on others can be used to illustrate characteristics of the individual being modeled because of pragmatic necessity.

In the biological sciences, each of these levels is the focus of a particular research program denoted by the fields of cell biology, genetics, anatomy, ethology etc. Research programs specific to each level of complexity generate a body of data specific to that level, and these data, like the laws of physics and chemistry, function as background knowledge for the investigator. These levels, however, are interdependent. Many causal relations between levels lead to new emergent properties as the levels build upon one another. For example, the properties of a particular organ system will depend on the properties of the organs constituting that system, and the properties of the organs themselves depend on the tissues constituting them, and so on. No single level, or isolated group of levels, can provide a holistic explanation for the entire organism, because of the causal extension of their properties to levels higher or lower in the hierarchy. Because of this causal overlap, the goal of modeling organisms is to construct a model that incorporates and synthesizes the causal nexus of all the levels. Such a model will give a holistic explanation of the organism.

1.2.2. Methodology

A model of an organism is equivalent to a hypothesis in that it seeks to provide a testable explanation of its functional morphology. Following the terminology of Bock & von Wahlert (1965), an organism's functional morphology is composed of faculties. Faculties are morphological characters that allow the organism to interact with its environment. A faculty is the combination of the form and function of a particular feature or group of features. Features are descriptive properties of an organism, and they occupy the predicate of a descriptive sentence for an organism. They are characterized by form(s) and function(s). The form of a feature is its shape, and the function is its action. The combination of a feature's form and function may produce one or multiple faculties. Each faculty is explained by a sub-model, and each form and

function of a feature are explained by sub-sub-models. In highly complex features, the form and function can be subdivided into sub_n-models. These sub-models, or auxiliary models, coalesce to create a holistic model with the auxiliary models arranged hierarchically to handle the levels of complexity in organisms.

There are two distinct phases in model construction for organisms: (1) The formation of a protomodel; and (2) the formation of a holistic model.

1.2.2.1. The Protomodel

The first step in modeling an organism is the assembly of a protomodel (Jeffrey Roland, pers. comm.). Investigators in the biological sciences possess a basic level knowledge of biology. This knowledge stems from the biological disciplines dedicated to specific levels of complexity. Subsidizing this basic knowledge of biology, investigators also have particular knowledge concerning particular organisms. The organismal, biological and general scientific knowledge (i.e., physics, chemistry, etc.) constitute an investigator's background knowledge. From this background knowledge a protomodel is constructed. This protomodel is an attempted explanation of the organism's functional morphology prior to the collection of empirical data by the investigator. Because protomodels are based on background knowledge, their characteristics (i.e., the properties of the auxiliary models and their relation to one another and the holistic model) will depend on the extent and synthesis of the knowledge possessed by an investigator. This makes protomodels unique to each investigator, because there will be variation on how the auxiliary models composing the protomodel are causally connected. For example, two investigators may assemble their individual models using the same background information, but they may emphasize different causal relationships between the auxiliary models.

Protomodels are usually considered unsatisfactory because they are limited by the current knowledge about an organism. A lack of understanding in one or more aspects of an organism will weaken the explanatory power of the protomodel. One or more auxiliary protomodels may lack sufficient data to provide an adequate explanation of a faculty, or the causal nexus connecting the auxiliary models may be incoherent. This inadequacy motivates the investigator to expand the protomodel and amend its incompleteness and incoherence. The expansion of the protomodel transforms it eventually into a holistic model.

1.2.2.2. The Holistic Model

The holistic model is based on both the background knowledge and the empirical observations of the investigator. The first empirical datum gathered in light of an auxiliary model marks the birth of the holistic model from the protomodel. Holistic models are explanations of the entire functional morphology of the organism, but they are not directly empirically based. Rather, the auxiliary models are constructed from empirical observations, and therefore they are the most crucial aspects in the construction of the holistic model.

A prescriptive methodology for building auxiliary models has been put forth by Homberger (1988), and it has been followed here with slight modifications. First, the investigator identifies an inadequately explained faculty of an organism. The inadequate explanation may concern the biomechanical function of the faculty, the feature-faculty causal relationship, the feature's form(s) or function(s), or any combination of these. Once the problem has been identified, detailed empirical data must be collected. The level of needed data will depend on the problem being addressed. For example: If the form of a particular feature, such as the shape of a muscle during contraction, is being investigated, then it will not be necessary to collect behavioral data.

However, data from the lower levels will be pertinent to the example, such as the biochemical and cellular structure of the muscle cells and the arrangement of the muscle cells from their origin to their insertion.

Once the relevant morphological data have been collected, they must be synthesized by connecting the causal relationship of each datum to one another. This synthesis will generate the auxiliary model. Keeping with the example above, integrating the causal connections between the muscle fiber bundles based on their individual cellular properties (i.e., fiber orientation, myoglobin content, etc.) will elucidate the shape the muscle will assume during contraction.

After each auxiliary model is satisfactorily constructed (see section **1.3.** for heuristic requirements), it must be integrated into the holistic model (Figure 1). This integration consists of drawing the causal connections between the newly constructed model and the other models that are already present. The synthesis of the causal connections will generate a higher level auxiliary model if not the holistic model itself. The integration is necessary even if the auxiliary models at first appear to stand in isolation. For example, it is not immediately clear how an auxiliary model explaining the biomechanical properties of limb function in a cow is causally connected to an auxiliary model explaining its reproductive system. However, upon closer inspection, it will become clear that the demand for nutrients by the reproductive system will adversely affect the contractile stamina of the limb muscles. The details of the causal effects will be contained in an auxiliary model explaining the organ systems. Neglecting to integrate the causal relations between auxiliary models would be akin to building a brick house without mortar.

1.3. Heuristic Requirements of a Morphological Model

1.3.1. Validity

A valid argument is one in which it is impossible for the conclusion to be false if the premises are true. Strictly, a holistic model is valid if, and only if, its composite auxiliary models are true. Heuristically, however, the notion of validity is a precautionary guide when assessing how well the holistic model follows from the causal network of its underlying auxiliary models. In this sense, holistic models can be described as having a measure of validity on a continuum from strictly false to strictly valid. In order to ensure the maximum validity of a holistic model and its composite auxiliary models, there are three specific requirements that act as heuristic devices in model building. These are accuracy, coherence, and completeness.

1.3.2. Accuracy

Accuracy applies mainly to auxiliary models, because the accuracy of the holistic model is based on the accuracy of the auxiliary models. The accuracy of an auxiliary model is a measure of the correctness of the empirical data contained within the model as compared to reality. For example, the observation that hemoglobin is red is accurate if in fact hemoglobin is red. Accuracy can explicitly be defined as follows:

> An auxiliary model, M_A, which seeks to explain a faculty, form, or function, F, of an organism's feature will be considered accurate to a degree if, and only if, the description of F provided by M_A is in fact the case with F.

If an auxiliary model is constructed to explain a feature's faculty, but fails to incorporate observations from the pertinent levels of complexity composing the feature, then it will be

considered inaccurate. Accuracy is obtained by detailed observations at every level of complexity relevant to the holistic model or auxiliary model. The accuracy of a model is dependent on the accuracies of the observations used to construct it. Accuracy is directly related to improvements in investigative tools, such as microscopy, biochemical analyses, and tissue staining procedures, as well as the thoroughness, expertise, and experience of the investigator.

1.3.3. Coherence

Coherence applies to the relationship among auxiliary models, and it is a measure of the level of harmony between their causal relations. In order for auxiliary models to be coherent with one another, they must be consistent with one another. However, because auxiliary models explain causally overlapping properties of organisms, consistency is not sufficient. For example: A model explaining the biomechanical function of a muscle-bone interaction and a second auxiliary model explaining the orbits of Jupiter's moons are consistent with one another because they do not logically conflict. However, the model of Jupiter's moons is causally irrelevant to the model of the muscle-bone system of a dog leg. Coherent models mutually support one another through their causal relationships. Therefore, the causal relationships between auxiliary models must be relevant and synthesizable at the organismal scale. Coherence can be explicitly defined as follows:

> Auxiliary models M_{A1}, M_{A2}, …, M_{An} are considered coherent with each other if, and only if, the systems described by M_{A1}, M_{A2}, …, M_{An} are relevant to the organism and are causally integrated.

To ensure coherence, each newly generated auxiliary model should be immediately integrated with the others in the holistic model. The holistic model will be internally coherent if, and only

if, its auxiliary models are coherent. The ability to attain internal coherence will depend partially on the accuracy of the observations and the completeness (see **1.3.4.** for completeness) of the auxiliary models.

1.3.4. Completeness

Completeness refers to the adequate inclusion of relevant data contained in an auxiliary model and to the number of the relevant auxiliary models composing a holistic model. Since an organism is an integrated entity composed of parts, a holistic model will be an integrated whole composed of auxiliary models. Neglecting to include auxiliary models in the holistic model is akin to leaving out parts of an organism; the outcome of the integration may be significantly affected. All auxiliary models that can be generated must be included in the holistic model. Completeness can be explicitly defined as follows:

> A holistic model, M_H, is considered complete if, and only if, it includes all possible auxiliary models, M_{A1}, M_{A2}, …, M_{An} relative to the object of the holistic model.

The level of completeness will partially depend on the accuracy of the observations, and the ability of the investigator to recognize each step in the causal chain from the lowest levels of complexity to the highest. Incompleteness may result in incoherent auxiliary models because the missing data may be a key component in establishing causal relations.

1.4. Testing Models

Both the protomodel and the holistic model can be tested using the heuristic requirements above. However, these requirements should not be taken as absolute tests. In principle, these are valid requirements for a model, but in practice their satisfaction cannot be absolute. The inherent

limitations of human observation and conceptualization will make all models incomplete, incoherent, and inaccurate to a certain degree and, as a consequence, in need of continuing revision and perfection (Dominique G. Homberger pers. comm.). Nevertheless, these requirements can be satisfied to a sufficient degree in practice and should be used to build models with ever increasing explanatory power. For example, if an investigator cannot identify an instance of causal incoherence, then the model will be deemed coherent. These heuristic devices serve as standards for models and model building, and they can also be used to assess the value of competing models.

1.4.1. Testing Protomodels

The testing of a protomodel is limited to coherence and completeness because accuracy is only testable through renewed or independent observation. Protomodels cannot be falsified, but can be considered fictional, realized, or realizable. Fictional protomodels describe organisms that do not exist. For example, a protomodel of a unicorn is considered fictional because unicorns are not known to biology and, therefore, cannot be a source of empirical data. This does not preclude the fictional protomodel from being based on scientific data, since the unicorn is a composite of a horse and an incisor tooth of a narwhal whale.

Realized protomodels can potentially become holistic models, because they describe extant organisms that are sources of empirical data. A realized protomodel is tested by evaluating the extent of coherence and completeness of the auxiliary protomodels. The coherence and completeness of the protomodels will depend on the availability and synthesis of the background knowledge possessed by the investigator. The testing of protomodels will usually reveal them to be incomplete, and this will motivate their expansion into holistic models. Incoherence may

indicate either the need for expansion or the inability of the investigator to integrate properly the causal relationships of the background knowledge. The specific shortcomings in the background knowledge, upon which the protomodel is based, will direct the investigator to the areas that need to be expanded.

Models of fossilized organisms are neither fictional nor realized, but they are realizable. They are not fictional because they were once living organisms, and they are known to biology as such. However, they are a source of only limited empirical data, and they provide only a limited amount of data for many levels of complexity. Because fossilized organisms are partially preserved, their models are partially realizable, with some models of fossilized organisms being more realizable than others. They function as hybrids between protomodels and holistic models because models of fossil organisms are based on an equal, if not greater, amount of subjective interpretation than empirical data.

1.4.2. Testing Holistic Models

The testing of holistic models determines whether they are confirmed or disconfirmed. These classifications are rendered by testing the composite auxiliary models at multiple levels as described by Homberger (1988). Auxiliary models are tested for the accuracy of their constituent data. If an auxiliary model can be transferred one-to-one with empirical observations without contradiction, then it will be considered confirmed. For example, a model describing muscle cells as consisting of actin and myosin will be confirmed, because muscle cells do in fact consist of actin and myosin. If the model cannot be transferred one-to-one with the observations without contradiction, then the model will be disconfirmed. For example a model describing muscle cells as *not* consisting of actin and myosin will be disconfirmed.

Auxiliary models can also be tested for coherence among each other by assessing how well each component auxiliary model fits the causal nexus emergent from other auxiliary models. For example, a model that explains the functioning of a limb must be able to integrate the antagonistic forces of all muscles and bones while allowing for the function of the circulatory and nervous systems explained by other auxiliary models. If the causal relations between auxiliary models explaining a complex system, such as a limb, cannot be established, then the model of the complex system will be deemed incoherent and disconfirmed.

Holistic models can be tested internally and externally. Internal tests assess the coherence and completeness of all auxiliary models composing the holistic model. Incoherence is sufficient for disconfirmation, but incompleteness is not. Incomplete holistic models may adequately explain the causal connections of their auxiliary models and can be confirmed as each new auxiliary model is added as long as incoherence is avoided, because incoherence between auxiliary models arises due to inaccuracies. Incoherence disconfirms a holistic model, but incompleteness only demands it be expanded.

External tests of holistic models are in the form of natural comparisons and they are the most epistemically valuable. There are two kinds of natural comparisons which can be used to assess holistic models: (1) Model *versus* living organism which provides a test of the holistic model; and (2) cross comparisons between models of two individuals in a population, which provides a test of how well a holistic model of an individual can be adapted to other individuals.

The causal nexus between auxiliary models can be tested by analyzing the validity of the prediction or corollaries emanating from the auxiliary models so that the validity of the holistic model can be determined. This type of analysis assesses whether the model explains the

independently observed behavior of the organism in the wild and asks, "Can the organism do what the holistic model claims?" For example, if a model of a lamprey does not allow the buccal funnel to generate a suction force as it actually does in a living lamprey, then the model will have failed the test for validity, and its auxiliary models will have to be reassessed. Tests of this type are important because the holistic model explains the faculties of a particular individual or representative of a group (i.e., a population or species) and, therefore, will explain any uniqueness possessed by that individual. This prevents hasty generalization that would assume the validity of a model of an individual would be valid for all individuals in a population.

Cross comparisons are made between two holistic models of two different individuals of a population. Intrapopulational comparisons test the functional malleability of models because the differences represent variations within a population. They determine to what extent the causal nexus of the holistic model can be altered and still provide a coherent system. These comparisons are important for addressing questions concerning the evolution through natural selection among variants of a population.

1.5. Bridge Techniques

As previously described, lower level auxiliary models explain the form and function of features, and the higher level auxiliary models explain the faculty or faculties that arise from the features. Testing the coherence of auxiliary models assesses the validity of the integration of the causal relationships among the lower level auxiliary models and the higher level auxiliary models. In practice, this integration of the models of features with the models of faculties is performed by bridge techniques. Bridge techniques are investigative tools, or data collection devices, that are applicable to the level of complexity between a feature and a faculty. For example, cell biology

synthesizes models of molecular structures that compose cells with their faculties. This synthesis can be performed by multiple bridge techniques in cell biology, such as electron micrographs, glass microneedles, fluorescence microscopy, and acoustic microscopy (Neville 1993; Janmey & Schmidt 2006). The bridge techniques for organismal biology include hypothesis-driven dissection, histology, immunohistochemistry, 3D imaging, scanning electron microscopy, and others. Among these, the hypothesis-driven dissection is the bridge technique that has the greatest capacity to provide the data necessary to integrate the form and function of features at the microscopic level with their macroscopic faculties.

1.6. Hypothesis-Driven Dissections

Faculties of organisms have functional morphological properties, which arise from their requisite features (Bock & von Wahlert 1965). Features are built upon lower levels of complexity, but these lower levels of complexity cannot causally account for the macroscopic faculties. The two properties of features, form and function, must be synthesized in order to understand the faculty, and this synthesis is done by hypothesis-driven dissections. For example, the form and function of muscle is synthesized by hypothesis-driven dissection to elucidate the origin and insertion of the muscle fiber bundles as well as the fiber direction, and these data tell what action the muscle will perform on the bones to which it attaches.

As stated earlier (section **1.2.2.**), models are equivalent to hypotheses. Therefore, hypothesis-driven dissection is equivalent to model-driven dissection. Denoting the dissection method as ‘hypothesis-driven’ rather than ‘model-driven’ captures the colloquial sense in how hypotheses and models are conceptualized by scientists. In biological parlance, the word ‘hypothesis’ implies a nascent explanatory proposition that is yet to be tested, whereas the word ‘model’

suggests something which is more complex, integrated, and suitable for providing explanations. The hypotheses in a hypothesis-driven dissection function as mini auxiliary protomodels, and, therefore, they are conceptually closer to the colloquial sense of what a 'hypothesis' is, rather than what a 'model' is.

Hypothesis-driven dissection is fundamentally a method of data collection, albeit at multiple levels. However, data collection cannot be aimless (Popper 2002). A question or hypothesis must precede empirical observation. Prior to observation, the investigator formulates a hypothesis of what emergent properties the form(s) and function(s) of a feature will give to the faculty; only then can data collection begin. As each new datum is collected, the hypothesis is either confirmed or disconfirmed. If disconfirmed, the hypothesis must be either abandoned or modified. If confirmed, the investigator is encouraged to continue collecting additional data. Once all available data have been collected, the hypothesis will be maximally either confirmed or disconfirmed. If the hypothesis is maximally confirmed, then it becomes the auxiliary model for the faculty and is subject to the three heuristic requirements listed above.

1.7. Modeling the Lamprey

In order to use the lamprey in evolutionary studies, it must be modeled following the theoretical approach described above. The first step is to generate a protomodel. Although lampreys have been systematically studied for over one hundred and fifty years (e.g., Müller 1856; Owen 1866; Hardisty 2006), the understanding of their functional morphology is still incomplete. As a consequence, a protomodel of the Atlantic Sea Lamprey, *Petromyzon marinus*, will be incomplete and incoherent because of inadequate auxiliary protomodels. The auxiliary protomodel of lamprey locomotion is one of the many incomplete auxiliary models.

Locomotion is a faculty of lampreys that allows them to interact directly with their environment. The auxiliary protomodel of lamprey locomotion is built upon knowledge of the feature(s) from which it arises. There are many features that contribute to locomotion, such as the muscular myomeres which generate force, the myosepta which subdivide the muscles into discrete units, the nerves which activates the muscle fibers of the myomeres, the notochord which stabilizes the length of the body, the shape of the body which affects fluid dynamics, and the skin which must adapt to changes in the body shape while containing the internal organs and which interacts directly with the surrounding waters.

In this report, only the myomeres and myosepta will be analyzed. They have a close causal relationship to one another, and they are a fundamental feature for locomotion, because they transmit force to propel the lamprey through the water.

The biochemistry, cell biology, and histology of the myomeres and myosepta in lampreys have been extensively described (Jasper 1967; Teravainen 1971; Rozhkova 1972; Nakao 1975; Hardisty 1979; Hardisty & Rovainen 1982; Vandekerckhove & Weber 1984; Bone 1989; Luther *et al.* 1996; Vogel & Gemballa 2000; Kusakabe *et al*. 2004), as has their biomechanical function (Nursall 1955; Alexander 1969; Hardisty 1979; Hardisty & Rovainen 1982; Altringham & Ellerby 1999; Vogel & Gemballa 2000; Gemballa & Vogel 2002). However, the anatomical relationship between the myomeres and myosepta has not been adequately established. This deficiency inhibits a proper explanation of the causal relationship between myomeres and myosepta. A hypothesis-driven dissection can provide the needed data and simultaneously establish causal connections between the myomere-myoseptum feature and the locomotive faculty.

2. Materials and Methods

2.1. Materials

One adult Atlantic Sea Lamprey, *Petromyzon marinus*, and one adult Pacific Lamprey, *Entosphenus tridentatus*, were acquired from the Anatomical Collection of Dr. Dominique G. Homberger where they had been stored in 1% 2-phenoxyethanol. The Atlantic Sea Lamprey had been preserved in a clockwise coiled position (in a ventral view) to fit in the bottom of a storage bucket (Figure 2). The Pacific Lamprey had been preserved in a straight position (Figure 3). The original fixation fluid for both specimens is unknown, although it can be surmised that it was formalin.

The total length of the specimen of the Atlantic Sea Lamprey, from the tip of the snout to the end of the tail, is 710mm. In its branchial region, the widest area of the body, the dorso-ventral diameter is 60mm, and the transverse diameter is 45mm. In contrast, the total length of the Pacific Lamprey is 640mm. In its branchial region, the widest area of the body, the dorso-ventral diameter is 45mm, and the transverse diameter is 34mm. The sex of both specimens is unknown.

2.2. Methods

2.2.1. Dissection tools

The specimens were dissected with a pair of hand-sharpened Watchmaker's forceps (Carolina Biological Supply Company, Burlington, North Carolina) under a stereo teaching microscope (WILD M3 TYP 355110, Heerbrugg, Switzerland) fitted with a ring light and a light polarizing filter. Light to the microscope was provided by a Volpi Intralux 5000 light box *via* a fiber optic

cable. Purple nitril powder free gloves were worn when handling the specimens. The specimens were placed on an absorptive liner during dissections.

2.2.2. Specimen Selection

The larger specimen of the Atlantic Sea Lamprey was selected for focused microdissection because its larger anatomical features are more easily observable than those in the smaller Pacific Lamprey. The skin had been removed from the specimen near the head and from the midtrunk by a previous investigator. The axial musculature was damaged at the middorsal line caudal to the branchial region. Some muscle fiber bundles near the dissected region had also been torn during the straightening procedure of the lamprey.

The specimen of the Pacific Lamprey, whose skin and musculature were intact, was dissected systematically from the epidermis to the muscle fiber bundles of the myomeres.

Because the axial musculature did not differ in the two lamprey species, the observations on the two specimens were combined for the modeling process.

2.2.3. Specimen Preparation

The Atlantic Sea Lamprey was dissected both in its original coiled and in a modified straightened position. To straighten this specimen, it was fastened to two straight rods of high density polyethylene (HDPE) by three rubber bands. The rods could be shifted circumferentially to allow access to different parts of the body for dissections. The Pacific Lamprey was not modified in any way because it was preserved in the preferred straight position.

2.2.4. Dissection

The dissection focused in both specimens on the cranial myomeres of the trunk immediately caudal to the branchial region.

In the Atlantic Sea Lamprey, the skin, fat, and connective tissue were removed from about six myomeres for approximately one centimeter on either side of the midventral line to expose the muscle fiber bundles of the myomeres and the myosepta between them.

In the Pacific Lamprey, the skin and underlying layers were removed systematically to expose the muscle fiber bundles of about ten myomeres from the middorsal line to the midventral line (Figure 4).

2.2.5. Photography

Macroscopic photographs of the specimens of the Atlantic Sea Lamprey and the Pacific Lamprey were made using a SPOT Insight Color Closed-Circuit TV Camera and SPOT Advanced Imaging Software (Diagnostic Instruments, Sterling Heights, Michigan). The camera was mounted on a Bencher copy stand *via* an Illuma System Light Control. Attached to the copy stand were 4 Sun-Lite UL E196460 Portable Luminaires, each containing a GE Reveal 120 watt, 120 volt incandescent bulb with a neodymium coating, which filters out yellow light. The two lamprey specimens were photographed using a 6.5mm Computer TV lens 1:1.8 with an object to lens distance of 250-775mm and a field of view of 378mm x 630mm to 816mm x 1360mm. The Pacific Lamprey specimen was also photographed using a 12.5mm Goldinar TV lens 1:1.3 with an object to lens distance of 190-1000mm and a field of view of 138mm x 230mm to 522mm x 870mm. The overhead fluorescent ceiling lights were turned off when taking pictures to avoid

glare and flickering. Glare reduction was also achieved by placing diffusers consisting of Plexiglas sheets that were covered with a sheet of visqueen plastic in front of the light bulbs.

Microscopic photographs of the specimen of the Atlantic Sea Lamprey were taken using a SPOT Insight Firewire Camera mounted on a Leica MZ6 microscope. The images were acquired using ImagePro version 4.5 Software. The microscope, the mounted camera, and the lamprey specimen to be photographed were placed on a Micro-g anti-vibration table Model 63-551 to prevent blurring of the image during exposure.

3. Results

3.1. Morphology

3.1.1. Integument

The integument of lampreys consists of four main layers. The thin epidermis lies atop a layer of organized connective tissue, the dermis. A solid pigment layer underlies the dermis and covers a thick layer of fat and loose connective tissue (Figure 4). The muscle fiber bundles of the myomeres lie directly below this layer of fat and connective tissue.

3.1.2. Myomeres and Myosepta

The axial musculature is arranged in dorso-ventral muscular units termed myomeres, which are separated by connective tissue termed myosepta (Figure 4). Each myomere is composed of muscle fiber bundles, termed muscle blocks or muscle units, which are encased in connective tissue sheets. The muscle fiber bundles are slightly angled relative to the midlateral line. Dorsal

to the midlateral line, the muscle fiber bundles run from cranioventral to caudodorsal, whereas ventral to the midlateral line, they run from craniodorsal to caudoventral (Figure 4).

The myosepta are composed of so-called belt-like tendons, which run from the middorsal line to the midventral line (Figure 4). Above and below the midlateral line, the belt-like tendons curve cranially describing an inverted "C" (Figure 4) and approaching the midventral and middorsal lines, respectively, at an increasingly shallow angle (Figures 5 & 6). At the midventral and middorsal lines, the belt-like tendons are oriented in the same direction as the muscle fiber bundles on the opposite side of the midventral and middorsal lines, respectively (Figures 5 & 6). This establishes a myo-tendinous continuity across the midventral line (Figure 7).

The belt-like tendons are composed of small individual tendons. These individual tendons enter the belt-like tendons obliquely, extending from muscle fiber bundles of adjacent myomeres (Figure 8). Because the muscle fiber bundles of a myomere are angled, their tendons enter the cranial side of a belt-like tendon and leave the caudal side of the belt-like tendon by turning towards the midlateral line.

4. Discussion

4.1. Justification for the Use of Two Lamprey Species

The objective of this study is to provide preliminary data on the anatomical relationship between the myomeres and myosepta in the Atlantic Sea Lamprey, *P. marinus*. However, the Pacific Lamprey, *E. tridentatus*, was used to illustrate the gross morphological construction of the myomeres and myosepta, because the Atlantic lamprey specimen was partly damaged. The structures of the Pacific Lamprey are identical to those of the Atlantic lamprey at the gross

morphological level. Future work will show whether the microscopic structures are also identical in the two species.

4.2. Hypothesis-Driven Dissection

Lampreys are studied every year in comparative anatomy courses at universities, but the techniques used consist mostly of sagittal and transverse sectioning (see, for example, Fishbeck & Sebastiani 2001; Homberger & Walker 2004; De Iuliis & Pulera 2007; Kardong & Zalisko 2009). Sections, however, are limited in their power to provide insight in the three-dimensional relationships among the various anatomical structures. The last comprehensive anatomical study of lampreys was performed in 1954 by Marinelli & Strenger. Their study has since served as the standard source of information on lamprey muscle morphology (Hardisty & Rovainen 1982), and subsequent investigations have focused only on particular functions or forms of lamprey features, such as the biochemical composition of their muscle fibers and the fiber direction of their myosepta (Nursall 1955; Jasper 1967; Alexander 1969; Teravainen 1971; Rozhkova 1972; Nakao 1975; Hardisty 1979; Hardisty & Rovainen 1982; Vandekerckhove & Weber 1984; Bone 1989; Luther *et al.* 1996; Altringham & Ellerby 1999; Vogel & Gemballa 2000; Gemballa & Vogel 2002; Kusakabe *et al*. 2004). However, Marinelli & Strenger (1954) described only the lamprey's major muscles, especially in the cranial region, and did not perform a microdissection on the myomeres and myosepta and, therefore, did not describe the structural relationships between the two structures. The present study of the microanatomical relationships between the myomeres and myosepta will serve to illustrate how to integrate data collected at the lower levels of complexity with general anatomical studies.

Formulation of the first hypothesis that initiated the present dissection was limited to descriptions of the myomere-myoseptum relationship presented in the literature (e.g., Nursall 1955; Nakao 1975; Hardisty 1979; Gemballa & Vogel 2002; Bone & Moore 2008). From the descriptions in the literature, it was hypothesized that the muscle fiber bundles of the myomeres inserted directly onto connective tissue sheets formed by the myosepta. This hypothesis warranted testing because a recent microdissection of the myomeres and myosepta of the Spiny Dogfish Shark (*Squalus acanthias*) had cast new doubts as to the actual relationship of myomeres and myosepta in fishes with segmentally arranged axial musculature (Andermann 2009). A hypothesis-driven dissection performed on the musculature of lampreys would collect the data necessary to confirm or disconfirm the previous studies.

Microdissection began with the removal of the skin, followed by the removal of the fat and connective tissue from the axial musculature on the ventral side of the lamprey. The serial arrangement of the myomeres along the long axis of the body and their muscle fiber direction as described by Nursall (1955) and Gemballa & Vogel (2002) were confirmed. Lamellae, described by Vogel & Gemball (2000) as intermuscular sheets of connective tissue, which separate the muscle fiber bundles into distinct units termed muscle blocks, were also observed. However, the belt-like tendons observed on the surface of the myosepta had not been described previously in the literature.

The only myoseptal tendons reported by previous investigators were located on myosepta in the tail region (Vogel & Gemballa 2000; Gemballa & Vogel 2002). Although the initial hypothesis driving the dissection did not contain an explanation for these belt-like tendons, their presence was not grounds for disconfirmation of the initial hypothesis. However, the hypothesis that muscle fiber bundles insert onto the connective tissue of the myosepta was disconfirmed when it

was observed that the belt-like tendons were composed of individual tendons arising from the superficial muscle fiber bundles of the myomeres. The junction of the muscle fiber bundles with the belt-like tendon instead of their insertion on the connective tissue of the myosepta disconfirmed the initial hypothesis, because this observation contradicted the initial prediction.

A new hypothesis was formed that integrated the new empirical observations with the non-contradictory background knowledge taken from the literature. The new hypothesis predicted that the individual tendons arising from the muscle fiber bundles of a myomere would form a junction either with the muscle fiber bundles of the myomere on the opposite side of the belt-like tendon, with a rigid structure at the dorsal and ventral ends of the belt-like tendons, or a combination of both. The first possibility was confirmed as the muscle fiber bundles on both sides of the belt-like tendon were cleaned and as the belt-like tendon was followed ventrally.

Removing the fat and connective tissue from the myomeres on either side of the belt-like tendon revealed that muscle fiber bundles of both flanking myomeres contributed individual tendons to the belt-like tendon. As the belt-like tendon was followed ventrally, it became apparent that its width was uniform. This could not be possible if both flanking myomeres contributed tendons, because the width of a belt-like tendon would grow increasingly wider as more and more tendons joined the belt. Therefore, an equal number of tendons had to leave the belt-like tendon as would enter it. The individual tendons leaving the belt-like tendon would form a myotendinous junction with the muscle fiber bundles of a myomere on either the caudal or cranial side of the belt-like tendon, and the tendons joining the belt-like tendons would originate from the muscle fiber bundles of the myomere on the opposite side of the belt-like tendon. However, which side, caudal or cranial, served as the origin of the tendons and which side served as the termination of the tendons would be explained by the model. In this configuration, the belt-like tendons are

formed by a gathering of tendons connecting to the muscle fiber bundles of adjacent myomeres. This observation implies that the muscle fiber bundles not only do not insert on the connective tissue of the myosepta, but more importantly that the muscle fiber bundles are continuous from one myomere to the next *via* the belt-like tendons (Figure 9).

The belt-like tendons were followed ventrally to see if they inserted on a rigid structure. The characteristics of this rigid structure were unknown, since no structure similar to a *Linea alba* or ventral skeletal component has been described in the literature (see, e.g., Hardisty 1981; Homberger & Walker 2004). It was discovered that at the midventral line, the belt-like tendons do not insert on a rigid structure. Instead, they form a junction with the muscle fiber bundles of the myomeres on the opposite side of the midlateral line. This relationship between the myomeres and myosepta on opposite sides of the midventral line has also not been described previously in the literature.

The continuous orientation of the belt-like tendons on one side of the midventral line and the muscle fiber bundles of the myomeres (Figures 6 & 7) on the opposite side suggests that muscle fiber bundles are continuous across the midventral line *via* the tendons of the belt-like tendon. The continuity described earlier between muscle fiber bundles of adjacent myomeres and the continuity between muscle fiber bundles of myomeres on opposite sides of the body has interesting biomechanical implications, and these implications directly affect the causal relationship between the myomere-myoseptum feature and the locomotive faculty. The data gathered from the hypothesis-driven dissection allows the construction of a more accurate and coherent model to explain lamprey locomotion. On the basis of the observations made thus far, a working model can be constructed to drive further dissection.

4.3. The Helical Contraction Model

4.3.1. A Tail of Two Helices

The empirical data collected were interpreted and used to create a coherent model, which would generate predictions to further test and assess the model's validity. Using the anatomical observations gathered through dissection, combined with the background knowledge from the literature, the Helical Contraction Model is constructed to explain the functional relationship between the myomeres and myosepta. The rationale for constructing this model is detailed as follows:

1) The dorsal muscle fiber bundles of myomeres run cranioventral to caudodorsal, and the ventral muscle fiber bundles run craniodorsal to caudoventral (Figure 4).

2) The belt-like tendons form an increasingly acute angle as they approach the middorsal and midventral line, thus assuming a single orientation with the muscle fiber bundles on the opposite side of the middorsal and midventral lines, respectively (Figures 5 & 6).

3) The muscle fiber bundles of adjacent myomeres are connected *via* their tendons to the belt-like tendons (Figures 8 & 9).

4) A contractile unit is formed by a muscle fiber bundle and its tendon traversing a belt-like tendon and continuing as a muscle fiber bundle of the next myomere, and so on (Figure 10).

5) The contractile units dorsal to the midlateral line are angled relative to the midlateral line and run from cranioventral to caudodorsal, and the contractile units ventral to the

midlateral line are angled in the opposite direction and run from craniodorsal to caudoventral (Figure 10).

6) The dorsal contractile units originate from the belt-like tendons at the midlateral line and run dorsally towards the middorsal line, and the ventral contractile units originate from the belt-like tendons at the midlateral line and run ventrally towards the midventral line (Figure 10).

7) The dorsal contractile units form a junction with the belt-like tendons that arise on the opposite, or contralateral, side of the middorsal line, and the ventral contractile units form a junction with the belt-like tendons that arise on the opposite, or contralateral, side of the midventral line (Figure 7).

8) Activation of a dorsal contractile unit transmits a force, F_1, across the middorsal line to the contralateral belt-like tendon with which it forms a junction.

9) The force, F_1, is then transmitted through the belt-like tendon to a ventral contractile unit.

10) Activation of a ventral contractile unit transmits a force, F_2, across the mid ventral line to the contralateral belt-like tendon with which it forms a junction.

11) The force, F_2, is then transmitted through the belt-like tendon to a dorsal contractile unit.

12) The series of contractile units and belt-like tendons generates a helical pattern of contractile forces.

13) One turn of the helix around the longitudinal axis leads from a dorsal contractile unit to the dorsal portion of a belt-like tendon and a ventral contractile unit, and finally to the ventral portion of another belt-like tendon (Figure 11).

14) Lampreys are bilaterally symmetrical and hence have a left side and a right side.

15) Starting with a dorsal contractile unit on the right side generates a pattern of forces in a left-handed helical configuration, and starting with a dorsal contractile unit on the left side generates a pattern of forces in a right-handed helical configuration.

16) The two helices contract alternately to produce the sinusoidal curves observed in the lamprey body during locomotion (Altringham & Ellerby 1999).

The Helical Contraction Model is the first attempt at explaining the faculty of lamprey locomotion as an auxiliary model on the basis of a hypothesis-driven dissection. Its formulation is a first step in the process of developing a holistic model of the lamprey's functional morphology, and it is a novel approach to synthesize the vast amount of data on lampreys at the lower and higher levels of complexity in a systematic and logical way. A holistic model of the lamprey will also direct future research that will expand and amend the auxiliary models that were identified as incomplete, incoherent, or inaccurate. Incoherent auxiliary models will decrease the accuracy and completeness of the holistic model, but they will not contribute to its disconfirmation. Future research will be guided by the model's testable predications listed below.

4.3.2. Testable Predictions of the Helical Contraction Model

The Helical Contraction Model provides new predictions that can be tested by further hypothesis-driven dissections.

1) The Helical Contraction Model predicts that the muscle fiber bundles from one myomere to the next are connected by a tendon that becomes part of a belt-like tendon for a certain distance and leaves it on the other side. This prediction can be tested by following a single muscle fiber bundle and its tendon through several belt-like tendons.

2) The model predicts that the muscle fiber bundles form distinct contractile units from the midlateral line to the midventral and the middorsal lines. This prediction can be tested by following a contractile unit from the midlateral line to the midventral line, and from there to the middorsal line.

3) The model predicts that, at the midlateral line, the ventral belt-like tendons form a junction with the muscle fiber bundles dorsal to the midlateral line, and that the dorsal belt-like tendons form a junction with the muscle fiber bundles ventral to the midlateral line. This prediction can be tested by following the belt-like tendons to the midlateral line and by tracing the junctions of the individual tendons.

4) The model predicts that if the belt-like tendons form a junction with the muscle fiber bundles at the midlateral line and the myomeres are part of contractile units, then the transfer of force will proceed in a helical configuration. This prediction can be tested by following a contractile unit across the midventral line into the belt-like tendon on the opposite side and then to the next contractile unit.

5) The model predicts that the muscle fiber bundles deep to the superficial muscle fiber bundles will also form a helical contractile configuration. This prediction can be tested by dissecting deep to the superficial muscle fiber bundles and repeating tests 1-4.

The alternation between contractile units and belt-like tendons cannot occur indefinitely along the entire body of finite length. At a certain point, the helices must begin and end. If the helices are not anchored at both ends, their contractile forces will not act upon the lamprey body and, instead, will act to collapse the helices without producing curvature in the lamprey body Presumably, the caudal ends of the helices terminate in the tail region where the myomeres end (Marinelli & Strenger 1954). Their origin would presumably be in the cranial region, but the configuration of the helices is not as clear since the myomere-myoseptum pattern extends well into the gill region which involves more complex structural relationships (Marinelli & Strenger 1954). The helices must be followed to their cranial and caudal points of termination to fully understand their biomechanical role over the entire body of the lamprey.

If the Helical Contraction Model has not been disconfirmed after having been subjected to the tests listed above, then it must be integrated coherently with the auxiliary models that explain other features relevant to lamprey locomotion. These other features include the mechanical properties of the notochord, the role of the arcualia for the myosepta attachment, and the neurophysiology of the contractile units. Models of each of these features will also produce additional data about the form and function of the myomeres and myosepta that the Helical Contraction Model must be able to explain.

4.4. 3-D Visualization Modeling

Because of new developments in 3-D imaging software and x-ray scanning hardware, morphological investigations are no longer limited to microdissection, histology, and electron microscopy. Visualization software, such as Avizo®, allows the integration of data taken from computed tomography (CT) scans with the data gathered from microdissections. CT scans of organisms allow the identification of anatomical structures without compromising their three-dimensional relationship because the scan preserves the intact organism, whereas dissection does not. However, CT scans are limited, because they present data based only on differential x-ray absorption and, therefore, do not clearly differentiate soft tissues, which must be resolved by manual segmentation using the anatomical data acquired through microdissection as background knowledge.

CT scans and their 3-D visualization can be valuable for modeling the myosepta and myomeres of lampreys. Using the data from microdissection, the myosepta can be identified in the CT scan and digitally marked with Avizo®. Through anatomical CT data integration, the myosepta can be reconstructed three-dimensionally along the entire length of the body making it possible to visualize their orientation in the specimen being dissected. The orientation of the superficial muscle fiber bundles of the myomeres may then have to be drawn by hand between the myosepta on the 3-D model of the lamprey. It may be possible to reconstruct the position of the larger muscle fiber bundles in the myomeres from CT data, because these muscle fiber bundles are often separated by some less dense tissue that appears black in the CT data. The reconstructed myosepta record the positions of superficial muscle fiber bundles, thereby allowing the dissection of the deeper muscle fiber bundles without losing their morphological relationship with the superficial muscle fiber bundles. Such an approach can lead to a digital representation

of the myomeres and myosepta of a lamprey that can be virtually dissected over and over again while still having it.

4.5. From Reality to Model to Reality

Understanding the functional morphology of the lamprey is important for assessing their evolutionary history as well as the significance of their characteristics as adaptations to their environment. Models of the functional morphology of lampreys can be used to address broad evolutionary concepts, such as sympatric speciation (Hardisty & Potter 1971; Salewski 2003; Docker 2009), the evolution of complex systems (Mallatt 1984, 1996), and the genotype-phenotype relationship (Cohn 2002; Escriva *et al*. 2002; Fried *et al*. 2003).

The modeling of lampreys, like the modeling of any organism, is an attempt to understand the functional and behavioral dynamics of living lampreys. Organisms are complex entities, and models enable biologists to grasp their complexity in ways that allow a conceptualization of an organism. Without modeling, biologists would be faced with a mountain of factual data that cannot be comprehended in a manner to allow a synthesis into a meaningful explanation.

Model building is a process that transforms the empirical observations of the world as it is to conceptualized ideas of the world. The testing of models is an important component in model building because it strengthens the epistemic connection between the way the world is and our conceptualizations of the world. Conceptualizations that do not pass the tests are abandoned as inaccurate and false. Those conceptualizations that pass the tests are causally integrated, and this integration generates new emergent hypotheses that can be tested on larger scales. In this way, models can become new sources of knowledge, and can provide new insights otherwise

unattainable. These new insights can lead to more coherently integrated explanations for the adaptation and evolution of organisms.

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6. Literature Cited

Alexander, R.M. (1969). The orientation of muscle fibers in the myomeres of fishes. Journal of Marine Biology 49:263-290.

Altringham, J. & D. Ellerby (1999). Fish swimming: Patterns in muscle function. Journal of Experimental Biology 202:3397-3403.

Andermann, R. (2009). Functional morphology of the superficial branchial musculature of the Dogfish Shark, *Squalus acanthias*. Undergraduate Honor's Thesis, Louisiana State University and A&M College, Baton Rouge.

Bock, W.J. (1999). Functional and evolutionary explanations in morphology. Netherlands Journal of Zoology 49(1):45-65.

Bock, W.J. & G. von Wahlert (1965). Adaptation and the form-function complex. Evolution 19(3):269-299.

Bone, Q. (1989). Evolutionary patterns of axial muscle systems in some invertebrates and fish. American Zoologist 29:5-18.

Bone, Q. & R.H. Moore (2008). Biology of Fishes, 3rd ed. Taylor & Francis Group: New York.

Cohn, M. (2002). Lamprey Hox genes and the origin of jaws. Nature 416:386-387.

De Iuliis, G. & D. Pulera (2007). The Dissection of Vertebrates. Academic Press: Boston.

Docker, M.F. (2009). A review of the evolution of nonparasitism in lampreys and an update of the paired species concept. Pp. 71-114 *in* Biology, Management, and Conservation of

Lampreys in North America (L.R. Brown, S.D. Chase, M.G. Mesa, R.J. Beamish & P.B. Moyle, eds.). American Fisheries Society Symposium 72.

Escriva, H., L. Manzon, J.H. Youson & V. Laudet (2002). Analysis of lamprey and hagfish genes reveals a complex history of gene duplications during early vertebrate evolution. Molecular Biology and Evolution 19(9):1440-1450.

Fishbeck, D.W. & A. Sebastiani (2001). Comparative Anatomy: Manual of Vertebrate Dissection. Morton Publishing Company: Englewood, CO.

Forey, P.L. (1995). Agnathans recent and fossil, and the origin of jawed vertebrates. Reviews in Fish Biology and Fisheries 5:267-303.

Fried, C., S. Prohaska & P. Stadler (2003). Independent Hox-cluster duplications in lampreys. Journal of Experimental Biology 229:18-25.

Gemballa, S. & F. Vogel (2002). Spatial arrangement of white muscle fibers and myoseptal tendons in fishes. Comparative Biochemistry and Physiology Part A 133:1013-1037.

Goodwin, B. (2009). Beyond the Darwinian paradigm: Understanding biological forms. Pp. 299-312 *in* Evolution: The First Four Billion Years (M. Ruse & J. Travis, eds.). The Belknap Press of Harvard University Press: Cambridge, Mass.

Grotmol, S., H. Kryvi, R. Keynes, C. Krossoy, K. Nordvik & G.K. Totland (2006). Stepwise enforcement of the notochord and its intersection with the myoseptum: An evolutionary path leading to development of the vertebra? Journal of Anatomy 209:339-357.

Hardisty, M.W. (1979). Biology of Cyclostomes. Chapman and Hall: London.

Hardisty, M.W. (1981). The skeleton. Pp. 333-376 *in* The Biology of Lampreys, Vol. 3 (M.W. Hardisty & I.C. Potter, eds.). Academic Press: London.

Hardisty, M.W. (2006). Lampreys: Life without Jaws. Forrest Text: Cardigan, United Kingdom.

Hardisty, M.W. & I.C. Potter (1971). The general biology of adult lampreys. Pp. 127-206 *in* The Biology of Lampreys, Vol. 1 (M.W. Hardisty & I.C. Potter, eds.). Academic Press: London.

Hardisty, M.W. & C.M. Rovainen (1982). Morphological and functional aspects of the muscular system. Pp. 137-231 *in* The Biology of Lampreys, Vol. 4A (M.W. Hardisty & I.C. Potter, eds.). Academic Press: London.

Heimberg, A.M., R. Cowper-Sallari, M. Semon, P.C.J. Donoghue & K.J. Peterson (2010). microRNAs reveal the interrelationships of hagfish, lampreys, and gnathostomes and the nature of the ancestral vertebrate. Proceedings of the National Academy of Sciences 107(45):19379-19383.

Homberger, D.G. (1988). Models and tests in functional morphology: The significance of description and integration. American Zoologist 28:217-229.

Homberger, D. G. & W. Walker (2004). Vertebrate Dissection, 9th ed. Thomson Learning, Inc: Belmont, CA.

Horigome, N., M. Myojin, T. Ueki, S. Hirano, S. Aizawa & S. Kuratani (1999). Development of cephalic neural crest cells in embryos of *Lampetra japonica*, with special reference to the evolution of the jaw. Developmental Biology 207:287-308.

Janmey, P. & C. Schmidt (2006). Experimental measurements of intracellular mechanics. Pp. 18-49 in Cytoskeletal Mechanics: Models and Measurements (M.R.K. Mofrad & R.D. Kamm, eds.). Cambridge University Press: Cambridge.

Janvier, P. (1998). Early Vertebrates. Clarendon Press: Oxford.

Janvier, P. (2008). Early jawless vertebrates and cyclostome origins. Zoological Science 25:1045-1056.

Jasper, D. (1967). Body muscles of the lamprey: Some structural features of the T system and sarcolemma. Journal of Cell Biology 32(1):219-227.

Jefferies, R.P.S. (1986). The Ancestry of the Vertebrates. The Cambridge University Press: London.

Kardong, K.V. & E.J. Zalisko (2009). Comparative Vertebrate Anatomy: A Laboratory Dissection Guide, 5th ed. McGraw-Hill Companies, Inc.: Boston, MA.

Kluge, B., N. Renault & K.B. Rohr (2005). Anatomical and molecular reinvestigation of lamprey endostyle development provides new insight into thyroid gland evolution. Development, Genes, & Evolution 215:32-40.

Kuraku, S. & S. Kuratani (2006). Time scale for cyclostome evolution inferred with a phylogenetic diagnosis of hagfish and lamprey cDNA sequences. Zoological Science 23:1053-1064.

Kuratani, S. (2008). Evolutionary developmental studies of cyclostomes and the origin of the vertebrate neck. Development, Growth, & Differentiation 50:189-194.

Kusakabe, R., M. Takechi, S. Tochinai & S. Kuratani (2004). Lamprey contractile protein genes mark different populations of skeletal muscles during development. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution 302B:121-133.

Kusakabe, R. & S. Kuratani (2005). Evolution and developmental patterning of the vertebrate skeletal muscles: perspectives from the lamprey. Developmental Dynamics 234:824-834.

Luther, P.K., J.M. Squire & P.L. Forey (1996). Evolution of myosin filament arrangements in vertebrate skeletal muscle. Journal of Morphology 229:325-335.

Mallatt, J. (1984). Early vertebrate evolution: Pharyngeal structure and the origin of gnathostomes. Journal of Zoology London 204:169-183.

Mallatt, J. (1996). Ventilation and the origin of jawed vertebrates: a new mouth. Zoological Journal of the Linnean Society 117:329-404.

Marinelli, W. & Strenger, A. 1954. Vergleichende Anatomie und Morphologie der Wirbeltiere. Franz Deuticke: Wien.

Müller, M. (1856). On the development of lampreys. Annals and Magazine of Natural History 18:298-301.

Nakao, T. (1975). Fine structure of the myotendinous junction and "terminal coupling" in the skeletal muscle of the lamprey, *Lampetra japonica*. The Anatomical Record 182:321-338.

Neville, A.C. (1993). Biology of Fibrous Composites: Development Beyond the Cell Membrane. Cambridge University Press: Cambridge.

Nursall, J.R. (1956). The lateral musculature and the swimming of fish. Proceedings of the Zoological Society of London 126:127-143.

Ohtani, K., T. Yao, M. Kobayashi, R. Kusakabe, S. Kuratani & H. Wada (2008). Expression of Sox and fibrillar collagen genes in lamprey larval chondrogenesis with implications for the evolution of vertebrate cartilage. Journal of Experimental Zoology 310B:596-607.

Osorio, J. & S. Retaux (2008). The lamprey in evolutionary studies. Development, Genes, and Evolution 218:221-235.

Owen, R. [1866] (1973). On the Anatomy of Vertebrates, Vol. I. AMS Press: New York.

Popper, K. [1963](2002). Conjectures and Refutations: The Growth of Scientific Knowledge. Routledge: London.

Rozhkova, E.K. (1972). Characteristic of cholinoreception in somatic muscle of the lamprey, *Lampetra fluviatilis*. Comparative and General Pharmacology 3:410-422.

Salewski, V. (2003). Satellite species in lampreys: A worldwide trend for ecological speciation in sympatry? Journal of Fish Biology 63:267-279.

Teräväinen, H. (1971). Anatomical and physiological studies on muscles of the lamprey. Journal of Neurophysiology 34(6):954-973.

Vandekerckhove, J. & K. Weber (1984). Chordate muscle actins differ distinctly from invertebrate muscle actins: The evolution of the different vertebrate muscle actins. Journal of Molecular Biology 179:391-413.

Vogel, F. & S. Gemballa (2000). Locomotory design of 'cyclostome' fishes: Spatial arrangement and architecture of myosepta and lamellae. Acta Zoologica 81:267-283.

Whiting, H.P. (1972). Cranial anatomy of the ostracoderms in relation to the organization of larval lampreys. Pp. 1-20 in Studies in Vertebrate Evolution (K.A. Joysey & T.S. Kemp, eds.). Winchester Press: New York.

Wright, G.M., F.W. Keeley, P. Robson (2001). The unusual cartilaginous tissues of jawless craniates, cephalochordates and invertebrates. Cell Tissue Research 304:165-174.

Youson, J. (1997). Is lamprey metamorphosis regulated by thyroid hormones? American Zoologist 37:441-460

Figures

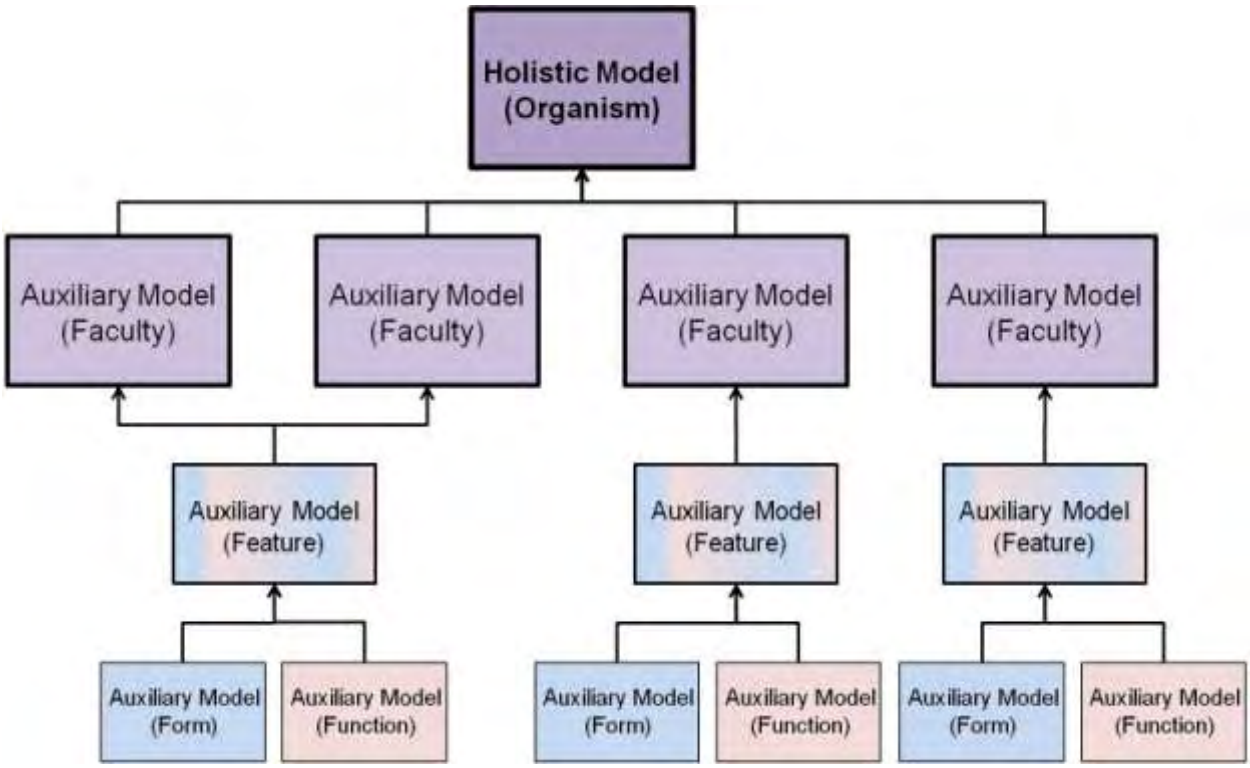

Figure 1. Methodological flow chart depicting the hierarchy of auxiliary models that explain features and their forms and functions. Features can give rise to faculties. All auxiliary models causally coalesce to generate the holistic model.

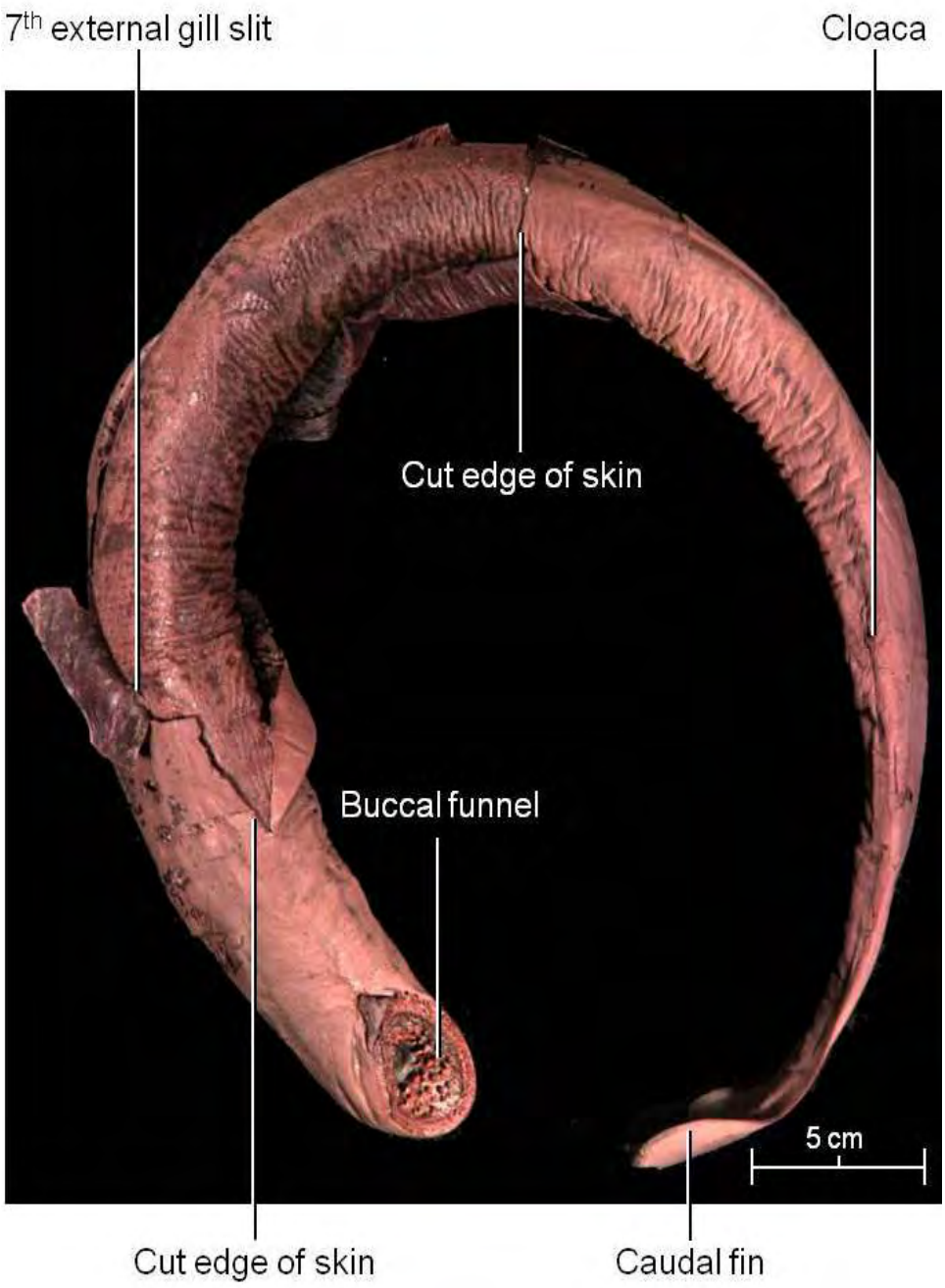

Figure 2. Ventral view of the morphological features of an Atlantic Sea Lamprey, *Petromyzon marinus*, which was preserved in a coiled position.

Figure 3. Lateral view of general morphological features of a Pacific Lamprey, *Entosphenus tridentatus.*

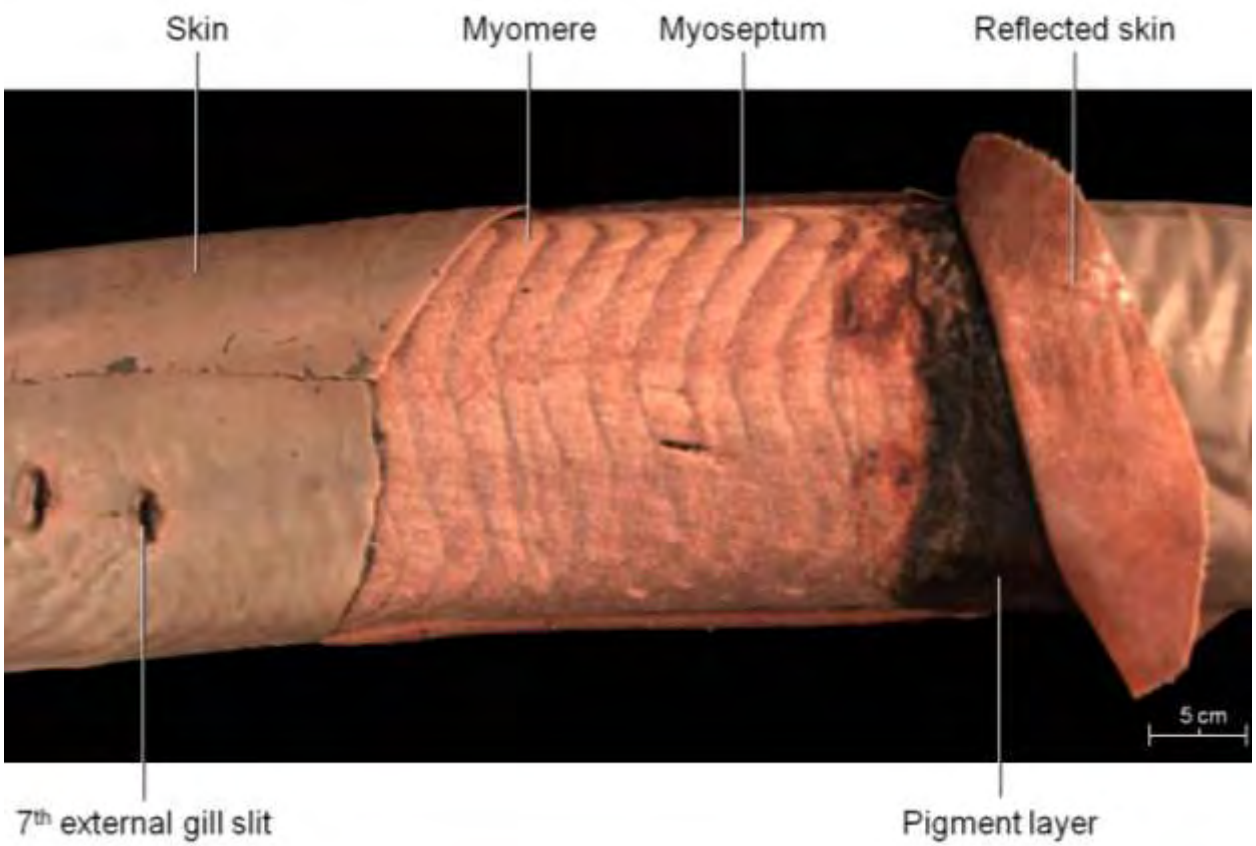

Figure 4. Lateral view of a Pacific Lamprey, *Entosphenus tridentatus*, to show the reflected skin and pigment layer as well as the surface configuration of the myomeres and myosepta. Cranial is to the left.

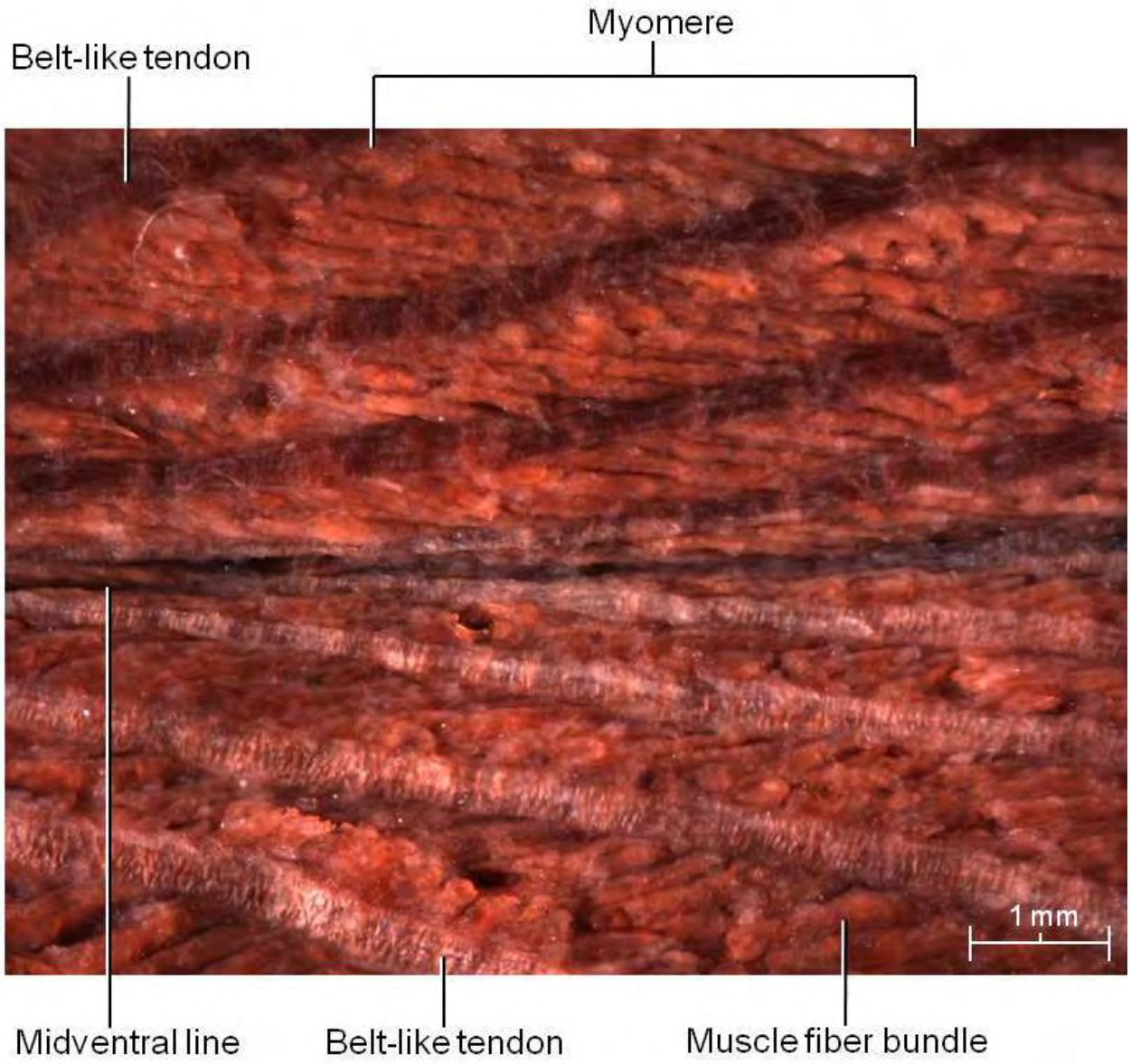

Figure 5. Magnified optical stereomicroscope image of the midventral region of an Atlantic Sea Lamprey, *Petromyzon marinus*, to show the convergence of the belt-like tendons at the mid-ventral line and the corresponding myomeres. The muscle fiber bundles on one side of the midventral line and the belt-like tendons on the contralateral side have the same orientation. Cranial is to the left.

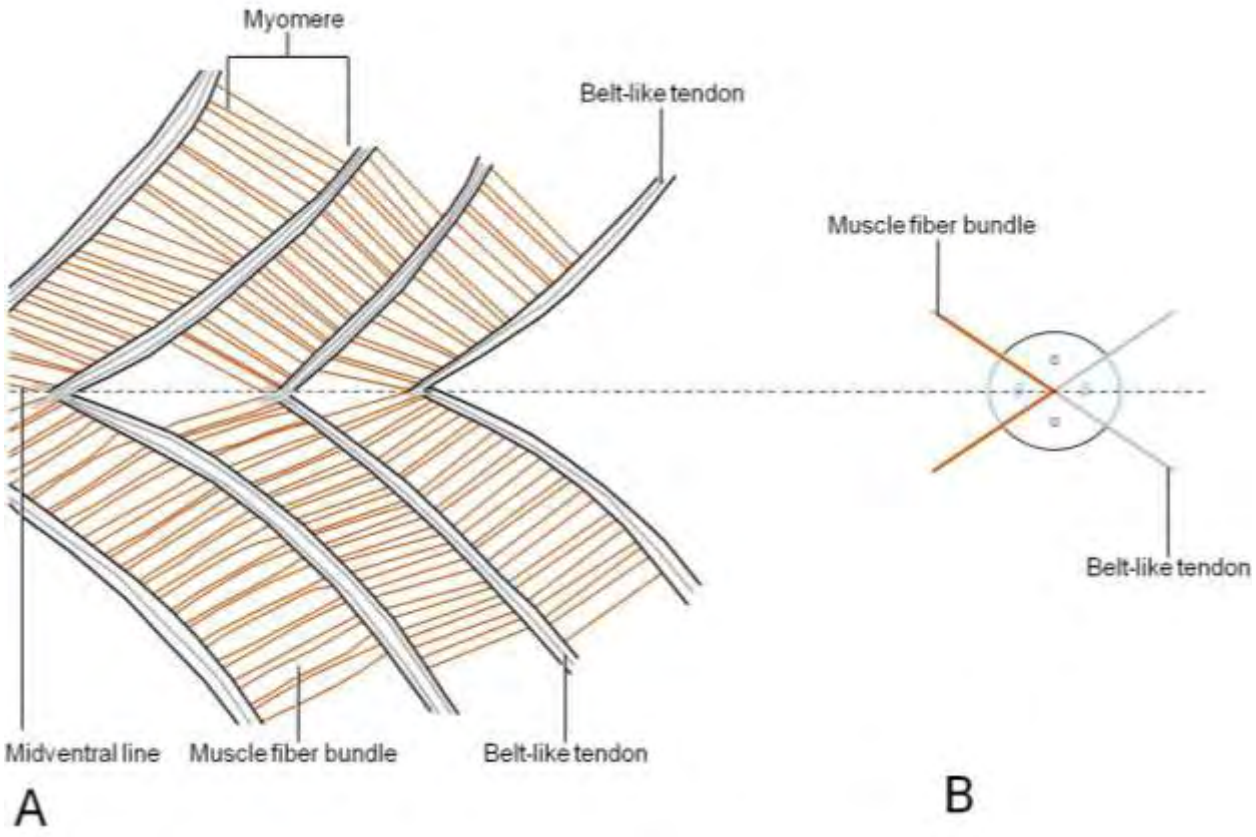

Figure 6. Diagrams depicting the convergence of the belt-like tendons at the midventral line and how the muscle fiber bundles on one side of the midventral line and the belt-like tendons on the contralateral side have the same orientation as observed in an Atlantic Sea Lamprey, *Petromyzon marinus*. (A) Semidiagram of the configuration. (B) Geometric diagram of the relationship between belt-like tendons and muscle fiber bundles of contralateral myomeres. Cranial is to the left.

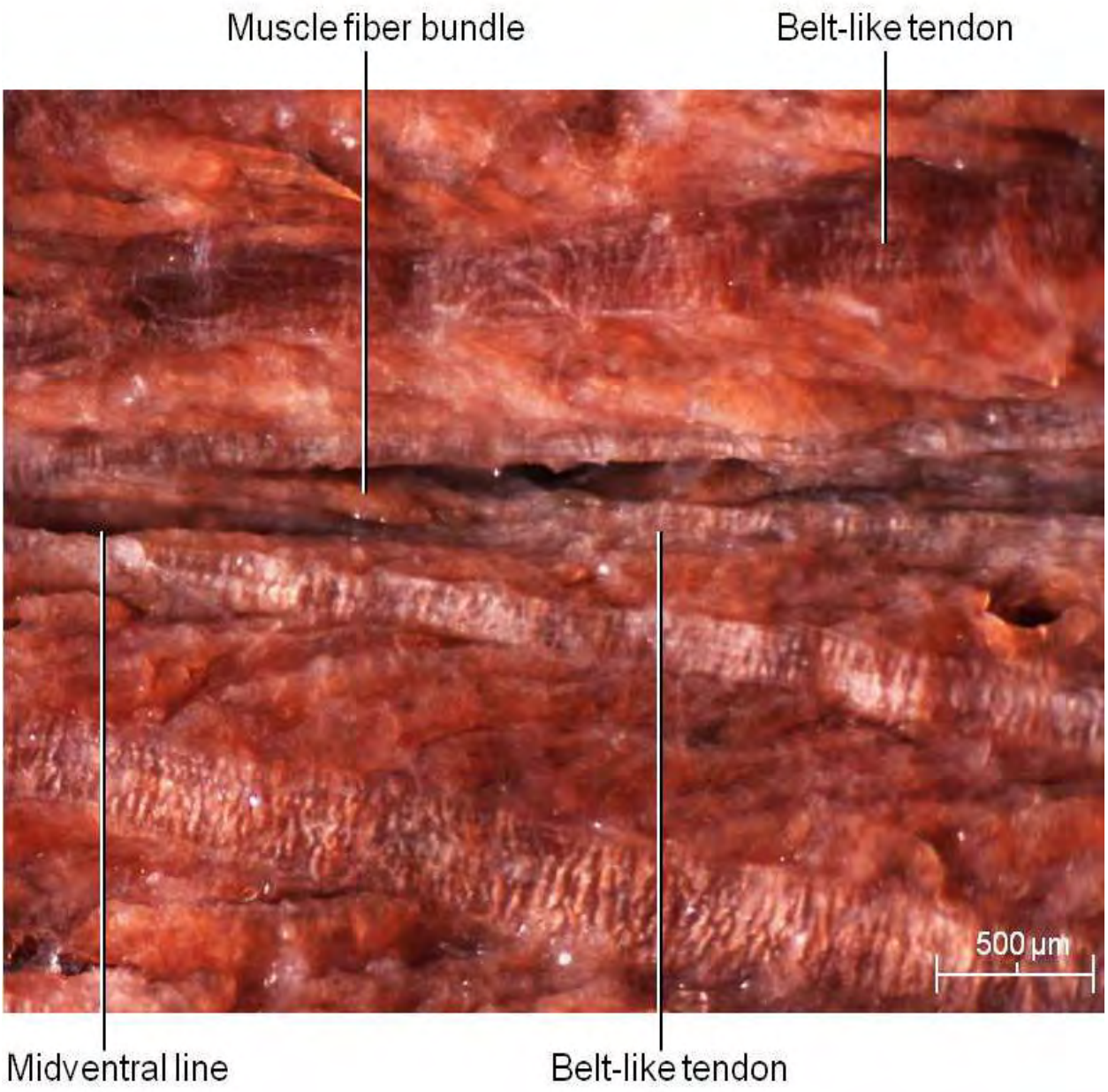

Figure 7. Magnified optical stereomicroscope image of midventral region of an Atlantic Sea Lamprey, *Petromyzon marinus*, to show the junction of a belt-like tendon to a muscle fiber bundle on the opposite side of the midventral line. Cranial is to the left.

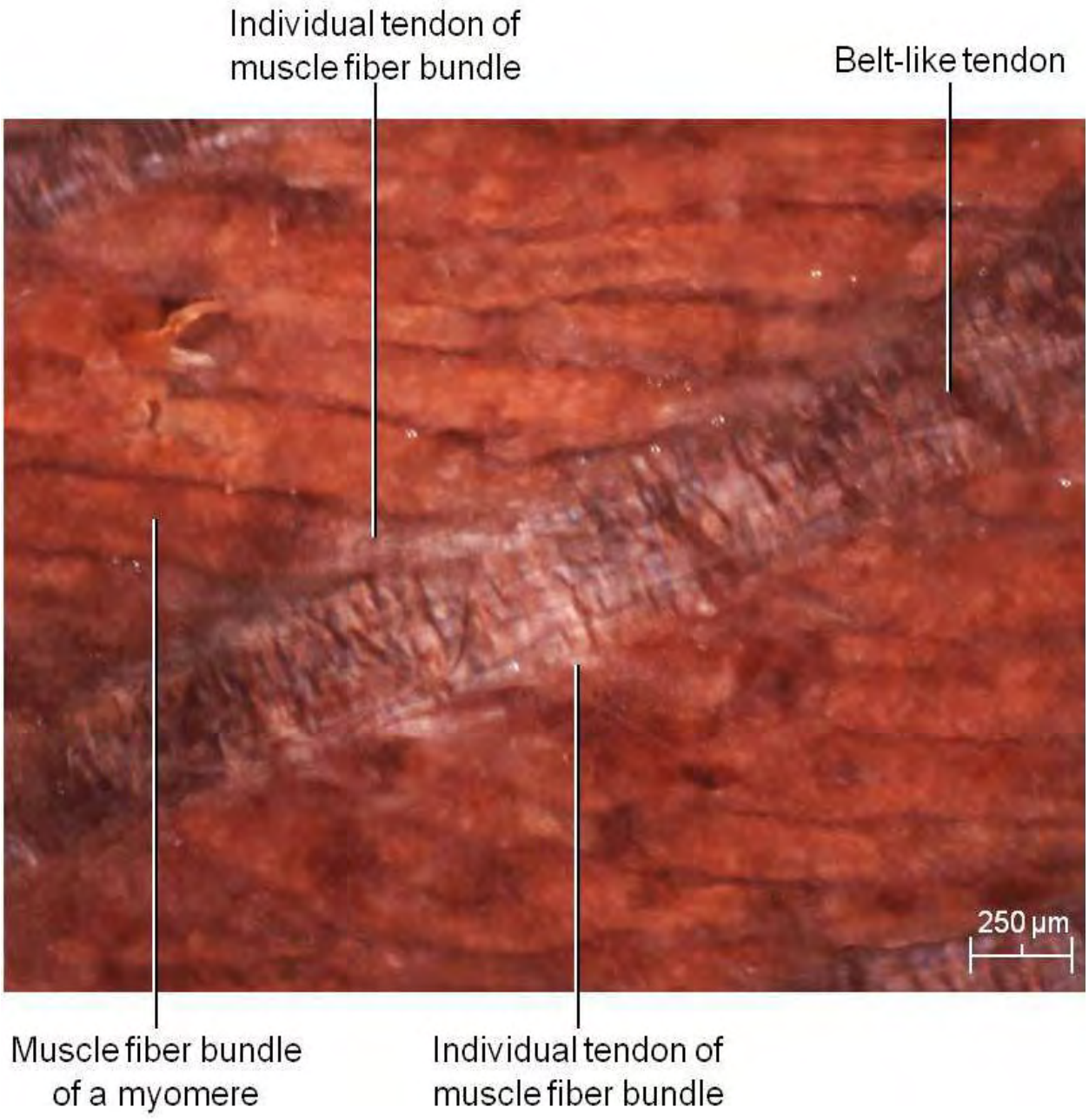

Figure 8. Magnified optical stereomicroscope image of a belt-like tendon and the junctions of individual tendons muscle fiber bundles of adjacent myomeres of an Atlantic Sea Lamprey, *Petromyzon marinus*. Cranial is to the left.

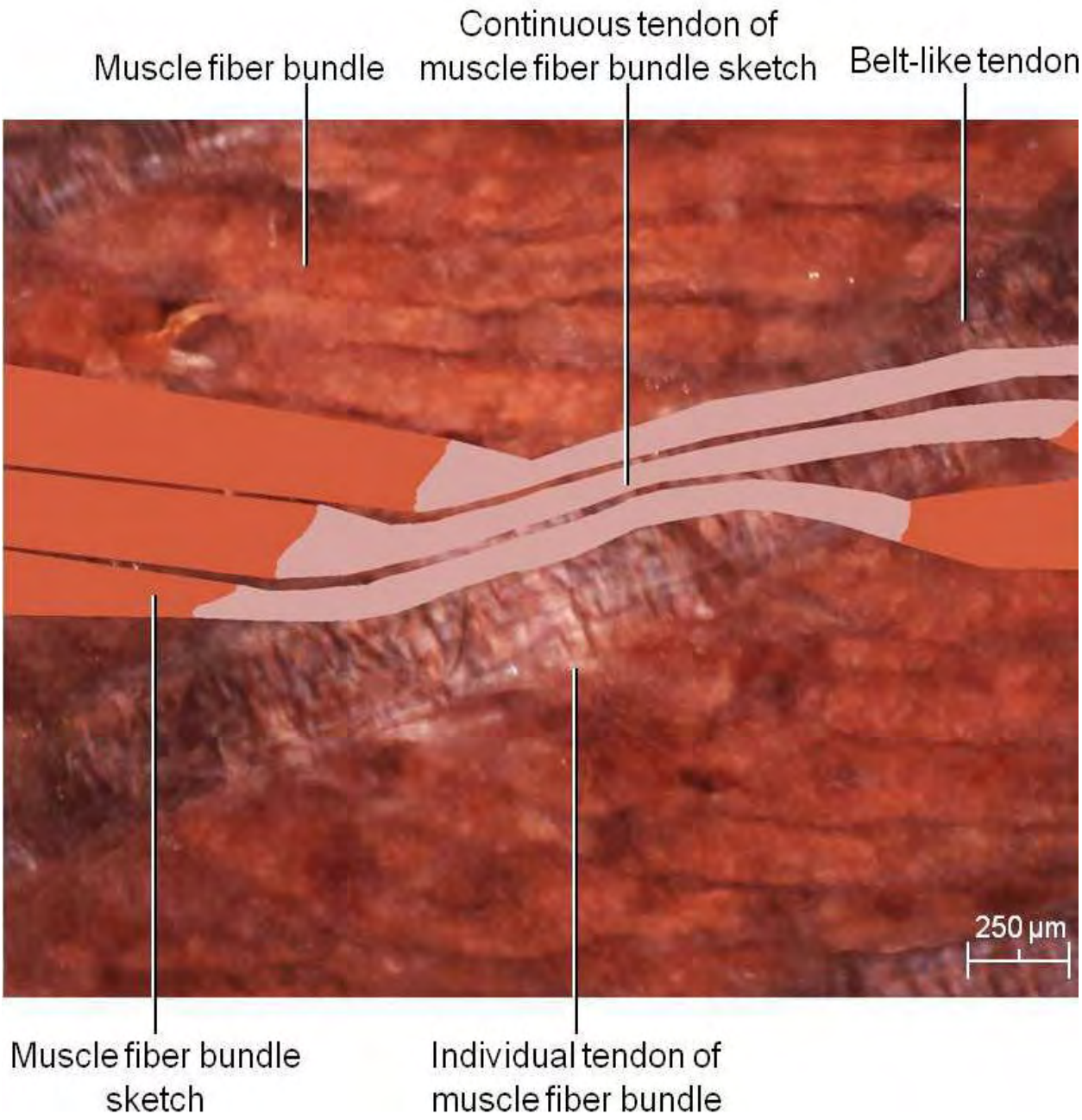

Figure 9. Semidiagram depicting the hypothesized connection between the muscle fiber bundles of adjacent myomeres *via* individual tendons becoming part of belt-like tendons for a certain extent as modeled in an Atlantic Sea Lamprey, *Petromyzon marinus*.

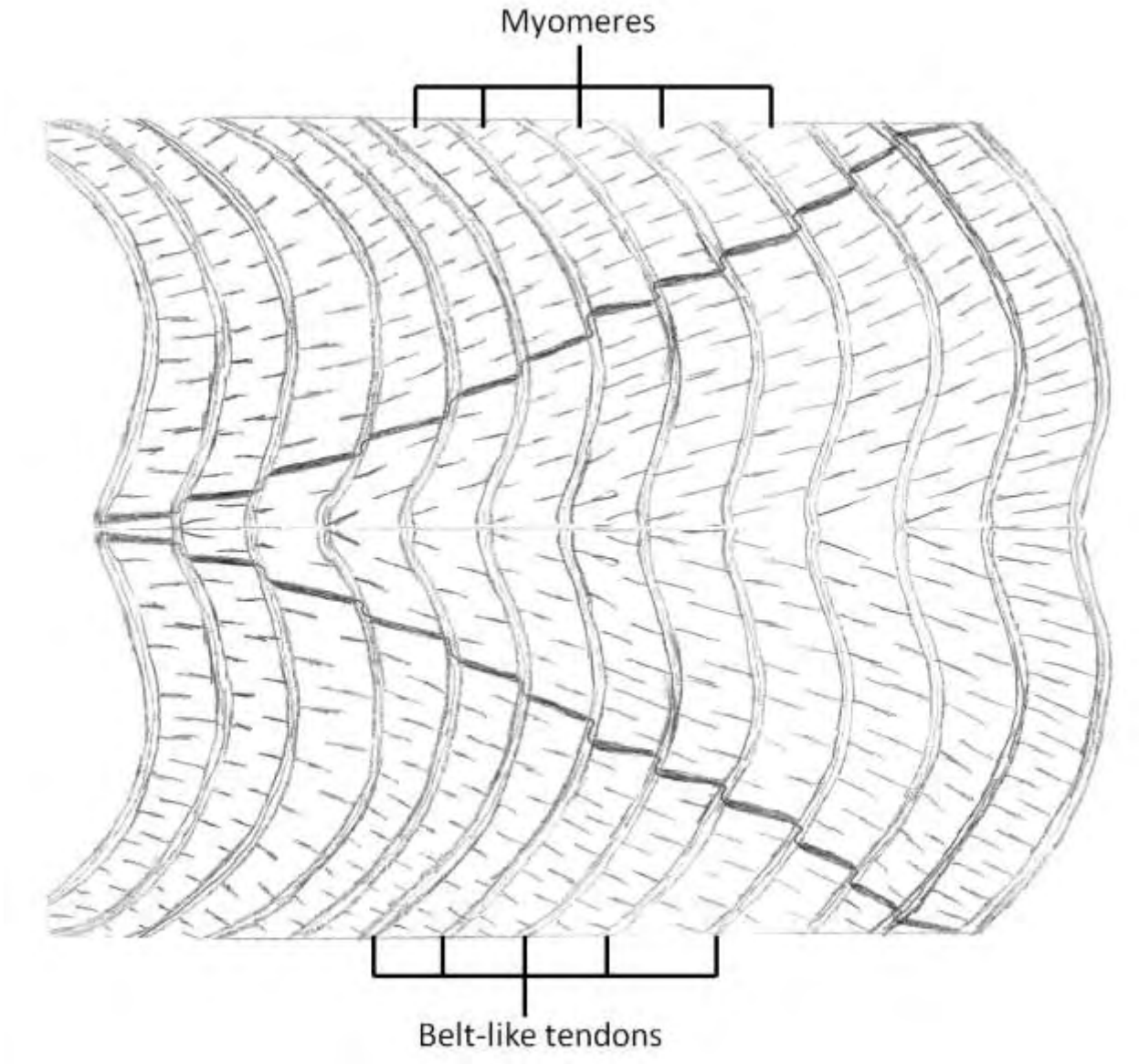

Figure 10. Diagram depicting hypothesized individual dorsal and ventral contractile units (highlighted in dark) converging at the midlateral line of an Atlantic Sea Lamprey, *Petromyzon marinus*. Cranial is to the left.

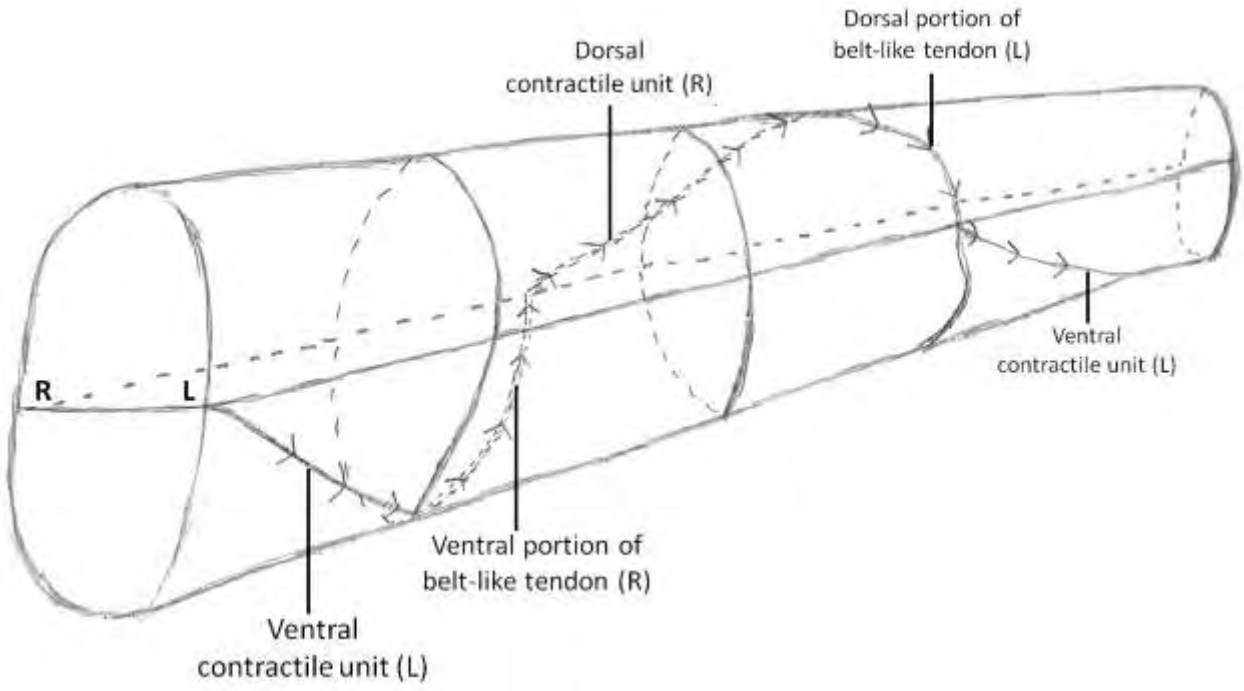

Figure 11. Diagram depicting the hypothesized helical configuration formed by contractile units and belt-like tendons. A single turn of the helix is pictured. The cranial end of the lamprey is to the left. Symbols: R = right side; L = left side.