Syst. Biol. 46(3):441–463, 1997

A MODEL SYSTEM OF STRUCTURAL DUPLICATION: HOMOLOGIES OF ADDUCTOR MANDIBULAE MUSCLES IN TETRAODONTIFORM FISHES

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Abstract.—We critically reviewed the homologies of the jaw muscles in tetraodontiform fishes (Triacanthoidea, Balistoidea, Tetraodontoidea), as first described in Winterbottom's phylogenetic monograph (1974, Smithson. Contrib. Zool. 155:1–201), as a case study in structural duplication. Within this order of teleost fishes, the two main adductor mandibulae muscles, A1 and A2, are duplicated one or more times in some subclades. The number of descendant A1 and A2 muscles ranges from as few as the original two muscles in triplespines to as many as eight muscles in some filefishes. As first pointed out by Winterbottom, the homologies of some muscles are unclear, particularly in comparisons between the superfamilies Balistoidea (boxfishes, triggerfishes, filefishes) and Tetraodontoidea (pursefishes, molas, puffers, porcupinefishes). We reassessed the homologies (orthologs and paralogs) of these A1 and A2 muscles based on their origins, insertions, and relative masses in representative taxa and their congruence with a phylogeny for these taxa. *New names that reflect the homologies of these muscles are presented. Ten muscle duplications* by subdivision and three phylogenetic losses of muscles have occurred in this system. No relationship was found between the number of separate muscles and the relative masses of the A1 or A2 muscles, suggesting that muscle duplication events essentially repackage existing muscle tissue. However, both A1 and A2 muscle masses are correlated with each other and with the feeding ecology of these fishes. Durophagous taxa have relatively larger A1 and A2 muscles, whereas planktivores and benthic grazers have relatively smaller A2 muscles. [Homology; morphological duplications; muscle evolution; orthology; paralogy; Tetraodontiformes.]

The concept of structural duplication has played a central role in the search for general patterns and repeating themes in the evolution of organismal design (Vermeij, 1973a, 1973b; Liem, 1980b; Lauder, 1981, 1990; Liem and Wake, 1985; Schaefer and Lauder, 1986; Emerson, 1988; Lauder and Liem, 1989). The central idea is that phylogenetic increases in the number of structural units with the same primitive function promote functional diversification by several routes. Repeated elements increase the overall complexity of design, which may underlie an increase in morphological and functional diversity within a clade (Vermeij, 1973a; Liem, 1980b; Schaefer and Lauder, 1986, 1996). The functional redundancy created by duplication of morphology or molecules may also release constraints on one of the primitive elements, permitting it to evolve a new form or function (Lauder, 1981). For example, redundancy is thought to be a primary mechanism in the evolution of gene function (Ohno, 1970; Ohta, 1989), the evolution of the protein structural diversity (Chothia, 1994), and the evolution of some developmental mechanisms in vertebrates (Holland et al., 1994; Holland and Garcia-Fernàndez, 1996).

Although numerous examples exist of repeated elements in animals and plants (e.g., appendages, blood vessels, body segments, hairs, leaves, muscles, scales), relatively few cases have been developed to the point that specific consequences of structural duplication can be tested within a rigorous phylogenetic framework (Lauder, 1990). Here, we describe a system of structural duplication in the jaw musculature of tetraodontiform fishes. Within this clade, the A1 and A2 adductor mandibulae muscles have been duplicated repeatedly in different subclades and extant taxa may possess from two to eight separate A1 and A2 muscles. Our primary purpose here is to review the homology and phylogenetic history of these tetraodontiform jaw muscles and present a nomenclature that reflects our hypotheses of homologies. We

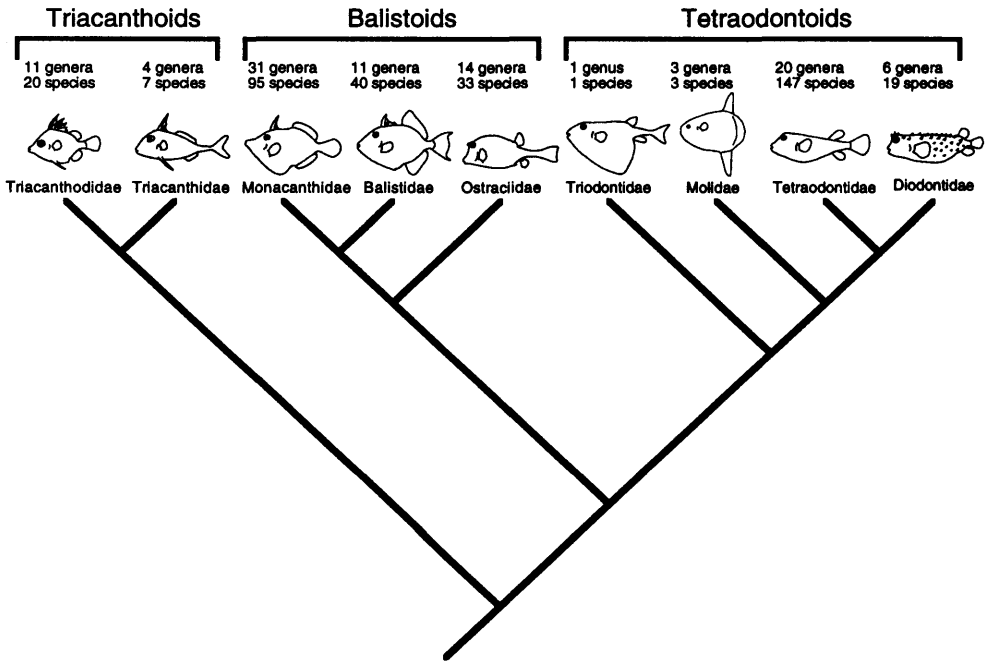

FIGURE 1. Phylogeny of extant tetraodontiform families based on the work of Winterbottom (1974b), Matsuura (1979), Tyler (1981), Lauder and Liem (1983), and Winterbottom and Tyler (1983). Images represent the general body form of fishes in these families. The number of genera and species are from Nelson (1994).

also present initial data on the functional consequences of muscle duplication through an analysis of muscle sizes.

Adductor Mandibulae Muscles of Tetraodontiform Fishes

The teleost order Tetraodontiformes is a diverse group of primarily marine fishes broadly distributed throughout the tropical and temperate regions of the world. This order is represented today by nine families containing approximately 101 genera with 365 species (Fig. 1; Nelson, 1994). The tetraodontiform phylogeny used in this study was proposed by Winterbottom (1974b), was corroborated by Lauder and Liem (1983), and is supported by additional data provided by Matsuura (1979), Tyler (1980), and Winterbottom and Tyler (1983). Tetraodontiforms are divided into three large clades. The relatively basal superfamily Triacanthoidea contains the Triacanthodidae (spikefishes) and Triacanthidae (triplespines). These triacanthoids are the sister taxon to the more familiar tetraodontiform fishes placed in the other two superfamilies. The superfamily Balistoidea contains the Ostraciidae (boxfishes, cowfishes), Balistidae (triggerfishes), and Monacanthidae (filefishes). Their sister group, the superfamily Tetraodontoidea, contains the Triodontidae (pursefishes), Molidae (ocean sunfishes), Tetraodontidae (puffers), and Diodontidae (porcupinefishes).

One of the most distinctive features of most tetraodontiforms are their robust teeth and powerful oral jaws. Unlike most other bony fishes, tetraodontiform fishes use their oral jaws not only to capture but also to process prey (Turingan and Wainwright, 1993; Wainwright and Turingan, 1993; Turingan, 1994). As in other fishes, the muscles responsible for closing and generating the biting forces of the oral jaws are those of the adductor mandibulae complex (Lauder, 1985). In most teleost fishes, this muscle complex consists of at least four muscles, A1, A2, A3, and Aw, which are distinguished based on their relative

origins and insertions on the jaws (Winterbottom, 1974a). All of these muscles originate on the palatal arch and are innervated by branches of the fifth cranial nerve. Winterbottom (1974a) hypothesized that all adductor mandibulae muscles of teleost fishes plesiomorphically inserted solely on the lower jaw and A1 has arisen phylogenetically by encroachment of muscle fibers upon the maxilla-mandibular ligament, which connects the upper and lower jaws. Thus, all adductor mandibulae muscles except A1 retain this plesiomorphic insertion on the lower jaw, and A1 may retain a close connection with A2 in basal tetraodontiforms such as triacanthoids. In most teleost fishes including all tetraodontiforms, A1 and A2 are the most easily observed jaw muscles given their large size and relatively superficial positions. A3 and Aw lie deep to both A1 and A2 and always insert on the lower jaw.

Within tetraodontiforms, the number of A1 and A2 muscles has increased in some clades, whereas the A3 and Aw muscles when present are always singular (Winterbottom, 1974b). The number of A1 muscles ranges from a single undivided A1 in all triacanthoids and triodontids to as many as six separate A1 muscles in some monacanthids. Similarly, the number of A2 muscles varies from a single A2 in triacanthids to as many as three separate A2 muscles in balistids and tetraodontids. This duplication of adductor mandibulae muscles, along with variation in their origins and insertions, has made identifying homologous muscles between families and particularly superfamilies difficult. Winterbottom's (1974b) classic monograph on the myology of tetraodontiform fishes documents the diversity of adductor mandibulae muscles in this clade and provided the first nomenclature for these muscles. During initial research on a representative balistoid (the gray triggerfish, *Balistes capriscus*) and a tetraodontoid (the southern puffer, *Sphoeroides nephalus*), we identified muscles following Winterbottom (1974b). However, like Winterbottom, we had difficulty identifying some muscles in *Sphoeroides* and in comprehending the transformations necessary to explain apparent morphological differences between "homologous" muscles in both taxa. Thus, we began to question these homologies, particularly in comparisons between balistoids and tetraodontoids.

In addition to reviewing the morphology described by Winterbottom (1974b), we examined additional taxa and here provide data on the relative masses of the A1 and A2 muscles in representatives of six of the nine tetraodontiform families. Mass is generally reflective of the force-producing capacity of skeletal muscles (Powell et al., 1984), and we analyzed the general patterns of adductor mass in light of this functional interpretation. We asked two specific questions. First, as the adductor muscles are duplicated, does the overall mass of the adductor muscle complex increase or are new muscles essentially carved out of existing tissue? Second, are patterns of diversity in adductor muscle size reflective of differences among species in feeding habits?

Homologies of Duplicated Muscles

Our review and new hypotheses of muscle homologies are based on the assumption that all the duplicated A1 and A2 muscles in this clade of fishes have been produced by physical subdivision of preexisting muscles. Specifically, all A1 and A2 muscles have arisen in a phylogenetic sense by repeated subdivision of singular A1 and A2 muscles present in the common ancestor of all tetraodontiforms and in an ontogenetic sense by repeated subdivision of singular A1 and A2 muscles present during the development of an individual fish. We favor this model of muscle evolution over a de novo model in which a new jaw muscle could arise from a novel condensation of myogenic tissue. Although we do not regard the de novo origin of muscles as an impossibility, a subdivision model is consistent with the repeated observations of incompletely divided muscles in some taxa (e.g., A1 muscles of ostraciids and A2 muscles of monacanthids). Such intermediate morphologies would not be produced by a de novo model but might

be expected with a subdivision model. Although no ontogenetic data on tetraodontiforms is available, study of the adductor mandibulae complex in other teleost fishes does support a subdivision model (Adriaens and Verraes, 1996).

Another important aspect of the subdivision model is that it produces a hierarchical pattern of ancestral and duplicated descendant muscles. Such a phylogenetic framework for muscles, like a phylogeny for taxa, allows for explicit predictions about the descendant muscles based on pleisiomorphic features (i.e., functions, motor patterns, physiology, etc.) inherited from the ancestral muscle. Muscles that appear de novo, by definition, have no clear homology to any preexisting muscle and thus no predictable pleisiomorphic features when they first appear.

We concur with Lauder (1990) that duplication of morphological structures such as jaw muscles of tetraodontiforms is analogous to the more familiar phenomenon of gene duplication. Granted, this analogy is not exact because duplication of genes is explained only by phylogeny and duplication of morphological structures is explained by both phylogeny and ontogeny (Patterson, 1987). Nevertheless, duplication of structures (i.e., genes or morphology) should require the recognition of both paralogs and orthologs if possible (Fitch, 1970; Patterson, 1987, 1988). Simply put, paralogs are different copies of a structure, derived from a single ancestral structure, that are present in the same or different individuals (e.g., α and β hemoglobin genes, A1α and A1β muscles; Fig. 2a), whereas orthologs are the same particular copy in different individuals (e.g., α hemoglobin genes, A1α muscles; Fig. 2a). Properly identifying orthologs in different taxa is critically important in any comparative or phylogenetic study involving duplicated structures (Patterson, 1987; Doyle, 1992; Hillis, 1994).

In practice, the identification of paralogs and orthologs of morphological structures is often not possible. Problems in homologizing duplicated structures both between individuals (i.e., orthology) and within individuals (i.e., paralogy) have been pointed out by Bateson (1892, 1894), Danforth (1930), Van Valen (1982), Roth (1984), Wagner (1989a, 1989b), Mabee (1993), and others. For example, it may be difficult if not impossible to identify orthologs when one particular copy of a duplicated structure is not morphologically distinct relative to

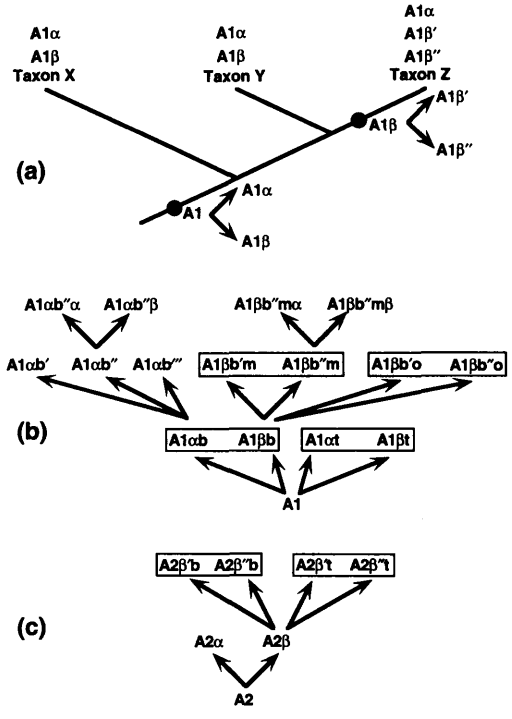

FIGURE 2. Muscle duplication by subdivision. (a) Muscle duplication events mapped onto a phylogeny of three hypothetical taxa. An ancestral muscle, A1, is subdivided into two descendant muscles, A1α and A1β, in the common ancestor of taxa X, Y, and Z. A1β is later subdivided into two additional descendant muscles, A1β′ and A1β″, in the ancestor of taxon Z. Different copies of a duplicated muscle in the same individual or in different taxa are paralogous muscles (e.g., A1α and A1β in taxa X and Y). The same copy of a duplicated muscle in different taxa are orthologous muscles (e.g., A1α in taxa X, Y, and Z; A1β in taxa X and Y). (b). Subdivision pattern of A1 muscles in tetraodontiform fishes. Muscle names reflect the historical relationships between duplicated muscles. Boxes at the same level in the figure and suffixes b (balistoids or balistids), m (monacanthids), o (ostraciids), and t (tetraodontoids or tetraodontids) denote muscles produced by parallel subdivision events in different clades. (c) Subdivision pattern of A2 muscles in tetraodontiform fishes.

others in different individuals (e.g., a scale on the body of a fish vs. another scale on a different fish) or when the total number of duplicated structures and thus the relative position or identity of any one copy differs between individuals (e.g., vertebra 15 in a fish with 50 total vertebrae vs. vertebra 15 in another fish with 55 total vertebrae). Furthermore, in most cases of serial homology (e.g., body segments, vertebrae, fin rays, teeth), homonomy (e.g., hairs, scales, blood cells), and redundant functional linkages (e.g., jaw opening ligaments in teleost fishes; Lauder and Liem, 1989), the duplicated structures cannot be homologized with a single structure in a hypothetical ancestor or outgroup taxon (McKitrick, 1994).

In contrast, the duplicated jaw muscles described in this study can be homologized with single ancestral muscles just like duplicated genes can be homologized with single ancestral genes. The general concepts of homology between muscles within an individual and the evolution of muscles via subdivision have previously been suggested by McKitrick (1994). Here, we build upon these concepts and present the first attempt to explicitly distinguish orthologs and paralogs for duplicated morphological structures.

Materials and Methods

This review is based in part on morphology described by Winterbottom (1974b). In addition, we examined the jaw musculature of 24 species from seven of the nine families. Taxa examined, taxon abbreviations used in figures, number of individuals examined, and standard length are as follows: one triacanthodid, *Parahollardia lineata* (1, 120 mm); two triacanthids, *Triacanthus biaculeatus* (TB, 2, 174–225 mm) and *Trixiphichthys weberi* (TW, 2, 124–143 mm); two ostraciids, *Acanthostracion quadricornis* (AQ, 2, 51–141 mm) and *A. polygonius* (AP, 1, 128 mm); three balistids, *Balistes capriscus* (BC, 2, 124–256 mm), *B. vetula* (BV, 2, 155–187 mm), and *Xanthichthys ringens* (XR, 2, 147–168 mm); four monacanthids, *Aluterus schoepfi* (AS, 2, 127–169 mm), *Cantherhines pullus* (CPU, 1, 143 mm), *C. macrocerus* (CM, 1, 325 mm), and *Monacanthus hispidus* (MH, 2, 93–215 mm); eight tetraodontids, *Arothron manilensis* (AM, 1, 185 mm), *Canthigaster rostrata* (CR, 1, 61 mm), *Chelonodon patoca* (CPA, 1, 115 mm), *Torquigener hicksi* (TH, 1, 83 mm), *Lagocephalus lagocephalus* (LL, 1, 506 mm), *Sphoeroides maculatus* (SM, 2, 130–172 mm), *S. nephalus* (SN, 3, 205–235 mm), and *S. testudineus* (ST, 1, 164 mm); and four diodontids, *Chilomycterus antennatus* (CA, 1, 130 mm), *C. schoepfi* (CS, 1, 96 mm), *Diodon holocanthus* (DHO, 1, 107 mm), and *D. hystrix* (DHY, 1, 235 mm). Fishes were initially fixed in 10% formalin and then transferred to 70% ethanol prior to dissection. All specimens except *Parahollardia lineata* (FMNH 46686) are from the laboratory collection of P. C. Wainwright at Florida State University. One or two individuals of each species were dissected along with an additional size series of 10 striped burrfish, *Chilomycterus schoepfi* (41–166 mm). These additional burrfish were included to obtain preliminary data on the allometry of the A1 and A2 muscles in tetraodontiform fishes. Fishes were weighed to the nearest gram prior to dissection, and all A1 and A2 muscles were carefully dissected and removed from one side of the head from all specimens except *Parahollardia lineata*. Individual muscles were gently patted dry to remove excess fluid and weighed to the nearest 0.01 g. Most of the anatomical illustrations that supplement our descriptions were produced from camera lucida drawings of representative specimens dissected in this study.

All mass data were log transformed before statistical analyses were performed. The effect of body size was removed by regressing muscle mass on body mass and calculating residuals. Any apparent correlations between the raw data points or residuals could be due to the confounding effects of phylogeny because species are not statistically independent data points (Felsenstein, 1985; Garland et al., 1992). To determine whether such correlated character evolution was real, we also calculated phylogenetically independent contrasts with the computer program CAIC (Purvis

and Rambaut, 1995). Using an incompletely resolved phylogeny and an assumption of equal branch lengths, 19 standardized contrasts were generated. All regressions involving contrasts were forced through the origin as required (Garland et al., 1992).

To reflect our hypotheses of muscle homologies, we produced a new nomenclature for the A1 and A2 muscles of tetraodontiform fishes. We tried to maintain current names when possible. Orthologous muscles have the same unique name in all taxa possessing them, and cases of parallel muscle evolution are always clearly identified (Figs. 2b, 2c). To preserve the historical relationships among adductor mandibulae muscles, each subdivision event requires new names for all descendant muscles. There has been a tendency to retain the ancestral muscle name for the one descendant muscle that diverges least from the ancestral muscle morphology. Although this practice reduces the number of new names needed, it obscures the homologies between the descendant muscles and their ancestral muscle.

In our new nomenclature, muscle names begin with either A1 or A2 followed by suffixes to reflect the transformational histories of these muscles. The descendant muscles of a primary subdivision event are designated by α and β, those of a subsequent secondary subdivision event by single, double, or triple primes if necessary (as in monacanthids), and those of a subsequent tertiary subdivision event by α and β again. In addition, the suffixes b, m, o, and t, for balistoids or balistids, monacanthids, ostraciids, and tetraodontoids or tetraodontids, respectively, are used to distinguish descendant muscles produced by parallel (i.e., nonhomologous) subdivision events in different clades. This nomenclature and hierarchy of muscles is summarized in Figures 2b and 2c. The correspondence between old and new muscle names is summarized in the Appendix. Abbreviations are used in figures for nonadductor mandibulae muscles and other elements: adductor arcus palatini (AAP), dentary (DEN), dilatator operculi (DO), erectores dorsalis (ERD), hypertrophied branchiostegal ray (HBR), levator arcus palatini (LAP), levator operculi (LO), maxilla (MX), palatine (PAL), premaxilla (PMX), protractor hyoidei (PHY), ramus mandibularis (RMD), and retractor arcus palatini (RAP). All osteological terms follow Tyler (1980).

Because the ultimate test of any hypothesis of homology is that of congruence with the homologies of other characters (Patterson, 1982, 1988), we used a variation of this test to argue for our new set of homologies. We compared transformation series for the A1 and A2 muscles, one based on current homologies and the other based on our new homologies, for the same phylogeny of tetraodontiform families (Fig. 1). Although this phylogeny was originally based in part on Winterbottom's homologies of A1 and A2 muscles, it is supported by other characters presented by Winterbottom (1974b), Matsuura (1979), Tyler (1980), and Winterbottom and Tyler (1983). The same phylogeny was produced by parsimony analysis of all available data minus any A1 and A2 characters, so there was no circularity in optimizing these muscle homologies on this phylogeny.

Transformation series were optimized using assumptions of both accelerated (ACCTRAN) and delayed (DELTRAN) transformation in MacClade (Maddison and Maddison, 1992). These options do not change the number of character steps but can reveal equally parsimonious transformation series. The ACCTRAN option maximizes reversals by favoring early gains of muscles near the root of the phylogeny and later losses of muscles. In contrast, the DELTRAN option maximizes parallelisms by favoring independent gains of muscles near the tips of the phylogeny. This procedure allowed us to determine the minimum number of evolutionary steps necessary to explain the observed morphology in the terminal taxa given a particular set of muscle homologies and to compare competing hypotheses of homologies. The preferred set of homologies was the one that required the fewest steps and least amount of homoplasy.

RESULTS

This review is divided into four sections: homologies of A1 muscles within balistoids, homologies of A1 muscles between balistoids and tetraodontoids, homologies of A2 muscles between balistoids and tetraodontoids, and comparisons of the relative masses of A1 and A2 muscles in representative taxa. Because the first three sections deal with competing hypotheses of homologies, it is necessary to use the two different nomenclatures. By convention, in the morphological descriptions the more familiar names of Winterbottom (1974b) are listed first and are immediately followed by the new names in parentheses, e.g., A1α (=A1αb'). In all other sections and in all figures, muscle names follow the new nomenclature, which is summarized in Figures 2b and 2c and the Appendix.

Homologies of A1 Muscles in Balistoids

Most triacanthoids retain the plesiomorphic A1 condition for all tetraodontiforms. There is a single superficial A1, dorsal to A2, which originates beneath the eye and inserts on both the upper and lower jaws (Fig. 3a). The only exceptions to this pattern are the long-snouted triacanthodid genera *Macrorhamphosodes* and *Halimochirurgus*, in which A1 shares a tendon with A2 and inserts solely on the lower jaw (Winterbottom, 1974b). In all triacanthoids, A1 is separated anteriorly from A2 by the path of the ramus mandibularis of the trigeminal nerve (Fig. 3a). However in triacanthids, A1 is not completely separated from A2 posteriorly (Fig. 3a) as it is in triacanthodids.

In contrast to the condition in triacanthoids, A1 is duplicated one or more times in all balistoids and all tetraodontoids except triodontids. Some of these A1 muscles in both balistoids and tetraodontoids were considered orthologs by Winterbottom (1974b).

In ostraciids, A1 is superficially covered by A2 muscles and is incompletely subdivided into three sections: a large dorsal division, A1β" (=A1αb), and two smaller ventral divisions, A1β (=A1βb'o) and A1β' (=A1βb"o) (Fig. 3d). The separation between these two ventral muscles is slight in some taxa (including the *Acanthostracion* examined here) but is well developed in other ostraciids examined by Winterbottom (1974b). All three A1 sections insert via a common tendon on the distal portion of the maxilla.

In balistids, most of A1 is superficially covered by A2 muscles and A1 is subdivided once into a superficial muscle, A1α (=A1αb) and a deeper muscle, A1β (=A1βb) (Figs. 3e, 3f). A1α (=A1αb) originates in front of the eye along the ethmoid region and narrowly inserts on the distal end of the maxilla. A1β (=A1βb), in contrast, originates relatively ventrally, on the metapterygoid, and inserts narrowly on the distal end of the maxilla. Both A1 muscles insert on the upper jaw via a common tendon.

This balistid pattern is modified in monacanthids by further duplication of A1 muscles. Most monacanthids have at least two superficial and three deep A1 muscles (Figs. 3g, 3h). The superficial A1 muscles include A1α (=A1αb'), which inserts on the maxilla, and A1α' (=A1αb"), which inserts on both the maxilla and upper lip. The deep A1 muscles include A1β (=A1βb'm) and A1β' (=A1βb"m), which both insert on the maxilla, and A1γ (=A1αb"'), which uniquely inserts on the palatine. Variations of this monacanthid pattern occur in a few genera. A1α' (=A1αb") is absent in *Aluterus*, and A1β' (=A1βb"m) is absent in *Anacanthus*. A1α" (=A1αb'β) is uniquely present in *Oxymonacanthus* dorsal to A1α' (=A1αb'α) and inserts on the maxilla. A1β" (=A1βb"mβ) is uniquely present in *Paraluteres* dorsomedial to A1β' (=A1βb"mα) and inserts on the maxilla.

Based on the pattern of A1 muscles in balistoids, Winterbottom (1974b) hypothesized that A1 was subdivided into a superficial A1α (=A1αb) and a deep A1β (=A1βb) in the common ancestor of all balistoids. In addition, his recognition of A1β' in both ostraciids (=A1βb"o) and monacanthids (=A1βb"m) could be construed as indicating that these muscles are

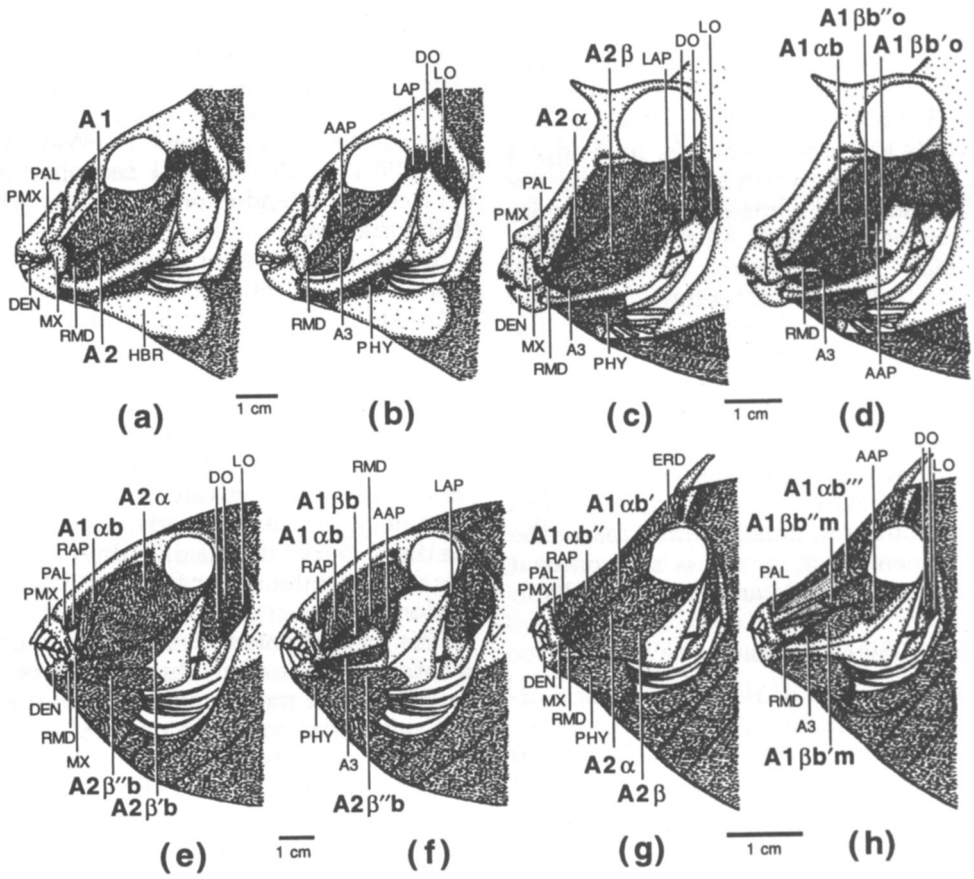

FIGURE 3. Jaw musculature of representative balistoids. See text for muscle and other element abbreviations. (a) *Triacanthus biaculeatus*, superficial view; A1 does not overlie A2, and both muscles are incompletely subdivided posteriorly. (b) *T. biaculeatus*, deep view of jaw musculature with A1 and A2 removed. (c) *Acanthostracion quadricornis*, superficial view; A2 is subdivided into A2α and A2β, and these two muscles lie superficial to the A1 muscles. (d) *A. quadricornis*, deep view with A2α and A2β removed; A1 is subdivided into A1αb, A1βb″o, and A1βb′o. (e) *Balistes capriscus*, superficial view; A2 is subdivided into A2α, A2β′b, and A2β″b, and these three muscles lie superficial to the A1 muscles. (f) *B. capriscus*, deep view with A2α and A2β′b removed; A1 is subdivided into A1αb and A1βb. (g) *Monacanthus hispidus*, superficial view; A2 is subdivided into A2α and A2β, and these two muscles lie superficial to all A1 muscles except A1αb′ and A1αb″. (h) *M. hispidus*, deep view with A1αb′, A1αb″, A2α, and A2β removed; note the three deep A1 muscles, A1αb‴, A1βb′m, and A1βb″m.

orthologs and that A1β′ was present in the common ancestor of all balistoids. This A1 pattern for the common ancestor of balistoids requires the following transformations to explain the morphology observed in extant taxa. Subsequently in the lineage leading to ostraciids, A1α (=A1αb) was lost and A1β′ (=A1βb″o) was subdivided to form A1β″ (=A1αb). Apparently, A1β′ was lost in the lineage leading to balistids. In the lineage leading to monacanthids and within this clade, A1α′ (=A1αb″) and A1α″ (=A1αb′β) have subdivided from A1α, A1β″ (=A1βb″mβ) has subdivided from A1β, and A1γ (=A1αb‴) has arisen with uncertain affinities to any other A1 muscles. This transformation series for A1 muscles in balistoids, using Winterbottom's homologies and nomenclature, is mapped on a phylogeny of tetraodontiforms in Figure 4a.

Our reexamination of adductor mandib-

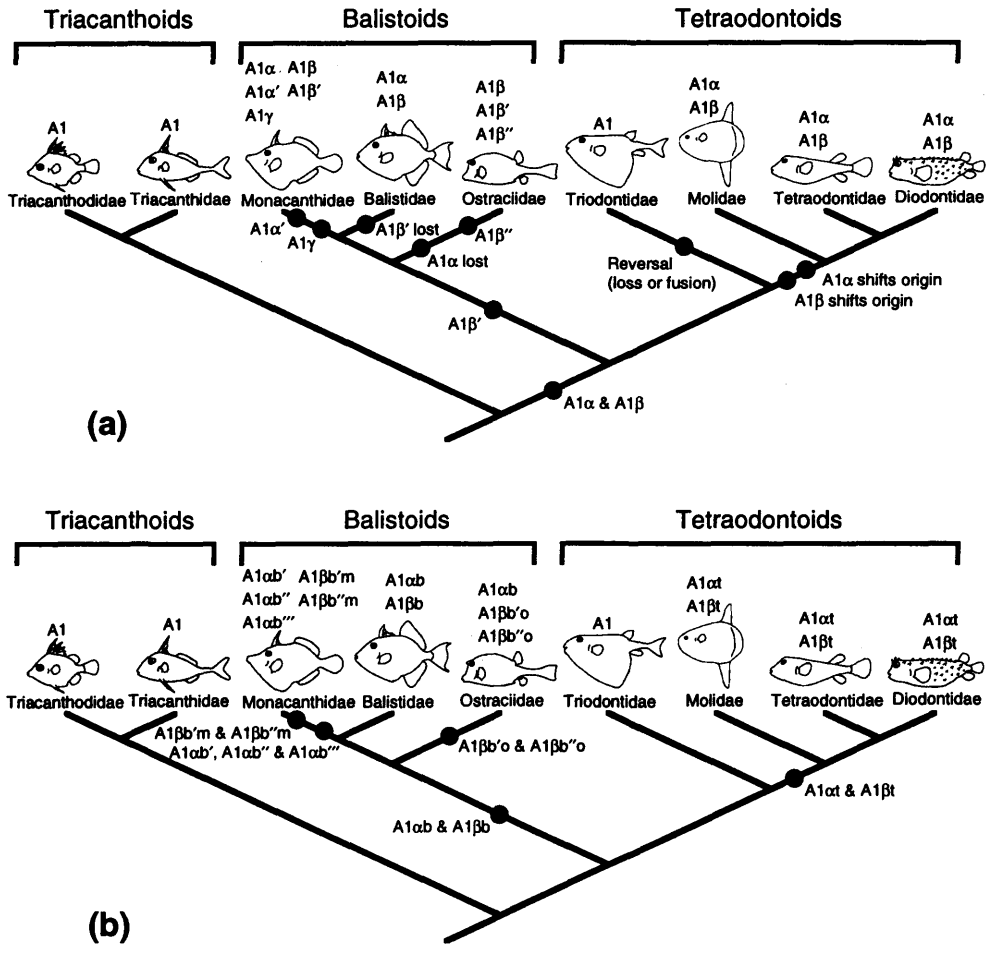

FIGURE 4. Competing hypotheses of the evolution of A1 muscles in tetraodontiform fishes. (a) Transformation series (ACCTRAN) based on muscle names and homologies proposed by Winterbottom (1974b). This series requires a minimum of 10 steps (five gains, two shifts in the relative position of muscles, three reversals by loss or fusion). (b) Alternative transformation series (ACCTRAN and DELTAN) based on new muscle names and homologies described here. This series requires a minimum of five steps (all muscle duplications by subdivision). Note the parallel evolution of A1α and A1β in balistoids and tetraodontoids and A1β′ and A1β″ in ostraciids and monacanthids.

ulae muscles suggests an alternative scenario of evolution of A1 muscles within balistoids. We hypothesize that the ancestral A1 condition for balistoids was not like that of balistids but was instead a partially subdivided A1 like that of ostraciids. We consider Winterbottom's (1974b) A1β″ of ostraciids and A1α of balistids and monacanthids to be orthologs, which we rename A1αb. This hypothesis of homology is suggested by the relative position and mass of this A1 muscle as compared with other A1 muscles. In all balistoids, A1αb is the dorsalmost A1 muscle and always lies anterodorsal to the path of the ramus mandibularis branch of the trigeminal nerve. In addition, new data on the relative masses of adductor mandibulae muscles reveal that A1αb is consistently the largest A1 muscle in all balistoids.

In our scenario of A1 evolution within balistoids, we hypothesize that A1αb completely separated from the ventral portion of A1, A1βb, and shifted more anterodor-

sally to a superficial position in the common ancestor of balistids and monacanthids. We do not consider Winterbottom's (1974b) A1β or A1β′ of ostraciids and monacanthids to be orthologs. Instead, we hypothesize these muscles have arisen independently through separate subdivision events in both clades and thus have renamed them A1β′o and A1β″o in ostraciids and A1β′m and A1β″m in monacanthids, respectively.

Several additional duplication events and losses of muscles have produced a complex pattern of A1 muscles in monacanthids. It is unclear whether A1αb′, A1αb″, and A1αb‴ have been produced by a single subdivision event or by two sequential subdivision events because all three muscles appear simultaneously on the phylogeny. Although other subdivision events in tetraodontiforms have produced only two descendant muscles, it is more parsimonious to assume that a single subdivision event of A1αb produced these three muscles. The absence of A1αb′ in *Aluterus* and of A1βb″m in *Anacanthus* is interpreted as reversal by loss or fusion. Both genera are relatively derived monacanthids and these muscles are present in their sister taxa (Matsuura, 1979). A1αb″α and A1αb″β in *Oxymonacanthus* and A1βb″mα and A1βb″mβ in *Paraluteres* have clearly arisen through two additional duplication events within these genera. This transformation series for A1 muscles for balistoids, using the new homologies and nomenclature, is mapped on a phylogeny of tetraodontiforms in Figure 4b.

Homologies of A1 Muscles between Balistoids and Tetraodontoids

In triodontids, the sister group to all other tetraodontoids, A1 is an undivided superficial muscle that originates beneath the eye and inserts broadly on the proximal portion of the maxilla (Fig. 5a). In contrast, A1 in molids, tetraodontids, and diodontids is subdivided into a superficial ventral muscle, A1α (=A1βt), and a superficial dorsal muscle, A1β (=A1αt) (Figs. 5b–e). These muscles are not covered by A2 muscles, and the relative dorsoventral positions of the A1 muscles is reversed as compared with balistoids. A1α (=A1βt) originates superficially over the A2 muscles and inserts broadly on the distal end of the maxilla. A1β (=A1αt) originates in front of the eye and inserts broadly on the proximal portion of the maxilla.

Winterbottom (1974b) did not explicitly state whether the undivided A1 of triodontids is a plesiomorphic condition as in triacanthoids or a derived condition (i.e., a reversal by either loss of one muscle or fusion of both). He did however explicitly homologize A1α (=A1αb) in balistoids (Figs. 3e, 3f) and A1α (=A1βt) in tetraodontoids (Figs. 3b, 3d) (Winterbottom, 1974b:74). This interpretation of A1 homologies implies that A1 was subdivided into A1α (=A1αb and A1βt) and A1β (=A1βb and A1αt) in the common ancestor of balistoids and tetraodontoids. However, Winterbottom (1974b:74) went on to state that A1β (=A1βb) of balistoids and A1β (=A1αt) of tetraodontoids were probably not homologous. These statements create a paradox because any subdivision event produces at least two pairs of orthologous muscles in descendant taxa. There are only two alternatives; either both A1α and A1β are orthologs in balistoids and tetraodontoids or neither of them are.

If one were to accept that A1α (=A1αb) in balistoids and A1α (=A1βt) in tetraodontoids are orthologs, then A1 must have been subdivided in the common ancestor of balistoids and tetraodontoids. This hypothesis then requires the loss of a muscle or fusion of both muscles to explain the undivided A1 in triodontids and apparent absence of A1α (=A1αb) in ostraciids. Such a scenario for A1 evolution, using Winterbottom's (1974b) names and homologies, is optimized (ACCTRAN) on a phylogeny of tetraodontiforms in Figure 4a. Based on this set of homologies, at least 10 steps are required (five gains of muscles, two shifts of muscle origins, and three reversals via loss or fusion of muscles) to explain the origin of the A1 muscles in the common ancestors of each tetraodontiform family. The only difference under DELTRAN is that A1β′ is gained independent-

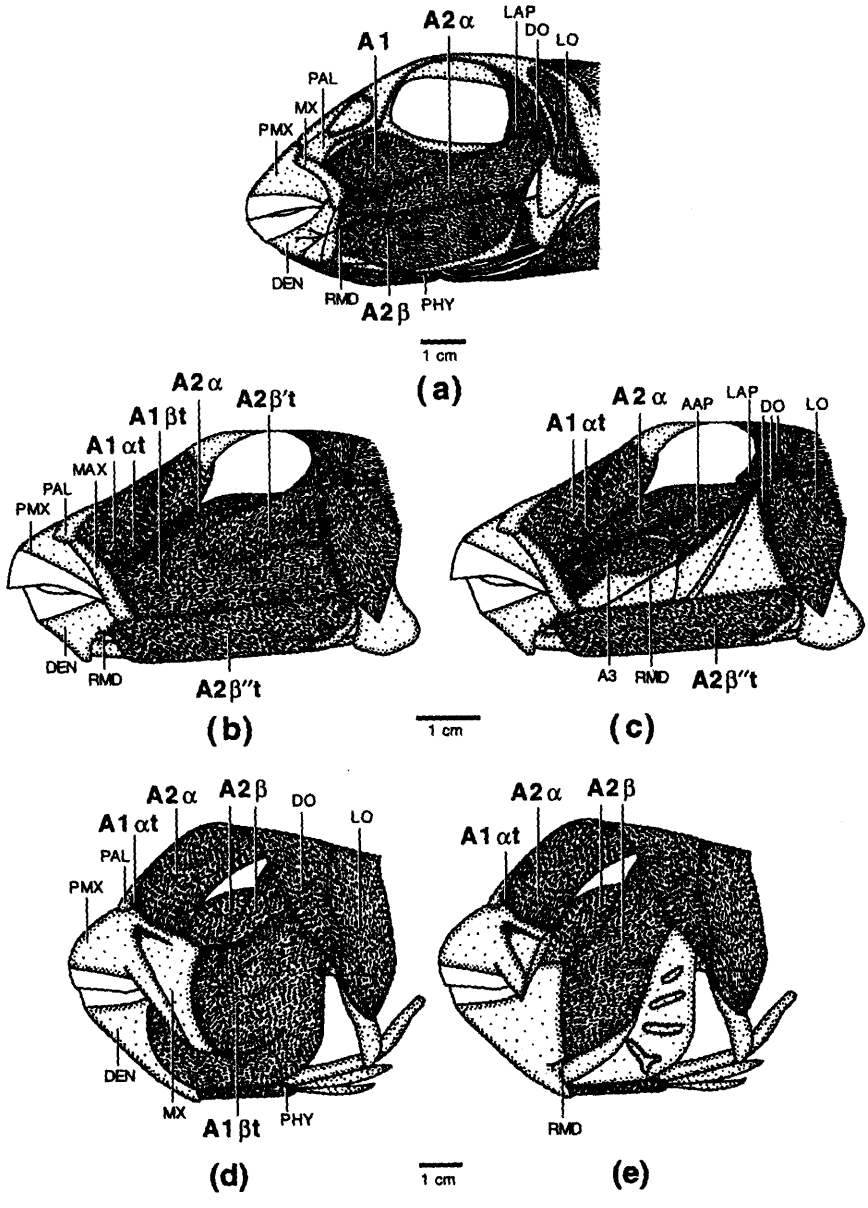

FIGURE 5. Jaw musculature of representative tetraodontoids. See text for muscle and other element abbreviations. (a) *Triodon macropterus*, superficial jaw musculature (adapted from original; Winterbottom, 1974b); A1 is undivided and A2 is subdivided into A2α and A2β. A2β passes medial to the path of the ramus mandibularis. (b) *Sphoeroides nephalus*, superficial view; A1 is subdivided into A1αt and A1βt, A1βt lies superficial to some A2 muscles, and A2 is subdivided into A2α, A2β't, and A2β"t. A2β"t passes lateral to the path of the ramus mandibularis. (c) *S. nephalus*, deep view with A1βt and A2β't removed; A2α inserts on the maxilla. (d) *Chilomycterus schoepfi*, superficial view; A1 is subdivided into a small A1αt and a large A1βt. A1βt lies superficial to some A2 muscles. (e) *C. schoepfi*, deep view with A1βt and part of the maxilla removed. The ramus mandibularis passes entirely within A2β from the neurocranium to the lower jaw.

ly in both monacanthids and ostraciids rather than being gained in the common ancestor of balistoids and later lost in balistids.

Alternatively, we hypothesize that the undivided A1 of triodontids is the plesiomorphic condition for tetraodontids because A1 was undivided in the common ancestor of both balistoids and tetraodontoids. Therefore no duplicated A1 muscles of tetraodontoids are orthologs of any duplicated A1 muscles of baslistoids. This hypothesis is completely consistent with our scenario for A1 evolution within balistids. Subsequently, A1 was subdivided in the common ancestor of molids, tetraodontids, and diodontids to form A1αt and A1βt. A1αt has remained in the relatively dorsal position of A1 in triodontids while A1βt has expanded ventrally over the A2 muscles. A transformation series for A1 evolution, using our nomenclature and homologies, is optimized on a phylogeny of tetraodontiforms in Figure 4b. Our scenario requires only five steps (all gains by muscle duplication by subdivision) to explain the origin of these A1 muscles in the common ancestors of each tetraodontiform family. This transformation series is the same under ACCTRAN or DELTRAN.

Homologies of A2 Muscles in Balistoids and Tetraodontoids

As in other teleost fishes, A2 is the main section of the adductor mandibulae musculature, which plesiomorphically inserts on the lower jaw. In tetraodontiforms, this muscle may be undivided or may consist of up to three separate muscles. To identify orthologous muscles in taxa with more than a single A2 muscle, Winterbottom (1974b) proposed using their positions relative to the path of the ramus mandibularis of the trigeminal nerve to the lower jaw.

In most triacanthodids, A2 is represented by two muscles, a posterodorsal A2α and an anteroventral A2β, which lies medial to the RMD (Winterbottom, 1974b). Gosline (1986) suggested that A2α in triacanthodids may in fact be a portion of A1. However, this is not the case in the triacanthid examined in this study (*Parahollardia*), and we followed Winterbottom's proposal. Exceptions to this triacanthodid pattern are the long-snouted genera, *Macrorhamphosodes* and *Halimochirurgus*, which have an undivided A2 (Winterbottom, 1974b). The path of the RMD in these two genera is unknown. As in these specialized triacanthodids, all triacanthids have an undivided A2 muscle, which lies medial to the path of the RMD (Fig. 3a).

In balistoids, A2 is always represented by at least two muscles. In ostraciids, both a dorsal A2α and a ventral A2β are present, and both muscles pass lateral to the path of the RMD (Fig. 3c). Plesiomorphically, these muscles are completely separated in ostraciids but are fused posteriorly in one ostraciid subclade, the tribe Aracanini (Winterbottom and Tyler, 1983). In balistids, there are three A2 muscles in a dorsoventral sequence, A2α, A2β (=A2β'b), and A2γ (=A2β"b), respectively (Fig. 3e). In most balistids, A2α passes lateral to the path of the RMD, whereas both A2β (=A2β'b) and A2γ (=A2β"b) are medial to the path of this nerve. Variation is found in the balistid *Balistipus undulatus*, where the RMD passes through A2α before passing lateral to A2β (=A2β'b) (Winterbottom, 1974b). In monacanthids, only two A2 muscles, A2α and A2β, are present (Fig. 3g). In some genera (e.g., *Chaetoderma*, *Monacanthus*, *Paramonacanthus*, *Paraluteres*, *Pervagor*, *Stephanolepis*), the ventral portion of A2β may be slightly differentiated (Fig. 3g) but is never completely separated as is A2γ (=A2β"b) in balistids (Figs. 3e, 3f). In all monacanthids, A2α passes lateral and A2β passes medial to the path of the RMD (Fig. 3g).

Like balistoids, all tetraodontoids also have at least two A2 muscles. In triodontids, A2 is represented by a dorsal A2α and a ventral A2β, and the RMD passes medial to A2α and lateral to A2β much like it does in most balistids and all monacanthids (Winterbottom, 1974b) (Fig. 5a). This triodontid A2 pattern differs however from that of all other tetraodontids. In molids, tetraodontids, and diodontids, A2α (=A2β or A2β't and A2β"t) and A2β (=A2α) are present but the relative origins

of these muscles have apparently shifted if A2β is identified as the A2 muscle medial to the path of the RMD (Figs. 5b–e). In these three families, A2α (=A2β or A2β't and A2β"t) now lies ventral to A2β (=A2α) and is in the same relative position as A2β in triodontids.

In tetraodontids, A2α (=A2β) has two distinct heads (=A2β't and A2β"t) (Winterbottom, 1974b). The ventral head (=A2β"t) is present in all tetraodontid genera examined here, but its separation from the dorsal head (=A2β't) may be partially obscured in superficial view by A1α (=A1βt). Furthermore, the insertion of A2β (=A2α) has shifted from the lower jaw to the maxilla in the three species of *Sphoeroides* examined.

The path of the RMD varies in tetraodontids and diodontids and differs from the clear pattern in balistids, monacanthids, and triodontids. The RMD may start medial to A2α (=A2β't and A2β"t) but passes through the anteroventral portion (A2β"t) just before it reaches the lower jaw (e.g., *Arothron, Canthigaster, Chelonodon, Sphoeroides*) or it may travel entirely within A2α (=A2β) before reaching the lower jaw (e.g., *Chilomycterus, Diodon*).

Based on the pattern of A2 muscles, Winterbottom (1974b) hypothesized that A2 was subdivided in the common ancestor of all tetraodontiforms and the undivided A2 of some triacanthoids had arisen through two separate character reversals by loss or fusion, once in triacanthids and once in the common ancestor of *Macrorhamphosodes* and *Halimochirurgus*. Winterbottom used the path of the RMD to distinguish A2α and A2β in most balistoids and all tetraodontoids. This hypothesis requires at least one shift of the RMD in ostraciids and a relative shift of the origins of both A2 muscles in the ancestor of molids, triodontids, and diodontids to maintain these homologies. A separate A2γ (=A2β"b) has arisen in the lineage leading to balistids by subdivision of A2β. Winterbottom however did not provide a separate name for an analogous subdivision of A2β, which occurs in tetraodontids. A transformation series for A2 muscles, using Winterbottom's names and homologies, is optimized (ACCTRAN) on a phylogeny of tetraodontiforms in Figure 6a. This scenario requires a minimum of six steps (two gains, one shift in the path of the RMD, two shifts of muscle origins, and one reversal by loss or fusion) to explain the origin of A2 muscles in the common ancestors of each tetraodontiform family. The only difference under DELTRAN is that subdivision of A2 into A2α and A2β has occurred independently in triacanthids and in the common ancestor of balistoids and tetraodontoids.

We also consider A2 to have been subdivided in the common ancestor of all tetraodontiforms and the undivided A2 in some triacanthodids and all triacanthids to be independent reversals. However, this hypothesis is tentative because under DELTRAN it is equally parsimonious to assume that the undivided A2 of triacanthids is plesiomorphic and the divided A2 of most triacanthodids is independently derived from that of balistoids and tetraodontoids. In either case, the A2 homologies we propose for balistoids and tetraodontoids are unaffected.

Our scenario of A2 evolution differs from Winterbottom's (1974b) mainly because we hypothesize that A2α and A2β have retained their relative positions in all tetraodontiforms while the path of the RMD has shifted at least twice. Based on our observations, variation in the path of the RMD between species is greater than originally suggested by Winterbottom. Thus, we consider the path of the RMD an unreliable landmark for identifying descendant A2 muscles. Use of this criterion leads to the misidentification of the orthologous A2 muscles in molids, tetraodontids, and diodontids.

A transformation series for the evolution of A2 muscles based on our names and homologies is optimized (ACCTRAN) on a phylogeny of tetraodontiforms in Figure 6b. As with Winterbottom's (1974b) homologies, the only difference under DELTRAN is that the subdivision of A2 into A2α and A2β has occurred independently in triacanthids and the common ancestor

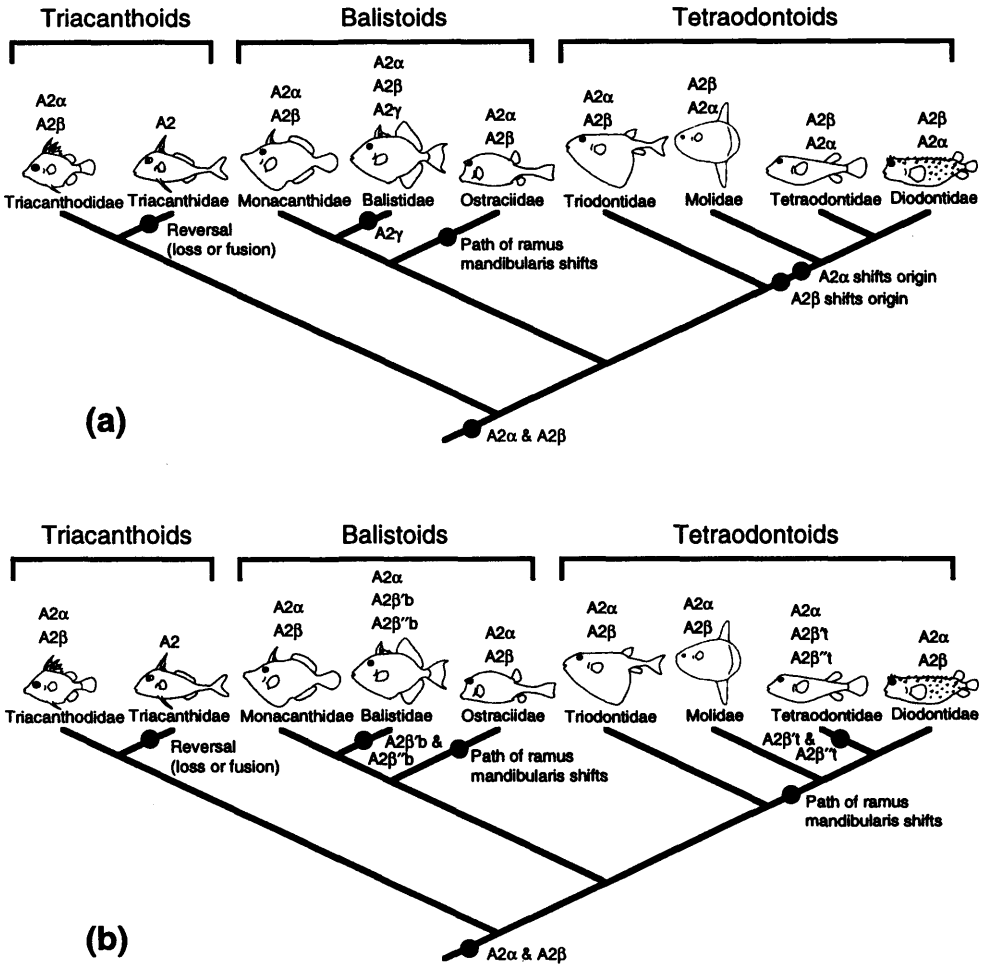

FIGURE 6. Competing hypotheses of the evolution of A2 muscles in tetraodontiform fishes. (a) Transformation series (ACCTRAN) based on muscle names and homologies proposed by Winterbottom (1974b). This series requires a minimum of six steps (two gains, one shift of the ramus mandibularis, two shifts in the relative positions of muscles, one reversal by loss or fusion). (b) Alternative transformation series (ACCTRAN) based on new muscle names and homologies described here. This series requires a minimum of six steps (three duplications by muscle subdivision, two shifts in the path of the ramus mandibularis, one reversal by loss or fusion). This series is more parsimonious than that based on the homologies proposed by Winterbottom (1974b) because it explains the origin of additional muscles not previously recognized in tetraodontids.

of balistoids and tetraodontoids. We hypothesize that the ancestor of balistoids and tetraodontoids had a dorsal A2α and ventral A2β with the RMD passing lateral to A2α. In the lineage leading to balistids and monacanthids, A2β had a slightly differentiated ventral section. This ventral section completely subdivided to form A2β'b and A2β"b in the common ancestor of balistids. In the ostraciid lineage, the path of the RMD shifted to pass medial to both A2α and A2β. Triodontids retain the relatively plesiomorphic A2 pattern for tetraodontiforms. This pattern was modified in the common ancestor of molids, tetraodontids, and diodontids when the path of the RMD again shifted to pass medial to A2β. In the tetraodontid lineage, A2β independently subdivided to form A2β't and A2β"t. In total, our scenario requires

six steps (three gains, two shifts in the path of the RMD, and one reversal by loss or fusion) to explain the origin of A2 muscles in the common ancestors of each tetraodontiform family and includes a step for the origin of A2β't and A2β"t in tetraodontids, which were not recognized as separate muscles by Winterbottom. Thus, the scenario based on our new homologies is more parsimonious than that based on Winterbottom's homologies.

Relative Masses of A1 and A2 Muscles

Based on an ontogenetic series of 10 burrfish, *Chilomycterus schoepfi* (41–166 mm standard length), most A1 and A2 muscles showed slight positive allometry (slopes of least squares regressions of log muscle mass on log body mass: A1αt = 0.936; A1βt = 1.126; A2α = 1.169; A2β = 1.126). Only A1αt differs from other A1 and A2 muscles by having slight negative allometry. This muscle is extremely atrophied in all diodontids examined (e.g., Figs. 5d, 5e). Interspecifically, the A1 and A2 muscles of 22 other tetraodontiform species also showed slight positive allometry. An example of this relationship is shown in Figure 7a, where the log of the mass of all A1 muscles is plotted against the log of body mass for 10 *C. schoepfi* along with single representatives of 22 other species. A least squares regression fitted to the log-transformed data of all species has a slope of 1.04, a *y*-intercept of −2.51, and an r^2 of 0.80. The only clear outliers to this trend are the ostraciids, *Acanthostracion polygonius* and *A. quadricornis*, and one triacanthid, *Trixiphichthys weberi*. Standardized independent contrasts of A1 mass and body mass also show a strong positive correlation (slope = 1.06; r^2 = 0.89) (Fig. 7b).

The percentage of adductor muscle mass comprised by the A1 muscle varies considerably among tetraodontiforms, ranging from 23% in one tetraodontid, *Sphoeroides nephalus*, to 61% in one balistid, *Xanthichthys ringens*. Thus, there is considerable variation in the amount of muscle tissue devoted to moving the upper or lower jaws. There is also considerable overlap in variation between most families, with the greatest intrafamilial variation in tetraodontids and balistids.

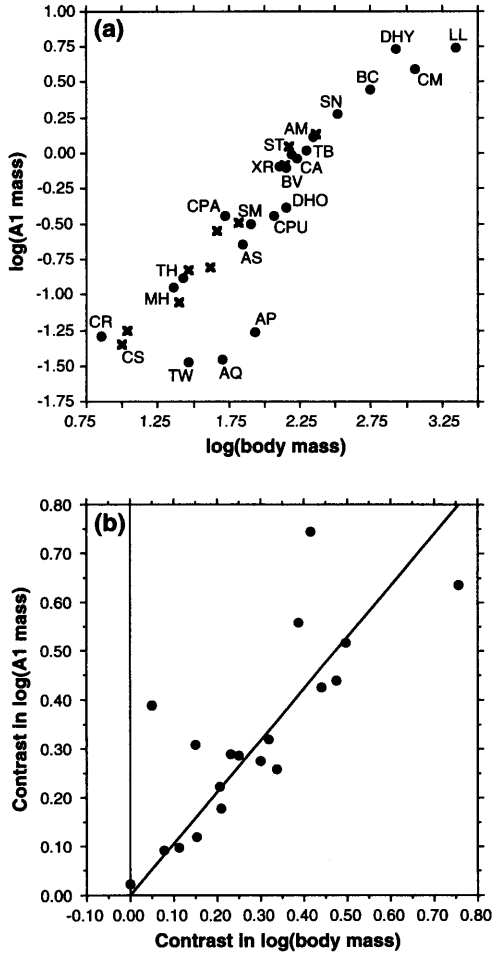

FIGURE 7. Slight positive allometry of A1 muscle mass for most tetraodontiform fishes. (a) Plot of log(A1 mass) and log(body mass) for 10 individuals of *Chilomycterus schoepfi* (✖) plus single individuals of 22 tetraodontiform species (●). See text for species abbreviations. (b) Plot of contrasts in log(A1 mass) and contrasts in log(body mass) for 19 independent contrasts. A line fitted to these contrasts and forced through the origin has a slope of 1.06 and an r^2 of 0.89.

A pattern does emerge when the residuals of log A1 mass and log A2 mass regressed against log body mass were plotted against each other (Fig. 8a). There was a strong tendency for species with relatively large A1 muscles to also have relatively large A2 muscles and for species with rel-

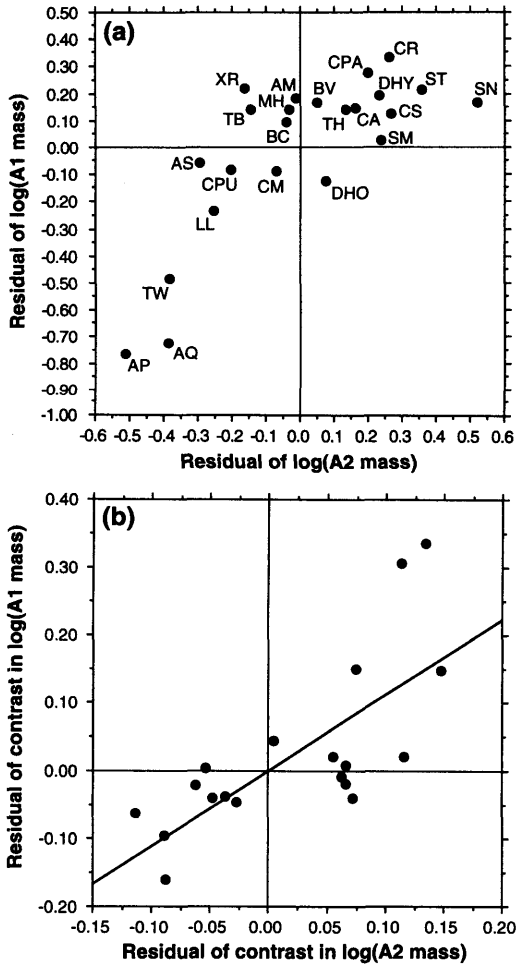

FIGURE 8. Positive correlation between A1 mass and A2 mass in tetraodontiform fishes. (a) Plot of residuals of log(total A1 mass) and the residuals of log(total A2 mass) both regressed against log(body mass) for single individuals of 23 tetraodontiform species. An individual located at the intersection of both axes would represent a fish with average A1 and A2 masses for all tetraodontiforms of that body size. See text for species abbreviations. (b) Plot of residuals of contrasts in log(A1 mass) and log(A2 mass) both regressed against contrast in log(body mass) for 19 independent contrasts. A line fitted to these contrasts and forced through the origin has a slope of 1.12 and an r^2 of 0.55.

atively small A1 muscles to have relatively small A2 muscles. This positive correlation is supported by residuals of standardized contrasts for A1 and A2 mass regressed against standardized contrasts of body mass (slope = 1.12; r^2 = 0.55) (Fig. 8b). Those taxa that have relatively large adductor muscles (most tetraodontids, diodontids, and *Balistes vetula*) are durophagous and feed on prey such as molluscs, echinoderms, and crabs (Randall, 1967). Species with relatively small A1 and A2 muscles (ostraciids, several monacanthids, one triacanthid) tend to be grazers that feed on algae and relatively soft-bodied invertebrates (Randall, 1967). Morphological outliers tend to be trophic outliers as well. For example, the tetraodontid *Lagocephalus* is a pelagic puffer that feeds largely on soft prey such as squid (Wheeler, 1969). The triggerfish *Xanthichthys* is a zooplanktivore and has a relatively small A2 muscle (Turingan, 1994).

No relationship was seen between the number of adductor mandibulae muscles possessed by a species and the relative size of the adductor mandibulae complex (Fig. 9a). Many taxa with only four or five A1 and A2 muscles, such as balistids, tetraodontids, or diodontids, have much larger residuals than some monacanthids, with as many as seven A1 and A2 muscles. This lack of correlation is also supported when residuals (standardized contrasts of log[A1 and A2 mass] regressed against standardized contrasts in log[body mass]) are plotted against standardized contrast in the number of A1 and A2 muscles (slope = 0.03; r^2 = 0.01) (Fig. 9b).

The relative contributions of individual A1 descendant muscles to total A1 mass for eight representative species are shown in Figure 10a. In all balistoids examined (ostraciids, balistids, monacanthids), A1αb is the largest A1 descendant muscle, and this relationship is maintained if its descendant muscles, e.g., A1αb′, A1αb″, and A1αb‴ in monacanthids such as *Monacanthus*, are pooled together. This observation that A1αb is the largest A1 muscle in balistoids is also consistent with our hypothesis that Winterbottom's (1974b) A1β″ of ostraciids (e.g., *Acanthostracion*) is the ortholog to A1αb of balistids and monacanthids.

Unlike balistoids, the relative masses of A1 descendant muscles in tetraodontoids

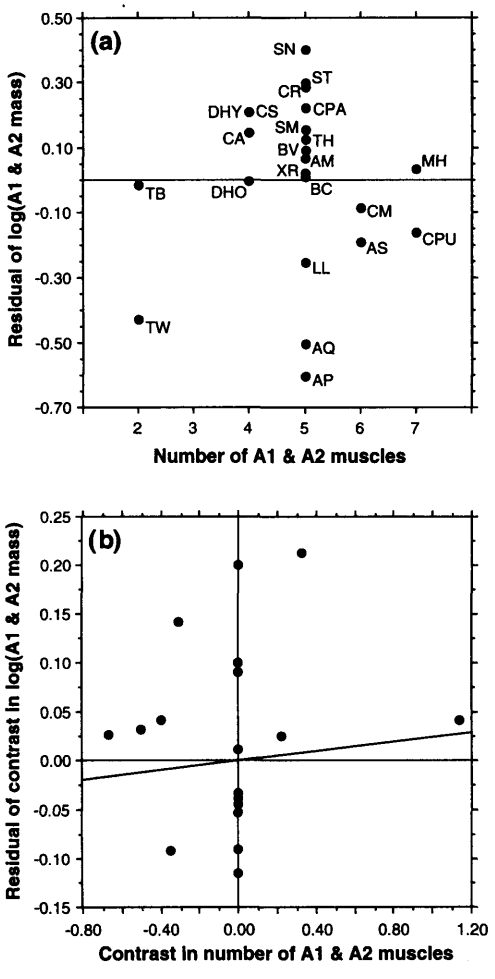

FIGURE 9. No correlation between muscle mass and number of jaw muscles in tetraodontiform fishes. (a) Plot of residuals of log(A1 mass + A2 mass) regressed against log(body mass) and total number of A1 and A2 muscles for single individuals of 23 tetraodontiform species. Data points for *Diodon hystrix* (DHY) and *Chilomycterus schoepfi* (CS) overlap on this plot. See text for other species abbreviations. (b) Plot of residuals of contrasts in log(A1 mass + A2 mass) regressed against contrasts in log(body mass) and total number of A1 and A2 muscles for 19 independent contrasts. A line fitted to these contrasts and forced through the origin has a slope of 0.025 and an r^2 of 0.01.

examined (tetraodontids, diodontids) vary considerably between taxa, which is consistent with our hypothesis that the A1 descendant muscles of tetraodontoids have arisen independently in this clade and are not orthologs of any A1 muscles of balistoids. At one extreme is the tetraodontid *Lagocephalus* with a large A1αt and a small A1βt, and at the other extreme are diodontids such as *Chilomycterus*, which have a minute A1αt and an enormous A1βt. Other tetraodontids examined in this study have relatively equal-size A1 descendant muscles such as those of *Sphoeroides* (Fig. 10a).

The relative contributions of individual A2 descendant muscles to total A2 mass for the same eight species are shown in Figure 10b. In most balistoids and tetraodontoids, A2β is larger than A2α. This pattern is reversed in all monacanthids such as *Monacanthus* and in a single balistid, *Xanthichthys*, where A2α is larger than A2β. This reversal is more likely due to a reduction in A2β rather than to an increase in A2α because these same taxa have negative A2 residuals as compared with other tetraodontiforms (Fig. 8). One other notable difference is the relatively small A2α and large A2β't and A2β"t of *Sphoeroides* as compared with other tetraodontids. This pattern may be associated with the fact that A2α uniquely inserts on the upper jaw in this genus.

DISCUSSION

The adductor mandibulae system of tetraodontiform fishes provides a clear example of phylogenetic repetition of morphological elements. This system is well suited to an analysis of the functional consequences of structural duplication for several key reasons. First, there is a well-corroborated phylogeny of the major lineages of tetraodontiform fishes. Second, the new muscle nomenclature presented here will permit specific comparisons to be made between the functional attributes of an ancestral muscle and the descendants that result from a splitting event. Third, including muscle evolution within tetraodontiform families, we recognize a total of 10 cases of muscle subdivision and 4 cases of reversals by either loss or fusion of muscle subdivisions in this system. This large number of independent changes in muscle number makes it possible to repeatedly test specific hypotheses about the conse-

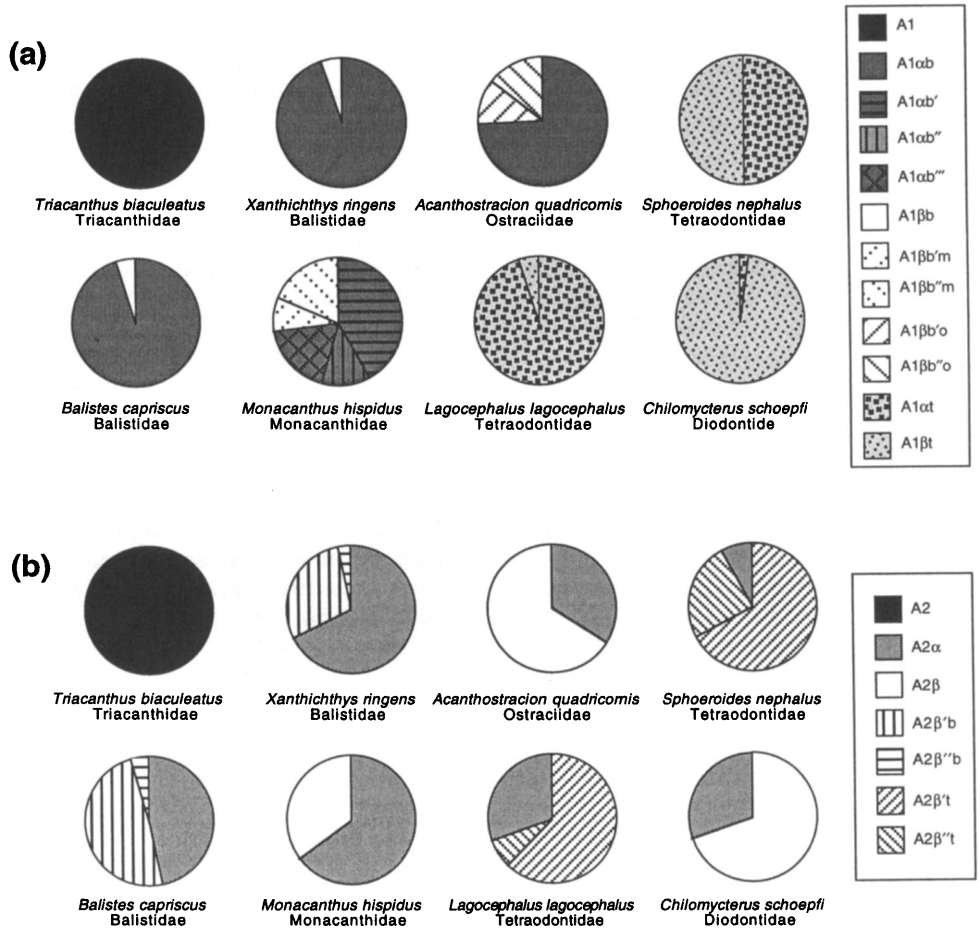

FIGURE 10. Pie charts of muscle masses for individuals of eight species from six tetraodontiform families. (a) A1 muscles. All descendant A1α muscles in balistoids are shaded the same. The A1α and A1β muscles have arisen independently in both balistoids and tetraodontoids. (b) A2 muscles. The A2β′ and A2β″ muscles have arisen independently in both balistids and tetraodontids.

quences of subdivision. Because muscle subdivision is not a phylogenetically or ontogenetically unique event, it is possible to generate statistically useful sample sizes, thus addressing a problem that has plagued comparative studies (Garland et al., 1992).

Muscle Homologies

This review of the A1 and A2 muscles of tetraodontiforms revealed that several muscles currently identified by the same name in different subclades are not orthologs and thus require new names to reflect new hypotheses of homology. Transformation series based on these new homologies are more parsimonious for both A1 and A2 muscles than are those based on homologies proposed by Winterbottom (1974b).

We hypothesize that a minimum of 10 separate duplication events have produced the diversity of A1 and A2 muscles in these fishes. This review revealed two trends in the evolution of jaw muscles in tetraodontiforms. First, the origins of A1 and A2 muscles and the relative positions of descendant muscles to each other are

conservative features in this clade. For these fishes, position is a more reliable criterion for identifying muscles than is the path of the RMD, which has been used in other studies (Winterbottom, 1974b; Gosline, 1986). The path of this nerve is a reliable landmark for A1 muscles in balistoids but not for A2 muscles in tetraodontoids.

Second, duplication of muscles by subdivision is more common whereas secondary loss of duplicated muscles is less common than previous studies have suggested. A similar trend in phylogenetic increases in muscle number was reported by Gosline (1986), who surveyed higher groups of teleost fishes and found that muscles produced by primary subdivision of the adductor mandibulae were seldom secondarily lost. Some of the differences in the muscle gain versus loss ratio for the transformations series examined in this study are expected because homoplasy that was originally due to secondary losses of "homologous" muscles is now explained instead by parallel gains of "nonhomologous" muscles through additional subdivision events. We recognize at least three such cases of parallel muscle evolution: A1αb and A1βb of balistoids versus A1αt and A1βt of tetraodontoids, A1βb'o and A1βb"o of ostraciids versus A1βb'm and A1βb"m of monacanthids, and A2β'b and A2β"b of balistids versus A2β't and A2β"t of tetraodontids.

Duplication of jaw muscles by subdivision has undoubtedly occurred several times within teleost fishes. According to Gosline (1989) "A1" and "A2" muscles have arisen once in ostariophysan fishes (Cypriniformes, Characiformes, Siluriformes, Gymnotiformes) and again indepenently in higher teleost fishes (Beryciformes, Zeiformes, Perciformes, Scorpaeniformes, Tetraodontiformes). Other more complex adductor mandibulae subdivisions similar to those described here for tetraodontiforms have occurred in loricarioid catfishes (Trichomycteridae, Nematogenyidae, Callichthyidae, Scoloplacidae, Astroblepidae, Loricariidae) (Howes, 1983; Schaefer and Lauder, 1986, 1996) and parrotfishes (Scaridae) (Bellwood and Choat, 1990; Bellwood, 1994). Loricarioids and scarids could potentially serve as other model cases of duplication because phylogenies are available for both groups (Howes, 1983; Schaefer, 1987; Bellwood, 1994). Complex patterns of duplicated jaw muscles occur in clades of fishes that have highly derived oral jaw morphologies and novel feeding modes. Most bony fishes are ram or suction feeders and use their oral jaws for prey capture but not processing (Liem, 1980a; Lauder, 1985). In contrast, tetraodontiform fishes and parrotfishes use their robust oral jaws to both capture and process food items, which are often quite hard (e.g., molluscs, corals) (Bellwood and Choat, 1990; Turingan and Wainwright, 1993; Wainwright and Turingan, 1993; Turingan, 1994). In loricarioid catfishes, the oral jaws are highly modified to scrape food items such as algae off objects or to rasp plant material (Schaefer and Lauder, 1986, 1996).

Our recognition of homologies different from those of Winterbottom (1974b) is due to two main factors. First, our homologies are based on an explicit model of muscle evolution by subdivision. This model produces paralogs, which we distinguished from their common ancestral muscle and each other. Although Winterbottom also suggested that new muscles are produced by subdivision, he often retained an ancestral muscle name for the one descendant muscle that resembled the ancestral muscle in outgroup taxa. This practice ultimately obscured the homologies between these muscles.

Second, Winterbottom's (1974b) analysis predates the widespread availability of computer programs for analyzing data and investigating alternative optimizations of characters. As Winterbottom stated (1974b: 73), his analysis methods precluded the possibility of any explanations based on parallel or convergent evolution. Although the topology of his "hand generated" phylogeny is identical to one produced using a computer algorithm and the same data, it lacked explicit character transformations such as those investigated in this study. A

posteriori analysis of the transformations series for homoplastic characters can sometimes reveal alternative and possibly more parsimoniously interpreted homologies. Although these new homologies may not change the phylogeny itself, they may radically affect any hypotheses based on the refuted homologies.

Masses of A1 and A2 Muscles

A correlation between adductor muscle mass and diet has been previously reported for other fishes. Bellwood and Choat (1990) found that parrotfishes that bite and excavate pieces of hard coral skeletons while feeding had relatively larger jaw bones and greater total adductor mandibulae mass than did parrotfishes that only scrape the algae-encrusted surfaces of old coral skeletons while feeding. Similarly, Turingan (1994) found that durophagous tetraodontiforms such as balistids, tetraodontids, and diodontids had relatively massive A2 muscles, jaws, and suspension elements as compared with a single grazing monacanthid examined. However, he did not find any significant differences in A1 mass between taxa, as we have found here with the larger number of nondurophagous taxa (i.e., triacanthids, ostraciids, and several monacanthids) examined. Although some monacanthids had A1 residuals similar to those of balistids, tetraodontids, and diodontids, most did not.

Our sample of 23 tetraodontiform species from six families shows no support for the idea that overall adductor mass will increase as new muscles evolve (Figs. 9a, 9b). During phylogenetic increases in the number of muscles, the existing muscle tissue apparently is subdivided not only in a qualitative sense, as our model states, but also in a quantitative sense. One interpretation of this pattern could be that constructional constraints (Barel, 1983) on the space available to adductor muscle tissue limit increases in total adductor muscle mass. However, this factor is probably not limiting the ability of the adductor muscle to change in overall size, as indicated by the existing diversity in overall adductor mass that clearly varies with trophic habits (Fig. 8a). Constructional constraints will undoubtedly place some upper boundary on the size of the adductor mandibulae musculature, but the flexibility that is indicated by ecomorphological patterns suggests that increases in the number of adductor muscles could theoretically influence the amount of muscular tissue.

Although there have been numerous muscle duplications in this system, most descendant muscles retain their primitive attachment to a particular jaw element (i.e., A1 muscles insert on the maxilla, and A2 muscles insert on the mandible). Only two examples were found where muscle insertions had changed to different bony elements. In monacanthids, the insertion of A1αb‴ has shifted from the maxilla to the palatine bone (Fig. 3g) (Winterbottom, 1974b). In tetraodontids of the genus *Sphoeroides*, A2α insertion has shifted from the mandible to the maxilla (Fig. 5c). In monacanthids, this shift is associated with a subdivision event, whereas in *Sphoeroides*, the shift clearly occurred after the subdivision event.

Currently, we are further exploring the analogy drawn in this study between duplications of genes and duplications of morphological elements. A major paradigm in molecular evolution is that gene duplication leads to divergence in nucleotide sequence and ultimately divergence in the function of paralogous genes (Ohno, 1970; Ohta, 1989; Chothia, 1994; Holland et al., 1994; Holland and Garcia-Fernàndez, 1996). Thus, is duplication of muscles also associated with divergence in function, and if so, are there general patterns in muscle evolution? To address these questions, we are using electromyography to quantify one aspect of muscle function, the individual activity patterns of muscles during feeding events. Because paralogous muscles are derived from the same ancestral muscle, they should have the same plesiomorphic function (i.e., activity pattern) unless functional divergence has occurred. If one or more of the duplicated muscles examined in this study have functionally diverged, we should find differences in the

relative timing (i.e., onset and duration) and/or intensity of muscular activity between paralogous muscles. Such differences in activity patterns should have functional consequences for the feeding mechanisms in these fishes.

ACKNOWLEDGMENTS

We thank J. Grubich, K. Ralston, and T. Thys for thoughtful comments on a draft of this manuscript and R. Winterbottom for his feedback on several aspects of this research. We also thank R. Turingan for providing some of the specimens used in this study and M. Westneat for the loan of specimens from the Field Museum of Natural History. Support for this research was provided by NSF grant IBN 9306672 to P. C. Wainwright.

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Received 16 July 1996; accepted 20 February 1997
Associate Editor: Paula Mabee

APPENDIX. Correspondence between the jaw muscle names used by Winterbottom (1974b) and the new names used in this study of tetraodontiform fishes.

Family	Winterbottom (1974b)	This study
Triacanthodidae	A1	A1
	A2α	A2α
	A2β	A2β
Triacanthidae	A1	A1
	A2	A2
Ostraciidae	A1β	A1βb′o
	A1β′	A1βb″o
	A1β″	A1αb
	A2α	A2α
	A2β	A2β
Balistidae	A1α	A1αb
	A1β	A1βb
	A2α	A2α
	A2β	A2β′b
	A2γ	A2β″b
Monacanthidae	A1α	A1αb′
	A1α′	A1αb″ or A1αb″α[a]
	A1α″	A1αb″β
	A1γ	A1αb‴
	A1β	A1βb′m
	A1β′	A1βb″m or A1βb″mα[a]
	A1β″	A1βb″mβ
	A2α	A2α
	A2β	A2β
Triodontidae	A1	A1
	A2α	A2α
	A2β	A2β
Molidae	A1α	A1βt
	A1β	A1αt
	A2α	A2β
	A2β	A2α
Tetraodontidae	A1α	A1βt
	A1β	A1αt
	A2α	A2β′t and A2β″t
	A2β	A2α
Diodontidae	A1α	A1βt
	A1β	A1αt
	A2α	A2β
	A2β	A2α

[a] Muscle duplications unique to some genera within this family.