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Tecidos mineralizados em Characiformes:
estudo sistemático da variação morfológica
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Resumo

A dentição é um complexo de caracteres reconhecido por ser altamente informativo em estudos sistemáticos para a ordem Characiformes, como consequência a dentição foi amplamente explorada em estudos sistemáticos das linhagens que compõem a ordem. No entanto, estudos sistemáticos detalhados que discutam a variação observada na dentição em um contexto da ordem como um todo são escassos. De maneira semelhante, apesar do amplo conhecimento existente sobre o esqueleto dos representantes adultos dos Characiformes, informações detalhadas sobre o desenvolvimento deste complexo anatômico assim como sobre a sequência de ossificação completa para representantes de Characiformes ainda são incipientes e, até hoje, existe apenas uma sequência completa de ossificação disponível na literatura. Apresentamos aqui um estudo detalhado sobre a dentição dos Characiformes contemplando a morfologia dentária, o modo de implantação e a posição da implantação, disposição dos dentes em cada osso, modo de formação dos dentes de substituição e padrão cronológico da substituição. Descrições detalhadas são fornecidas para 78 espécies de Characiformes, com representantes de todas as famílias reconhecidas, exceto para a família Tarumaniidae recentemente descrita. Cinquenta e nove caracteres são propostos, incluindo caracteres reinterpretados já propostos na literatura, juntamente com caracteres originais. A variação e distribuição dos caracteres propostos em Characiformes é discutida juntamente com a proposta de novas interpretações da identidade e homologia das fileiras de dente no pré-maxilar em representantes da ordem. São fornecidas descrições de variações ontogenéticas da dentição em espécies de Characiformes selecionadas. Possíveis sinapomorfias adicionais relacionadas à dentição são propostas para os Anostomidae, Heterocharacinae e Serrasalminae. Dois padrões de substituição dentro dos Characoidei foram identificados e descritos em detalhes. Interpretação alternativa para a identidade e homologia das três fileiras de dentes na pré-maxila presentes em *Brycon*, *Triportheus* e *Chalceus* é apresentada juntamente com uma proposta de homologia da única fileira de dentes pré-maxilares presentes nos Characoidei e Citharinoidei. Também estudamos aqui o desenvolvimento esquelético do caracídeo *Astyanax lacustris* e do ctenoluciídeo *Ctenolucius hujeta*, apresentando a sequência de ossificação do esqueleto completo para ambas as espécies. O estudo foi baseado em 111 exemplares de *A. lacustris* (2,9 mm NL a 27,6 mm SL) e 100 exemplares de *C. hujeta* (3,5 mm NL a 35,5 mm SL). Espécimes

foram diafanizados e corados, a presença de uma ossificação foi pontuada sempre que a mineralização foi detectada. Para ambas as espécies, as primeiras ossificações a aparecer foram as placas dentárias associadas ao ceratobranquial 4 e faringobranquial 4, o cleitro e o opérculo em 2,9 mm NL para *A. lacustris* e 3,5 mm NL para *C. hujeta*. Análises comparativas foram realizadas para as sequências de *A. lacustris*, *C. hujeta* e a sequência já publicada de *S. brasiliensis*. As sequências são comparadas dentro de complexos osteológicos para a sequência dentro desse complexo, bem como no contexto de toda a sequência. Observaram-se diferenças entre as três sequências quanto à sequência de ossificação dentro de um complexo osteológico; os intervalos relativos entre a ossificação do primeiro e do último elemento em um complexo osteológico, bem como o deslocamento de todo um complexo osteológico na sequência completa.

Abstract

The dentition is a character complex recognized for being highly informative for systematic studies for the order Characiformes, hence the dentition was widely explored in systematic studies of several lineages within the order. However, detailed systematic studies that discuss the observed variation in the context of the order as a whole are still scarce. In a similar manner despite the wide knowledge produced about the adult skeleton of the representatives of the Characiformes, detailed information about the development of this anatomical complex, as well as about the sequence of ossification of the complete skeleton of representatives of the Characiformes are still scarce and to this date there is only one complete sequence of ossification available in the literature. We herein present a detailed study on the dentition of the Characiformes contemplating tooth morphology, tooth implantation mode and implantation position, arrangement of teeth on each bone, formation mode of replacement teeth and the chronological pattern of the replacement. Detailed descriptions are provided for 78 species of Characiformes. with representatives from all recognized families, except for the recently described Tarumaniidae. Fifty-nine characters are proposed, including reinterpreted characters previously proposed in the literature, along with original characters. The variation and distribution of the proposed characters in the Characiformes is discussed along with the proposal of new interpretations of the identity and homology of tooth rows in the premaxilla in representatives of the order. Descriptions of ontogenetic variation of

dentition in selected characiform species are provided. Possible additional synapomorphies related to the dentition are proposed for the Anostomidae, Heterocharacinae and Serrasalminae. Two patterns of replacement within the Characoidei are identified and described in detail. Alternative interpretation for the identity and homology of the three tooth rows on the premaxilla present in *Brycon*, *Triportheus* and *Chalceus* is presented along with a proposal of homology of the single premaxillary tooth row present within the Characoidei and Citharinoidei. Also, we herein studied the skeletal development of the small sized characid *Astyanax lacustris* and the ctenoluciid *Ctenolucius hujeta* presenting the ossification sequence of the complete skeleton for both species. This study was based on 111 specimens of *A. lacustris* (2.9 mm NL to 27.6 mm SL) and 100 specimens of *C. hujeta* (3.5 mm NL to 35.5 mm SL). Specimens were cleared and double stained and the presence of an ossification was scored whenever mineralization was detected. For both species the first ossifications to appear were the tooth plates associated to ceratobranchial 4 and pharyngobranchial 4, the cleithrum and the opercle at 2.9 mm NL for *A. lacustris* and 3.5 mm NL for *C. hujeta*. Comparative analyses were conducted for the sequences of *A. lacustris*, *C. hujeta* and the already published sequence of *S. brasiliensis*. The sequences are compared within osteological complexes for the sequence within that complex and in the context of the entire sequence. Differences were observed regarding the sequence of ossification within an osteological complex; the relative intervals between the ossification of the first and last element in an osteological complex, as well as shift of an entire osteological complex in the complete sequence.

Introduction

The order Characiformes constitute a dominant group of fresh-water fishes with over 2200 species distributed in the Americas and Africa (Fricke *et al.* 2019). Most of its diversity is in the Neotropics, with approximately 2000 species present from the river basin on the south of the United States until the central region of Chile and Argentina. Number and composition of the recognized families vary within the order (Reis, *et al.* 2003; Oliveira *et al.*, 2011; Mirande, 2019), the variation mainly due to differences in relation to the limits of the family Characidae the most species rich family within the order with almost 1200 species. It is in this family that most taxonomical and phylogenetic issues still reside. The relatively small size, the high number of species, and the generalized morphology make it difficult to elucidate the taxonomical and phylogenetic questions within the family.

The current knowledge on the phylogenetic relations within the Characiformes derives mostly from studies based on morphological characters, mainly focused on the osteology. However, the utilization of alternative source of characters has become important in order to reevaluate previously proposed phylogenetic hypotheses as well as to further refine the knowledge on the relations of several taxa. Among the alternative source of characters, the use of molecular characters (Ortí & Meyer, 1997; Calcagnotto *et al.*, 2005; Oliveira *et al.*, 2011; Arcila *et al.* 2017; Betancur-R *et al.*, 2019; Mirande 2019); reproductive biology characters (Weitzman *et al.*, 2005); sperm ultrastructure (Gusmão-Pompiani *et al.*, 2009; Baicere-Silva *et al.*, 2011) along with cephalic myology (Datovo & Castro, 2012; Mattox & Toledo-Piza, 2012) and cephalic lateral-line system (Pastana *et al.*, 2019).

The most utilized source of characters for systematic studies of fishes is the skeleton, being the main source of information for most phylogenetic studies based on morphological characters (Mirande, 2010; Sidlauskas & Vari, 2008; Stiassny & Jensen, 1987; Tyler *et al.*, 2003; Westneat, 1993; Wiley & Johnson, 2010; Winterbottom, 1974; Zanata & Vari, 2005). The dentition, a subset of the skeletal system, is notably variable in the Characiformes, among adult specimens several tooth morphologies are observed including conical, incisiform, molariform, multicuspid (with variable number and arrangement of cusps) and mamilliform. This morphological diversity is associated to the several different trophic strategies adopted by different lineages within the order including piscivores, herbivores, detritivores and even lepidophagous taxa (Géry, 1977).

Roberts (1967) stated that the trophic diversification had an important role on the diversification of the major lineages within the order. Roberts (1967) also stated that “in any broad studies of the biology, or systematics, or phylogeny, or evolutionary processes in the characoids, not only the morphology of the teeth in adults, but also tooth formation, tooth replacement and ontogenetic changes in dentition will have to be taken into account”.

Papers like those of Monod (1950) and Roberts (1967) surveyed several aspects such as the morphology of the functional teeth, morphology and arrangement of the developing replacement teeth, morphology of the bony cavities in which the replacement teeth are formed, histological aspects of the implantation of the functional teeth and of the developing replacement teeth along with the replacement patterns. Both authors had a relatively large representativeness of the diversity of the order. Little was produced on the characiform dentition with an ample sampling within the order since Roberts (1967). The most prevalent papers are those focusing in a single or few taxa investigating replacement patterns and ultrastructure (Berkovitz, 1975; 1980; Shellis & Berkovitz, 1976; Berkovitz & Shellis, 1978). Berkovitz & Shellis (2017) presented a review of the information in the literature on characiform dentition regarding tooth morphology and its relation to the diet of different lineages within the family and the different observed replacement patterns.

Information on the ontogenetic shifts in Characiformes dentition are also scarce, Azevedo and Vieira (1938) report ontogenetic modification on the dentition of *Prochilodus argenteus*, such modifications were later confirmed by Castro & Vari (2004). Azevedo *et al.* (1938) reported the presence of small conical teeth on the larvae of representatives of the Curimatidae and Tetragonopterinae. Trapani (2005) investigated the transition from unicuspid to multicuspid dentition in *Astyanax mexicanus* and concluded that the two dentitions are independent, since they present distinct origins (multicuspid teeth are formed intraosseously, whereas unicuspid teeth are formed extraosseously), diverging from what was proposed by Roberts (1967), that multicuspid teeth were the result of the fusion of several conical elements at their base, presumably by some kind of bone tissue.

Information on teeth implantation mode for characiforms *Hepsetus odoe*, *Paracheirodon simulans* and *Rhoadsia altipinna* was presented by Fink (1981) in his survey on the ontogeny and phylogeny on tooth implantation mode across the Actinopterygii. The position of developing replacement teeth was reported for

representatives of the Alestidae, Anostomidae, Cynodontidae and Serrasalminidae by Trapani (2001) in his paper on the variation of intra- and extraosseous development of replacement teeth in Teleostei.

Papers that deal with the dentition in a broader taxonomical context are those that use this anatomical complex as a source of characters (e.g. Malabarba, 1998; Zanata & Vari, 2005; Mirande, 2010; Ferreira *et al.*, 2011; Mattox & Toledo-Piza, 2012), however not in great detail. The only recent paper on characiform dentition with detail encompassing the implantation mode, histological and ultrastructural aspects with satisfactory taxonomical sampling is the one from Scharcasky & Lucena (2008) with representatives of the families Anostomidae, Curimatidae, Prochilodontidae, Chilodontidae, Hemiodontidae and Parodontidae. Along with the descriptive approach the authors discussed the observed variations in the context of the knowledge of the phylogenetic relationships of these families. Despite the great detail the authors evaluated only part of the obtained data in the phylogenetic context.

In the past few years the interest in fish dentition appear to be going through a relative renaissance (Fraser *et al.* 2012; Conway, 2015; Bemis & Bemis, 2015; Bemis *et al.*, 2019), and the Characiformes were not left out, Guisande *et al.* (2013) in their study on the ecological factors that could have driven the diversification among Neotropical Characiformes identified 14 different tooth morphologies and presented an ancestral character state reconstruction of six of those morphologies on the topology obtained by them based on mitochondrial and nuclear gene sequences. The authors stated that tooth morphology presented a strong phylogenetic signal, especially for the Anostomidae and Prochilodontidae. Atukorala & Franz-Odenaal (2014) explored spatial and temporal events in the tooth development of *Astyanax mexicanus* comparing cave-dwelling and surface forms. Kolmann *et al.* (2019) investigated the patterns of articulation among adjacent teeth the replacement pattern observed in the Serrasalminidae along with inferences on the evolution of these characters in the family.

As stated above, the skeleton of adult specimens is the major source of information for phylogenetic studies of the Characiformes, and to this date little is known about the development of the skeleton of representatives of the order. The study of ontogeny of the skeleton is not only a promising source of characters, but also a valuable resource utilized by comparative anatomists in order to understand in more detail the nature and homology of several morphological characters, and complements information based on the examination of adult specimens (Nelson, 1978; 1985; Britz & Johnson, 2002; 2005;

Fraser et al., 2012). In addition, the study of ossification sequences is important in order to identify and understand heterochronic events that might be present in cases of miniaturization (Britz & Conway, 2009; Britz *et al.*, 2009; Mattox *et al.* 2016).

Among the Ostariophysi studies on the skeletal development and ossification sequence were carried out for the Gonorhynchiformes (Taki *et al.*, 1986; 1987; Kohno *et al.*, 1996; Arratia & Bagarinao, 2010), non neotropical Siluriformes (Adriaens *et al.*, 1997; Adriaens & Verraes, 1998) and Cypriniformes (Vandewalle *et al.*, 1992; Cubbage & Mabee, 1996; Bird & Mabee, 2003; Engeman *et al.*, 2009; Conway *et al.*, 2017), the available information is scarce for Gymnotiformes and neotropical Siluriformes (Geerinckx *et al.*, 2007) and Characiformes.

Studies on the ontogeny of the skeleton of the Characiformes are still scarce and most of them deal with only part of the skeleton. Bertmar (1959) published a monograph on the development of the neurocranium and visceral arches of *Hepsetus odoe* (Hepsetidae), the author focused on the development of chondral elements, rarely referring to ossified structures. Vandewalle *et al.* (2005) presented a study on the chondrogenesis and osteogenesis of the cranial skeleton of *Brycon moorei*. More recently, Walter (2013) presented a study on the chondrogenesis and osteogenesis of the cranium of the characid *Moenkhausia sanctaefilomenae* and Carvalho and Vari (2015) presented a study on the development of the mandibular, hyoid and branchial arches of *Prochilodus argenteus* (Prochilodontidae) with a discussion on the basal developmental patterns in Ostariophysi. Mattox *et al.* (2014) were the first to present a study on the development of the complete skeleton within the Characiformes, the authors proposed a sequence of ossification of the complete skeleton for *Salminus brasiliensis*.

The proposal of additional sequences of ossification for the complete skeleton constitutes an important source of information not only on the development of the selected taxa, but also enable the comparison among sequences of ossification of the complete skeleton of other representatives of the Characiformes and possibly identifying differences with phylogenetic significance.

The current study aims to document in detail the dentition including the aspects involved in the replacement for the Characiformes and to discuss this variation in a phylogenetic framework. Also, this study proposes complete sequences of ossifications of the characid *Astyanax lacustris* and the ctenoluciid *Ctenolucius hujeta*, along with a first attempt of a comparative analysis of complete sequences of ossification of representatives of Characiformes.

Conclusions

Detailed examination of the dentition, an already vastly explored character complex, resulted in the refinement of characters related to dentition and resulted in the proposition of possible additional synapomorphies for the Heterocharacinae (unicuspid teeth with abruptly tapering cusp), Serrasalminae (replacement teeth of the dentary formed in completely enclosed bony cavities) and Anostomidae (pleurodont implantation on premaxilla and maxilla).

Unilateral replacement patterns were observed in several taxa with multicuspid dentition including representatives of the Characidae, Chalceidae, Alestidae, Serrasalminae, Iguanodectidae, Bryconidae and Agoniistidae. Furthermore, two distinct patterns within the unilateral pattern were identified the simultaneous unilateral replacement pattern observed in *Serrasalmus* and *Astyanax* and the sequential unilateral pattern observed in *Brycon* and *Chalceus*.

Based on differences in the modes of formation of replacement teeth we propose (i) an alternative interpretation of the identity and homology of the three premaxillary tooth rows observed in *Brycon*, *Triportheus* and *Chalceus*; (ii) the identification of two tooth rows for taxa in which this differentiation is problematic such as *Mimagoniates* and *Bryconamericus lethostigmus*; (iii) a proposition of homology between the single row of teeth present in taxa within the Characoidei and the inner row of taxa with two or three tooth rows on the premaxilla; (iv) the proposition of homology of the single row of teeth present in taxa within the Citharinoidei with the outer row of *Xenocharax spilurus*.

The comparative analysis of complete ossification sequences for representatives of Characiformes presented herein showed that there is a considerable amount of variation among the sequence of ossification of *Astyanax lacustris*, *Ctenopoma hujeta* and *Salminus brasiliensis*, anatomical complexes with the same ossification sequence across the three species were a minority. The observed variation includes: (i) different sequence of ossification within an osteological complex. In those cases the differences within a complex can be coupled with alterations in the timing of the ossification of the complex in the context of the entire sequence (*e. g.* observed for the olfactory region of the neurocranium) or not (*e. g.* hyopalatine arch); (ii) different relative intervals between the ossification of the first and last element in an osteological complex, despite identical sequences within the complex (*e. g.* relative interval of the elements of the occipital region of the neurocranium in *C. hujeta*); (iii) shift of an entire osteological complex in the

complete sequence, despite similar sequences and relative intervals across the three species (*e. g.* Weberian apparatus).

The observed degree of variation suggests that the study of ossification sequences is a promising source of information to be explored in a phylogenetic context. Comparative studies employing ossification sequences of the entire skeleton could point to heterochronic changes between taxa that could be explored in a functional anatomy perspective, as well as in a developmental genetic approach. In addition, information from complete ossification sequences could be used as characters in a phylogenetics systematics framework in a similar way other character complexes are used.

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