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Macroevolutionary consequences of karyotypic changes in the neotropical Serrasalmidae fishes (Ostariophysi, Characiformes) diversification

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Abstract

In the Neotropical region, one of the most diverse families of freshwater fishes is the monophyletic Serrasalmidae. Karyotypically, the family shows high diversity in chromosome numbers (2n = 54 to 64). However, little is discussed about whether the chromosomal changes are associated with cladogenetic events within this family. In the present study, we evaluated the role of chromosomal changes in the evolutionary diversification of Serrasalmidae. Our phylogenetic sampling included 36 species and revealed three main clades. The ancestral chromosome number reconstruction revealed the basic number 2n = 54 and a high frequency of ascending dysploid events in the most derived lineages. Our biogeographic reconstruction suggests an Amazonian origin of the family at 48–38 Mya, with independent colonization of other basins between 15 and 8 Mya. We did not find specific chromosomal changes or increased diversification rates correlated with the colonization of a new environment. On the other hand, an increase in the diversification rate was detected involving the genus *Serrasalmus* and *Pygocentrus* in the Miocene, correlated with the stasis of 2n = 60. Our data demonstrate that chromosomal rearrangements might have played an important evolutionary role in major cladogenetic events in Serrasalmidae, revealing them as a possible evolutionary driver in their diversification.

Keywords Neotropical fishes · Piranhas · Ascending dysploidy · Karyoevolution

Introduction

The study of chromosomal changes in a macroevolutionary framework via Phylogenetic Comparative Methods is gaining substantial interest within the cytogenetics field (Martinez et al. 2015; Lume et al. 2017; Sader et al. 2019). These integrated studies discuss chromosomal polymorphisms in a temporal, spatial and phylogenetic context, clarifying the role of these karyotypic variants in cladogenetic events driving biodiversity (Jacobina et al. 2016; Cioffi et al. 2018; Costa et al. 2020). A central organizing component of genome architecture is the chromosome number (2n), and changes in this trait play a key role in evolutionary processes (Schubert 2007; Freyman and Höhna 2018). Numerical and structural chromosomal alterations may have important evolutionary consequences, affecting recombination rates, increasing reproductive isolation between lineages, and driving diversification between species (Ratomponirina et al. 1988; Yoshida and Kitano 2021). These changes are usually detected in comparative studies, in the search for karyotypic trends among phylogenetically related groups (Jacobina et al. 2016; Sader et al. 2019).

In fish, chromosomal rearrangements such as centric fusion and fission (Robertsonian translocation), and pericentric inversions have been some of the most determinant mechanisms of chromosomal alterations, differentiating both marine and freshwater lineages (Bertollo et al. 2000; Galetti et al. 2006; Jacobina et al. 2013; Sember et al. 2020). Fusions and fissions are readily identified in comparative karyotype studies, as both result in concomitant changes in chromosome morphology and chromosome number (Sember et al. 2020). On the other hand, pericentric inversions change only the chromosomal morphology, without altering

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chromosome number (Molina 2007; Jacobina et al. 2013). When discussing the significance of these chromosomal alterations to the diversification processes, it is common to attribute their importance to post-mating barriers, progressive isolation from populations, and incipient species (Ayala and Coluzzi 2005). Another trait that decisively impacts diversification rates is the colonization of new environments (Costa et al. 2020). This is mainly due to the morphological differentiation that often follows the arrival of a lineage in a new habitat (Friedman et al. 2020). These macroevolutionary changes are often associated with the absence of predation/competition in the new environment leading to the exploration of new niches and consequently speciation (Liem 1973; Schluter 2000). In this context, biogeographic changes may or may not be associated with chromosomal polymorphisms (Rosa et al. 2014; Costa et al. 2020; Nirchio et al. 2019).

In the Neotropical region, Characiformes fish represent one of the most diverse orders in terms of taxa, with more than 2150 species (Fricke et al. 2022). In addition, they present a high variation in chromosome number and morphology (Bertollo et al. 2000; Nakayama et al. 2012). Within this order, one of the most diverse families is the monophyletic Serrasalmidae, popularly known in Brazil as pacus, piranhas and tambaquis (Cione et al. 2009). This group, which includes about 101 valid species and 16 genera, has a high morphological diversity, with elevated bodies, laterally compressed, with abdominal spines and long dorsal fins (Kolmann, et al. 2021). Based on molecular hypotheses, the family is currently divided into two subfamilies, Colossomatinae and Serrasalminae, with Serrasalminae composed of two tribes: Myleini (comprising most of pacus species) and Serrasalmini (represented by *Metynnis*, *Catoprion*, and remaining piranha's genera) (Mateussi et al. 2020). They inhabit almost all the continental basins of South America (Jégu 2003; Fricke et al. 2022) and a variety of lotic and lentic environments (Goulding 1980), where they perform ecological functions, and support important continental fisheries (Araujo-Lima and Goulding 1997). Ecologically, they are generally divided into two lineages, one composed of herbivores (pacus and tambaquis), and another more derived group, composed of carnivorous piranhas [*Pygocentrus* and *Serrasalmus*] (Géry 1977; Goulding 1980; Correa et al. 2007). However, in recent years, studies of diets in these species have reinforced that they are considerably more diverse than previously predicted (Kolmann et al. 2021).

Regarding chromosomal aspects, representatives of this family have shown diversity in the chromosome (2n = 54 to 2n = 64) and fundamental (FN = 108 to FN = 122) numbers (Nirchio et al. 2003; Nakayama et al. 2001). In previous studies, many karyotypes have been described in several species of serrasalmids (Almeida-Toledo et al. 1987; Cestari and Galetti 1992; Nakayama et al. 2000, 2001, 2002, 2008,

2012; Centofante et al. 2002; Nirchio et al. 2003; Gaviria et al. 2005). A recent comparative cytogenetic analysis of Serrasalmidae based on classical and molecular cytogenetic techniques revealed the distribution of heterochromatin predominantly in pericentromeric regions in all species (Favarato et al. 2021). The cytogenetic data, when superimposed on the phylogeny of the family, revealed a tendency to increase the diploid chromosome numbers from 54 to 62 chromosomes, which occurred in a nonlinear manner and is the result of several chromosomal rearrangements (Favarato et al. 2021). However, little is discussed about whether the chromosomal changes detected are associated with the cladogenetic diversification of this family, especially considering their phylogenetic and biogeographic context. Chromosomal information from an evolutionary phylogenetic perspective can shed light on plesiomorphic and apomorphic states in different lineages (Mezzasalma et al. 2016). Thus, comparative phylogenetic methods, integrating phylogenetic and ecological traits, have sought to clarify the evolutionary systematic relationships between fish lineages (Kolman et al. 2021). In addition, they can clarify the evolutionary trends of related groups, and their role in species diversification (Aprea et al. 2013). In the present study, we evaluated the role of chromosomal changes in the evolutionary diversification of the Serrasalmidae family from a phylogenetic and biogeographic perspective. We seek to clarify and discuss the powerful evolutionary forces that boosted its diversity in the Neotropical region. We tested the hypothesis that the diversification rate of a lineage can be increased following the arrival in a new environment (e.g. Costa et al. 2020) and associated with chromosomal rearrangements (e.g. Sader et al. 2019; Costa et al. 2020; Martinez et al. 2015).

Materials and methods

Phylogenetic analyses and divergence time

A total of 36 species from the family Serrasalmidae and two outgroups (*Schizodon vittatus* and *Steindachnerina argentea*) were utilized for the phylogenetic analysis (see Supplementary Material 1). We aligned the sequences of the genes cytochrome oxidase subunit I gene COI (580pb), ribosomal DNA 16S (518pb) and 12S (318pb), and the recombination activating protein gene Rag1 (1246 pb) and Rag2 (1031 pb) using ClustalOmega as a plugin implemented in Geneious v.7.1.9 (Kearse et al. 2012; Supplementary Material 1). We then used the SeaView4 software (Gouy et al. 2010) to concatenate the different loci; the final matrix comprised 3,693 bp. A concatenated alignment containing was imported into BEAST v. 1.10.1 (Drummond and Rambaut 2007; Drummond et al. 2012). The Bayesian analysis was conducted considering all partitions simultaneously under

the most general substitution model (GTR + G). Uncorrelated relaxed lognormal clock (Drummond and Rambaut 2007) and Birth–Death speciation model (Gernhard 2008) were applied. One run of 50,000,000 generations was performed, sampling every 5000 generations. In order to verify the effective sampling of all parameters and assess the convergence of independent chains, we examined their posterior distributions in TRACER v.1.6. (Rambaut et al. 2014). The MCMC sampling was considered sufficient at effective sampling sizes higher than 200. After removing 25% of samples as burn-in, the independent runs were combined and a maximum clade credibility (MCC) tree was constructed using TreeAnnotator v.1.8.2. (Drummond et al. 2012). For divergence time estimates, we used four calibration points, employing a standard deviation of 10% of the node age. Three secondary calibrations were done, one according to Betancur et al. (2015), which presented a phylogeny of 1407 ray-finned fish, dated with over 200 fossil records. For the other calibrations, we used fossils, one of pacu teeth described by DeCelles and Horton (2003) (minimum age = 38.0 Ma/offset, mean = 6.75). Another calibration points for *Mylopus* in the Miocene (minimum age/offset = 11.2 Ma, mean = 9.0), (Roberts 1975; Dahdul 2004). And finally, another with *Megapiranha paranensis* (Cione et al. 2009), to calibrate all piranha genera (minimum age = 6.8 Ma/offset, mean = 10.4).

Chromosome number reconstruction

A priori chromosomal information was obtained from the Arai checklist book (2011) and was later updated until 2022 (for more details, see supplementary material 2). The MCC tree obtained in the BEAST analysis was used for the reconstruction of the haploid chromosome number with the ChromEvol software (Glick and Mayrose 2014). This software uses a Maximum Likelihood Estimate (MLE) approach to infer ancestral chromosome numbers along a phylogeny. This is done under customizable models that use different weights for events of polyploidy (whole genome duplication), demiploidy (1.5 × genome increase) and disploidy (gain or loss of a chromosome). The analysis ran for 10,000 simulations under all eight pre-existent chromosome evolution models available on the software. The Akaike information criterion was used to assess the best-fitted model (Mayrose et al. 2010).

Ancestral area reconstruction

To investigate the historic biogeography of Serrasalmidae, we employed a model-based likelihood approach implemented in the R package BioGeoBEARS (Matzke 2013). First, the MCC tree yielded by BEAST was pruned to exclude the outgroup using the function "drop.tip"

implemented in the R package phytools (Revell 2012). Our samples were drawn from the following three ecoregions: (A) Amazon and Orinoco basins, (B) São Francisco basin and Atlantic Coastal drainages and (C) Paraguay, Parana, and Uruguay basins. These areas were chosen based on the distribution of Serrasalmidae species in river basins according to Fishbase information (Froese and Pauly 2022). The distribution data was retrieved from the Global Biodiversity Facility (GBIF) database. We remove duplicates and records with obvious georeferenced errors. This procedure excluded occurrences in the ocean or outside the Neotropical region, those without country names, coordinates with zero latitude or longitude, and coordinates annotated on the coarse-scale grid without decimal precision (Supplementary Material 2).

We used the pruned maximum credibility tree for ancestral range estimation to test likelihood implementations of three different biogeographic models in BioGeoBEARS. The DEC model treats dispersal and extinction as anagenetic events (modeled as free parameters) and sympatry, subset sympatry, and vicariance as cladogenetic events (modeled as fixed parameters) (Ree and Smith 2008). The DIVA model is similar, but it allows widespread vicariance as a possible cladogenetic event (Ronquist 1997). The BAYAREA model assumes that cladogenetic events are not accompanied by changes in geographic areas (Landis et al. 2013). Each of these models was also tested with the addition of the free parameter j, which treats jump dispersal as a cladogenetic event and has been shown to improve model likelihood (Matzke 2014). We compared the results of the models with and without the parameter j using likelihood ratio tests; the model weights were calculated under the Akaike information criterion (AIC). In order to measure the numbers of dispersal, vicariance, and sympatry events, we conducted 100 stochastic mapping replicates under the best model yielded by BioGeoBEARS. Each stochastic map represents a possible biogeographic history considering the chosen model and the estimated parameters (Duplin et al. 2017).

Diversification rate analysis

Shifts in diversification rates were calculated using speciation/extinction model type analysis in BAMM (Rabosky et al. 2014). For this, we used the same pruned phylogeny of the previous analysis. The missing taxa per tip (subgenus) in the phylogenetic tree was estimated according to the total number of species reported for each genus on the FishBase database (Froese and Pauly 2022). We divided the genera in three clades based on the MCC tree to state the percentage of species informed by clade: Clade I, subfamily Colossomatinae (*Colossoma* + *Mylossoma* + *Piaractus*), Clade II, tribe Myleini (*Myleus* + *Myloplus* + *Mylesinus*) and Clade III (subclade a *Metynnis*) and (subclade

b *Catoprion + Pristobrycon + Pygopristis + Pygocentrus + Serrasalmus*), tribe Serrasalmini.

Priors for the BAMM control file were generated using the dated phylogenetic tree input into the function set BAMM priors in the package BAMM tools v. 2.5.0 implemented in R. The control file was set for 1,000,000 generations and the analysis was run twice as recommended, returning similar results. Resulting MCMC Log likelihoods were tested against generation number using the CODA package (Plummer et al. 2006) implemented in R. All remaining outputs contained in the event data file were analysed using BAMMtools in R.

Results

Phylogenetic analyses and divergence time

The MCC tree yielded by BEAST (Fig. 1) showed the family Serrasalmidae as monophyletic with a high support (pp = 0.97) with estimated origin around 48 Mya. Within the family, the genera *Serrasalmus* and *Pygocentrus* were paraphyletic, forming a well-supported clade (pp = 1) that originated approx. 16.9 Mya. The individuals of genera *Catoprion*, *Pygopristis* and *Prystobrycon* formed a monophyletic group with approx. 21.5 My (pp = 0.99). The monophyletic

genus *Metynnis* (pp = 0.99) was found to be the sister of these subclades, with an estimated origin of approx. 25.9 Mya a. These three subclades together were named here as Clade III. The Clade II (pp = 0.99) was formed by genera *Myleus* and *Mylesinus*, diverging around 28.9 Mya. Lastly, Clade I was composed of the monophyletic genus *Piaractus* (pp = 0.99), the paraphyletic genus *Mylossoma* and the representant from genus *Colossoma* (pp = 0.98) and sister to the remaining Serrasalmidae clades (Fig. 1).

Phylogenetic comparative methods

We reconstructed the haploid chromosome number across the phylogeny of subfamily Serrasalminae based on Maximum Likelihood using ChromEvol. After all runs were completed, the AIC scores were assessed for choosing the best-fitted model. The models M1 (CONST_RATE), M2 (CONST_RATE_DEMI), and M4 (CONST_RATE_NO_DUPL) presented lower AIC scores, all very similar (91.32, 91.32, and 89.32 respectively). These three models presented almost identical results when plotted on the phylogeny, therefore the simplest model (M1) was chosen for the discussion of the results (Fig. 2). The ancestral haploid chromosome number of the subfamily was inferred to be n = 28 (pp = 0.5), with a very close probability of n = 29 (pp = 0.43). All the main clades
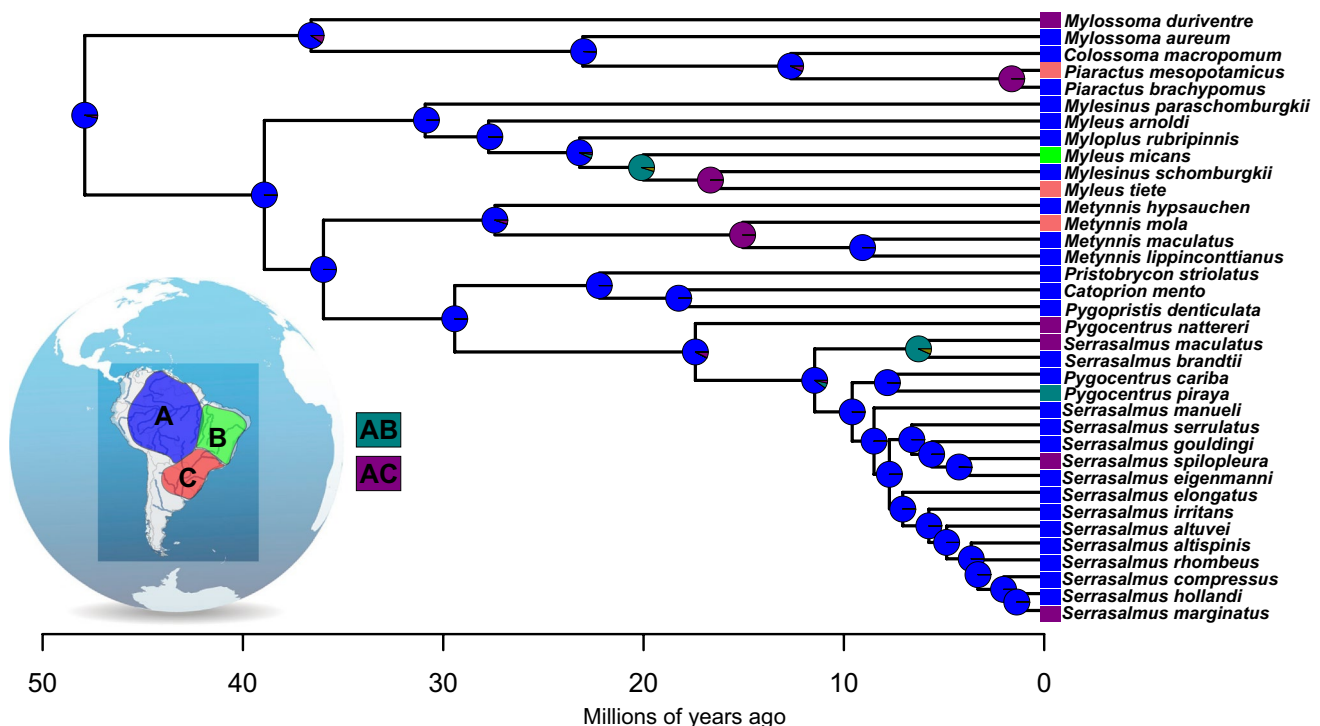
Fig. 1 Ancestral area reconstruction inferred by the DIVALIKE model implemented on BioGeoBEARs. Pies at the nodes represent the posterior probability of a given area, with colors coded as informed on the map on the lower left corner. The axis scale represents the time of divergence in millions of years
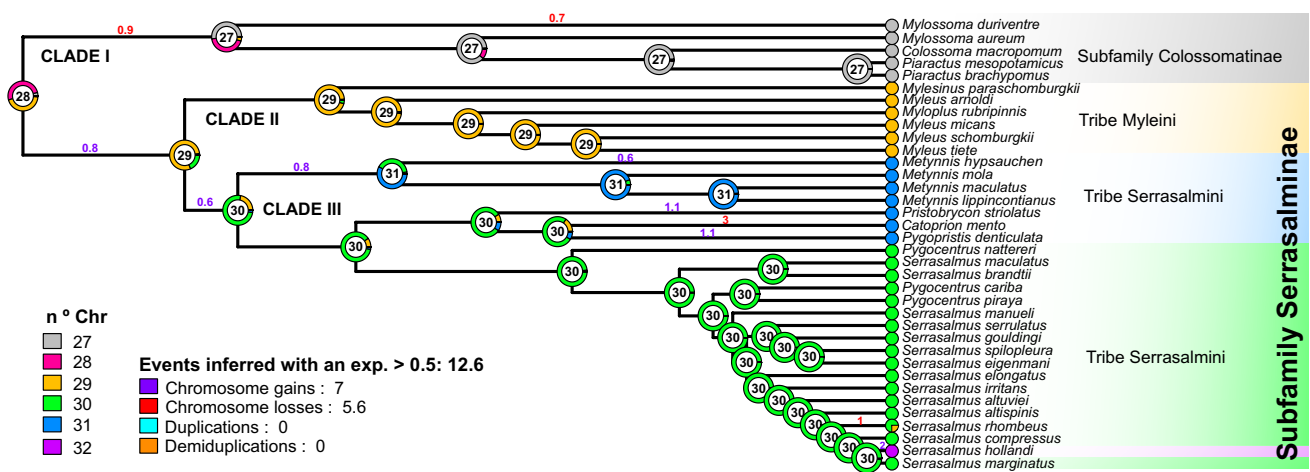
Fig. 2 Ancestral chromosome number reconstruction on Serrasalminae using the model M1 of ChromEvol. Pies at the nodes represent the posterior probability of inferred chromosome number, with the most probable number written at the center of the pie. Numbers above the branches represent the probability of one of the four chromosome number change events (gains, losses, duplication or demiduplication) given by maximum likelihood estimation

presented different ancestral chromosome numbers. Clade III presented n = 30 (pp = 0.87), with independent disploidy events responsible for n = 31 in *Pristobrycon* and *Pygopristis*, and n = 32 in a few *Serrasalmus* species. Clade II presented n = 29 (pp = 0.95), without further number changes. Clade I presented n = 27 (pp = 0.51) with a close possibility of n = 28 (pp = 0.43). According to the results, chromosome evolution on this group was mainly directed by ascending and descending disploidy (exp. of 7 and 5.6 respectively), with no incidences of polyploidy or demipolyploidy.

We used BAMM to detect heterogeneity in evolutionary rates across Serrasalmidae phylogeny. The 95% credible set of rate shift configurations sampled with BAMM presented three distinct shift configurations. The set with the highest probability (f = 0.4, Fig. 3) presented one shift of diversification on the ancestral node of the clade formed by *Serrasalmus* and *Pygocentrus* at around 11 Mya.

We tested whether this increase in the rate of diversification in clade III was associated with biogeographic colonization of new environments. To this, we used BioGeoBEARS for ancestral area reconstruction based on the Bayesian topology. The DIVALIKE model presented the lowest AIC score (90.87), being considered the best-fitted model (Fig. 1). The cladogenetic events were majorly sympatry events (83.9%) with only a few vicariance events (16.1%) mainly at the more recent nodes of the phylogeny. The whole subfamily and each of the main clades was confirmed to have originated in the Amazon region of Brazil, with the first incursions to other regions occurring ~20 Mya on Clade III.

Discussion

Our phylogenetic analyses provided molecular support for the recognition of three main clades in Serrasalmidae, congruent with previous studies (Ortí et al. 2008; Cione et al. 2009; Thompson et al. 2014; Mateussi et al. 2020; Favarato et al. 2021). The previous phylogenies currently subdivide Serrasalmidae into two subfamilies: Colossomatinae [Clade I] and Serrasalminae (split into the tribes Myleini [CladeII] and Serrasalmini [Clade III]) (Mateussi et al. 2020). When evaluating the ancestral karyotype in our topology, it was revealed that during the cladogenesis of this family, there were two distinct events of chromosomal rearrangements. The first one, leading to a descending dysploidy to n = 27 (2n = 54) in the subfamily Colossomatinae (Clade I), and the other, an ascending dysploidy in the subfamily Serrasalminae with Myleini and Serrasalmini tribes (Clade II and III), showing n = 29 (2n = 58), n = 30 (2n = 60), 31 (2n = 62) and n 32 (2n = 64) respectively. Regarding the first diverging lineage of the family, some studies have suggested n = 27 as the most plesiomorphic karyotype, due to its presence in older groups, such as the genera *Mylossoma*, *Brachypomus*, *Colossoma* and *Piaractus* (Nakayma et al. 2012). The chromosome number 2n = 54 was also detected in other representatives of the order Characiformes, such as the families Anostomidae and Prochilodontidae, which have a high degree of chromosomal conservation (Vicari et al. 2006; Aguilar and Galleti 2008). However, upon conducting the ancestral chromosomal reconstruction, we did not obtain evidence that would support this hypothesis. During the early
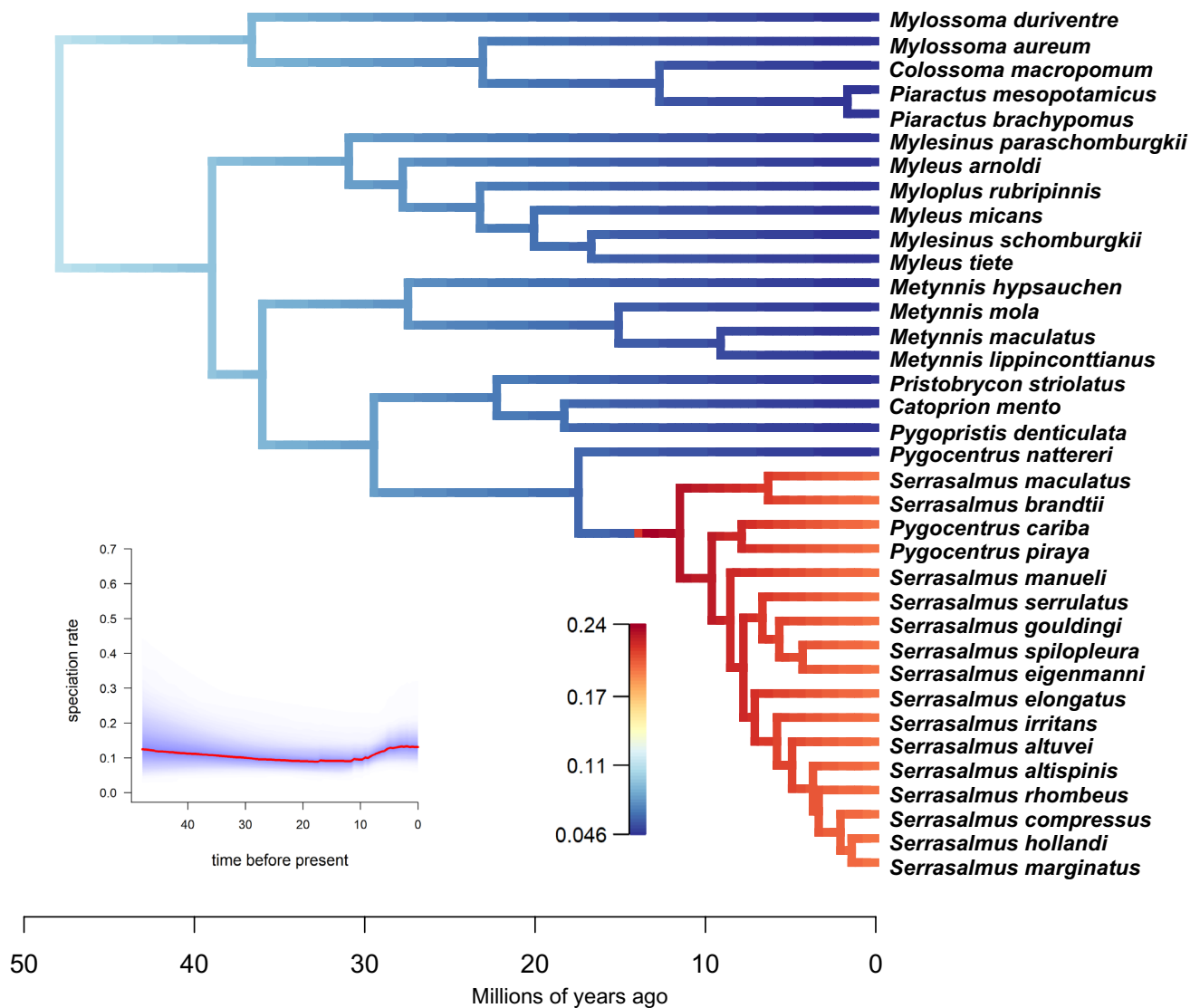
Fig. 3 Best credible set of rate shift configuration as estimated in the BAMM analysis. Warmer branch colors represent higher rates of net diversification as informed on the legend bellow the tree. On the lower right corner, we present a speciation rate x time of divergence (in My) for the subfamily

divergence of the Serrasalmidae family, we observed two distinct trends: one characterized by chromosomal conservation and the other by variation, resulting in an increase in the chromosomal number.

Throughout our chromosome number reconstruction, it was possible to identify ascending dysploid events (increasing the chromosome numbers) in the most derived genera, such as *Mylesinus*, *Myleus* and *Myloplus*, which present 2n = 58. This increase becomes accentuated in representatives of the most diverse genera, *Pygopristis*, *Pygocentrus* and *Serrasalmus* with 2n = 60, 62 and 64. The differential morphology of karyotypes with more derived chromosome numbers, such as an increase in acrocentric chromosomes, indicates that chromosomal fission rearrangements drive the

karyoevolution of the Serrasalmidae family (Nakayama et al. 2012). Centric fissions lead to karyotype diversity within a population, consequently increasing the probability of genetic isolation and speciation (Perry et al. 2004). Indeed, these processes appear to have been one of the main mechanisms in cladogenetic events in this family.

Our biogeographic reconstruction and molecular dating also allowed us to understand the phylogenetic relationships in a temporal context and discuss geographic distribution across space and time. Regarding the geographic distribution, most of the representatives of Serrasalmidae occur in the Amazon basins, which suggests that this region is the center of origin of the group. On the other hand, much is discussed about the evolutionary origin of the family, which

has often been controversial. Some authors suggest an older origin, around 66–56 Ma, during the Middle Paleocene, but with the beginning of its diversification around 45 Ma, in the late Eocene (Thompson et al. 2014; Burns and Sidlauskas 2019). Another study pointed to a younger age, between 42 and 38 Ma (Kolmann et al. 2021). Our analyses point to a scenario between 48 and 38 Ma, within the threshold of previous studies, associated with the uplift of the Andes (Armijo et al. 2015). In this context, the diversification scenario has been congruent with these studies (Burns and Sidlauskas 2019; Kolmann et al. 2021).

The cladogenetic events that separate the three major clades are related to the late Eocene and early Oligocene (38 to 30 Mya) in our study, which coincides with the great division of the West–East Amazon drainage, with the origin of the Purus Arc and a period of mega-wetland formation in the proto-Orinoco-Amazonas (Lundberg et al. 1998; Albert and Reis 2011). The uplift of this arc is due to an orogenetic response to the initial elevation of the Andes Mountain range, which consequently may have caused allopatric speciation in some fish species (Armijo et al. 2015). This context of allopatry is reinforced by the presence of fossils of *C. macropomum*, known as pacus, in the Magdalena River basin in Colombia, which today is an inhabitant of the Amazon and Orinoco rivers. This has suggested that this taxon inhabited ancient systems that connected the Amazon and Magdalena River basins, today separated by the Andes Mountain range (Lundberg et al. 2009). Ecological factors have also been associated with the diversification of frugivorous pacus, which coincides with the diversification of fruit plants during the Eocene (Correa et al. 2015). However, with regard to chromosomal aspects, the subfamily Cossolomatinae was the group that presented descending dysploidy (2n = 54), which has not presented an increase in chromosomal diversification, contrary to the subfamily Serrasalminae, constituted by an ascending to dysploidy with 2n = 58 to 64.

During the Lower Eocene and Upper Miocene (30–20 Mya), the uplift of the Andes in the North and Central region culminated in a change in the course of several rivers (Lundberg et al. 1998). These orogenetic processes significantly altered the hydrography in South America, leading to fracturing processes in several basins, with redirection of river courses and headwater capture events (Hoorn et al. 2010; Evenstar et al. 2015). These processes may have provided ecological opportunities and colonization of Serrasalmideos for new habitats, providing speciation events (Melo et al. 2018, 2022; Roxo et al. 2019; Ochoa et al. 2020). Together with these cladogenetic mechanisms, they may have triggered chromosomal rearrangements with a tendency towards ascending dysploidy. However, our biogeographic reconstruction demonstrated that dispersal/ vicariance events apparently did not accompany karyotypic changes.

As the ancestral chromosome reconstruction shows, ascending dysploidy lead to higher chromosome numbers at the most derived lineages of Serrasalminae, culminating in the 2n = 60 karyotype being predominant in the *Serrasalmus* and *Pygocentrus* genera. In addition to this, our data revealed an increase in the diversification rate during the Miocene (11–8 Mya), involving these genera. These findings are in agreement with other studies, which also pointed to rapid and recent radiation involving this group of piranhas, which have diversified greatly in the plains of South America (Hubert and Renno 2006; Hubert et al. 2007). The apparent correlation between the 2n = 60 karyotypes and the increase in diversification rate, coupled with the tendency for ascending dysploidy in the family, may point to a scenario in which high chromosome numbers are associated with species diversification and evolutionary success. However, causation would be hard to infer until further and more detailed genomic studies for these groups are provided.

During the late Miocene and Pliocene, hydrological and paleogeographic events may have driven changes in the diversification rates of this group. In relation to other river basins, the Amazon separated from the Paraná-Paraguay system by 10 Ma, leading to the separation of the ichthyofauna in these systems (Hubert and Renno 2006). Headwater capture events have also been identified between the Upper Paraná and São Francisco basins around 10 Mya (Hubert and Renno 2006). Dispersions such as separations between watersheds, provided by tectonic activations and changes in the course of rivers (geodispersion), may have provided a certain advantage for carnivorous piranhas, in dispersing species and conquering new habitats, reducing predation and competition. These processes can lead to increased rates of speciation and diversification, as happened in the subfamily Hipostominae (Cardoso et al. 2012). In addition to orogenetic movements, sea level fluctuations may also have further contributed to promoting this diversification (Hubert and Renno 2006). In the last 10 Mya, the sea level has varied from 35 m above to 122 m below the current level and may also have contributed to accelerate this process (Hubert et al. 2007). In this context, in addition to allopatric speciation, processes of sympatric speciation have also been detected in some sister lineages of piranhas, associated with habitat heterogeneity. As is the case of *S. compressus* n = 30 and *S. hollandi* n = 32, which live in the Madeira River and diverged in the last 2 Ma (Hubert et al. 2007).

All the discussed scenarios highlight that historical and ecological processes seem to have shaped this family's genetic and phylogenetic diversity, involving several types of chromosomal rearrangements. In this context, ascending dysploidy seems to have driven the karyotypic evolution of some lineages towards higher chromosome numbers,resultin in highly diversified persistent lineages across different hydrographic basins of South America.

These data, combined with previous studies (Correa et al. 2015), demonstrate that the rate of diversification in Serrasalmidae is not correlated with biogeographic changes, but that it could possibly be linked to ecological processes that lead to morphological changes (Kolmann et al. 2021), as well as karyoevolutionary differentiation by dysploidy. Generally, dysploidy does not necessarily imply changes in DNA content, only in the structure of chromosomal rearrangements that can occur in the genome. These processes, so far, have been considered to have a neutral effect in relation to the diversification of evolutionary processes over the long term (Escudero et al. 2014).

Final considerations

Our data demonstrate that chromosomal rearrangements played an important evolutionary role in major cladogenetic events in Serrasalmidae, revealing them as a powerful evolutionary driver in family diversification. Our results support the hypothesis that ascending dysploidy acted as one of the main drivers in the chromosomal evolution of the Serrasalminae family and seems to be more correlated with diversification patterns than biogeographic history. In this context, we suggest the importance of integrating cytogenetic studies to evaluate the systematic aspects of the Serrasalmidae family. In addition, we highlight the importance of correctly interpreting the karyotype in a phylogenetic and biogeographic context.

Author contributions All authors wrote the main and reviewed text the manuscript.

Declarations

Competing interests The authors declare no competing interests.

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