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Systematic Reassessment of the Serrasalmidae Family Using Molecular Markers

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Abstract

Using partial sequences of two mitochondrial genes, Cytochrome b and Cytochrome c Oxidase I, the current study analyzed the genetic and evolutionary relationships between seven piranha species belonging to the genera Serrasalmus and Pygocentrus. The study was conducted in the Paraná-Paraguay, São Francisco, and Tocantins River basins. Bayesian inference and maximum likelihood phylogenetic analysis were techniques that were utilised to determine between these species. The findings showed that Serrasalmus rhombeus and Serrasalmus marginatus, as well as Serrasalmus maculatus, Serrasalmus brandtii, and Serrasalmus eigenmanni, had more genetic similarities than the two Pygocentrus species (P. nattereri and P. piraya). High levels of intraspecific genetic diversity were seen in Pygocentrus nattereri, S. rhombeus, and S. maculatus. Each species has at least two distinct mitochondrial lineages, now found in allopathy (P. nattereri and S. maculatus) or sympatry (S. rhombus). Studies on species delimitation and the sizeable genetic variability between populations of S. rhombus and S. maculatus suggested that each species might be a complex cryptic species. The fact that S. maculatus and S. rhombus are not monophyletic gives credibility to the theory. The geographic distribution and genetic differentiation pattern of the piranha species investigated here is explored in connection to the geological and hydrological processes that happened in the hydrographic basins.

Keywords: Systematic Reassessment (SR), Serrasalmidae Family (SF), Molecular Markers (MM)

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Introduction

The manufacture of evidence in a scholarly manner on a topic which can be elaborated in a very unique way by the application of complicated methods to define, elaborate and approach the research on that specific topic comes under the category of a systematic review. The extraction and elaboration of data from the studies that are done by scholars already on that specific topic is called a systematic review. In this procedure, analysis, elaboration, admiring that topic critically and then summing up that elaboration into a clear outcome that is also based on evidence(Dahruddin, 2019). Let us understand this topic with the help of an example, a systematic review of the trial that is controlled randomly is a unique way that can be applied to the summarization and implementation of that medicine which is based on evidence. Systematic review is a term that can also be applied to the fields of biomedical and health care. Its utilization can also be seen where analysis of the matter which needs to be defined in a precise manner can make the understanding in that respective field at an advanced level. In the review of systematic type clinical tests, interventions about public health and environmental interventions can be seen(Garavello et al., 2021). Certain other interference can also be involved in it which can be social intervention, adverse effects, evidence synthesis of qualitative type, reviews based on methodological manner, reviews that are about the policy and the evaluations having a deep connection with the economic status. A close connection can be seen between systematic reviews and meta-analyses and sometimes both of these things can be combined(Souza et al., 2022). One of the differences between these two things is that meta-analyses utilize the methods of statistical types to impose a number that should be single from the set of data that needs to be pooled. Whereas a definition of systematic review is very strict and clear, this step must be excluded. When

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these things are done practically then it comes to know that when one thing is said to be done then the other thing will also be involved as the systematic review is taken by it to gather the information which is analyzed by meta-analysis(Bagley et al., 2019). People may also mention it as an example for systematic review, the component of meta-analytical Type is also involved in it. There are two reviews which are comparable with each other, one is the systematic review and the other one is the narrative review. A literature review is the actual thing which is done in both the systematic review and the literature review. But if the term literature review is mentioned in such a way that there is no further description is mentioned regarding it then it will be considered as a narrative review. The designation of literature review is done in such a way that it will provide a summary of current literature thoroughly that can be related to the research question too(Melo et al., 2022). In a systematic review, a very strict and clear approach can be seen for the synthesis of research. The aim should be assessment and by looking at attitude, the possibility minimizes the level of bias in the outcomes. A family of characiform fishes which are given the status of a family in recent times are named Serrasalmidae. More than 90 species are involved in it (Röst & Sadeghimanesh, 2023). The name Serrasalmidae represents the term serrated salmon family(Oliveira et al., 2011). This name is given to the fishes of this family due to the presence of serrated keel which is running along with the abdominal region of these fishes. Fishes which are given the names of Serrasalmidae are also given the names of pacu, piranhaa and silver dollar. These are the names which are usually given due to the designation of different characters of teeth and different styles of feeding. The size of Serrasalmids varies from medium to large size and the fishes should be of characiform type which can extend up to 1m long and can be equal to 3.3 feet. Another characteristic of these fishes is that their body is deep and compressed laterally along with the series of abdominal spines that can be midventral. Not necessarily spines that can also be scutes(Dahdul et al., 2010). A long dorsal fin is also found in them. A biological molecule which comes into the category of measurable indicator that is found in blood and many other fluids of the body or tissues comes under the category of molecular markers(Kolmann et al., 2021). A fragment of DNA that has a strong connection with a certain location within the territory of the genome is described under the term molecular marker. The role of molecular markers is the Creation of clear differences between base pairs that are found at a specific place which is also known as polymorphism within the pool that is of unknown DNA. The types of molecular markers can be of two types that may be PCR based or hybridization of DNA-based. Genes and all those micro RNAs which are mentioned in benign and cancerous cells come into the category of molecular markers. The utilization of molecular markers can be seen in the specimens of thyroid biopsy to diagnose the cancer cells or to determine the concept that the nodule is benign(Jacobina et al., 2023). Two tests of molecular markers which are very common include Afirma GSC and Thyroseq. All the studies which were done in previous times which are based on the foundation of DNA sequencing of mitochondrial rRNA genes represent three main groups that are found in the family of Serrasalmidae. The first one is the pacu which is the clade of herbivores (Young, 2023). The second one is the Myleus clade and the third one is the piranha clade. However, resolution is very poor which is found in each clade that can be obtained due to a very low level of changing among the rRNA gene sequencing(Ortí et al., 2008). Because ornamental fishes have been heavily exploited for many years, little is known about their biology and ecology, including population genetics and capacity for regeneration. We created Project Piaba as an integrated research program to continue the earlier Project Piaba efforts, and PRONEX, a Brazilian Program for Excellence Groups, just sanctioned it. Providing a framework for characterising the population genetics of ornamental fish wild populations in the Rio Negro basin and applying the data to practical management and conservation issues is the main goal of the fish genetics group. In contrast to current molecular genetic studies on Amazonian fish species, these analyses have not included many ornamental fishes. We must, however, highlight certain findings in Symphysodon spp. utilising the mtDNA gene 16S and 234 Jorge I. R. Porto et al. nuclear loci (I.P. Faris at the University of Amazonas) and in Paracheirodon axelrodi using D-loop sequences (Harris & Petry, in this book). To maximise the potential utility of the genetic information of the Amazon fishes, we believe there are still a few factors to take into account. First, collaborate with local fishermen to determine the precise spot where the ornamental fishes were captured. Second, consider the fish species' biogeography. 3. Collaborate with taxonomists. Fourth, compare all of the data with the various Project Piaba interfaces. Physiology, ecology, socioeconomics, and other fields are among them. Finding out which genetic traits could be involved in taxonomy, evolution, species management, and conservation challenges will only be feasible in this manner.

Research Objective

The main objective of doing this research is development of an understanding of the topic of Systematic Reassessment of the Serrasalmidae Family Using Molecular Markers. So that the role of molecular markers can be understood while investigating the family of Serrasalmidae.

Literature Review

Researchers reveal that the presence of Serrasalmus brandtii species is observed in the Francisco River. but it is introduced in some other aquatic environments to study its behavioral changes .serralsalmus species was first introduced in Dice River to understand the impact of this species on the native assemblages of other fish species(Assis et al., 2024) Studies explain that the approach of hybridization is adopted in the fish farming technique. new and optimized species have been produced through the process of hybridization.in certain circumstances, the improper management of the fish farming system results in the loss of hybrid species from the protected areas.to study the impact of hybrid species and their interaction with other fish species the process of molecular identification of hybrid species is carried out in the Parana River (Cavalcanti, 2024).studies suggest that the Amazon River has a diversified range of species that makes the richness of species in this region up to fifteen percent. The serralmidesa has a specific eating behavior. it eats herbivores as well as carnivore species. Most fruit-eating fish species are found in the flooded forest as they are considered the oldest (Coronado Franco, 2023).studies claim that species' diversity results from their genetic variation. the species of fish found in the neotropical river are at risk of decline because of the impact of various anthropogenic factors.to save such species requires formulation of proper conservation strategy(Delord et al., 2023).scholars reveal that the presence of Myxozoans species is indicated in the blood of fish species. in the Amazon River Myxozoans species is found in the blood of myetynnis lippincottianus.the blood of the myetynnis lippincottianus was taken through syringes and then ten percent EDTA solution was added to predict the presence of Myxozoans in the blood of this specie(Façanha et al., 2024)studies predict that estimation of the number of species and counting of specie is a process carried out through CNN. imaging technology based on the CNN approach is utilized for evaluating the number of Serrasalmidae species in the water(Fernandes et al., 2024).studies reveal that fish species found in the upper Paraguay River are conserved using effective conservation strategies Most of the important species are found in the river basin and thus require proper monitoring for their conservation(Fernando, 2024)studies conclude that the growth behavior of two different groups of Serrasalmidae specie is studied by giving them each with different feed.after a few months, the growth of different feeding Serrasalmidae species was determined. the result of the study predicts that hybrid as well as pure Serrasalmidae specie shows variation in growth patterns(Gervaz et al., 2023).studies reveal that freshwater aquatic species migrate form one ecosystem to another to maintain their biodiversity during time of climatic fluctuations. the flow of water in freshwater bodies allows fish species to migrate into another freshwater environment (Herrera-R et al., 2024).scholar reveals that the diversity of Serrasalmidae species is due to the high number of chromosomes present in their body. the macroevolutionary studies reveal that chromosomal number in Serrasalmidae species is one of the prominent factors that result in the species diversification process(Jacobina et al., 2023).scholarly studies reveal that certain pollutant substances harm the fish species present in the aquatic environment. The health of aquatic species gets negatively influenced due to the entry of pollutants from the environment Certain species' behaviors were assessed to determine the impact of pollutants on their health.the behavior of species belonging to the Serrasalmidae group was assessed using biomonitors (Loureiro et al., 2023).studies suggest that species present in the Philippines River show great biodiversity. information regarding the freshwater species present in the Parana River is low because of less number of studies made on freshwater species.to assess the species of San Augustin the use of net-based modified tools was used during the year 2016.this assessment provided information about the diverse number of freshwater aquatic organisms (Macalisang et al., 2023) Studies were made on Serrasalmidae to determine the closely related species of this family. the studies reveal the myloplus schomburgki species related to Serrasalmidae through the technique of DNA barcoding. also, morphological analysis of this species reveals that it is present in the Amazon River (Machado et al., 2024).scholars explain that in the freshwater of South America, the *piaractus mesopotamicus* species is found. this species has commercial importance and is found in aquaculture to make its growth optimized.the high-quality *Piaractus mesopotamicus* grown in aquaculture are sold in the market as excellent quality seafood (Mascali et al., 2024). The general uniformity in the amount of ribosomal sequence locations in each clade suggests that Serrasalmidae conserves its chromosome organisation. Even if the mapping of telomeric sequences did not show any rearrangement, differences in the number and location of rDNA sequences among species may indicate that these sequences have evolutionary independence, either within or between the genera of Serrasalmidae. For example, pair 7 of the Serrasalmus species contains the homeology of the 5S rDNA, and Metynnis possesses synteny in all three of the species that were studied. The number of diploid chromosomes is another example of this conservation; all species in the Colossomatinae have 2n = 54. In Serrasalminae, the number of diploid chromosomes rose; the study discovered 2n = 58 (Myleus, Myloplus, and Tometes). Metynnis species have 2n = 62 chromosomes, whereas Serrasalmus and Pygocentrus have 2n = 60. The ancestral number of the family was formerly thought to be 2n = 54 chromosomes, with a tendency to increase the 2n number from 54 to 62 chromosomes. Since all chromosomes in Colossomatinae have two arms (meta and submetacentric type), but certain pairs of chromosomes in the derived Serrasalmini have only one arm (acrocentric), chromosomal fission would have been the source of the increase in diploid number. studies show that in the river basin of the South Amazon River, the two species were found and observed. the first species was mylosssoma aurem and the second was mylossoma duriventre. the number of parasites observed in the blood of the second species was more than observed in the first one. the difference in the number of parasitic worms found in these species is because of their evolving taxonomic features(Pelegrini et al., 2024).studies elaborate that successful reproductive cycle of aquatic species gets negatively affected by certain polluting factors.the change in the habitat of fishes spices makes their survival difficult.to conserve the freshwater species the use of supplemental technological tools are employed in the aquatic management system(Ribolli et al., 2023).scholar studies suggest that the population of invasive species in Brazilian freshwater is increasing.the patterns shown by the aquatic Brazilian species are studied to distinguish native and nonnative species(Rocha et al., 2023)to understand the diverse taxonomic characteristics of Serrasalmini species studies were made on different species related to the Serrasalmini group. the chromosomal behavior of the three species of Serrasalmini was studied to understand the diversity of each species present in the Serrasalmini-related species group(Santos et al., 2023).studies suggest that the amount of seafood produced by aquaculture is huge .aquacultures are a source of production of sustainable seafood of high quality but sometimes parasitic diseases spread in the fish species of aquaculture to avoid massive species loss the use of chemotherapeutics is made for the species of aquaculture Parasite affecting the fish species are treated by IVM which has great therapeutic applications(Shaik et al., 2024).studies elaborate that suing molecular makers for studying genetic polymorphism in fish species holds importance. microsatellites are used as a source of molecular markers for performing assessment studies on aquatic species for the conservation of the aquatic population(Wenne, 2023).

Figure 1: Serrasalmidae

Serrasalmidae Family

Because they are regarded as a luxury food in South America, the species in this clade are the most commercially significant in this family. Members of this group are used to cultivate most of the native species produced in Brazil's continental farming. Aquaculture of these species has shown to be an effective way to meet consumer demand while reducing the harm that large-scale commercial fishing exploitation causes to natural population stocks. Interspecific hybridisation, a conventional method of genetic improvement in culture systems, has been used to make interspecific crosses, mostly for the species Colossoma macropomum, Piaractus mesopotamicus, and Piaractus brachypomus. These hybrids are produced because they have better meat quality, are more resistant to cold, and develop more quickly than the original species. In some regions of Brazil, hybrids outnumber their parent species. For instance, species P production is higher in the midwestern region of the country, even if "Tambacu" productivity surpasses 8359 tonnes. With about 2,000 species currently recognised from several drainages in the New World and Africa, the order Characiformes makes up one of the major components of the freshwater fish fauna worldwide. In the past ten years, more than 300 characiform species—mostly from the Neotropics—have been reported, and the rate at which new species are being described is not slowing down. The abundance of both species and supraspecific taxa in the characiform faunas on either side of the Atlantic Ocean show a clear imbalance. There are over 220 known species in the order's African components. They extend south from the Nile River basin in the North African deserts to much of the rest of the continent, with the highest variety found in the wetter areas such as the Congo River Basin, West Africa, and Lower Guinea. On the other side of the Atlantic Ocean, which extends from the southwest parts of the United States south to Mexico, Central America, South America, and central Chile and Argentina, there are now over 1,700 species known to exist. All of the main drainage basins in South America have sizable and taxonomically overlapping assemblages of characiform species. From the swift-moving rivers and streams of the Andean piedmont and Neotropical cordilleras to the lentic backwaters of lowland flood plains in the Americas and Africa, characiforms inhabit a wide range of habitats. There are hundreds of medium-sized to large characiform species in these environments, in addition to dozens of tiny and small species. Many of the bigger varieties have ecological and economic significance, and some of them dominate the overall biomass of fish in different drainages. In lowland river systems, this and other characiform species are important for material cycling, intra-ecosystem energy exchange, and ecosystem engineering.

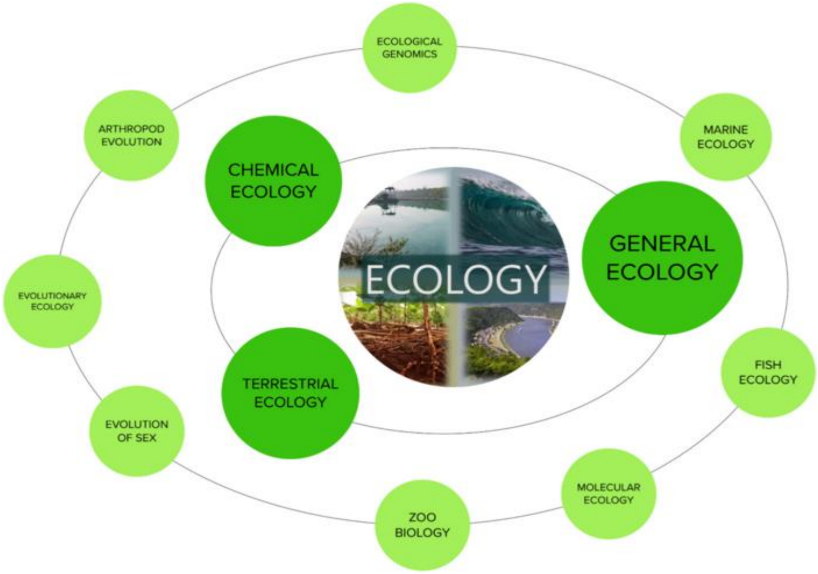

Figure 2: Ecology

Ecology

Along with seeds, fruits, leaves, and a range of invertebrate and vertebrate foods, the various serrasalmid fishes also consume fish flesh, scales, and fins. Authors often use the fruit-and-leaf-eating pacus and the more carnivorous piranhas to illustrate the variety of diets. Most members of the family are herbivorous, with the exception of piranhas. Piranhas, on the other hand, have long been thought to be strict carnivores. Depending on their age and the availability of resources, many animals alter their diet. According to phylogeny, the largely herbivorous (albeit omnivorous) Metynnis is also a member of the primarily carnivorous piranha group, which also includes the genera Catoprion, Pristobrycon, Pygocentrus, Pygopristis, and Serrasalmus. Based on ecology and, to a lesser degree, phylogeny, the surviving mostly herbivorous species can be categorised into two groups: Colossoma, Mylossoma, and Piaractus are primarily found in waterways that move slowly. They consume a lot of fruits, nuts, and seeds and are important seed dispersers. Mylesinus, Myleus, Ossubtus, Tometes, and Utiaritichthys inhabit fast-moving river segments and mostly eat aquatic plants, especially Podostemaceae. Myloplus mostly consumes plant stuff, and some of its species are phylogenetically related to the preceding group, despite the fact that this genus includes species of both slow- and fast-flowing rivers.

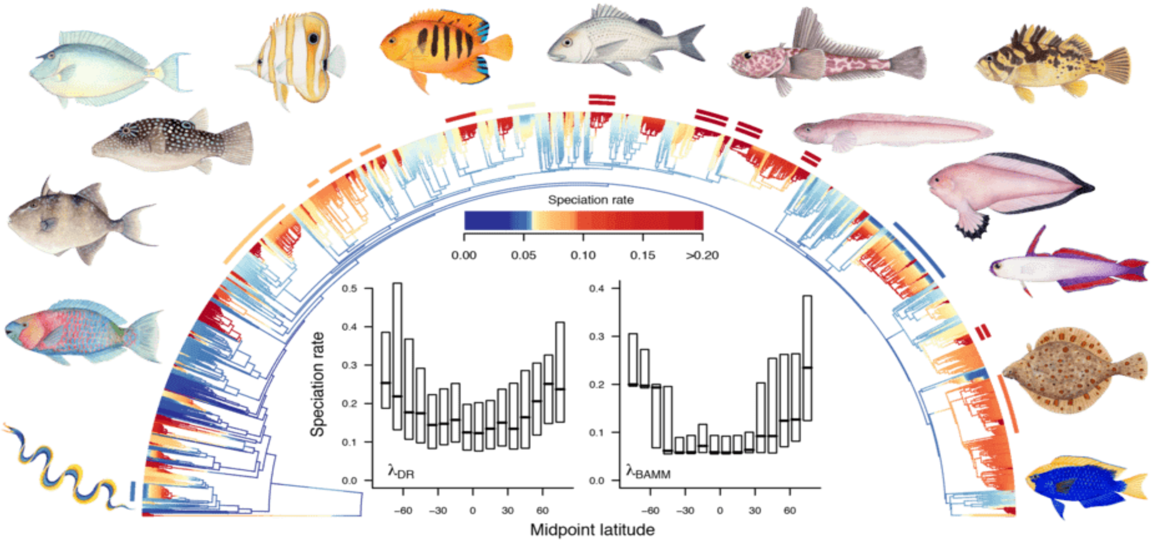

Figure 3: Serrasalmidae Texonomy

Taxonomy

The Serrasalmidae were recently assigned to the Characidae subfamily. Their relationship to other characiforms is yet unclear. Numerous uncertainties remain concerning the systematics and taxonomy of piranhas and their relatives. Because of this, many taxa struggle with phylogenetic position as well as species identification. The ongoing classification of these species is difficult and often contentious, though, because ichthyologists base ranks on characteristics that may occasionally overlap (see Cladistics). Ultimately, classification could be quite arbitrary.

Relationship to Humans

A number of pacus, including Piaractus and Colossoma, are commercially significant to commercial fisheries and aquaculture, while several serrasalmids are sought-after as ornamentals for aquariums. Although they are often sold for food in local markets and devoured by subsistence fishermen, piranhas are typically less valued. A small number of piranha species are periodically sold in aquariums, and dried specimens have been sold as souvenirs to tourists in recent decades. Although major attacks are uncommon and the threat to people has been overblown, piranhas do bite and occasionally damage swimmers and bathers. However, because they break nets and other equipment, steal bait, mutilate catch, and can attack when handled, piranhas pose a significant annoyance to both commercial and recreational fishermen.

Discussion

The current work expands our knowledge of Serrasalmidae cytogenetics, mainly with respect to Myleini, by including species of the genera Myloplus and Tometes for the first time and increasing the number of Metynnis species with chromosomal information within Serrasalmidae to five. Understanding the evolution of the following tribe members is important since Metynnis was the first genus in Serrasalmini to separate. In order to better understand the phylogenetic relationships at the intergeneric level, especially with regard to paraphyletic genera like Myleus, Myloplus, Tometes, and Pristobrycon, eight species had species-specific karyotype formulas and/or fundamental numbers. These can be helpful when paired with molecular markers. We discovered that the pericentromeric and terminal portions of multiple chromosomes in Myleini and the species of Metynnis under investigation contain large heterochromatic blocks. Other Myleini and Serrasalmini species have already been found to have this pattern. Serrasalmus compressus Jégu, Leão & Santos 1991 and Serrasalmus elongatus Kner 1858 were also found to have totally heterochromatic short limbs, similar to those found in Myloplus schomburgkii and Myloplus lobatus. This observation may be connected to interspecific differences in Myloplus. These heterochromatic arms and blocks might be signs of chromosomal rearrangements, such as non-R or Robertsonian rearrangements, which may or may not alter 2n. Certain mapped ribosomal sequences are linked to heterochromatic blocks in the species examined in this study. The distinction among karyomorphs, where the chromosomes are mostly subtelocentric/acrocentric with 18S ribosomal cistrons, is C-band positive, was directly impacted by this relationship in the case of piranhas, such as Serrasalmus rhombeus. Heterochromatin may be producing spots that are vulnerable to chromosomal breaks and influencing the group's karyotype evolution. These chromosomes appear to have undergone rearrangements, resulting in differences in the 2n and karyotype formulae. The 18S and 5S rDNA sites found in Myloplus and Tometes species had single 5S and multiple 18S labelling, which is similar to the placement pattern previously reported in the family. All Serrasalmus species that have been examined so far have displayed this 5S rDNA localisation pattern. A taxonomic identifier may be created from this pattern of ribosomal sequences since both locations offer distinct markers for every species under study. Furthermore, all of the Serrasalmus species under study possessed this location in the interstitial area of pair 7, indicating that 5S rDNA was previously identified as a pertinent cytogenetic marker within the family. Therefore, we advise using 5S rDNA in conjunction with DNA barcoding, which has been widely used in recent years, in the family's integrated taxonomy method. Even with the numerous indications of new species utilising COI, none of the 14 new taxa that have been discovered in the previous ten years—despite the advancements in species description of Serrasalmidae—included a finding of Serrasalmus.

Conclusion

The morphological and chromosomal diversity of Serrasalmidae is great. Based on molecular theory, the family is currently divided into two subfamilies: Serrasalminae and Colossomatinae. The two tribes that comprise Serrasalminae are Myleini, which include most pacus species, and Serrasalmini, which comprises Metynnis, Catoprion, and the other piranha genera. This study aimed to analyse species of the tribes Myleini (Myloplus asterias, M. lobatus, M. rubripinnis, M. schomburgki, and Tometes camunani) and Serrasalmini (Metynnis cuiaba, M. hypsauchen, and M. longipinnis) using both classical and molecular cytogenetic methods in order to understand the chromosomal evolution of the family. The four species of the genus Myloplus and T. camunani had 2n = 58 chromosomes, while the species of Metynnis had 2n = 62. Heterochromatin was most common in the pericentromeric regions of all species. Tometes camunani and Myloplus species shared a single 5S rDNA site. Numerous 18S rDNA markers were discovered in T. camunani, M. asterias, M. lobatus, M. rubripinnis, and M. schomburgkii. In contrast, all three species exhibited 18S and 5S rDNA synteny for Metynnis, with M. longipinnis displaying an additional 5S marker. When compared to the family's phylogeny, these data point to a propensity for the number of diploid chromosomes to rise from 54 to 62. This tendency was nonlinear and the product of many chromosomal rearrangements. Furthermore, ribosomal sequence sites and other karyotype formulae can be utilised as cytotaxonomic markers to help identify species. Characiform taxa that lack a supraorbital bone and have a derived location of the hyoid artery's emergence from the anterior ceratohyal are included in the Characidae, a monophyletic collection that is firmly supported by all of our phylogenetic study. We suggest modifying the boundaries of a few families to acknowledge this and a number of other monophyletic groupings within the Characiforms. This will make it easier to do future research in the Characiforms, especially the Characidae. A novel phylogenetic framework for a speciose and morphologically varied group of freshwater fishes with major ecological and evolutionary significance throughout the Neotropics and parts of Africa is presented in this paper. Fish farming and seed production are the two complementing subsectors of fish culture in Brazil. Companies all around the nation produce anything from 4 to 50 million juvenile Serrasalmid seeds annually. A small percentage of fish farmers grow their own seeds. Due to the specific issue of morphological identification of Serrasalmid hybrid juveniles (Senhorini, personal communication), as well as of adult F1 Serrasalmid hybrids, the trade between seed providers and fish farmers is therefore a crucial link in the productive cycle. Brazil does not yet have any organizations or procedures for seed certification. Because it lowers productivity or crop value and might lead to the development of incorrect broodstocks, mixing of Serrasalmid stocks could thus pose a serious challenge to the aquaculture sector. Furthermore, two issues should be taken into account when discussing the possible environmental effects of Serrasalmid hybrids: the fertility of Serrasalmid hybrids and the escapes and releases of these hybrid individuals from fish farms. Nevertheless, research on the true genetic-ecological effects of Serrasalmid fish hybrids is still lacking. Fish hybrid introductions may generally have negative ecological impacts, and many populations and species have gone extinct as a result of hybridization's negative effects, mostly those brought on by introgression. It is important to reduce morphological identification issues in order to implement measures pertaining to the management and surveillance of Serrasalmid hybrids. Therefore, fish species and their hybrids may be identified almost perfectly using molecular techniques. However, the previously unreported 18S and 5S sequence synteny of the Metynnis species can be considered an unusual characteristic for the family. The number of markers for Me. maculatus (Kner 1858) and Me. lippincottianus, two congeners that look comparable but are not closely related based on molecular phylogenies, matched the number of 18S rDNA sites discovered in one extra chromosomal pair in addition to the syntenic pair. As noted for some genera of Julidini (Perciformes), the co-location of these cistrons in the three species appears to show that this condition is maintained and repeated within Metynnis, suggesting some adaptive benefit for preserving this arrangement in the genera. According to the rDNA data, the family's clades and genera appear to be well-organised and conserved. Two pairings of the 5S rDNA sequence are found in the pericentromeric areas of C. macropomum, Mylossoma spp., and Piaractus mesopotamicus (Colossomatinae). In contrast, this ribosomal site is marked by a single meta/submetacentric pair in Pygocentrus, Serrasalmus (Serrasalmini), Myleus micans, Myloplus, and Tometes (Myleini). On the other hand, 18S rDNA significantly increases the number of basal clade sites that lead to the derived clade. There are one to three pairs of 18S carriers in the species Myleini. All species of Pygocentrus and Serrasalmus have at least five pairs of these sequences, with acrocentric chromosomes clearly having the majority.

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