

Major biogeographic events in the Amazon shaped the diversification of *Acanthodoras* species (Doradidae: Acanthodoradinae): Chromosomal diversification and reconstruction of the ancient diploid number for the thorny catfishes

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Abstract

The fishes of the Doradidae family constitute one of the most diverse groups of Neotropical freshwater environments. Acanthodoradinae is the oldest lineage and sister group to all other thorny catfishes, and includes only the genus *Acanthodoras*. The diversity of *Acanthodoras* remains underestimated, with several putative species yet to be described, as well as others requiring revalidation. The development of complementary approaches, including genetic studies, is an essential step to better characterize the diversity and relationships among the species within the genus. Therefore, we conducted a comprehensive analysis using conventional cytogenetic techniques and mapping of seven repetitive DNA sequences in two sympatric species from the Negro River: *Acanthodoras cataphractus* and *Acanthodoras* cf. *spinosissimus*. The new set of genetic data generated in our study allowed us to realize the reconstruction of the ancestral chromosome number ($2n$) for Doradidae. The results revealed species-specific patterns that efficiently diagnose both species. The microstructural divergences are possibly a product from a complex interaction among chromosome rearrangements and repetitive DNA dispersal mechanisms, whose origins and fixation rates directly reflect the geomorphological history of the Amazon basin. The karyotypic data now available for the Acanthodoradinae subfamily confirms that the $2n = 58$ chromosomes is the plesiomorphic condition of Doradidae, a crucial step for understanding the chromosome evolution of thorny catfishes and closely related groups. This study contributes to the understanding of the mechanisms behind chromosome diversification and karyotypic evolution of the Doradidae family and highlights the importance of the Acanthodoradinae subfamily in the evolutionary history of thorny catfishes.

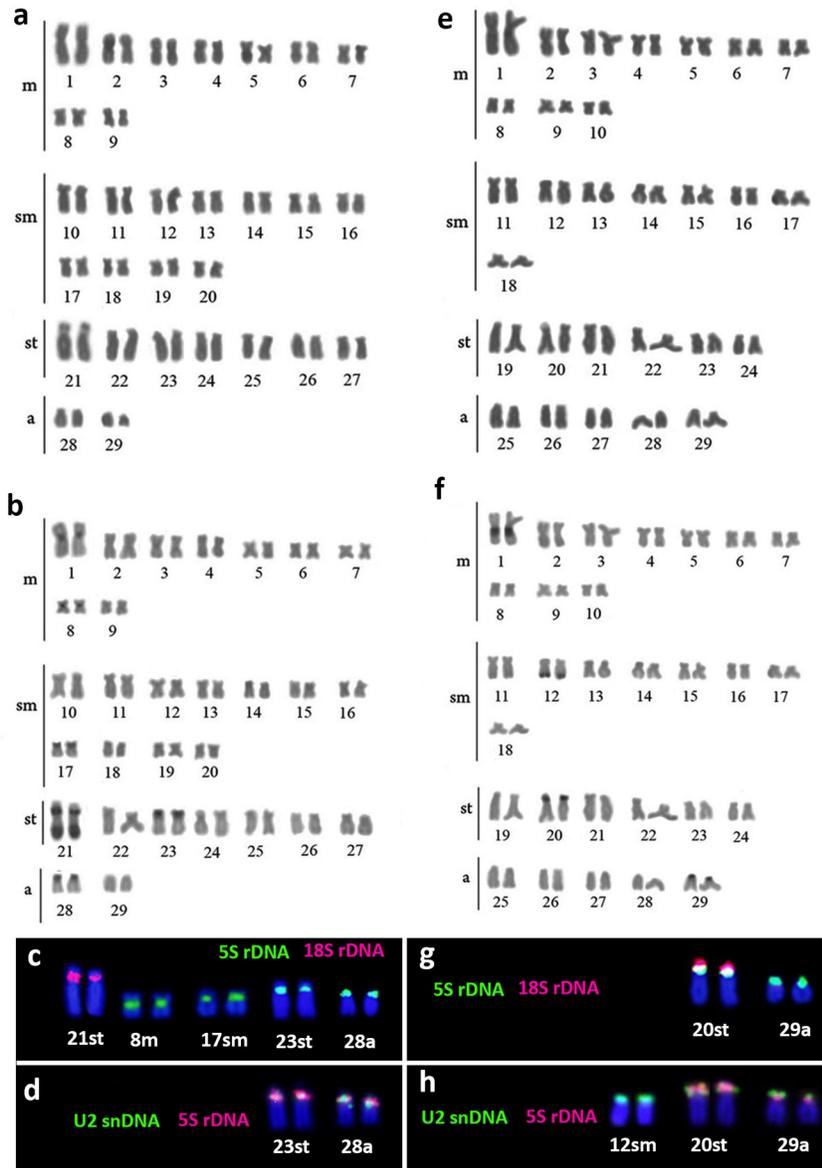


Figure 1: Karyotypes of *Acanthodoras* species after conventional and molecular cytogenetics techniques: *Acanthodoras* cf. *spinosissimus* (a) Giemsa staining; (b) C-banding; (c) FISH with 18S rDNA (pink) and 5S rDNA (green) probes; (d) FISH with U2 snDNA (green) and 5S rDNA (pink) probes. *Acanthodoras cataphractus* (e) Giemsa staining; (f) C-banding; (g) FISH with 18S rDNA (pink) and 5S rDNA (green) probes; (h) FISH with U2 snDNA (green) and 5S rDNA (pink) probes

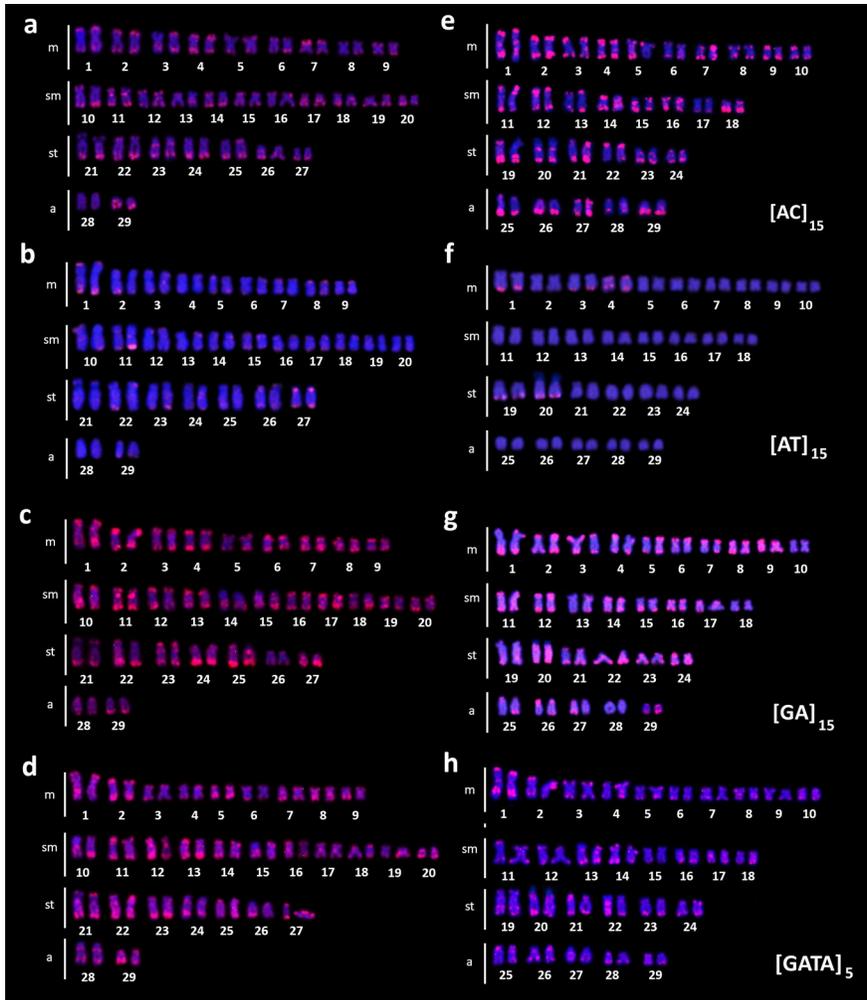


Figure 2: Karyotypes of *Acanthodoras* species after Physical mapping by FISH using several microsatellites motifs: *Acanthodoras* cf. *spinosissimus* (a) FISH with (AC)₁₅ (b) FISH with (AT)₁₅; (c) FISH with (GA)₁₅; (d) FISH with [GATA]₅. *Acanthodoras* *cataphractus* (e) FISH with (AC)₁₅ (f) FISH with (AT)₁₅; (g) FISH with (GA)₁₅; (h) FISH with (GATA)₅

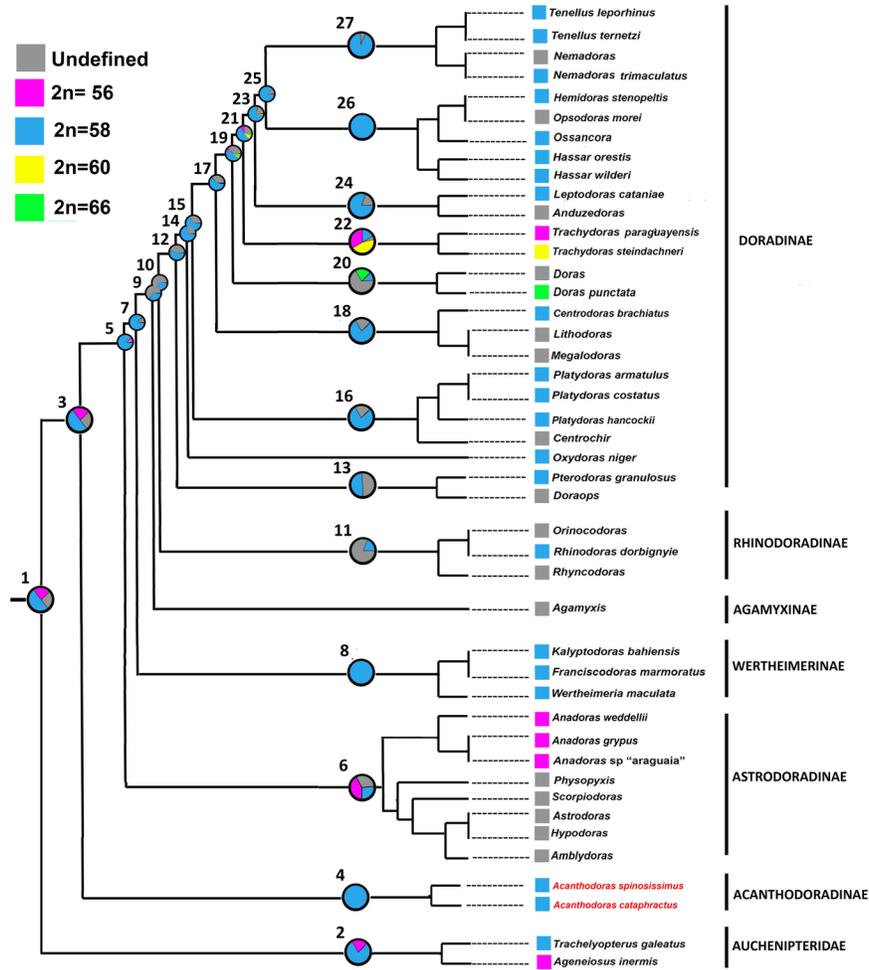


Figure 3: Consensus trees showing maximum likelihood ancestral state reconstructions of diploid numbers based on the Mk1 model using the Mesquite software. This evolutionary analysis integrated cytogenetic data available for Doradidae species (including the present study) and two Auchenipteridae species (sister group) with sequences of two mitochondrial DNA fragments (COI and 16S) and one nuclear DNA fragment (Rag 1) obtained from the molecular phylogeny of Sabaj and Arce (2022)