



Different roles of the Amazon-Orinoco barrier on the genetic structure of two sardine genera from the Western Atlantic Ocean

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Abstract Three sardine species of *Harengula* and one of *Opisthonema* (Clupeiformes, Clupeidae) are known in the Western Atlantic, where the Amazon-Orinoco plume has been recognized as a major biogeographic barrier, albeit permeable to larger and generalist species. Here we used mitochondrial *cox1* gene DNA sequences to check the lineage delimitation of both genera, testing the influence of the Amazon-Orinoco barrier (AOB) and marine provinces on their phylogeographic structure. Results indicate that the two genera are differently affected by the AOB,

including cryptic speciation in *Harengula* and population structure in *Opisthonema*. *Harengula* show a broad distribution in the Brazilian Province (BRA) distinct from *H. clupeola* and *H. jaguana* from the Greater Caribbean Region (GCR). Divergence time between *Harengula* from the GCR vs. BRA was estimated as about 2.4 Mya, which coincides with the period of increasing sediment and freshwater discharge of the Amazon River in the Atlantic. Results also indicate the existence of a single species of *Opisthonema*, albeit with population structuring related to the marine provinces. Since species of both genera are relevant to artisanal fisheries and the maintenance of oceanic ecosystems, these results may help in fisheries management of these important marine resources.

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Introduction

Taxonomic uncertainties and lack of proper stock delimitation can seriously compromise the management of fisheries resources (Carvalho & Hauser, 1994; Ward et al., 2005). Fisheries statistics are particularly sensitive to species misidentification (FAO, 2016), a situation that is common among pelagic and forage fishes. Additionally, molecular tools are useful in identifying hidden diversity, including species relevant to fisheries (Tourinho et al., 2012; Thomas et al., 2014; Lima et al., 2017). The use of mitochondrial DNA, despite its limitations, has already revealed cryptic species in several commercial fishes (Mat Jaafar et al., 2012; Wu et al., 2016; Durand et al., 2017; Jacobina et al., 2020), resulting in a refined understanding of species distributions and population structure.

Clupeids are among the most economically relevant forage fishes for both artisanal and industrial fisheries worldwide, such as sardines and herrings (Whitehead, 1985; Birge et al., 2021). In addition to serving as food, they are also often used as bait for fishing larger species (Whitehead, 1985; Lopes et al., 2017). They are most often marine and pelagic, but some species exclusively inhabit freshwaters, whereas others are euryhaline or anadromous (Whitehead, 1985). *Harengula* Valenciennes 1847 and *Opisthonema* Gill 1861 are two clupeid genera from the Atlantic and Pacific coasts of the New World. Both are represented by small to medium-sized schooling fishes that are usually found along the coast but also in estuaries and lagoons (Whitehead, 1985; Miller et al., 2005; Petry et al., 2016; Pinheiro et al., 2018). *Harengula* is also distinct in the Southwestern Atlantic since it is the only clupeid in the Brazilian oceanic islands of the Rocas Atoll, and

the Trindade-Martin Vaz and Fernando de Noronha archipelagos (Gasparini & Floeter, 2001; Sazima et al., 2006; Pinheiro et al., 2018).

The Atlantic thread herring *Opisthonema oglinum* (Le Sueur 1818) is the only species of the genus in the Western Atlantic (WA), distributed from the Gulf of Maine in USA to the estuary of la Plata River in Uruguay (Munroe et al., 2015a). In turn, three species of scaled-sardines of the genus *Harengula* are recognized in the WA: *Harengula clupeiola* (Cuvier 1829), *Harengula humeralis* (Cuvier 1829), and *Harengula jaguana* Poey 1865 (Whitehead, 1973, 1985; Fricke et al., 2024). While *H. humeralis* is reported from the eastern Florida (USA) to the French Guiana (Munroe et al., 2015b) and possibly in the north coast of Brazil (Cervigón, 1991; Robertson & Van Tassell, 2023), *H. clupeiola* and *H. jaguana* are broadly distributed from the east coast of USA to southern Brazil (Whitehead, 1985; Munroe et al., 2015c, 2019). *Harengula humeralis* can be easily distinguished from congeners based on morphology (Whitehead, 1985; Cervigón, 1991), but anatomical distinction between *H. clupeiola* and *H. jaguana* is challenging (Rivas, 1963; Berry, 1964; Whitehead, 1967, 1973, 1985). In the most recent revision of the genus, Whitehead (1985: 66) also indicated the likely existence of one or more subspecies of *H. jaguana* along its extensive range, as previously suggested by Rivas (1950). This might indicate population structuring or cryptic species in the genus, as extensively detected in several coastal species previously considered as having a wide distribution in the WA (Colborn et al., 2001; Rocha, 2003; Luiz et al., 2012; Rodríguez-Rey et al., 2017; Dias et al., 2019; Petean et al., 2020; Araujo et al., 2022). In Brazil, they are usually identified as *H. clupeiola*, but both species are reported, while in the oceanic islands a single morphotype is known but its identity is uncertain (Figueiredo & Menezes, 1978; Whitehead, 1985).

Genetic structuring of clupeids can be related to temperature, salinity, and depth, considering that these oceanographic features are known to influence other marine taxa with pelagic larvae (Palumbi, 1994; Floeter et al., 2008; Luiz et al., 2012; Stern et al., 2018; Jacobina et al., 2020). In the WA, the Amazon-Orinoco barrier (AOB) marks the limit between the Greater Caribbean biogeographic region (GCR), composed by the Carolinian and the Caribbean

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Provinces in the north, and the Brazilian Province (BRA) in the south (Floeter et al., 2008; Briggs & Bowen, 2012; Robertson & Cramer, 2014). The AOB is considered a biogeographic filter acting in the genetic structuring of several marine coastal fishes especially since the Pleistocene (Rocha et al., 2002, 2008; Rocha, 2003; Floeter et al., 2008; Luiz et al., 2012; Reis et al., 2016; Jacobina et al., 2020; Araujo et al., 2022; Quintão et al., 2022). The effectiveness of this barrier varied across taxa and with increased sedimentation and sea-level fluctuations through interglacial and glacial periods (Rocha, 2003; Figueiredo et al., 2009; Ludt & Rocha, 2015; Araujo et al., 2022). The AOB is also less effective in the isolation of larger species or those that are more tolerant to variations in salinity (Araujo et al., 2022; Giachini Tosetto et al., 2022).

The effectiveness of a barrier can be estimated by comparing the genetic structure of species with distinct biological attributes and from different marine provinces (Araujo et al., 2022). Herein we tested the role of the AOB in the genetic structure of two sardine genera along the WA based on DNA sequences of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene. Since both *Harengula* and *Opisthonema* are pelagic fish, we expect to find a genetic signature of the AOB on them. However, due to the smaller size (common length: 10–12 cm vs. 20 cm standard length, respectively, Whitehead, 1985) and putative subspecies along its distribution, *Harengula* must be more affected by the AOB and other oceanographic barriers than *Opisthonema*. Additionally, we provide information regarding taxonomy and fisheries stocks, including the identity of *Harengula* from Fernando de Noronha oceanic archipelago, which is in the center of a fisheries conflict in the island (Mendes et al., 2020).

Material and methods

DNA extraction, amplification, and sequencing

DNA sequences were obtained from specimens acquired in fish markets, collected using a 5 m-long beach-seine (5 mm mesh) or deposited in fish collections from 19 localities along the Brazilian coast and the Fernando de Noronha oceanic archipelago, off northeastern Brazil (Online Resource

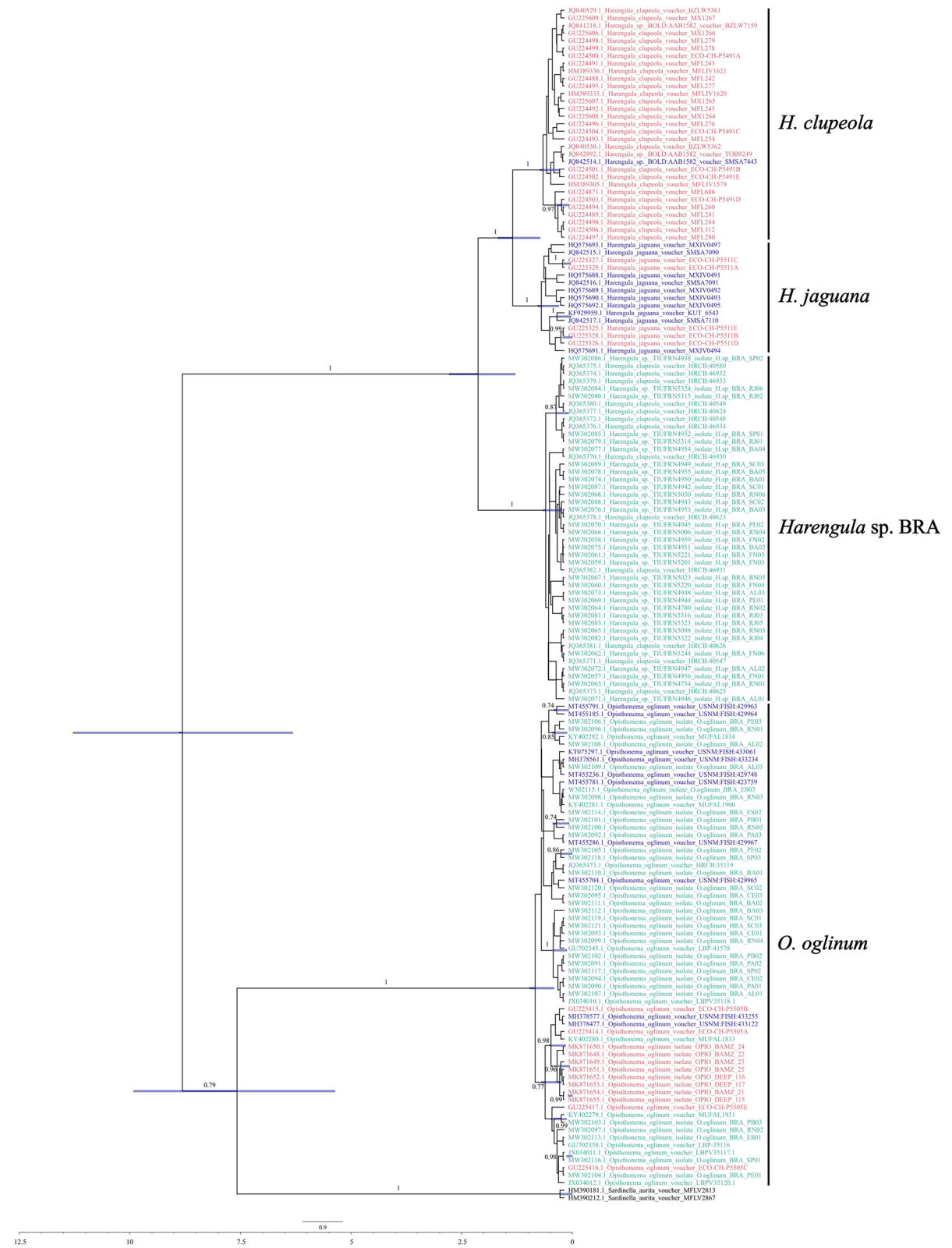
1). Collection of specimens in Fernando de Noronha was conducted under the System Authorization and Information on Biodiversity permit (SISBIO n° 67671-1). Tissue samples were stored in 98% ethanol, and the voucher specimens were fixed in a formaldehyde 4% solution, transferred to a 70% ethanol solution, and then deposited in the ichthyological collections of the Universidade Federal do Rio Grande do Norte (UFRN) and the Instituto de Biodiversidade e Sustentabilidade, Universidade Federal do Rio de Janeiro (NPM). Specimens were morphologically identified at the genus level based on Figueiredo and Menezes (1978) and Whitehead (1985).

Genomic DNA was extracted by saline protocol based on Bruford et al. (1998) with some modifications (Online Resource 2). DNA amplification by PCR was performed using the GoTaq® Green Master mix (Promega, USA) and the primers FISH-BCL (5'- TCAACYAATCAY AAAGATATYGGCAC) and FISH-BCH (5'- TAA ACTTCAGGGTGACCAAAAAATCA) of the mitochondrial gene *cox1* (Baldwin et al., 2009). PCR steps consisted in a first cycle of 2 min at 95 °C, 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 1 min, and a final cycle of 10 min at 72 °C, according to Baldwin et al. (2009). Amplicons were sequenced in both directions by Macrogen Inc (<https://dna.macrogen.com/>).

Additional sequences of specimens from USA, Mexico, and Caribbean were obtained from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) and aligned with our sequences (Online Resource 1). Three datasets were assembled: the first (*Harengula* dataset) includes sequences of *H. clupeiola*, *H. jaguana*, and *Harengula* sp. from the WA, with *Sardinella aurita* Valenciennes 1847 as outgroup; the second (*Opisthonema* dataset) includes sequences of *Opisthonema* from the WA, also with *S. aurita* as outgroup. Finally, the third dataset (combined dataset) is the merger of the two previously mentioned datasets.

Phylogenetic analysis and lineage delimitation

Forward and reverse sequences of *Opisthonema* and *Harengula* were edited, and consensus sequences of 555 bp were defined in SeqTrace v. 0.9 (Stucky,



◀**Fig. 1** Bayesian time-calibrated tree including sequences of *Harengula* and *Opisthonema* from the Western Atlantic (combined dataset). Clade colors represent Carolinian Province (blue), Caribbean Province (red), and Brazilian Province (green). Numbers on branches are posterior probability values. Blue bars over nodes are confidence intervals for dates of cladogenetic events. Scale in millions of years (Mya)

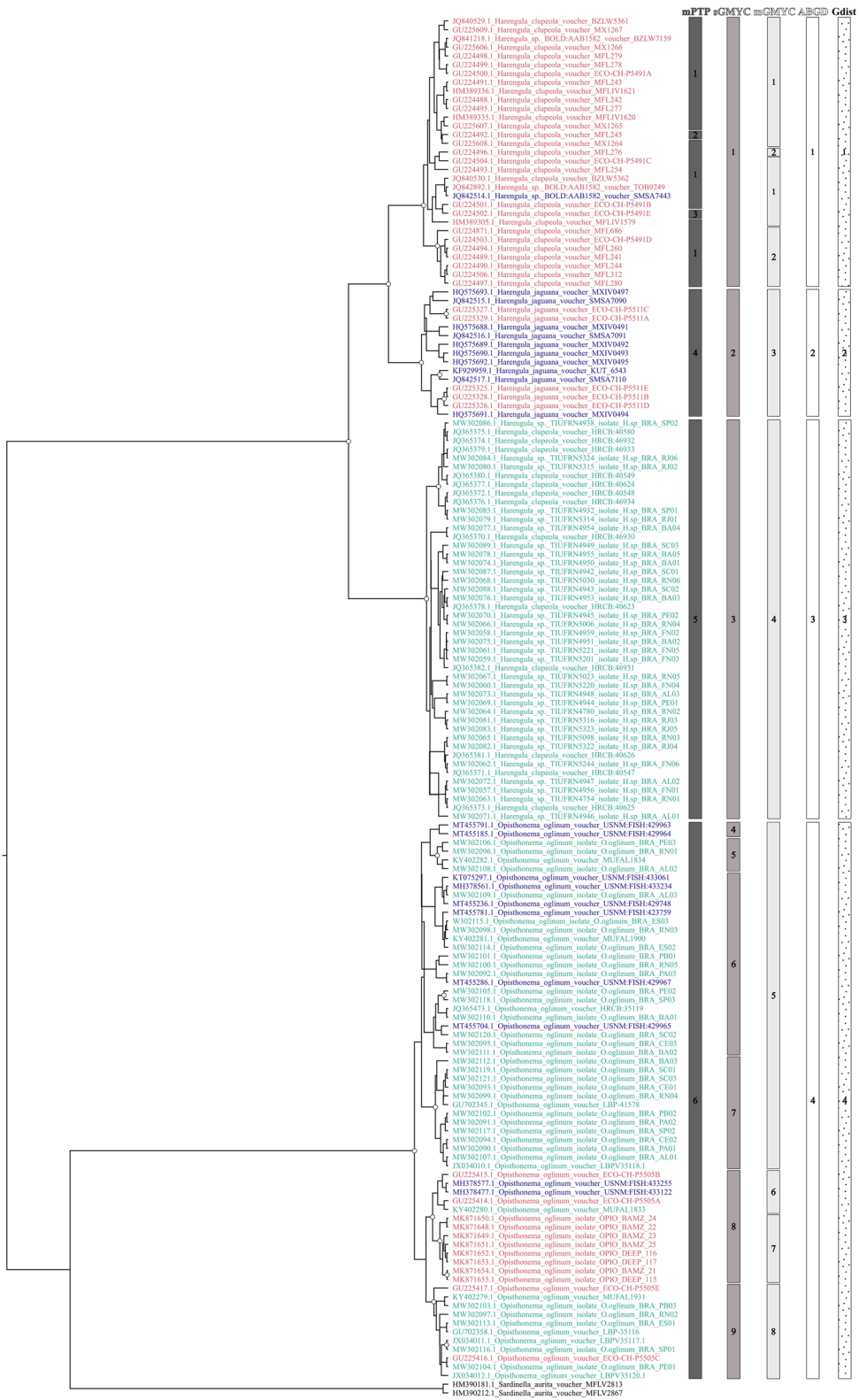
2012). The sequences obtained were deposited in GenBank (MW302057-MW32121, Online Resource 1). Sequences of the *Opisthonema*, *Harengula*, and the combined datasets were separately aligned using the MUSCLE algorithm (Edgar, 2004), and with the best evolutionary models selected in MEGA 11 (Tamura et al., 2021). Following the Bayesian Information Criterion, the evolutionary model used for the combined dataset was Kimura 2-parameter with invariant sites (K2P+I), while for the *Opisthonema* and *Harengula* datasets were K2P with gamma distribution (K2P+G).

Bayesian Inference (BI) was performed in BEAST v. 1.10.4 (Suchard et al., 2018) using the following parameters: substitution model as Hasegawa-Kishino-Yano with invariant sites (HKY+I) for the combined dataset, and HKY+G for the *Harengula* and *Opisthonema* datasets with base frequencies as all equal (since there is no K2P model in BEAST, the equivalent of it is HKY with base frequencies equal). The selected clock type was strict with normal distribution and mean of 0.01 mutations/Mya, a substitution rate suggested for fish mtDNA (Bermingham et al., 1997; Thomaz et al., 2015), and standard deviation of 0.001. The tree prior model was set as speciation with Yule process for the combined and *Harengula* datasets and coalescent with constant size for the *Opisthonema* dataset. The pInv prior (proportion of invariant sites parameter) selected was normal distribution with mean of 0.61 and standard deviation of 0.01 for the combined dataset. The alpha prior (gamma shape parameter) selected was normal distribution with mean of 0.24 and 0.17 and standard deviation of 0.01 for *Harengula* and *Opisthonema* datasets, respectively. The Markov chain Monte Carlo (MCMC) simulations were run with 20,000,000 generations and sampled every 2,000 generations in all datasets. Other parameters were set as default. To ensure quality of the MCMC simulations, ESS values of at least 200 were checked using Tracer v. 1.7.1 (Rambaut et al., 2018). TreeAnnotator v. 1.10.2 was used to summarize results of BEAST into a single

tree with burn-in of 20% and a posterior probability limit of 0.5. The final trees for each dataset were visualized and edited in FigTree v. 1.4.4 (Rambaut, 2018).

For lineage delimitations, we analyzed the *Harengula* and *Opisthonema* datasets using four single-locus methods: multi-rate Poisson Tree Processes (mPTP) (Kapli et al., 2017); single-threshold Generalized Mixed Yule-Coalescent (sGMYC), and multiple-threshold GMYC (mGMYC) (Fujisawa & Barraclough, 2013); and Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2012). A Maximum Likelihood tree generated on MEGA 7 (Kumar et al., 2016) with 1,000 replications, Nearest-Neighbor-Interchange with branch swap filter as moderate, was used as input tree for mPTP. The mPTP was performed on an online server (<https://mptp.h-its.org/#/tree>) using the default parameters. Ultrametric trees generated on BEAST from both the *Opisthonema* and *Harengula* datasets were used as input file for sGMYC and mGMYC. Both analyses were performed on an online server (<https://species.h-its.org/gmyc/>). ABGD distance-based analyses were run through the online server at <https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>, with the relative gap width of 1.0 and the remaining parameters set as default for all the distances available (Jukes-Cantor, Kimura, and simple distance). In this analysis, the delineation considered was the one with p-value of 0.01, as suggested by previous studies (Puillandre et al., 2012; Blair & Bryson, 2017). For the delimitation based on genetic distance, the genetic divergence (K2P) was calculated in MEGA 7 and a threshold value was set using the cut-off values of 2% of divergence for *cox1* (Ward, 2009).

To detect molecular structuring in *Opisthonema* and *Harengula*, we used GENELAND, which does not require a priori assignment of samples (Guillot et al., 2005). The GENELAND analysis was based on an uncorrelated frequency model, which is used to delimit clusters of possible distinct lineages (Pavón-Vázquez et al., 2018), with a minimum population number 1 and maximum population number 10. The spatial model was selected to infer the number of clusters in nine independent runs using 1,000,000 MCMC iterations, of which every 1,000 was retained. A burn-in of 200 was applied and the run with the highest mean logarithm of posterior probability was used to compute the posterior probabilities of



◀**Fig. 2** Bayesian Inference tree and lineage delimitation analyses of *Harengula* and *Opisthonema* from the Western Atlantic. Clade colors represent Carolinian Province (blue), Caribbean Province (red), and Brazilian Province (green). White circles over nodes indicate high posterior probability values (>0.85). Bars on the right side indicate lineages delimited by the following analyses: mPTP—multiple rate PTP; sGMYC—single-threshold of Generalized Mixed Yule-Coalescent; mGMYC—multiple-threshold GMYC; ABGD—Automatic Barcode Gap Discovery; Gdist—Genetic distance (K2P). Number inside the bars correspond to grouping

population membership. Additionally, a haplotype network was inferred using the TSC method in PopART software (Leigh & Bryant, 2015) to highlight the degree of divergence and spatial distribution of the molecular diversity of each taxa along the three marine provinces. To further investigate population structure, we performed Analyses of Molecular Variance (AMOVA) using Arlequin v. 3.5.2.2 (Excoffier & Lischer, 2010) to test the structuring hypotheses regarding the AOB, the marine provinces and those suggested by GENELAND, for each taxa. Significance was inferred using 1,000 random permutations. Gene flow (Φ_{ST}) was also accessed in AMOVA, as the variance among populations within groups.

Results

A total of 33 *cox1* sequences of *Harengula* and 32 of *Opisthonema* from the BRA were sequenced and edited, then aligned with 61 sequences of *H. clupeiola*, *H. jaguana*, and *Harengula* sp., and 32 sequences of *O. oglinum* available in GenBank. The Bayesian analysis of the combined dataset (Fig. 1) indicated a clearly distinct assemblage formed by all sequences *Harengula* sp. and *H. clupeiola* from BRA, including individuals from the Fernando de Noronha Archipelago, which is herein referred to as *Harengula* sp. BRA. This clade is sister of *Harengula* from the GCR, with most specimens identified as *H. clupeiola* restricted to the Caribbean Province (except for a single *Harengula* sp. from Florida, USA), and all *H. jaguana*, most of them from the Carolinian Province (10 in 15) in well-supported clades (posterior probability=1). The separation between *Harengula* sp. BRA and the clade formed by *H. clupeiola* and *H. jaguana* was estimated at approximately 2.5 Mya

(3.5–1.5 Mya), suggesting the Amazon-Orinoco plume as a barrier, but also a signature of the marine provinces in the genetic structuring. In *Opisthonema*, there are few well-supported clades, and the analysis overall failed to reveal a clear biogeographic pattern (Fig. 1), indicating a permeable role of the Amazon-Orinoco plume in this widely distributed species.

Similar results were obtained in the lineage delimitation analyses of *Harengula* (Fig. 2). The sGMYC, ABGD, and genetic distance (Gdist) also indicated three lineages, two of them formed by *H. clupeiola* and *H. jaguana* clades from the GCR, and another formed by *Harengula* sp. BRA (Online Resource 3). All analyses recovered both *H. jaguana* and *Harengula* sp. BRA clades. Lastly, sequences of *H. clupeiola* from GCR were subdivided in different lineages in the mPTP and mGMYC analyses (Fig. 2). For the *Opisthonema* dataset, delimitation analyses were also incongruent among themselves, with mPTP, ABGD, and genetic distance (Gdist) indicating a single lineage. However, the sGMYC and mGMYC analyses subdivided *O. oglinum* into several lineages that are not concordant with the AOB or the marine provinces (Fig. 2).

Molecular structuring analyses also recovered three main lineages of *Harengula* separated by the AOB and marine provinces, one formed by sequences of *H. jaguana* and *H. clupeiola* from Carolinian Province, other containing *H. jaguana* and *H. clupeiola* from Caribbean Province, and another restricted to BRA (Fig. 3). In *Opisthonema*, while three populations were indicated by the analysis, we were not able to visually determine these clusters based on the result map (Fig. 4).

The haplotype network of *Harengula* showed a deep structure, with 18 mutational steps (4.8% genetic divergence) between *Harengula* sp. BRA and the group formed by *H. clupeiola* and *H. jaguana* from GCR. Ten mutational steps (2% genetic divergence) also separate *H. clupeiola* and *H. jaguana* clades (Fig. 5). Individuals from the Fernando de Noronha Archipelago share the same haplotype from the northeastern Brazilian coast. The haplotype network of *Opisthonema oglinum*, in turn, failed to reveal any clear genetic structure, except for two haplotypes exclusively from the Bermuda Archipelago (GCR) and from a few localities in BRA.

The AMOVA results for both *Harengula* and *Opisthonema* agree with other results. In *Harengula*,

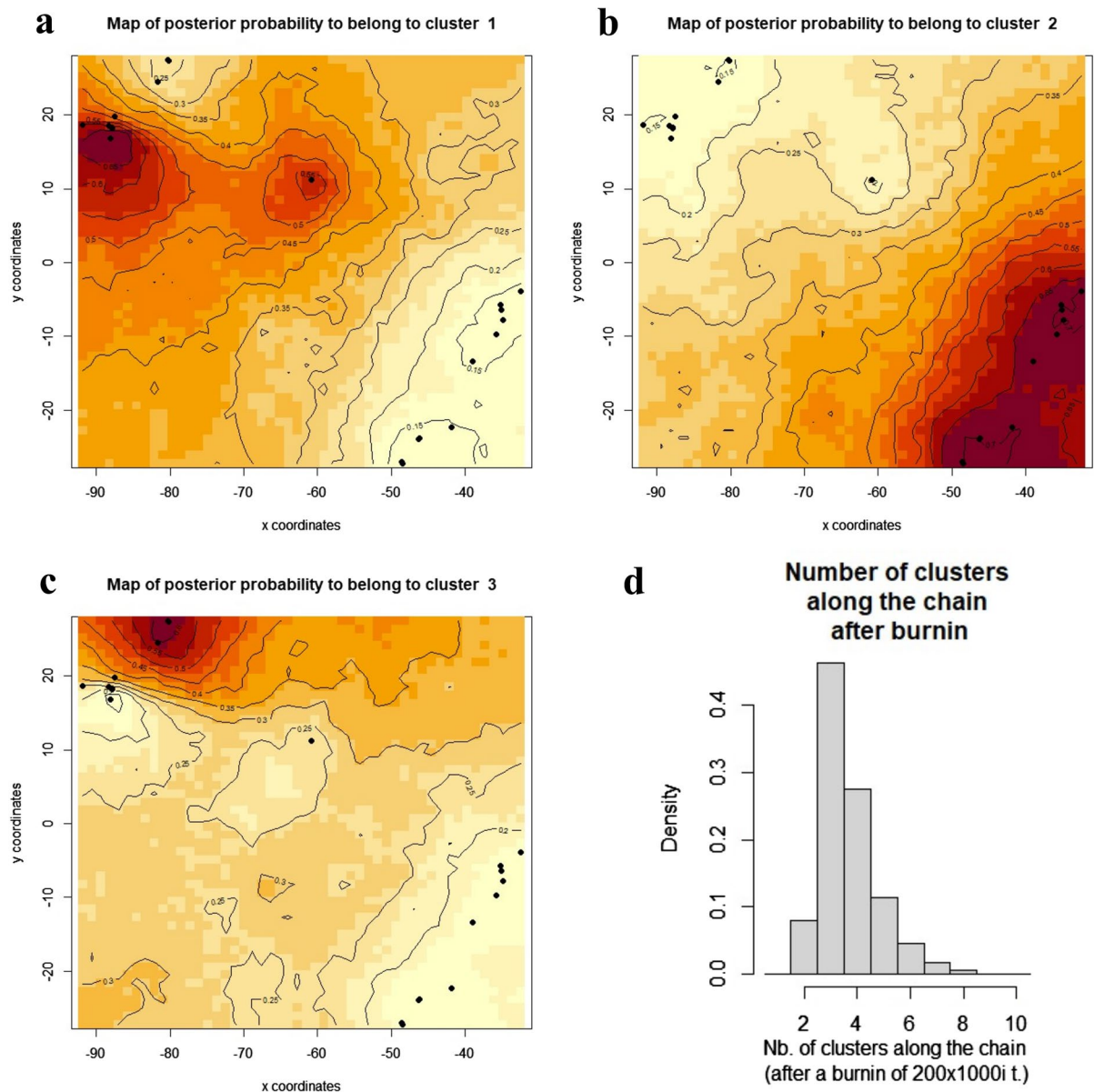


Fig. 3 Maps of posterior probabilities of population membership and spatial location of genetic discontinuities in *Harengula* from the Western Atlantic. Three main clusters ($K=3$) can be visualized (d): *Harengula clupeiola* and *H. jaguana* from Caribbean Province (a), *Harengula* sp. BRA

from the Brazilian Province (b), and *H. clupeiola* and *H. jaguana* from Carolinian Province (c). Darker colors indicate highest probabilities of membership and contour lines represent the spatial position of genetic discontinuities between lineages

it indicated that both AOB and marine provinces (the same of GENELAND hypothesis) were significantly structuring the genetic partitioning, explaining 30% and 27.4% of the variance (Table 1). In *Opisthonema*,

the AOB was not significant, but the marine provinces hypothesis was (12.6%) (Table 1). ϕ_{ST} were significant in all scenarios for *Harengula*, but not for *Opisthonema* (Table 1), suggesting additional structuring within the main lineages, as also indicated by a few lineage delimitation methods.

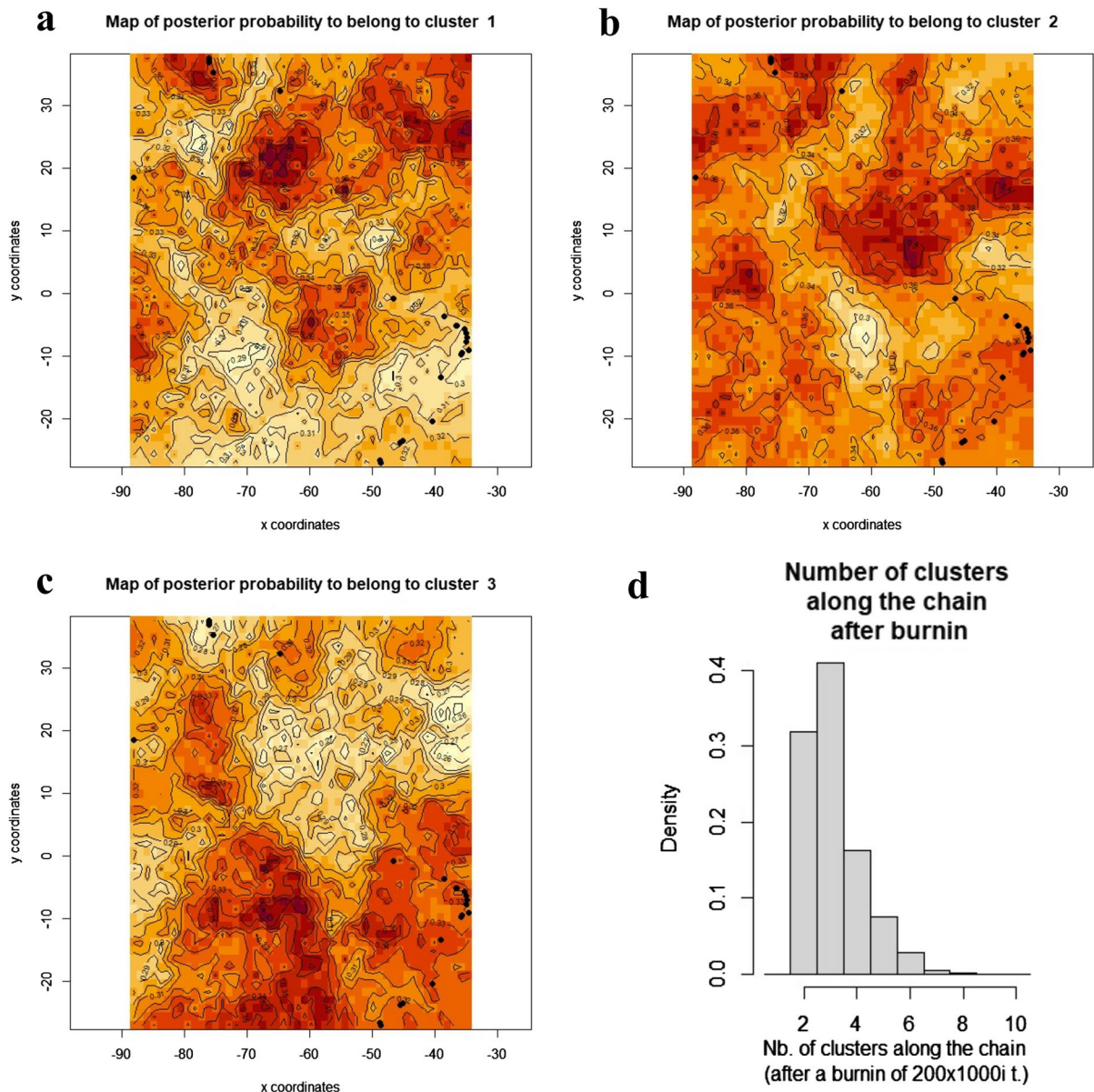


Fig. 4 Maps of posterior probabilities of cluster membership and spatial location of genetic discontinuities in *Opisthonema oglinum* from the Western Atlantic. Three main clusters ($K=3$) were recovered (d), but it is not possible to clearly

determine their geographical limits. Darker colors indicate highest probabilities of membership and contour lines represent the spatial position of genetic discontinuities between populations

Considering the size of both taxa, *Harengula* sp. BRA, *H. clupeola* (GCR) and *H. jaguana* (GCR) presented a smaller size and a stronger influence of the AOB, with different species in each side of the barrier, than in *Opisthonema oglinum*, larger and distributed along the WA (Fig. 6).

Discussion

Role of the Amazon-Orinoco barrier

Soft barriers, such as the AOB, can act as a filter to dispersal, which can promote speciation, but

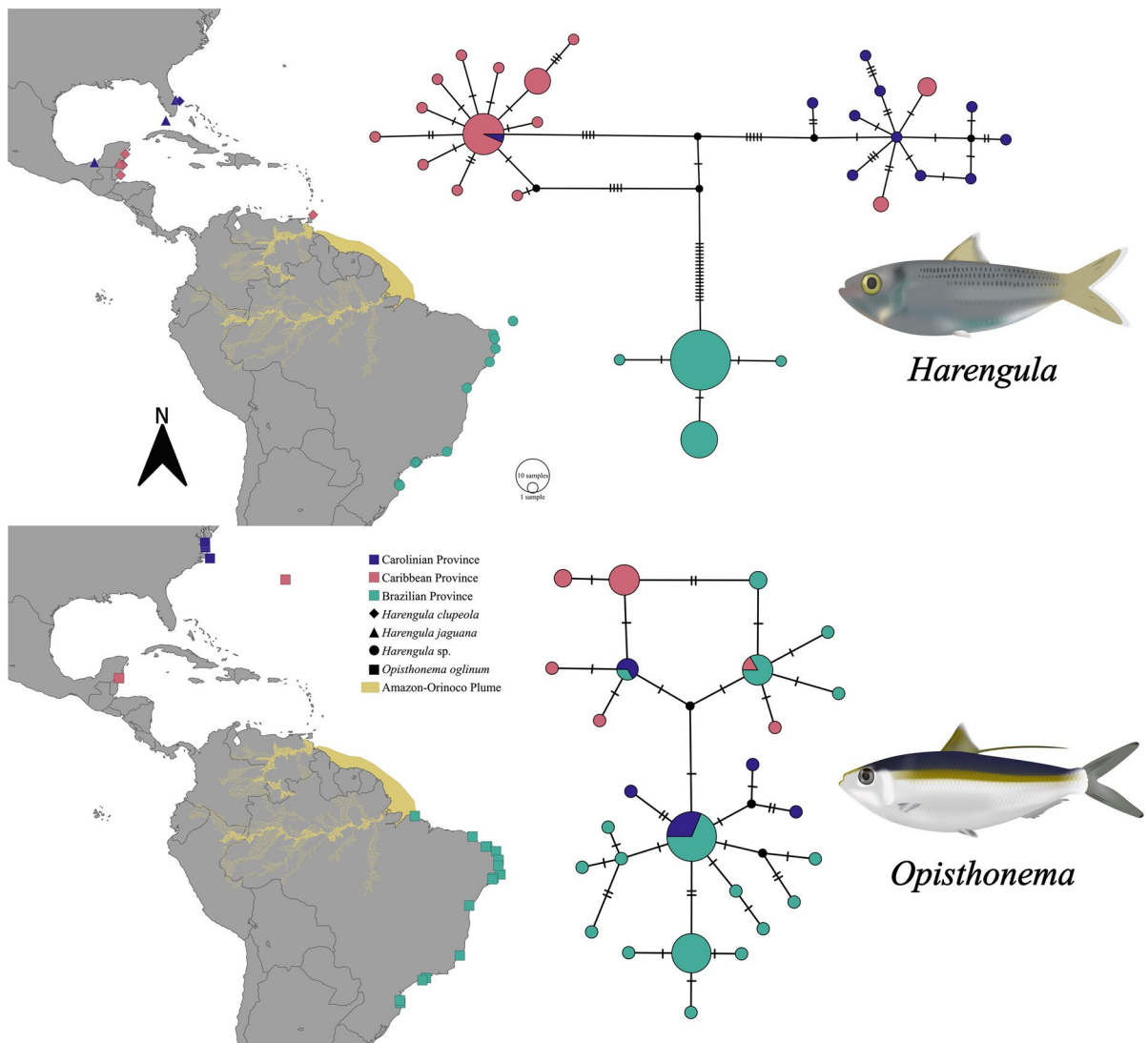


Fig. 5 Map distribution and *cox1* haplotype networks of *Harengula* and *Opisthonema oglinum* in the Western Atlantic. Bars over lines indicate mutational steps between haplotypes. Circle size represents number of specimens with the same haplotype

at the same time allow occasional crossings that may lead to the establishment of populations on the other side of the barrier or the maintenance of gene flow between both sides (Luiz et al., 2012; Araujo et al., 2022; Giachini Tosetto et al., 2022; Quintão et al., 2022). This situation was evidenced by the different phylogeographic patterns recovered in two sardine genera of the WA, suggesting cryptic speciation in *Harengula* and population structuring in *Opisthonema*. Surprisingly, the marine provinces hypotheses were also significant for both taxa (see AMOVA results), indicating additional

oceanographic barriers, as in the case of other reef fishes (e.g. *Haemulon aurolineatum* Cuvier 1830 and *Selene setapinnis* (Mitchill 1815)) along the WA (Araujo et al., 2022).

The Amazon River became a transcontinental river around 9.4–9 Mya (Gorini et al., 2014; Hoorn et al., 2017), with a substantial increase in freshwater and sediment discharges estimated at around 2.4 Mya (Figueiredo et al., 2009). The estimated divergence time of *H. jaguana* and *H. clupeiola* clades in the Greater Caribbean Region (GCR) vs. *Harengula* sp. BRA is at around 3.5–1.5 Mya, coinciding with

Table 1 Results of analysis of molecular variance (AMOVA) of *cox1* data of *Harengula* and *Opisthonema oglinum* from the Western Atlantic

Structure hypotheses	Percentage of variation	ϕ_{ST}
<i>Harengula</i>—Amazon-Orinoco Plume (n = 2)		
Brazilian Province/Carolinian and Caribbean Provinces		0.42126
Among groups	30.09*	
Among populations within groups	12.03*	
Within populations	57.87*	
<i>Harengula</i>—Provinces (n = 3)		
Brazilian Province/Carolinian Province/Caribbean Province		0.39461
Among groups	27.40*	
Among populations within groups	12.06*	
Within populations	60.54*	
<i>Opisthonema oglinum</i>—Amazon-Orinoco Plume (n = 2)		
Brazilian Province/Carolinian and Caribbean Provinces		0.14123
Among groups	4.51*	
Among populations within groups	9.62	
Within populations	85.88	
<i>Opisthonema oglinum</i>—Provinces (n = 3)		
Brazilian Province/Carolinian Province/Caribbean Province		0.17105
Among groups	12.57*	
Among populations within groups	4.54	
Within populations	82.90*	

Groupings were used to test different population structure hypotheses. Values with an asterisk have *p*-value < 0.05

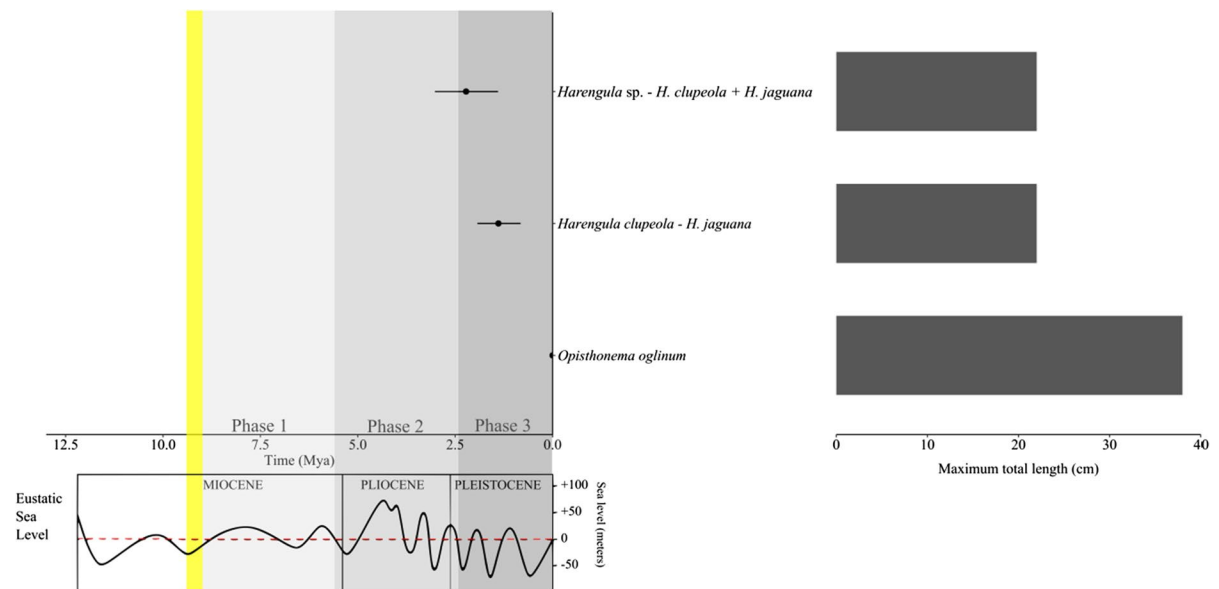


Fig. 6 Estimates of divergence times between the *Harengula* species and *Opisthonema oglinum* populations in the Western Atlantic and fluctuations in sea-level in the last 12.5 Mya, in relation to the three stages of increasing sediment and freshwater discharge of the Amazon River (in shades of gray: Phase 1, 9.4–5.6 Mya; Phase 2, 5.6–2.4 Mya; Phase 3, 2.4 Mya–present). Figure modified from Araujo et al. (2022). The red dotted line corresponds to current sea-level; sea-level curve

modified from Haq et al. (1987) and Johnson et al. (2006), following the timescale by Hilgen et al. (2012). The yellow stripe indicates the estimated time interval of the emergence of the Amazon River (9.4–9.0 Mya). Mean and 95% high posterior probabilities are indicated by the dots and lines in each species/population comparison, respectively. Timescale in millions of years (Mya)

the stage of increased sediment and freshwater discharge of the Amazon River, when relatively larger and pelagic species were more affected (Araujo et al., 2022). Interestingly, the AOB might also act as a barrier to *H. humeralis*, likely restricting its distribution to the north of the AOB, however, its occurrence in the north of Brazil is uncertain (Cervigón, 1991; Robertson & Van Tassell, 2023).

The effectiveness of an oceanographic barrier depends on the biology of each species (Rocha, 2003; Luiz et al., 2012; Araujo et al., 2022). Despite both being pelagic and forage fishes, species of *Harengula* and *Opisthonema* likely differ in key biological traits that might account for the distinct responses to the AOB. *Opisthonema oglinum* seems to be less sensitive to lower salinities when compared to species of *Harengula* (Paramo et al., 2003). *Opisthonema oglinum* is also a larger species, about 70% larger than *H. clupeiola* and *H. jaguana* (38 vs. 22.5–21.2 cm total length (TL) respectively; Cervigón et al., 1992; Da Costa et al., 2018), one feature that might also be important since body size is a key predictor to the dispersal capacity across the AOB (Luiz et al., 2012; Araujo et al., 2022). These differences are comparable to those seen in reef fishes during the last 2.4 Mya, the most intense sedimentation and freshwater discharge period, and average maximum TL of 50.6 cm, as in the population structure of *O. oglinum*, and the intermediary phase of the Amazon River (5.8–2.4 Mya) and 24.2 cm TL, as in the speciation of *Harengula* (Araujo et al., 2022).

Taxonomic accounts

Our molecular data of *O. oglinum* is in agreement with the literature in terms of taxonomy and distribution, however in *Harengula*, it suggests that *H. clupeiola* and *H. jaguana*, which are closely related, are restricted to the Carolinian and Caribbean Provinces. Meanwhile, in the Brazilian Province, *Harengula* sp. BRA may represent another species not formally recognized that may be limited by the AOB (Araujo et al. 2022), since both *H. clupeiola* (type locality, Martinique Island) and *H. jaguana* (type locality, Cuba) were described based on specimens from the GCR (Whitehead, 1985).

Harengula is the only sardine found in the oceanic islands of the Southwestern Atlantic. Perhaps surprisingly at first, our results indicate a single

population of *Harengula* inhabiting the northeastern Brazilian coast and the Fernando de Noronha Archipelago, located about 300 km off the coast. This suggests a higher dispersive potential across distant and deep marine regions or even a conservative molecular marker. *Harengula* is also known to inhabit the Rocas Atoll and Trindade-Martin Vaz Archipelago, which are located at about 240 and ~1,200 km off the coast, respectively (Gasparini & Floeter, 2001; Simon et al., 2013). The Fernando de Noronha Archipelago is part of the Fernando de Noronha Ridge, which also includes the Rocas Atoll and several seamounts along the northern portion of Brazil (Alberoni et al., 2020). An almost continuous series of seamounts is also present between the Trindade-Martin Vaz Archipelago and the central coast of Brazil (Pinheiro et al., 2018). This might explain the occurrence of *Harengula* in these distant oceanic islands since seamounts are known to act as steppingstones for some species (Pinheiro et al., 2017; Lima et al., 2022; Simon et al., 2022).

Some delimitation analyses (sGMYC and mGMYC) indicated further subdivisions of *H. clupeiola* and *H. jaguana*, a result that is congruent with previous studies that concluded that GMYC analyses tend to overestimate the number of lineages (Fujisawa & Barraclough, 2013; Hamilton et al., 2014). The phylogeographic structure detected may reflect some phenotypic differences in *H. jaguana* (six proposed subspecies), and *O. oglinum* (one) (Rivas, 1950, 1963; Whitehead, 1985). In Brazil, species of *Harengula* are either identified as *H. clupeiola* or *H. jaguana*, without the recognition of subspecies. *Harengula macrophthalmia* (Ranzani 1842), described for Brazil, is currently regarded as a junior synonym of *H. clupeiola* (Whitehead, 1985). However, the identity of the species of *Harengula* in the Brazilian Province remains to be elucidated in further studies, which would ideally include additional molecular markers and morphological tools in a taxonomic context.

Stock delimitation

Our results are also relevant for fisheries management of these sardine species in the Western Atlantic at different levels. In addition to their relevance to artisanal fisheries, there is a complex ongoing conflict involving the artisanal fishing of *Harengula*

in the Fernando de Noronha Archipelago, a Marine Protected Area (Freire & Pauly, 2015; Lopes et al., 2017; Mendes et al., 2020; Pauly et al., 2020). Our results are the first to shed light on the identity and geographic distribution of the species from the Brazilian Exclusive Economic Zone (BEEZ), indicating that Fernando de Noronha specimens belong to a putative undescribed species of *Harengula* from Brazil.

As climate change keeps intensifying, fisheries in Brazil are also at increasing risk of reducing productivity, which makes it urgent to correctly define and manage fishing stocks (Lam et al., 2020). Herrings and sardines are overall forage and low trophic level fishes, making them key actors in marine coastal ecosystems since they connect primary production and keystone predators (Pikitch et al., 2014). Future management and conservation plans for *Opisthonema oglinum* and *Harengula* sp. in BRA can benefit from the fact that these species apparently have a wide distribution in the extensive BEEZ. Additional phylogeographic studies, with more samples, localities, and variable markers, including genomics, must be done to correct delimit the fisheries stocks of *Harengula* in the BEEZ, as well as its irreplaceable occurrence in the oceanic islands.

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Data availability The edited and aligned DNA sequences used in this study are available on GenBank database under accession codes MW302057-MW302121. Raw DNA sequences can be shared upon request.

Declarations

Conflict of interest The authors declare no conflict of interest to declare that are relevant to the content of this article.

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