Cord I, Araujo GS, Silva FC, Kurtz YR, Rocha CR, Pinheiro HT, Rocha LA, Floeter SR

Biogeography and evolution of reef fishes on tropical Mid-Atlantic Ridge islands

Proceedings of the Royal Society B: Biological Sciences

DOI: 10.1098/rspb.2025.0756

ESM (Electronic Supplementary Material)

Supplementary Tables

Table S1. Characteristics of St Paul's Rocks, Ascension, and St Helena. Distances from the Western Atlantic (WA) and Eastern Atlantic (EA) are shown in kilometres, and islands' ages in millions of years before the present. Shallow reef areas obtained from Hachich et al. (2015). Prevailing surface currents were obtained from Stramma and England (1999), Coles et al. (2013), and Kough et al. (2013).

Island	Reef fish species	Single Island Endemics	Age (Ma)	Dist WA (km)	Dist EA (km)	Shallow reef area (km²)	Prevailing surface currents
St Paul's Rocks	73	7	9	1,175	1,995	3.5	Northern South Equatorial Current, Atlantic Equatorial Undercurrent (~ 100 metres deep)
Ascension	116	10	1.15	2,150	1,600	176.6	Central South Equatorial Current, South Equatorial Counter Current
St Helena	94	7	15	4,000	1,870	375.6	Southern South Equatorial Current

Table S2. Biogeographical definitions and acronyms of the sites where data were gathered for the presence/absence of reef fish in the Atlantic basin. Regions were defined based on Cord et al. (2022), Floeter et al. (2008), Kulbicki et al. (2013), Robertson & Cramer (2014), and Spalding et al. (2007)

Region	Sub-provinces	Geographical definition	Acronym
Western Atlantic			
Greater Caribbean	Carolinian	North Carolina State to Mérida (México)	CA
	Bermuda	Bermuda Is.	BE
	Central Caribbean	Central America and offshore islands (except mentioned elsewhere), Florida Keys	CC
	Southern Caribbean	Panama to Trinidad and Tobago	SC
Southwestern Atlantic			
Brazilian Province	North Brazil	Great Amazon Reef	NB
	Northeastern Brazil	Rio Grande do Norte to Southern Bahia State	NE
	Southeastern Brazil	Espírito Santo to Paraná States	SE
	South Brazil	Santa Catarina State	SB
Brazilian oceanic islands	Rocas Atoll		AR
	Fernando de Noronha		FN
	Trindade Is.		TR
Warm-temperate Argentinian Province	Argentinian	Rio Grande do Sul State, from Uruguay to Patagonia	PT
Mid-Atlantic Ridge	St Paul's Rocks		SP
	Ascension		AS
	St Helena		SH
Eastern Atlantic			
Northeastern Atlantic	Mediterranean	Levant to Gibraltar	MD
Lusitana	Azores		AZ
	Madeira		MA

	Canaries		CN
Tropical Eastern Atlantic	Northwest Africa	Gibraltar to Cape Verde (Senegal)	NT
	Cape Verde Is.		CV
	São Tomé & Príncipe	Includes Annobon	ST
	Tropical West Africa	Cape Verde to Moçamedes (Angola)	EA
Southwest Africa	Benguela	Moçamedes to Cape of Good Hope	BN
	Agulhas	Cape of Good Hope to Kei River	CP
Southwestern Indian Ocean	South Africa's Indian Ocean	From Kei River to Kosi Bay	IO

Table S3. Species, molecular markers, and accession numbers from the GenBank and Barcode of Life (BOLD) of the sequences utilized and generated in the present work.

Species	12S	16S	COI	CR	Cytb	ETS-2	RAG2	S7	TMO-4c4
Chaetodon dolosus	EF616855	EF616938	JF493094				EF617103		EF617186
Chaetodon guentheri	EF616868	EF616951	HQ956575				EF617116		EF617199
Chaetodon litus	MK100716		MK100733		MK100726		MK100747		MK100759
Chaetodon miliaris	EF616834	DQ278890	UKFBJ278-08		U23606	FJ167749	EF617082	FJ167813	EF617165
Chaetodon multicinctus	EF616838	EF616922	MG816672		U23597	FJ167750	EF617086	FJ167814	EF617169
Chaetodon sanctaehelenae					FJ167702	FJ167758		FJ167822	
Chaetodon sedentarius	EF616828	EF616912			FJ167703	FJ167759	EF617076	FJ167823	EF617159
Chromis cadenati			GQ341589						
Chromis chromis		FJ616425	KF564300	EF489542	AY208527				
Chromis limbata		EF489736		KT844434	EF392576				
Chromis lubbocki			MT726967						
Chromis sanctaehelenae			MT726969						
Ophioblennius trinitatis					PQ801055 - PQ801084				
Prognathodes aculeatus			KT883642						
Prognathodes basabei			KX783255 - KX783257						
Prognathodes brasiliensis			PV329840						
Prognathodes dichrous			PV329835 - PV329836 - PV329837 -						

	PV329838 - PV329839	
Prognathodes falcifer	GU581196	
Prognathodes guyanensis	PV329834	
	MG895423 -	
Prognathodes geminus	MG895424	
	PV329831 - PV329830 -	
Prognathodes marcellae	PV329829	
	PV329833 -	
Prognathodes obliquus	PV329832	
	KF268185 -	
Roa jayakari	KF268186	
	NC_065810	
Roa modestus		
		MK093832 -
Scartella caboverdiana		MK093834
		MK093764 -
Scartella cristata Brazilian Province		MK093798
		MK093809 -
Scartella cristata Caribbean		MK093813
		MK797744 -
Scartella cristata Gulf of Mexico		MK797746
Scartella cristata Mediterranean + Canaries		MK093799 -
		MK093808
		MK093814 -
Scartella cristata São Tomé & Príncipe		MK093818
		MK093839 -
Scartella emarginata		MK093840
		MK093819 -
Scartella itajobi		MK093823

	MK093828 -	
Scartella nuchifilis	MK093831	
	MK093824 -	
Scartella poiti	MK093827	
	MK093835 -	
Scartella springeri	MK093838	
		PQ800783 -
Stegastes lubbocki		PQ800787*
		PQ800792 -
Stegastes pictus		PQ801054
Stegastes		PQ800788 -
sanctaehelenae		PQ800791*

Table S4. Best nucleotide substitution models determined for each molecular marker using jModelTest 2.1, based on the Bayesian information criterion (BIC).

Genus	Gene	Substitution model
Chaetodon	12S	GTR + G
	16S	K80 + I
	COI	HKY + I
	CytB	K80 + I
	ETS-2	JC + G
	S7	JC
	RAG2	K80
	TMO-4c4	K80
Chromis	12S	HKY + I + G
	COI	HKY + G
	CytB	HKY + I
	Control Region	TrN + I
Ophioblennius	CytB	TN93 + I
Stegastes	CytB	HKY + I + G

Table S5. Calibration points employed in the analyses of divergence time implemented in BEAST.

Genus	Node age	Reference
Chaetodon	*Chaetodon multicinctus* + *C. litus* - *C. sedentarius*: 7.2 Mya	Delrieu-Trottin et al. 2019
Chromis	*Chromis lubbocki* - *C. chromis*, 11.4 Mya	McCord et al. 2021
Ophioblennius	*Cirripectes* - *Ophioblennius*, 20.5 Mya	Lin & Hastings, 2013
Priolepis	*Priolepis hipoliti* - *P. cincta,* 21.0 Mya	Thacker, 2015
Scartella	*Hypsoblennius* - *Scartella*, 27.0 Mya	Lin & Hastings, 2013
Sparisoma	*Sparisoma radians* + *S. cretense* - *S. strigatum*: 12.5 Mya	Siqueira et al. 2019
Stegastes	*Stegastes leucostictus* + *S. partitus* - *S. pictus*: 19.9 Mya	McCord et al. 2021
Thalassoma	*Thalassoma bifasciatum* + *T. noronhanum* - *T. ballieui*, 26.0 Mya	Rabosky et al. 2018

Table S6. Functional indexes.

	feve	fric	fdiv
MAR_END	0.707719	0.1832903	0.7953153
MAR	0.710690	0.4345241	0.8251451
ATL	0.631619	0.8597578	0.8503490

Table S7. Total number of: fish species; endemics (i.e. occurs in that region and nowhere else in the Atlantic); species shared with any of the Mid-Atlantic Ridge (MAR) islands; species shared exclusively with any of the MAR islands (i.e. occurs in the region and at least one of the MAR islands, but nowhere else in the Atlantic); species shared with Ascension and/or St Helena; species shared exclusively with Ascension and/or St Helena (i.e. occurs only in the region and/or Ascension and/or St Helena); species shared with St Paul's Rocks, and; species shared exclusively with St Paul's rocks (i.e. occurs only in the region and St Paul's Rocks). For the names in bold (Western and Eastern Atlantic), numbers are the sum of the species within those geographical regions. ASH = Ascension + St Helena; SP = St Paul's Rocks.

Region	Species	Endemics	MAR Shared	MAR "Exclusively shared"	ASH Shared	ASH "Exclusively shared"	SP Shared	SP "Exclusively shared"
Western Atlantic	973	805	113	47	87	26	56	25
Greater Caribbean + Bermuda	829	483 (58.21%)	94	1	83	1	39	0
Brazilian Province	448	77 (17.19%)	106	14	81	1	55	12
Patagonia	89	21 (23.60%)	15	0	14	0	5	0
Eastern Atlantic	537	416	76	10	70	9	32	1
Northeastern Atlantic	310	83 (26.77%)	53	0	51	0	22	0
Tropical Eastern Atlantic	419	131 (31.26%)	76	2	71	0	31	0
Southern Africa	238	169 (70.76%)	33	0	29	0	10	0
MAR islands	169	42 (24.85%)	84	17	-	-	-	-
St Paul's Rocks	70	7 (10%)	-	-	34	3	-	-
Ascension	113	10 (8.85%)	-	-	-	-	33	0
St Helena	94	7 (7.45%)	-	-	-	-	26	0
Ascension & St Helena	133	14 (10.53%)	-	-	-	14	-	-

Table S8. For Mid-Atlantic Ridge (MAR) endemic fishes: Geographical range, origin (i.e. route for colonising the Mid-Atlantic Ridge), putative sister-species (for genus *Bodianus*, *Centropyge, Stegastes,* and *Thalassoma*, we added both the "molecular" sister-species and, in parentheses, the ancestral species that likely first reached the Mid-Atlantic Ridge), sister species' geographic range, separation dates, and used references. For non-endemic MAR-fishes: Origin (i.e. route for colonising the Mid-Atlantic Ridge) and putative sister species (only provided in specific cases).

Species name	Endemics - geographical range	Origin	Putative sister species	Sister species' geographic range	Separation dates	References
MAR Endemics						
Amblycirrhitus earnshawi	AS	WA				
Apogon axillaris	AS, SH	WA				
Bodianus insularis	SP, AS, SH	WA	*B. pulchellus* (*B. rufus*)		> 1 Mya (~ 5 Mya)	da Motta-Neto et al. (2020) 10.1016/j.jcz.2020.03.004; Santini et al. (2016) 10.1016/j.ympev.2016.02.011
Bothus mellissi	AS, SH	WA				
Centropyge resplendens	AS	IO	**C. argi/C. aurantonotus* *unresolved (*C. acanthops*)	Great Caribbean (Indian Ocean)	> 0.5 Mya (> 0.5 Mya)	Bowen et al. (2006) 10.1093/jhered/esj006; Baraf et al. (2019) 10.1016/j.ympev.2019.106596
Chaetodon sanctaehelenae	AS, SH	IO	*C. dolosus*		0.66 Mya	Cord et al. (2025; Figure 2) 10.1098/rspb.2025.0756
Chromis sanctaehelenae	SH	EA	*C. cadenati*		2 Mya	Cord et al. (2025; Figure 2) 10.1098/rspb.2025.0756
Diplodus ascensionis	AS	EA				Summerer et al. (2001) 10.1111/j.1095-8649.2001.tb00227.x
Diplodus helenae	SH	EA				Summerer et al. (2001) 10.1111/j.1095-8649.2001.tb00227.x
Emblemariopsis sp.	SP	WA				
Enneanectes smithi	SP	WA				
Helcogramma ascensionis	AS, SH	IO				Brown et al. (2019) 10.11646/zootaxa.4543.2.1
Ichthyapus insularis	AS	WA				
Malacoctenus sp. (St Paul's Rocks)	SP	WA				Dias et al. (2019) 10.1007/s10750-019-3966-z
Ophioblennius aff. *atlanticus*	AS, SH	EA	Eastern Atlantic *Ophioblennius* sp.		8 Mya	Cord et al. (2025; Figure 3) 10.1098/rspb.2025.0756
Priolepis ascensionis	AS, SH	WA	*P. dawsoni*			Quintão et al. (2022) 10.1007/s00227-022-04082-3
Prognathodes dichrous	AS, SH	WA				Cord et al. (2025; Figure 2) 10.1098/rspb.2025.0756

Prognathodes obliquus	SP	WA			Cord et al. (2025; Figure 2) 10.1098/rspb.2025.0756
Scartella nuchifilis	AS	EA			Cord et al. (2025; Figure 2) 10.1098/rspb.2025.0756 Araujo et al. (2020) 10.1093/zoolinnean/zlz142
Scartella springeri	SH	EA			Cord et al. (2025; Figure 2) 10.1098/rspb.2025.0756; Araujo et al. (2020) 10.1093/zoolinnean/zlz142
Sparisoma strigatum	AS, SH	EA		1.8 Mya	Cord et al. (2025; Figure 2) 10.1098/rspb.2025.0756; McCord et al. (2021) https://doi.org/10.1101/2021.02.07.430149
Stegastes lubbocki	AS	WA	*S. sanctaehelenae* (*S. pictus*)	> 0.3 Mya (0.3 Mya)	Cord et al. (2025; Figure 3) 10.1098/rspb.2025.0756
Stegastes sanctaehelenae	SH	WA	*S. lubbocki]* (*S. pictus*)	> 0.3 Mya (0.3 Mya)	Cord et al. (2025; Figure) 10.1098/rspb.2025.0756
Stegastes sanctipauli	SP	WA	*S. rocasensis* (*S. fuscus*)	> 0.5 Mya	
Thalassoma ascensionis	AS	EA	*T. sanctaehelenae* (*T. newtoni*)	3.1 Mya (7.1 Mya)	Araujo et al. (2022) 10.1111/jbi.14398
Thalassoma sanctaehelenae	AS, SH	EA	*T. ascensionis* (*T. newtoni*)	3.1 Mya (7.1 Mya)	Araujo et al. (2022) 10.1111/jbi.14398
Non-endemic MAR fishes					
Abudefduf hoefleri		EA			
Acanthostracion notacanthus		EA			
Acanthurus bahianus		WA	*A. tractus*		Bernal & Rocha (2011) 10.11646/zootaxa.2905.1.5
Acanthurus chirurgus		WA			
Acanthurus coeruleus		WA			
Acanthurus monroviae		EA			
Amblycirrhitus pinos		WA			
Anthias asperilinguis		WA			
Apogon americanus		WA			
Apogon pseudomaculatus		WA			
Arothron firmamentum		IO			

Aulostomus strigosus	EA	*A. chinensis*	2.5 Mya	Bowen et al. (2001) 10.1111/j.0014-3820.2001.tb00619.x
Balistes vetula	WA			
Bathygobius brasiliensis	WA			
Callionymus bairdi	WA			
Cantherhines macrocerus	WA			
Caranx fischeri	EA			
Carapus acus	EA			
Cephalopholis fulva	WA			
Cephalopholis furcifer	WA			
Chaetodon striatus	WA			
Chromis enchrysura	WA			
Clepticus brasiliensis	WA			
Cryptotomus roseus	WA			
Dactylopterus volitans	WA			
Decapterus muroadsi	IO			
Echidna catenata	WA			
Enchelycore aff. nycturanus	IO			
Enchelycore carychroa	WA			
Entomacrodus vomerinus	WA			
Gnatholepis thompsoni	IO	*G. cauerensis*		Rocha et al. 2005 10.1111/j.1365-294X.2005.02698.x
Gymnothorax funebris	WA	*H. brasiliensis*		Joyeux et al. (2001) 10.1046/j.1365-2699.2003.00952.x
Halichoeres radiatus	WA			
Heteroconger camelopardalis	WA			
Hippocampus erectus	WA			
Holacanthus ciliaris	WA			
Ichthyapus ophioneus	WA			

Lutjanus jocu	WA		
Lutjanus purpureus	WA		
Lythrypnus sp.n.Noronha	WA		
Malacanthus plumieri	WA		
Muraena melanotis	EA		
Muraena pavonina	WA		
Myroconger compressus	EA		
Ophioblennius trinitatis	WA		
Pomacanthus paru	WA	*P. arcuatus*	Baraf et al. (2019) 10.1016/j.ympev.2019.106596
Quassiremus ascensionis	WA		
Scartella itajobi	WA		
Seriola lalandi	WA		
Sparisoma amplum	WA		
Sparisoma axillare	WA		
Sparisoma frondosum	WA		
Starksia multilepis	WA		
Stegastes rocasensis	WA		
Syacium papillosum	WA		
Thalassoma noronhanum	WA	*T. bifasciatum*	Joyeux et al. (2001) 10.1046/j.1365-2699.2003.00952.x
Trachinotus ovatus	EA		
Uraspis helvola	IO		Brown et al. (2019) 10.11646/zootaxa.4543.2.1
Uropterygius macularius	WA		
Xyrichtys sanctaehelenae	EA		

Supplementary Figures

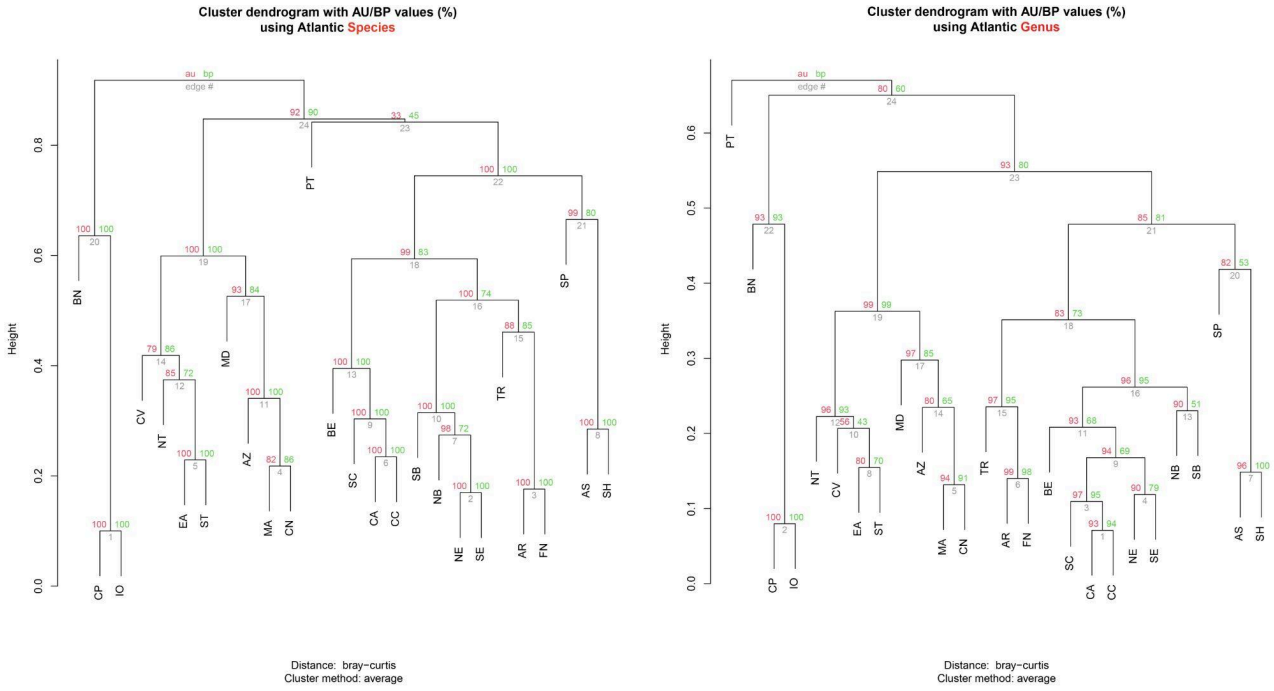

Figure S1. Bootstrapped cluster analyses, using Sørensen dissimilarity and UPGMA, showing fish species grouping units within the Atlantic Basin. Map and dendrogram branches are coloured according to Provinces (significant groups). Red numbers on the top left of the branches represent approximately unbiased p-values ("*au*"), and green numbers on the right are the bootstrap probability value ("*bp*") for each cluster. Two letter acronyms in the dendrogram refer to the 26 sampling units used for compiling reef fish distributions: Carolinian (CA); Bermuda (BE); Central Caribbean (CC); Southern Caribbean (SC); North Brazil (NB); Northeastern Brazil (NE); Southeastern Brazil (SE); South Brazil (SB); Rocas Atoll (RA); Fernando de Noronha (FN); Trindade Martin-Vaz Chain (TR); Patagonian Province (PT); St Paul's Rocks (SP); Ascension (AS); St Helena (SH); Mediterranean (MD); Azores (AZ); Canarias (CN); Madeira (MA); Cabo Verde (CV); Northeast Atlantic (NT); Tropical East Atlantic (EA); São Tomé (ST); Benguela (BN); Cape of Good Hope (CP); Southern Africa's Indian Ocean (IO).

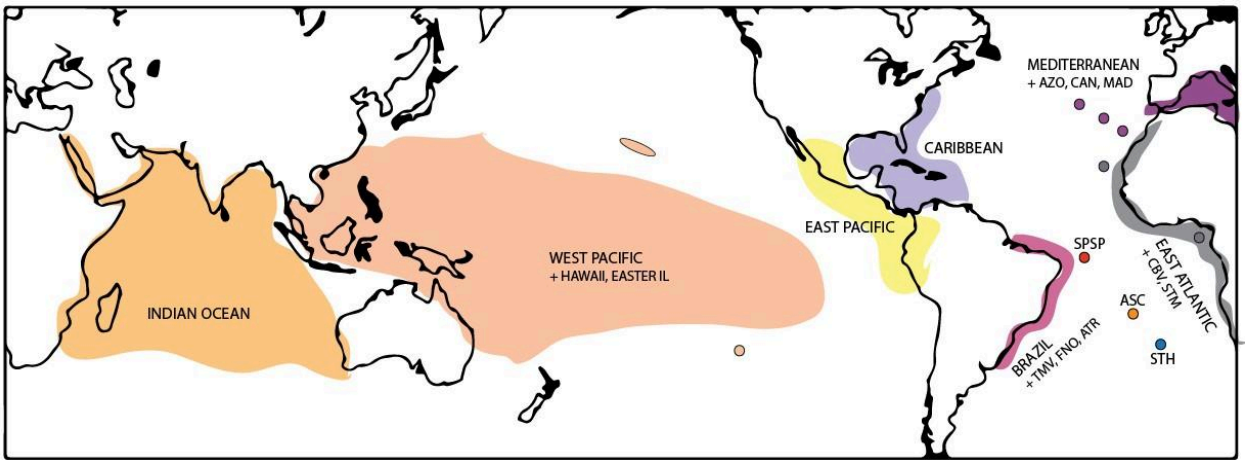

Figure S2. Biogeographic regions used in BioGeoBEARS analyses. Colored polygons on the schematic map indicate the areas defined for reconstructing ancestral ranges of ten Mid-Atlantic Ridge lineages. Areas were adapted from Briggs & Bowen (2013), Spalding et al. (2007), and Cord et al. (2022): West Pacific (includes Hawaii and Easter Island), East Pacific, Greater Caribbean, Indian Ocean, East Atlantic (includes Cabo Verde and São Tomé e Príncipe), Brazilian Province (includes Rocas Atoll, Fernando de Noronha, and Trindade), Mediterranean (includes Azores, Canaries, and Madeira), and each of the Mid-Atlantic Ridge islands—St Paul's Rocks, Ascension, and St Helena.

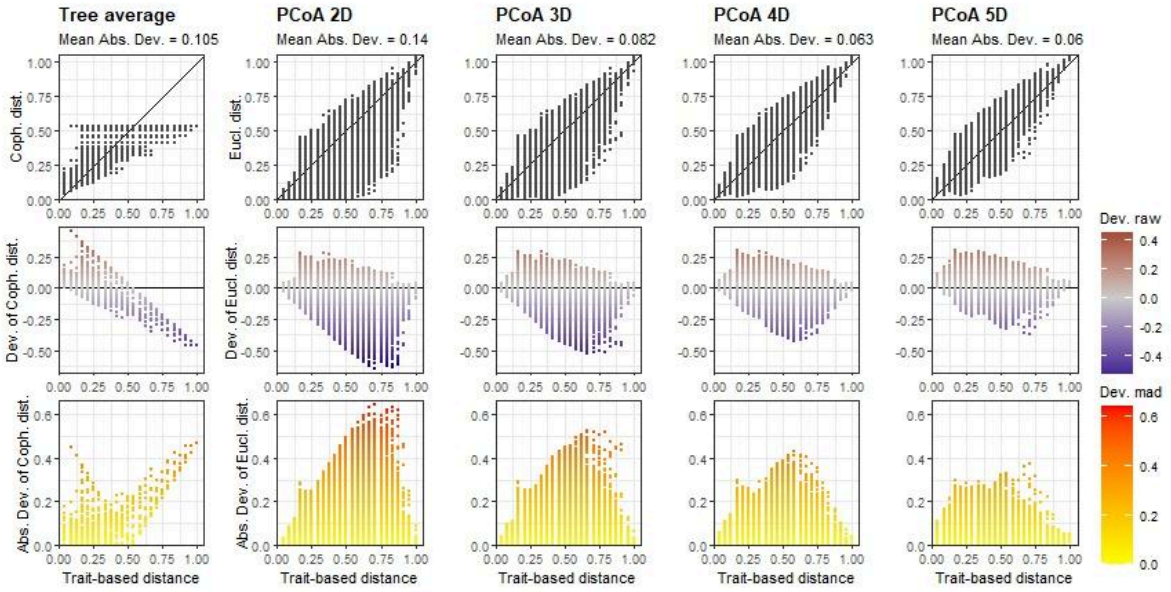

Figure S3. Quality of the functional spaces and the UPGMA dendrogram in a single plot, featuring three panels per space. Each column represents a distinct functional space, while the x-axis across all panels represents trait-based distances. The y-axis varies across rows: the first row displays species' functional distances in the multidimensional space, the second row shows the raw deviation of species distances in the functional space compared to trait-based distances, and the third row showcases the absolute deviation of distances in the functional space.

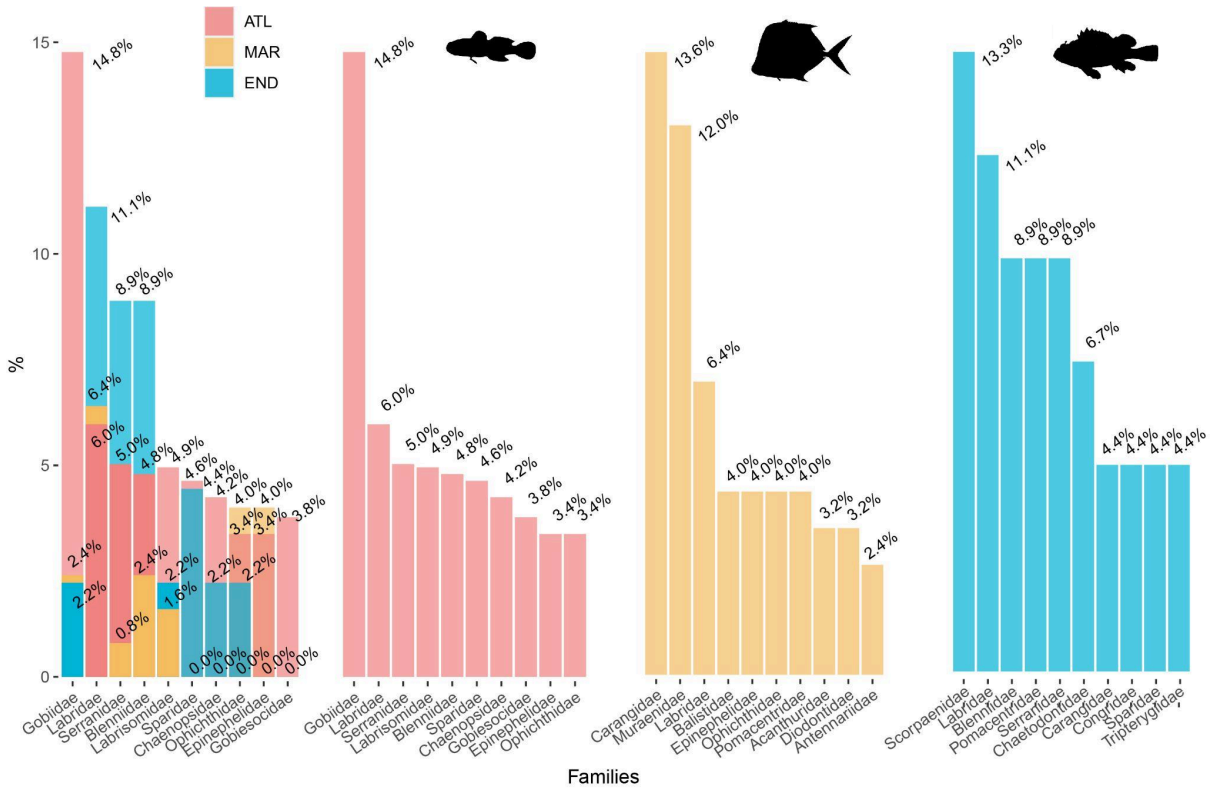

Figure S4. On the left, the plot shows the top 10 most speciose families in the Atlantic in all three regions. In red, ATL represents fishes that occur in the Atlantic but are not found in any of the tropical Mid-Atlantic Ridge (MAR) islands (St Paul's Rocks, Ascension, or St Helena); in yellow, MAR accounts for the species that occur on any of the three forenamed islands, but not exclusively so and, finally, in blue, the bar plot in blue represents species endemic to St Paul's Rocks, Ascension, and/or St Helena (i.e. are present in any of those islands and not found anywhere else).

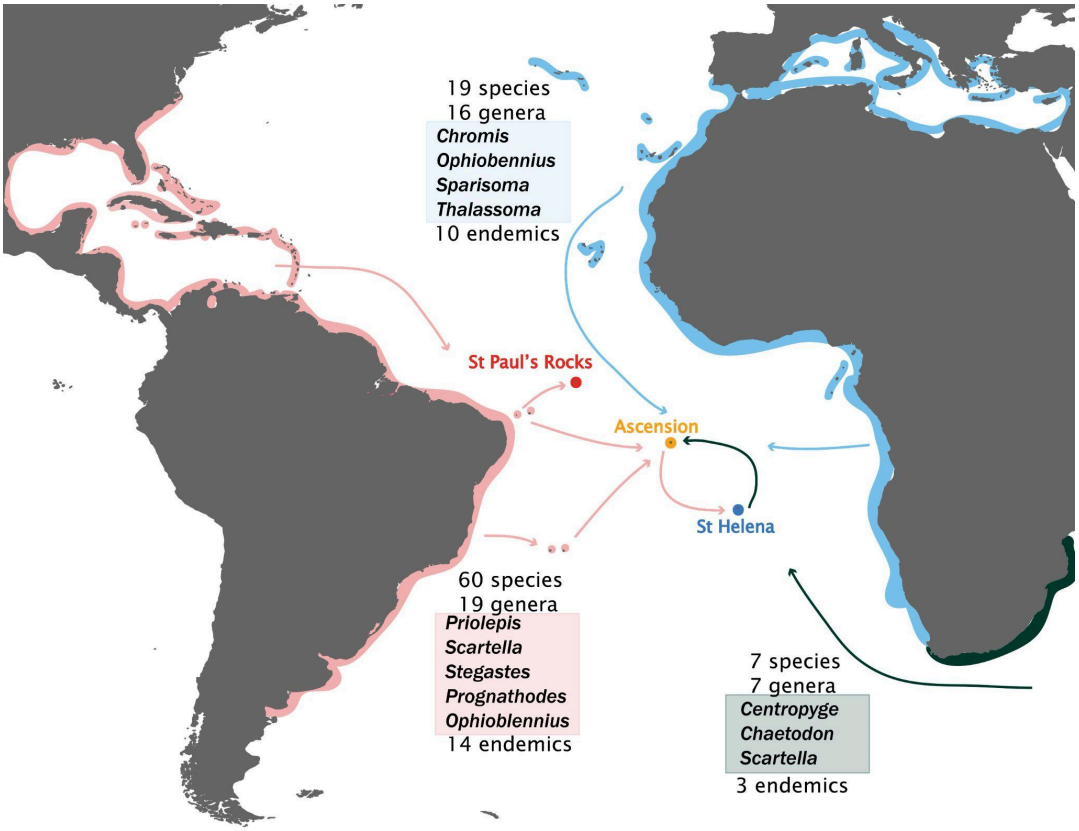

Figure S5. Map of the Atlantic Ocean with arrows representing likely dispersal routes for fishes present in at least one of the tropical Mid-Atlantic Ridge (MAR) islands. Colours represent the main origin areas for MAR-fishes, namely: Western Atlantic in pink, Eastern Atlantic in blue, and Indo-Pacific Ocean in dark green. Above the coloured squares are the numbers of reef fish species and genera whose routes were traced to each of the main areas; numbers below the squares are MAR-endemic species (i.e. found only in St Paul's Rocks, and/or Ascension, and/or St Helena). Inside each square are examples of genera that followed each possible route inferred from BioGeoBEARS or haplotype networks. For further details on the species and routes, see table S8.

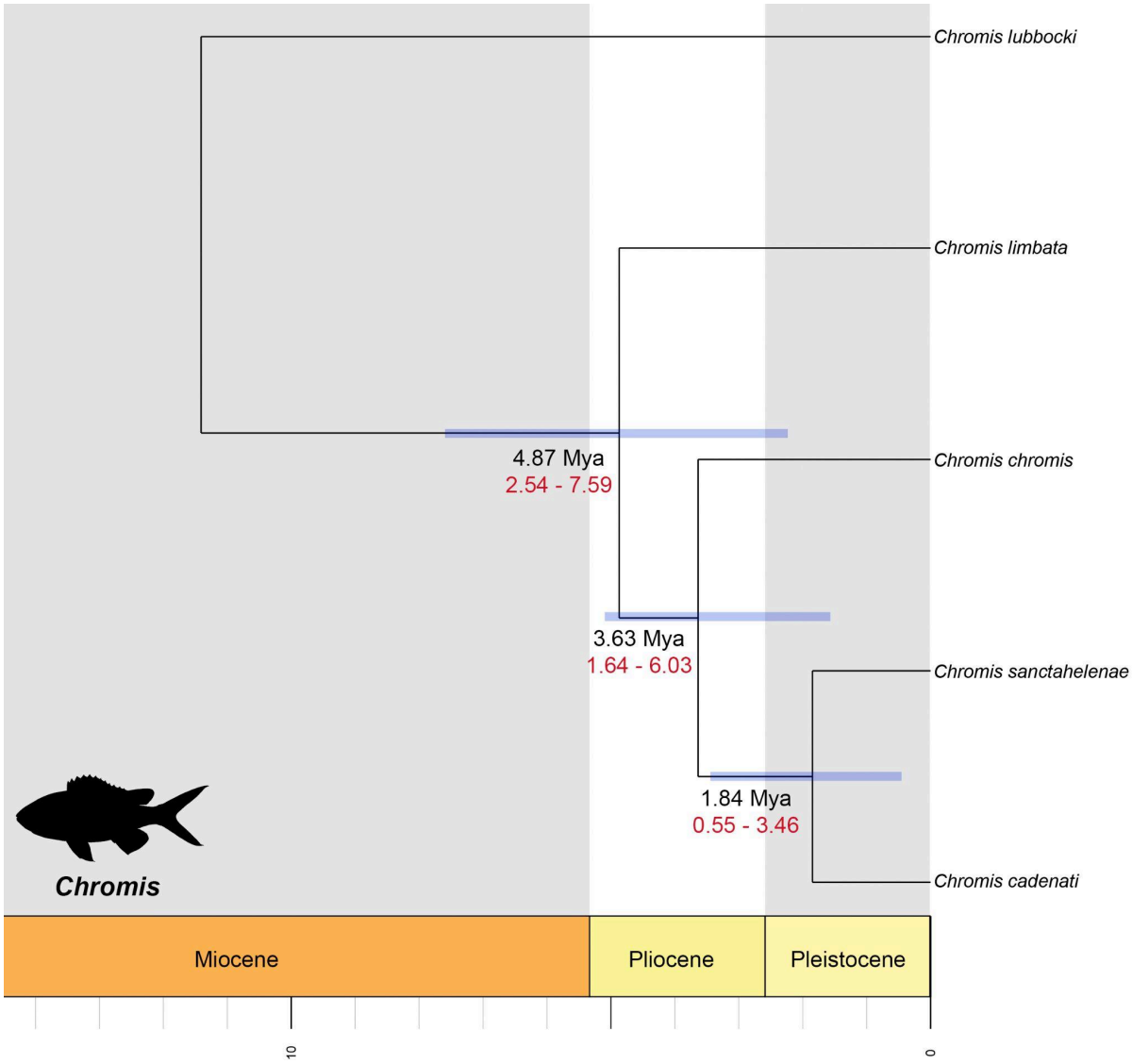

Figure S6. Estimates of divergence time within the clade of *Chromis* containing endemic species from the tropical Mid-Atlantic Ridge. Estimations are based on four molecular markers: 12S, COI, Cytb, and D-loop. Divergence times are shown in black. The red numbers indicate the 95% Highest Posterior Density (HPD) intervals. Timescale in millions of years before the present.

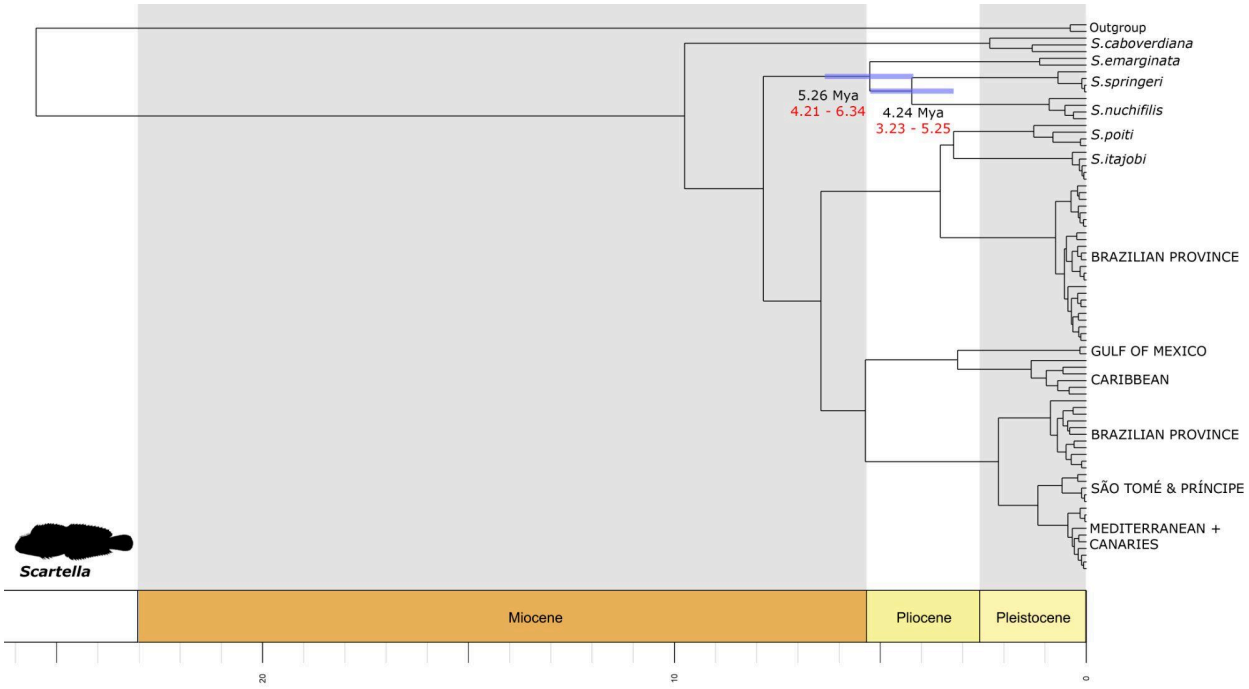

Figure S7. Estimates of divergence time within the clade of *Scartella* containing endemic species from the tropical Mid-Atlantic Ridge (Modified from Araujo et al. 2020). Estimations are based on the mitochondrial D-loop region. Divergence times are shown in black. The red numbers indicate the 95% Highest Posterior Density (HPD) intervals. Timescale in millions of years before the present.

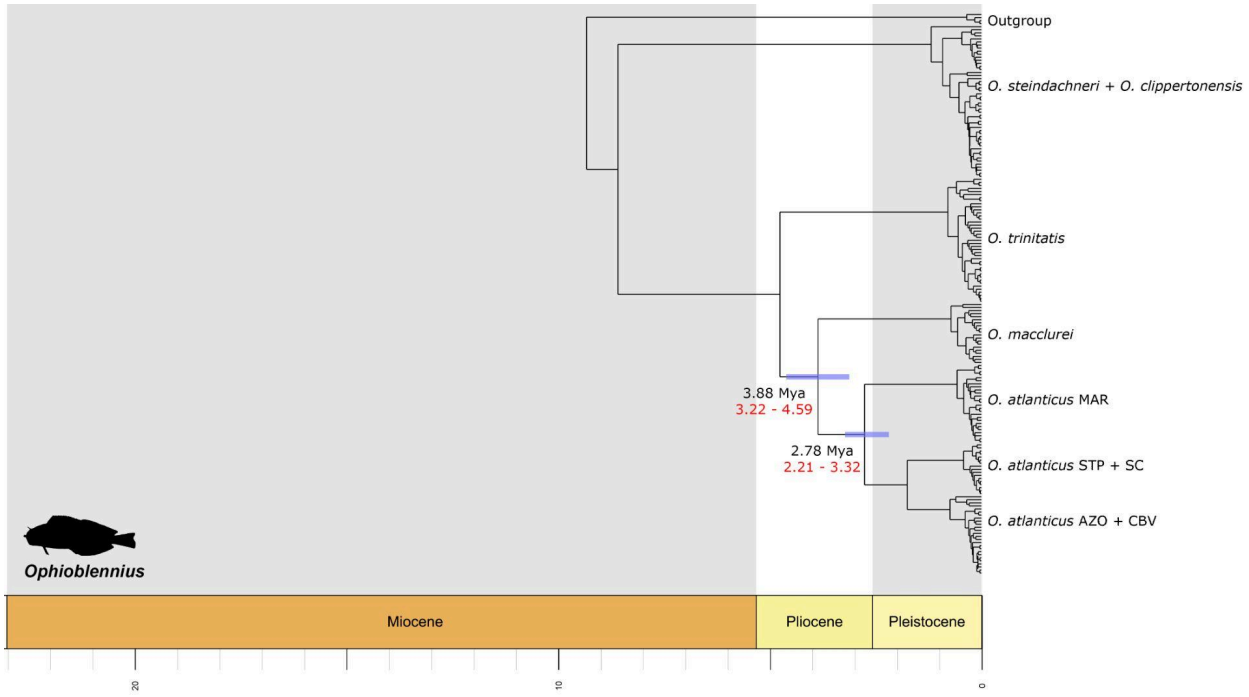

Figure S8. Estimates of divergence time within the clade of *Ophioblennius* containing endemic species from the tropical Mid-Atlantic Ridge. Estimations are based on the Cytb. Divergence times are shown in black. The red numbers indicate the 95% Highest Posterior Density (HPD) intervals. Timescale in millions of years before the present.

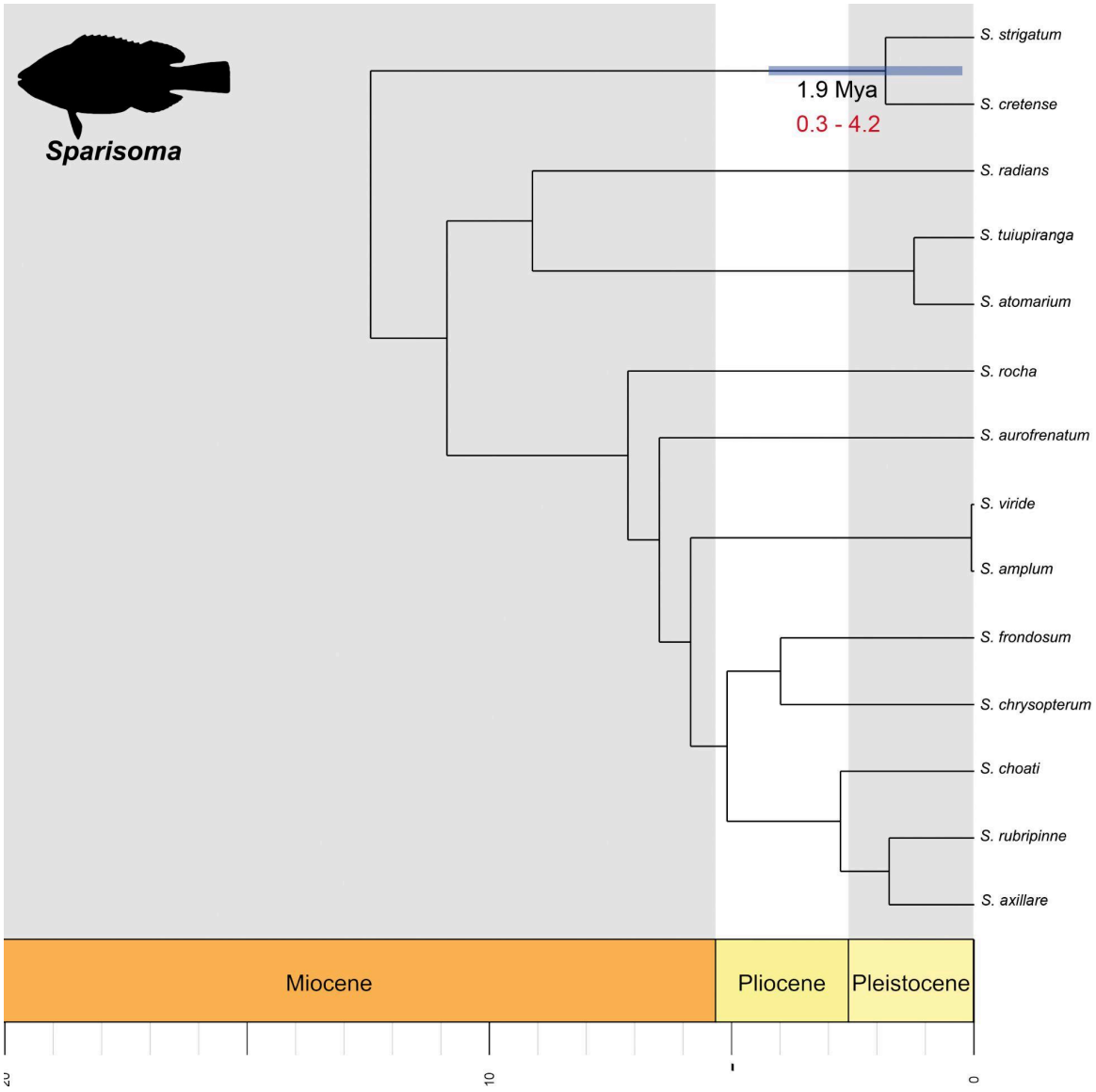

Figure S9. Estimates of divergence time within the clade of *Sparisoma* containing endemic species from the tropical Mid-Atlantic Ridge (modified from Siqueira et al. 2019). Estimations are based on 11 molecular markers: COI, Cytb, 12S, 16S, Control Region, Bmp4, Dlx2, Otx1, Rag2, S7I1, and TMO4C4. Divergence times are shown in black. The red numbers indicate the 95% Highest Posterior Density (HPD) intervals. Timescale in millions of years before the present.

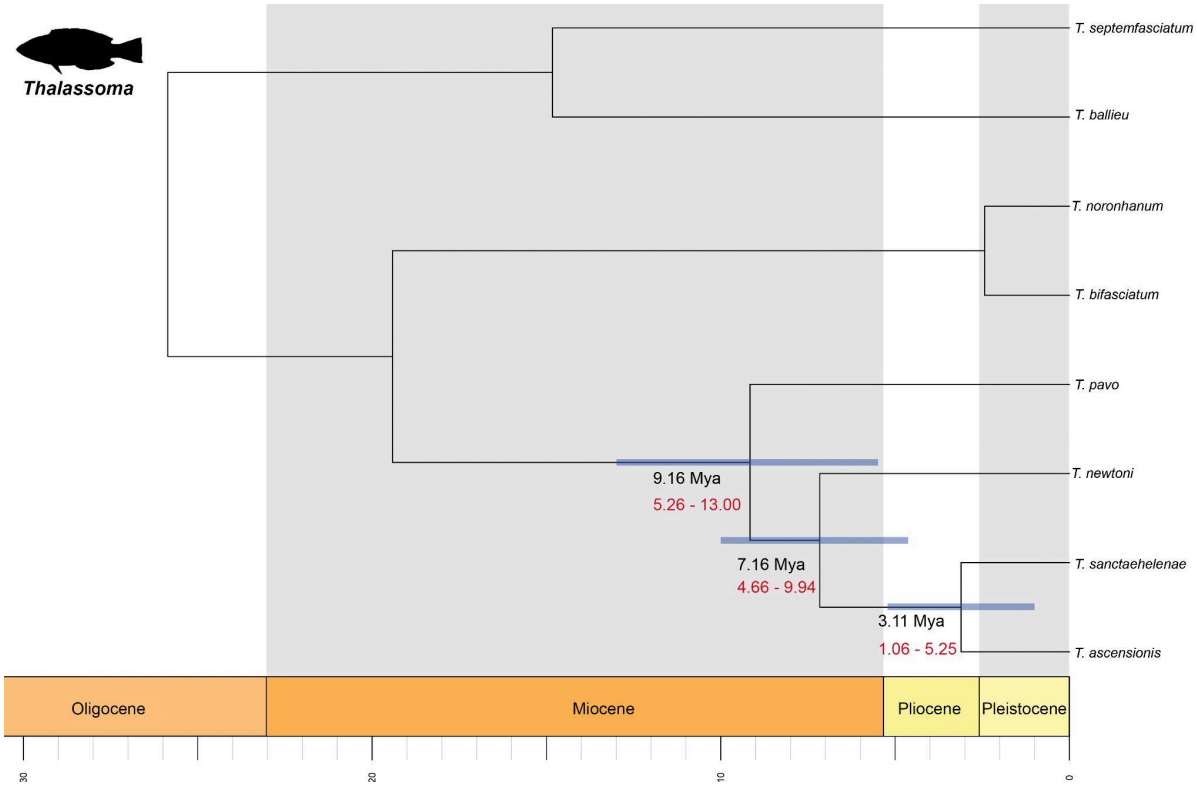

Figure S10. Estimates of divergence time within the clade of *Thalassoma* containing endemic species from the tropical Mid-Atlantic Ridge. Estimations are based on five molecular markers: COI, Cytb, 12S, Control Region, and S7. Divergence times are shown in black. The red numbers indicate the 95% Highest Posterior Density (HPD) intervals. Timescale in millions of years before the present.

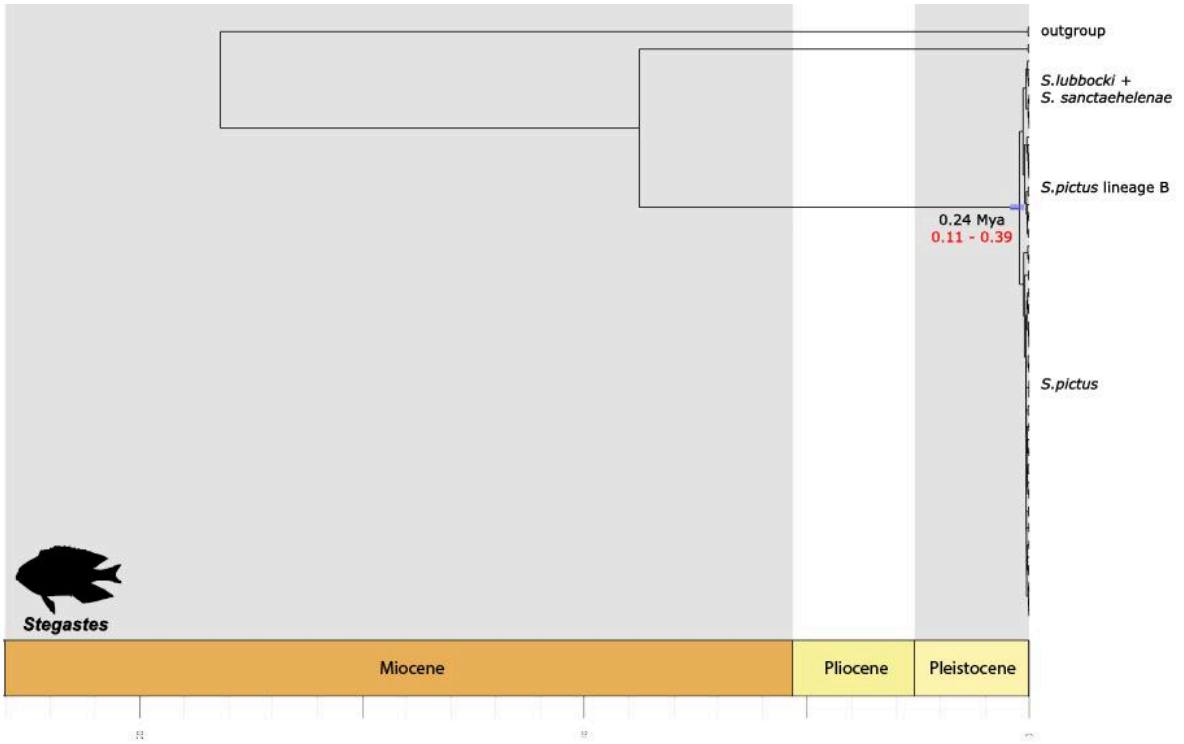

Figure S11. Estimates of divergence time within the clade of *Stegastes* containing endemic species from the tropical Mid-Atlantic Ridge. Estimations are based on Cytb. Divergence times are shown in black. The red numbers indicate the 95% Highest Posterior Density (HPD) intervals. Timescale in millions of years before the present.

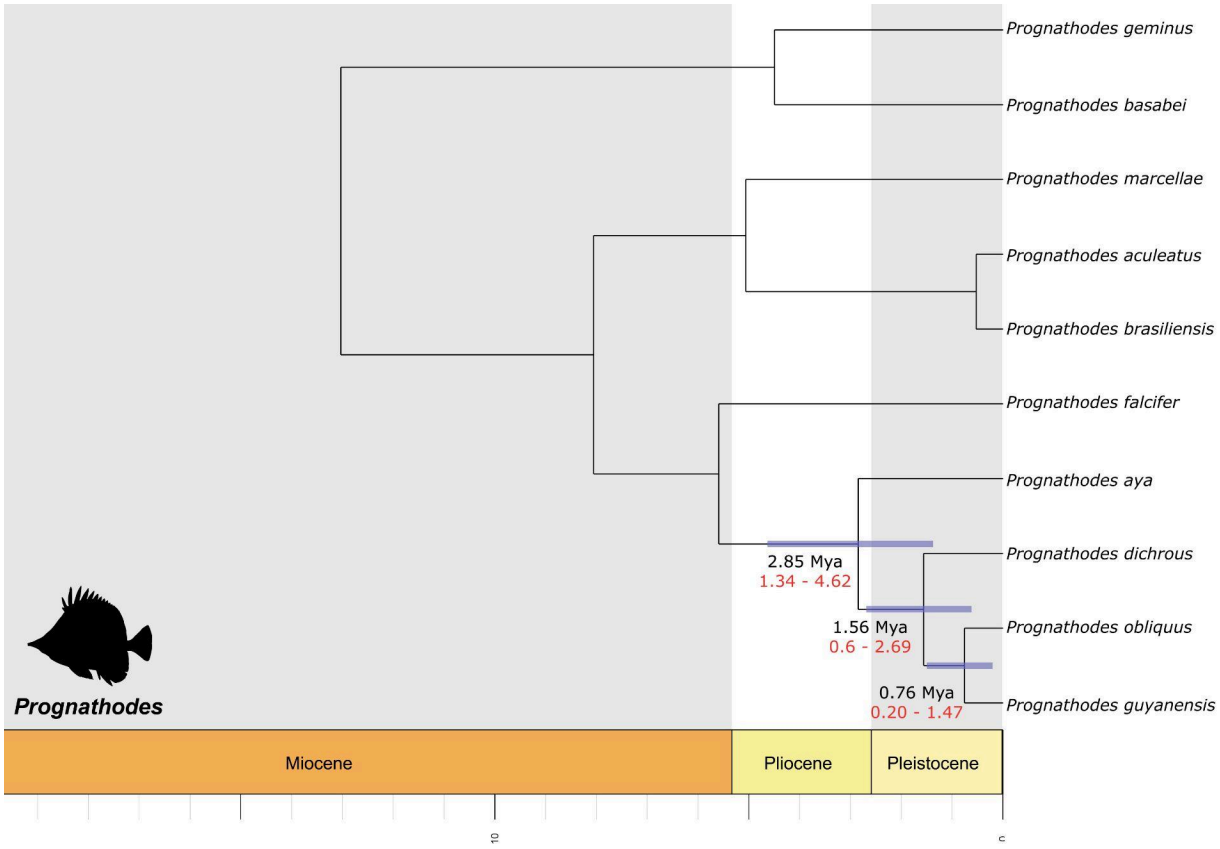

Figure S12. Estimates of divergence time within the clade of *Prognathodes* containing endemic species from the tropical Mid-Atlantic Ridge. Estimations are based on COI. Divergence times are shown in black. The red numbers indicate the 95% Highest Posterior Density (HPD) intervals. Timescale in millions of years before the present.

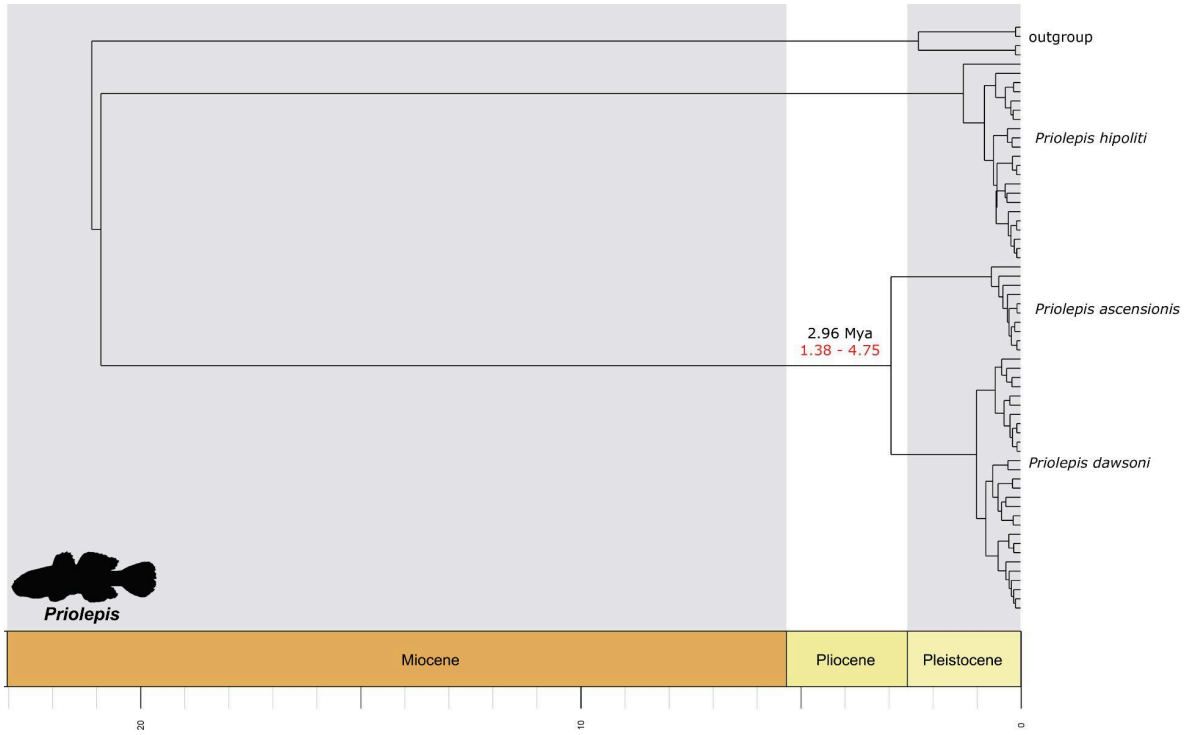

Figure S13. Estimates of divergence time within the clade of *Priolepis* containing endemic species from the tropical Mid-Atlantic Ridge. Estimations are based on COI. Divergence times are shown in black. The red numbers indicate the 95% Highest Posterior Density (HPD) intervals. Timescale in millions of years before the present.

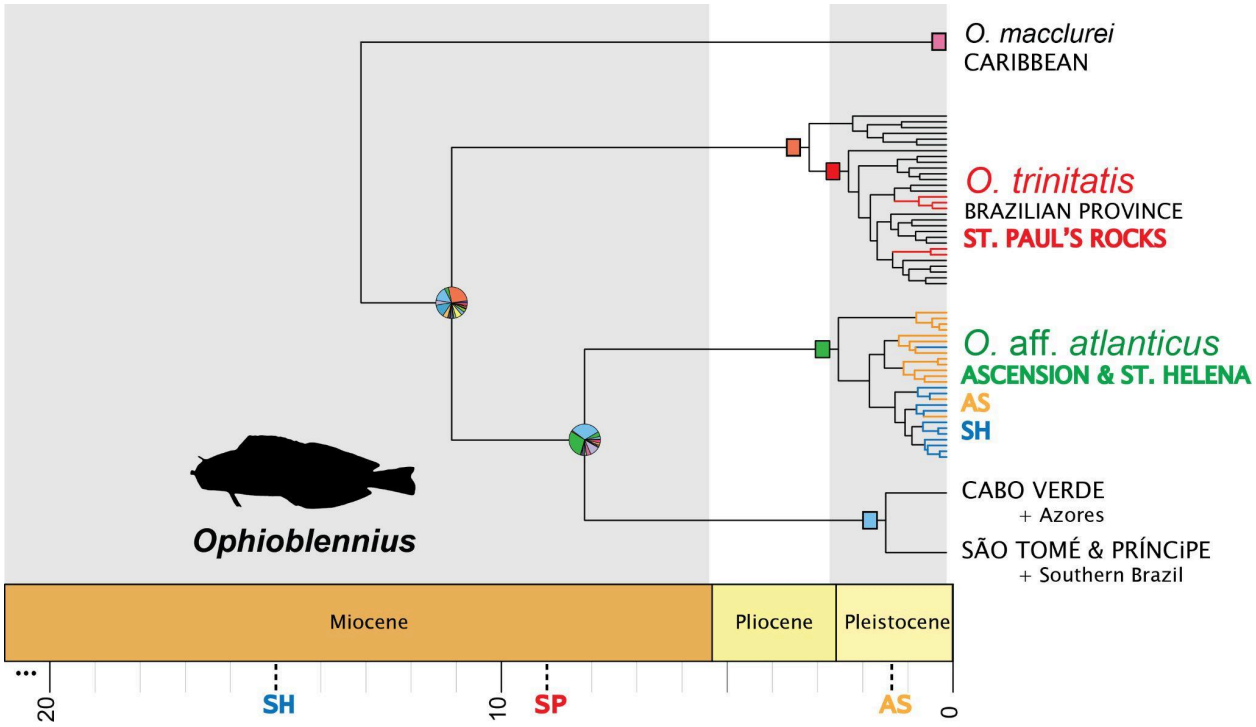

Figure S14. Ancestral area reconstruction over chrono-phylogeny for the *Ophioblennius* clade containing species from the tropical Mid-Atlantic Ridge islands. Pie charts represent the probability of ancestral geographic origins for each node. Timescale in millions of years before the present.