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ORIGINAL ARTICLE

Marine dispersal and barriers drive Atlantic seahorse diversification

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ABSTRACT

Aim To investigate how marine barriers shaped the demographic history of Atlantic seahorses (Syngnathidae: *Hippocampus*).

Location Atlantic Ocean.

Methods Range-wide sampling (n = 390) at mitochondrial and up to five nuclear DNA loci was carried out across the *Hippocampus erectus* species complex (*H. erectus* from the Caribbean/North America, *H. patagonicus* from South America and *H. hippocampus* from Europe and West Africa). Multi-species coalescent and approximate Bayesian computation (ABC) frameworks were used to estimate support of competing biogeographical hypotheses and demographic parameters, including lineage divergence times, effective population sizes and magnitudes of population size change.

Results We identified four distinct lineages within the *H. erectus* complex. A posterior probability of 0.626 and corresponding Bayes factors ranging from 3.68 to 11.38 gave moderate to strong support for a basal divergence between South American populations of *H. patagonicus* and Caribbean/North American populations of *H. erectus* coincident with the inter-regional freshwater outflow of the Amazon River Barrier (ARB). Estimates of historical effective population sizes and divergence times indicate that European and West African populations of *H. hippocampus* expanded after colonization from a more demographically stable Caribbean/North American *H. erectus.*

Main conclusions Our findings of trans-Atlantic colonization followed by isolation across a deep oceanic divide, and isolation across a freshwater barrier, may demonstrate a contrast in marine divide permeability for this group of rafters. Demographic inference supports the establishment of an ancestral population of the *H. erectus* complex in the Americas, followed by the ARB splitting it into Caribbean/North and South American lineages at a time of increased sedimentation and outflow. Our estimates suggest that following this split, colonization occurred across the Atlantic via the Gulf Stream currents with subsequent trans-Atlantic isolation. These results illustrate that rafting can be a means of range expansion over large distances, but may be insufficient for sustaining genetic connectivity across major barriers, thereby resulting in lineage divergence.

Keywords

Atlantic Ocean, coalescent, demographic history, *Hippocampus*, marine barriers, ocean currents, rafting, seahorse, Syngnathidae.

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INTRODUCTION

For marine taxa with freely mobile adults or larvae, mounting genetic evidence demonstrates that mechanisms other than strict vicariant speciation may drive diversification, including soft vicariance, parapatric speciation, ecological speciation and founder colonization (Rocha *et al.*, 2005; Lessios & Robertson, 2006; Hickerson & Meyer, 2008; Rocha & Bowen, 2008; Luiz *et al.*, 2012). These processes are undoubtedly affected by the permeability of marine divides, which are proposed to serve as 'biogeographical filters' rather than strict barriers (Vermeij, 1978; Floeter *et al.*, 2008). Hence, the interplay between ocean currents, the emergence of oceanic barriers, organismal dispersal potential and organismal environmental tolerances all contribute to patterns of diversification in the oceans (Joyeux *et al.*, 2001; Muss *et al.*, 2001; Floeter *et al.*, 2008; Rocha *et al.*, 2008; Hellberg, 2009).

Seahorses (Syngnathidae: *Hippocampus*; nomenclature follows Lourie *et al.*, 1999; Piacentino & Luzzatto, 2004) are presumed to have a low dispersal potential because they are poor swimmers and lack a pelagic larval phase, yet many species have vast distributions (thousands of kilometres) with variable genetic connectivity, which suggests moderate to high long-distance dispersal capabilities (Lourie *et al.*, 2004, 2005; Teske *et al.*, 2005; Woodall *et al.*, 2011). This apparent paradox probably arises from the ability to raft on floating mats of *Sargassum* and other macroalgae (Günther, 1870; Casazza & Ross, 2008; Woodall *et al.*, 2009), a mode of dispersal utilized by many fishes (Casazza & Ross, 2008; Luiz *et al.*, 2012) and invertebrates (Ingólfsson, 1995; Theil & Gutow, 2005; Fraser *et al.*, 2011). Rafting has been shown to be a strong general predictor of colonization for coral reef fishes across the deep oceanic barrier of the equatorial mid-Atlantic (Luiz *et al.*, 2012). Consistent with this trend, recent studies have concluded that diversification in *Hippocampus* may be driven by rafting-mediated founder colonization followed by levels of gene flow insufficient for maintaining genetic connectivity with the original population (Lourie *et al.*, 2005; Teske *et al.*, 2005, 2007). Therefore, rafting-mediated dispersal can be differentiated into two general scales: (1) regional gene flow that links demes by recruitment between spatially distinct populations; and (2) large-scale colonization that results in range expansion across marine barriers, followed by divergence when subsequent dispersal events are too rare to prevent isolation (i.e. < 1 migrating individual per generation) (Slatkin, 1985; Paulay & Meyer, 2002).

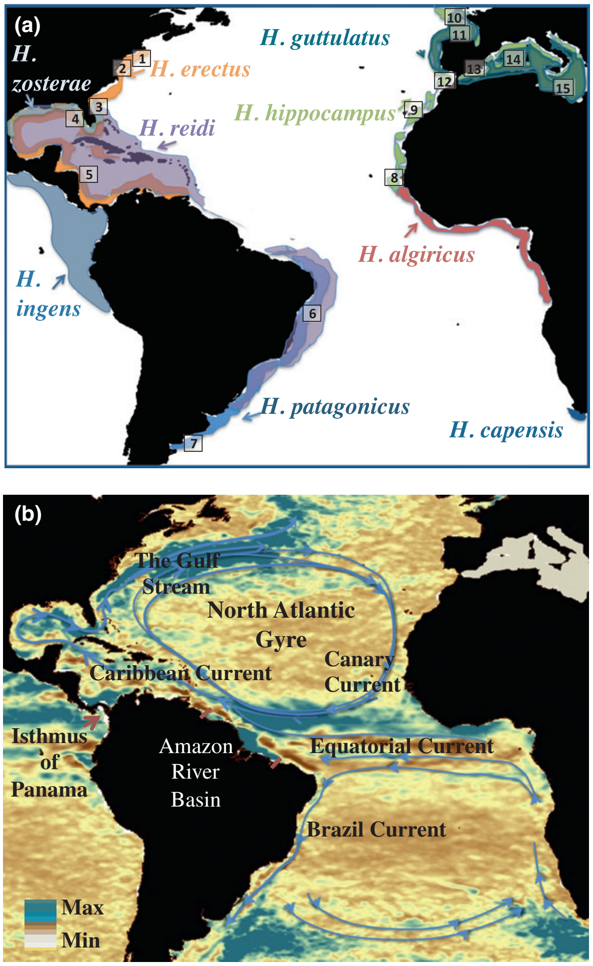

Figure 1 Atlantic seahorse distributions and major ocean currents. (a) Distributions modified from Teske *et al.* (2007) to include *Hippocampus patagonicus.* Small boxes 1–15 represent the collection locations of the *Hippocampus erectus* complex used in this study: (1) Hudson River Estuary and southern Long Island, New York ($n = 31$), Tuckerton and Little Bay, New Jersey ($n = 13$); (2) Chesapeake Bay ($n = 22$); (3) Atlantic Coast (Indian River and Jacksonville, Florida; $n = 10$); (4) Gulf Coast (Tampa Bay, south-east peninsula, offshore trawls; $n = 28$); (5) Caribbean Sea ($n = 10$); (6) Brazil ($n = 7$); (7) Argentina (Argentine Sea, San Antonio Bay and Mar de Plata; $n = 14$); (8) West Africa (Senegal; $n = 26$); (9) Canary and Porto Santo Islands ($n = 44$); (10) UK and Channel Islands ($n = 39$); (11) Bay of Biscay ($n = 32$); (12) South Iberia ($n = 31$); (13) southern coast, Spain ($n = 18$); (14) western Mediterranean ($n = 44$); and (15) eastern Mediterranean ($n = 44$). (b) Major ocean currents of North and South Atlantic gyre systems. The ocean environmental data layer is a five-year mean (1993–1997) of absolute velocity maximum (AVISO Remote Satellite data) transformed using Marine Geospatial Ecology Tools (mget) 0.7 (Roberts *et al.*, 2010) and visualized in ArcGIS 9.3 (ESRI, Redlands, CA). Blue colours represent current maxima, with arrows showing direction.

Here, we use population-level sampling to test four competing historical biogeographical hypotheses that involve divergences across oceanic divides and a major freshwater outflow in three widespread sibling species of seahorses: *H. erectus* from Caribbean/North America, *H. hippocampus* from Europe and West Africa, and *H. patagonicus* from South America (hereafter the *H. erectus* complex). The distribution of the *H. erectus* complex encompasses most of the tropical and warm-temperate coasts of the Atlantic (Fig. 1a), allowing us to study the impact of two types of major marine barriers on the diversification of this species complex: (1) the deep oceanic divides of the mid-northern and equatorial Atlantic; and (2) the Amazon River Barrier (ARB), the largest freshwater outflow in the world (Briggs, 1995; Rocha, 2003). Both barrier types are permeable, but to cross them poses considerable challenges, and may affect rafting dispersers in different ways. For oceanic crossing, currents can provide directionality to patterns of colonization from source regions (Gillespie *et al.*, 2012), and rafting vectors are predicted to disperse primarily with the prevailing asymmetrical clockwise surface currents of the North Atlantic Gyre system (Fig. 1b). For freshwater outflows, the extreme environmental shift created by the ARB acts as a break between some Caribbean and Brazilian faunas, while others show evidence of genetic connectivity (Joyeux *et al.*, 2001; Rocha *et al.*, 2002, 2005).

In order to investigate how these two types of barriers shaped diversification in *Hippocampus*, we use coalescent methods with multi-locus population genetic data (mitochondrial DNA and up to five nuclear DNA loci, from 390 individuals throughout the *H. erectus* complex range) to obtain the relative probabilities of the following four competing hypotheses whose branching order is depicted in Fig. 2.

1. **H_{EAO}** – western Atlantic lineages were the product of equatorial current dispersal from a basal north-eastern Atlantic population followed by ARB isolation between Caribbean/North American and South American lineages.
2. **H_{WAO}** – north-eastern Atlantic lineages were the product of Gulf Stream dispersal from the Caribbean/North American lineage subsequent to the ARB isolating Caribbean/North American and South American lineages.
3. **H_{CLK}** – north-eastern Atlantic lineages were the product of Gulf Stream dispersal from a basal Caribbean/North American lineage prior to a colonization of South American lineages from the north-eastern Atlantic.

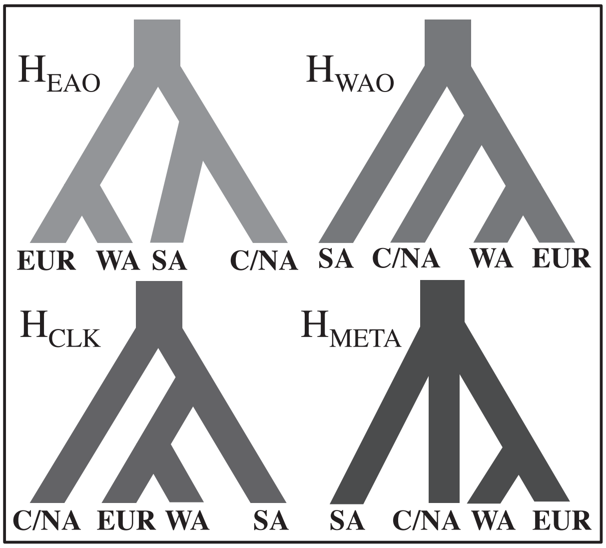

Figure 2 Four possible hypotheses explaining the branching history of the *Hippocampus erectus* complex: H_{EAO}, eastern Atlantic origin hypothesis; H_{WAO}, western Atlantic origin hypothesis; H_{CLK}, clockwise colonization hypothesis; H_{META}, metapopulation divergence hypothesis. C/NA, Caribbean/North America; SA, South America; WA, West Africa; EUR, Europe.

4. **H_{META}** – a large connected metapopulation spanning the Americas and the north-eastern Atlantic became isolated to form all contemporary *H. erectus* complex lineages.

To estimate the statistical support for each of the four hypotheses, we use an approximate Bayesian computation (ABC) framework. Importantly, this approach yields relative timeframe estimates for the corresponding demographic events, and patterns of population stability and instability within each modelled hypothesis. To put the inferences in a larger context, we also reconstruct the species tree for all Atlantic seahorse species. The majority of Atlantic *Hippocampus* species remain 'data deficient' under IUCN Red List evaluations (IUCN, 2012), and our study is therefore well timed to help inform conservation management, while providing insight into ecological and evolutionary processes that drive diversification of Atlantic fishes.

MATERIALS AND METHODS

Taxonomic background of study taxa and sampling

Many *Hippocampus* species have a high degree of intraspecific morphological plasticity linked to environmental gradients and habitats resulting in historical taxonomic confusion (Ginsburg, 1937; Lourie *et al.*, 2004). Lourie *et al.* (2004) resolved many of these taxonomic issues and recognized seven species of Atlantic seahorses. *Hippocampus erectus* was consolidated into a single species with a confirmed distribution along the coastal western Atlantic from Venezuela to Canada (Lourie *et al.*, 2004), with individuals as far south as Argentina tentatively included. Limited genetic data suggested that specimens from Argentina and Brazil appear distinct from north Atlantic specimens (Casey *et al.*, 2004; Lourie *et al.*, 2004; Luzzatto *et al.*, 2012). In addition, our findings of evolutionary lineage independence (Moritz, 2002) and morphological evidence further support the distribution of a second species named *H. patagonicus* (Piacentino & Luzzatto, 2004) and we treat the lineage south of the ARB as *H. patagonicus* throughout this study (Fig. 1b).

Individuals were sampled throughout the range of the *H. erectus* complex (390 samples; listed in Appendix S1 in Supporting Information). In addition, specimens of *H. capensis* ($n = 2$), *H. reidi* ($n = 4$), *H. zosterae* ($n = 3$), *H. guttulatus* ($n = 3$), *H. algiricus* ($n = 2$) and non-Atlantic *H. ingens* (eastern Pacific, $n = 4$) and *H. kelloggi* (Indo-Pacific, $n = 4$) were included in species tree (*BEAST) estimates (Heled & Drummond, 2010). Sequence divergence between samples of the transisthmian geminate species *H. ingens* and *H. reidi* were used to estimate nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) mutation rates. These species are assumed to have separated during the final rise of the Isthmus of Panama (3.2 Ma) (Coates *et al.*, 1992; Teske *et al.*, 2007), with genetic divergence occurring earlier (Hickerson *et al.*, 2006), thereby yielding a range of upper and lower bound temporal rate estimates. Data for all species were combined from direct sequencing and NCBI database specimens (Appendix S1). When possible, non-invasive sampling was conducted by removing a fin clip from the lower dorsal fin. In total, sequence data from 412 individuals were included in this study. Newly generated sequences were deposited in GenBank (Appendix S1).

Coalescent-based reconstruction of species trees and demographic history

Phylogenetic relationships among Atlantic seahorses were reconstructed in *BEAST 1.53 using a multispecies coalescent approach that co-estimates multiple gene trees embedded in a shared species tree, while accounting for the stochastic gene tree/species tree incongruence expected with genetic drift (Edwards, 2009; Heled & Drummond, 2010). Our aligned data set was 3840 bp from three partial mtDNA gene regions [cytochrome *b* (*cytb*): 696 bp; cytochrome *c* oxidase subunit I: 652 bp; and control region: 371 bp] as well as five nuclear loci (aldolase: 186 bp; myh6: 711 bp; rhodopsin: 438 bp; Tmo4c4: 464 bp; and a partial S7 intron: 322 bp). For details of PCR and sequencing methods, see Appendix S1.

*BEAST accounts for the independent coalescent histories of each sampled locus, with the mtDNA genes being treated as a single non-recombining locus. The number of gene copies per locus per population averaged 4.8, and each nDNA locus was assumed to be unlinked. All loci were partitioned using the closest models selected by the Akaike information criterion (AIC) in jModelTest 2 (Darriba *et al.*, 2012). *BEAST was run using a Yule prior with a strict molecular clock (estimated across loci) consisting of two independent chains each of 10^8 iterations and parameters logged every 10,000 samples. After discarding the first 2×10^7 iterations as burn-in, a 50% majority clade tree was obtained in TreeAnnotator 1.6.1 and visualized in FigTree 1.3.1 (Rambaut, 2012). Convergence was assessed with effective sample size (ESS) values (all values > 200) in Tracer 1.5 (Rambaut & Drummond, 2007). In addition, the final log file of the 335 most probable species trees after burn-in was visualized in DensiTree 1.45 (Bouckaert, 2010) to illustrate the statistical uncertainty associated with our species-tree estimate (Fig. 3). For independent gene trees, see Appendix S2.

In order to assess the relative probability of each of the four historical hypotheses for the *H. erectus* complex outlined in the Introduction (Fig. 2), we used an approximate Bayesian computation (ABC) approach deployed in the program DIYABC (Cornuet *et al.*, 2008, 2010). This approach compresses the molecular data into informative summary statistics that are then compared to simulated data under our modelled hypotheses. Following simulation, Euclidean distances between the observed and simulated data set were computed using a local linear or polychotomous regression, and the closest 2% of the simulated data to the observed data were retained to estimate the posterior distributions (Beaumont *et al.*, 2002; Cornuet *et al.*, 2008). This allows for the ranking of modelled hypotheses based on approximate marginal likelihoods that were statistically compared using

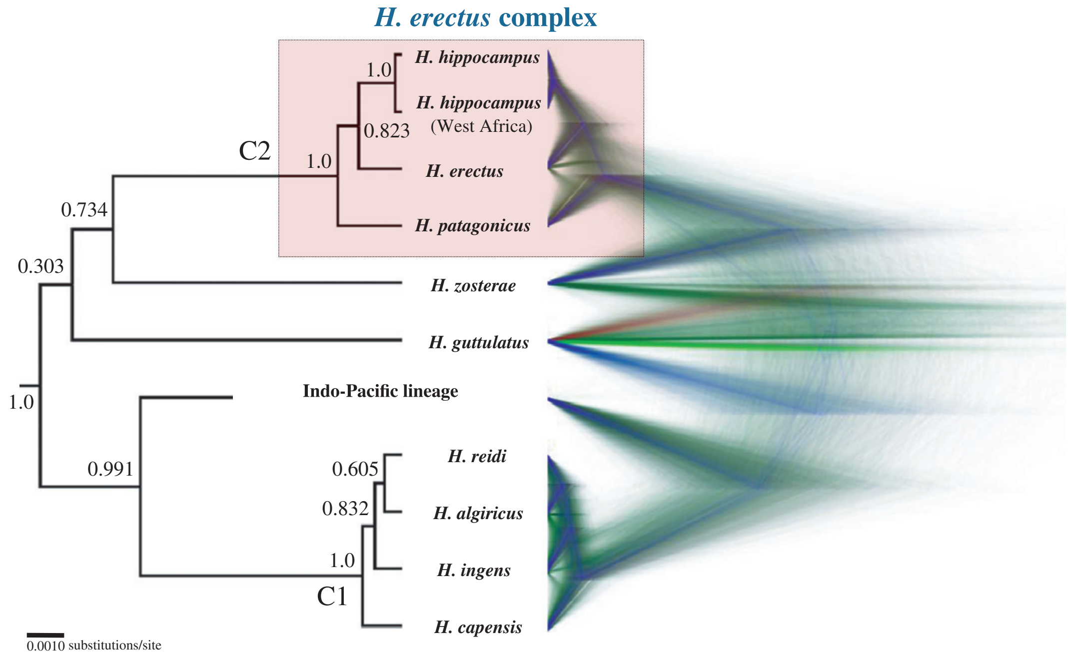

Figure 3 Species tree of the *Hippocampus erectus* complex and other Atlantic seahorses. Left: 50% majority-rule tree representing the probability of a given gene tree evolving within the branches of a species tree, generated in *BEAST. Right: 335 most probable topologies visualized in DensiTree 1.45 to illustrate the statistical uncertainty of our species tree estimation. Greater topological agreement is visualized by a higher density of trees, whereas uncertainty in the height and distribution of nodes are represented by increased transparency.

Bayes factors (Jeffreys, 1961) to classify the best-fit model. Following this step, Monte Carlo validation was used to demonstrate accuracy and precision in the model choice procedure (Robert *et al.*, 2011).

Within the ABC framework, four alternative hypotheses based on different species tree topologies (Fig. 2) were jointly simulated with population genetic parameters. These include: effective population sizes, divergence times and the timing and magnitude of changes in historical population size. Using sample sizes identical to those of the observed data, we generated 1 million simulations under each modelled hypothesis. Based on intraspecific analyses across all sampled populations (Appendix S3), Caribbean/North American *H. erectus* and South American *H. patagonicus* samples were considered independent evolutionary panmictic populations (Moritz, 2002), whereas eastern Atlantic *H. hippocampus* samples were regionally subdivided and constrained to be sister populations (West African and Europe). In all four historical scenarios, we explored three possible three-taxon population topologies plus a polytomy, each involving the split of an ancestral population into the three regional lineages with a subsequent split of the north-eastern Atlantic population into West African and European populations (Fig. 2).

As a preliminary check, we evaluated whether our models together with the chosen prior distributions were able to generate a subset of statistics close to our observed summary statistics. This was carried out by locating the observed value of each summary statistic within a principal components analysis (PCA) plane of 50,000 simulated data sets (Cornuet *et al.*, 2010) (Appendix S2). All summary statistics used in our final analysis showed a good fit across models, and we kept the following global summary statistics for downstream analyses: (1) number of segregating sites and (2) mean pairwise differences, which are well established as being jointly informative about past growth rates in the populations (Beaumont, 2010). Standard measures of allelic diversity included (3) the number of private segregating sites, and (4) the number of distinct haplotypes (mtDNA only). For pairwise interpopulation summary statistics, we included the mean of between-sample pairwise differences and the F_{ST} value between two samples (Appendix S3).

From the mtDNA locus, 390 individuals from the *H. erectus* complex were included (partial *cytb* – 391 bp) in the ABC analysis (Fig. 1a and Appendix S1). At the five autosomal loci, all sequences were phased probabilistically using the program PHASE in DnaSP 5 (Librado & Rozas, 2009). PHASE was run for 10,000 iterations, retaining results with a probability of > 95%. All base calls were unambiguously recovered, and final data sets included both phased gene copies from each sequenced individual (*H. erectus*, *n* = 8–46; *H. patagonicus*, *n* = 6–18; *H. hippocampus* West Africa, *n* = 6; and *H. hippocampus* Europe, *n* = 6–36). Genetic data under each of the four hypotheses were simulated under the exact number of gene copies per locus per population sample, which includes independent observed data sets for haploid (mtDNA) and diploid (nDNA) loci (Appendix S3). For the final analysis, we used uniform priors with a lower and an upper bound for population size of 10 to 2×10^6, and divergence times of 1×10^3 to 4×10^6 generations in the past for t1 and t2, and 1×10^3 to 7.25×10^6 generations in the past for t3. Generation time was assumed to be one year for all species, and all populations were allowed to vary to include a discrete size-change event. The mutation rate of each nuclear locus was drawn from a gamma distribution with 95% quantiles encompassing 1.6×10^9 to 1.6×10^{10} (in units of mutations per site per generation/lineage), while the mtDNA mutation rate was drawn from a uniform distribution with 95% quantiles encompassing 5.5×10^9 to 7.5×10^9 (in units of mutations per site per generation/lineage). Because there is great uncertainty in assumed mutation rates (Ho *et al.*, 2011), we emphasize relative time estimates.

We computed the posterior probability of each hypothesis by performing a weighted polychotomous logistic regression on 2% of simulated data sets closest to the observed data (Cornuet *et al.*, 2008, 2010). Subsequently, we used a local linear regression on 1% of the accepted closest simulated data sets conditional on the most likely hypothesis to estimate the posterior distributions of parameters. Due to the potential biases arising when using Bayes factors to conduct ABC model discrimination among non-nested models (Robert *et al.*, 2011), we evaluated the power and accuracy of our ABC model selection using simulation validation techniques (Cornuet *et al.*, 2008, 2010). Specifically, we simulated 100 test data sets (i.e. PODS; pseudo-observed data sets) under each of the four competing hypotheses and calculated the probability of type I and type II errors using the criteria of Cornuet *et al.* (2010), with conditions (i.e. tolerance, summary statistics and number of random prior iterations) and sample sizes identical to our empirically-based estimates and hypothesis choice.

RESULTS

Phylogenetic relationships of Atlantic seahorses

The *BEAST species tree recovered two well-supported clades (C1 and C2) (Fig. 3; posterior probability = 1.0), both of which are associated with trans-Atlantic biogeographical distributions, and bifurcations appear to follow major directional ocean currents (Fig. 1b) from basal to terminal species throughout Atlantic seahorse diversification (Fig. 3). The focal lineages in this study, *H. erectus*, *H. hippocampus* and *H. patagonicus* (C2), form a monophyletic group (posterior probability 1.0) (Fig. 3), clearly placing *H. patagonicus* within the *H. erectus* complex, with a within-clade posterior probability of 0.823. Atlantic *Hippocampus* is most likely to have originated from Indo-Pacific lineages roughly 15 Ma, coincident with an ancient split at the closure of the Tethyan Seaway, followed by trans-Atlantic expansion (Teske *et al.*, 2007). The oldest bifurcation between the north-eastern Atlantic *H. guttulatus* (Europe) and the north-western

H. zosterae (Florida, USA) lacks support (posterior probability 0.303) and additional loci may be required to resolve this divergence.

Consistent with Teske *et al.* (2007), C1 contains a trans-Atlantic sister-species relationship between *H. algiricus* and *H. reidi* across the equatorial mid-Atlantic, with a basal placement of *H. capensis* (posterior probability 1.0) and is linked to an ancestral Indo-Pacific lineage (Fig. 3) (Teske *et al.*, 2007). This topology is consistent with a 'colonization pathway' of dispersal around the southern tip of Africa that has been found for other species of Atlantic fishes with ancestral origins in the Indian Ocean (Bowen *et al.*, 2006). C1 also includes the transisthmian geminate species *H. ingens* that is believed to have separated from *H. reidi* after the final rise of the Isthmus of Panama (Teske *et al.*, 2007).

Demographic history inference and species tree concordance of the *Hippocampus erectus* complex

Both coalescent-based methods suggest the western Atlantic origin hypothesis (H_{WAO}; Fig. 2) for the *H. erectus* complex, in preference to the three other hypotheses. Posterior probability of support for H_{WAO} was 0.626 using DIYABC (model probabilities: H_{EAO}, 0.170; H_{CLK}, 0.055; H_{META}, 0.149), with corresponding Bayes factors ranging from 3.68 to 11.38 giving moderate to strong support for H_{WAO} over the three hypotheses (Appendix S2). Additionally, model choice validation using PODS indicate that adequate power exists for selecting the true hypothesis among competing hypotheses (Cornuet *et al.*, 2010) with an acceptable type I error rate (28%) and low type II error rate (8%).

Conditional on H_{WAO}, the ancestral population of the *H. erectus* complex was first isolated in the western Atlantic, supporting a divergence between South American populations (*H. patagonicus*) and Caribbean/North American populations (*H. erectus*) approximately 5.27 Ma [95% credibility intervals (CI): 2.74–7.25 Ma] (Table 1, Fig. 4). Subsequent to this isolation event, the European and West African populations (*H. hippocampus*) became isolated from Caribbean/North American populations around 3.35 Ma (95% CI: 1.98–5.73 Ma). This second isolation event probably involved a substantial reduction in population size with the founding of the eastern Atlantic region, the effective population size (N_e) being 54,300 individuals (95% CI: 38,000–1,610,000) before the split into West African and European populations 484 ka (95% CI: 268 ka to 1.39 Ma). Following this split, subsequent population expansions occurred in Europe with an N_e of 1.7 million individuals (95% CI: 1,470,000–1,940,000) and a West African N_e of 829,000 individuals (95% CI: 404,000–1,680,000). In both eastern Atlantic populations, the posterior probabilities support the timings of major expansions to be recent and substantial. In contrast, the N_e of the Caribbean/North American population (*H. erectus*) of 1.92 million individuals (95% CI: 1,730,000–1,980,000) is inferred to be more stable throughout the various spitting events with some expansion. In addition, the N_e of the Caribbean/North American population (*H. erectus*) is roughly 10 times larger than the South American population (*H. patagonicus*) of 191,000 individuals (95% CI: 86,000–691,000).

Table 1 Parameter estimates based on posterior probabilities of the western Atlantic origin hypothesis (H_{WAO}) for the *Hippocampus erectus* complex.

Parameter	Mode	Quantiles 5%–95%
Modern N_e		
C/NA	1.92×10^6	1.73×10^6 to 1.98×10^6
SA	1.91×10^5	8.62×10^4 to 6.91×10^5
EU	1.79×10^6	1.47×10^6 to 1.94×10^6
WA	8.29×10^5	4.04×10^5 to 1.68×10^6
Ancestral N_e at time of size change		
EUWAt1sc	NC	
EUt1sc	3.39×10^5	1.01×10^5 to 1.79×10^6
EUAnt2sc	5.43×10^4	3.78×10^4 to 1.61×10^6
SAsc	NC	
C/NAsc	8.18×10^5	2.10×10^5 to 1.88×10^6
Ancestral populations		
A2sc	1.39×10^6	1.53×10^5 to 1.90×10^6
A1sc	2.28×10^5	5.19×10^4 to 1.64×10^6
Divergence times		
t1	4.84×10^5	2.76×10^5 to 1.43×10^6
t2	3.35×10^6	1.98×10^6 to 5.73×10^6
t3	5.27×10^6	2.74×10^6 to 7.25×10^6
Time of size change		
t2NA	1.25×10^6	1.24×10^5 to 4.37×10^6
t1EU	5.88×10^4	2.65×10^4 to 1.07×10^6
t1EUWA	6.80×10^4	3.55×10^4 to 1.36×10^6
t3SA	NC	

C/NA, *H. erectus*; SA, *H. patagonicus*; EU, *H. hippocampus* (Europe); WA, *H. hippocampus* (West Africa); EUAnt2, *H. hippocampus* effective population size (N_e) after t2 divergence; EUWAt1, *H. hippocampus* (West Africa) N_e after t1 divergence; EU t1, *H. hippocampus* (Europe) N_e after t1 divergence; SAsc, *H. patagonicus* N_e after t3 divergence; C/NAsc, *H. erectus* N_e after t2 divergence; A2sc, ancestral N_e of *H. erectus* and *H. hippocampus*; A1sc, ancestral N_e to all lineages. NC, posterior probability curves were non-informative (uniform).

DISCUSSION

We detected two features that drove diversification in the Atlantic *H. erectus* complex: (1) a vicariant breakup of an ancestral population into relatively stable Caribbean/North American and South American lineages; and (2) a founder colonization of the north-eastern Atlantic from Caribbean/North America via the Gulf Stream. The timing of the first event (5.27 Ma; 95% CI: 2.74–7.25 Ma) coincided with a sustained period of increasing Amazon River sediment deposition approximately 2.6–6.4 Ma (Figueiredo *et al.*, 2009) and geological estimates of maximum freshwater outflow (Rocha, 2003) that isolated the regions of the Caribbean and Brazil (Fig. 4). The second set of events includes the trans-Atlantic dispersal and subsequent trans-Atlantic isolation (3.35 Ma; 95% CI: 1.98–5.73 Ma) consistent with an

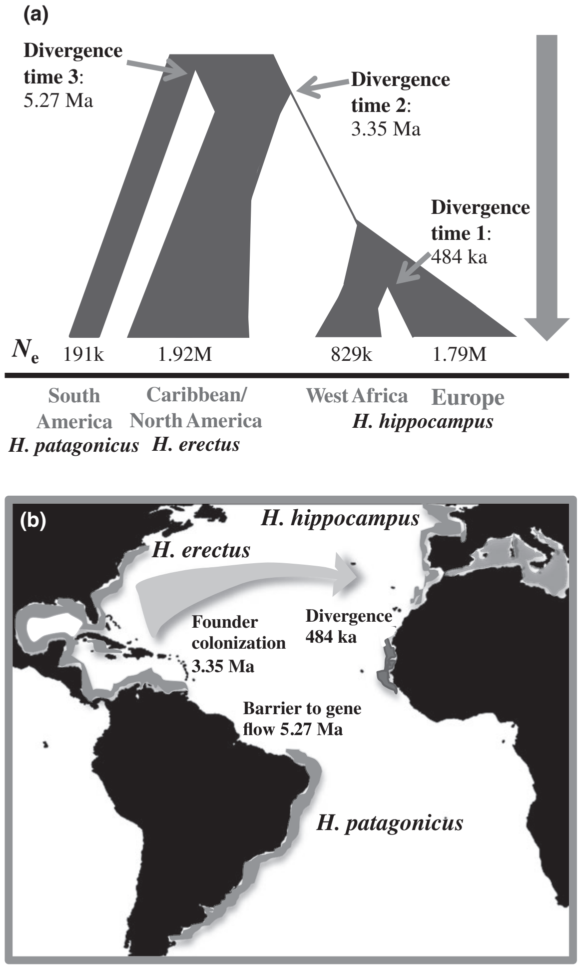

Figure 4 Most probable hypothesis (H_{WAO}) representing the demographic history of the *Hippocampus erectus* complex. (a) Tree illustration reconstructed from parameter values based on the mode of the posterior probabilities estimated from 1 million simulated data sets. Numerical values of effective population size (N_e) correspond to thousands (k) or millions (M) of individuals per population. (b) Visual representation of demographic inference.

acceleration and reorganization of circulation patterns and Gulf Stream currents starting roughly 4.6 Ma (Haug & Tiedemann, 1998), coincident with the rising of the Isthmus of Panama (Coates *et al.*, 1992; Muss *et al.*, 2001; Teske *et al.*, 2007; Lessios, 2008).

Allopatric divergence in the western Atlantic

All three species in the *H. erectus* complex appear to share similar widespread latitudinal distributions and tolerance across tropical to warm-temperate climate gradients, with limited genetic structuring within regions (Appendix S3). Our findings of relatively widespread gene flow within species (Caribbean to North America in *H. erectus*, and Brazil to Argentina in *H. patagonicus*) are in stark contrast to the ancient divergence between South American and Caribbean/North American populations (5.27 Ma; 95% CI: 2.74–7.25 Ma) coincident with the outflow of the Amazon River Barrier (ARB). The ARB accounts for over 2300 km of muddy coastline between the Amazon (Brazil) and Orinoco (Venezuela) rivers. The dramatic shift in substrate and oceanographical conditions coincides with the boundary between the Brazilian and the Caribbean biogeographical provinces (Collette & Rützler, 1977; Rocha *et al.*, 2005). The divergence between *H. erectus* and *H. patagonicus* is a common distribution pattern shared with many western Atlantic taxa, including numerous Brazilian fishes isolated from their Caribbean sister species (Joyeux *et al.*, 2001; Rocha, 2003; Briggs & Bowen, 2012). Although the soft sponge habitat below the ARB freshwater surface plume allows for connectivity in some fish species (Rocha *et al.*, 2002), the divergence we find in seahorses is consistent with their absence in marine surveys, suggesting that syngnathids may not utilize this passage to maintain trans-barrier connectivity (Collette & Rützler, 1977).

Geological estimates of the age of the ARB range from roughly 11.8 to 1.6 Ma (Rocha, 2003), with the earliest proposed date representing the initial presence of a large late Miocene submarine fan due to the Andean uplift (Hoorn *et al.*, 1995). However, recent evidence supports the idea that the ARB reached its present form in the late Pliocene, with three clear stages of advancement: an initial stage of low sedimentation rates from 11.8 to 6.8 Ma; an increase in sedimentation from 6.8 to 2.4 Ma; and very high sedimentation rates from 2.4 Ma to the present (Figueiredo *et al.*, 2009). Our divergence time estimates most closely align with the increase in ARB sediment deposition occurring 2.6 to 6.4 Ma, but it is likely that multiple determinants underlie the isolation of these lineages. These include the rise and fall of sea levels (Robertson *et al.*, 2006), and fast-flowing ocean currents that may further prevent rafting organisms from crossing the ARB. As westward-flowing equatorial currents meet eastern Brazil, the currents split into the north-westward-flowing Caribbean current and the southward-flowing Brazilian current (Fig. 1b). For seahorses, which are incapable of active long-distance migration, and are presumed to passively migrate by rafting, ocean current directionality may be particularly influential because Brazilian individuals would have to travel against the current before migrating westwards to bypass the ARB.

Founder colonization of the north-eastern Atlantic

Teske *et al.* (2007), provided strong phylogenetic evidence that rejected the hypothesis of trans-Atlantic vicariance from continental breakup 84 Ma for Atlantic species of *Hippocampus*, and instead proposed that trans-Atlantic sister species were the result of long-distance founder dispersal, following the final rise of the Isthmus of Panama. The closing of this land bridge isolated the western Atlantic from the eastern Pacific, contributing to a profound reorganization of ocean circulation and stability starting around 4.6 Ma (Haug & Tiedemann, 1998). Since closure, the North Atlantic Gyre's relatively stable currents have predominantly moved in an asymmetrical clockwise pattern (Fig. 1b), but with strong evidence of dynamic accelerations and decelerations, and latitudinal shifts that coincide with Pleistocene glacial and interglacial cycles (Kaneps, 1979; Keffer *et al.*, 1988; Haug & Tiedemann, 1998). Accelerations could facilitate long-distance dispersal, whereas decelerations and latitude shifts could have isolated migrants at a time period consistent with our findings of trans-Atlantic divergence between *H. erectus* and *H. hippocampus* populations (3.35 Ma; 95% CI: 1.98–5.73 Ma).

Counter to the typical east–west pattern of post-Pleistocene colonization found in temperate North Atlantic invertebrates (Wares & Cunningham, 2001; Ilves *et al.*, 2010; Waltari & Hickerson, 2012), Briggs (1995) suggested that most trans-Atlantic species originated from west-to-east dispersal-driven diversification, although evidence supports the western Atlantic as an area of both origin and accumulation of biodiversity (Floeter *et al.*, 2008; Rocha *et al.*, 2008). For the *H. erectus* complex, whose distribution is associated with tropical to warm-temperate waters, the circularity of the North Atlantic Gyre system could have allowed dispersal to occur both westwards and eastwards across the Atlantic, presenting a possibility for multi-directional exchange that complicates the inference of ancestral source populations. However, we find the highest probability of support for western Atlantic origins ($\mathrm{H}_{\mathrm{WAO}}$; Figs 2 & 4). Under this scenario, historical population size estimates suggest an eastward founder colonization of the eastern Atlantic *H. hippocampus*, which expanded into present-day European and West African populations subsequent to isolation from the more demographically stable western Atlantic *H. erectus* populations. Our estimates of a much smaller founder population in the eastern Atlantic supports typical patterns of source-to-recipient dispersal dynamics, and although a large amount of inherent uncertainty exists when using ancestral area inference with phylogenetic results, our historical demographic parameter estimates add additional support for eastward expansion and founder colonization.

An alternative interpretation may also explain the inferred patterns of dispersal under the western Atlantic origin hypothesis. Rather than suggesting ARB vicariance followed by trans-Atlantic colonization of Europe/West Africa (Fig. 4), this history could have been characterized by widespread or intermittent ancestral connectivity (by way of circular belt-like dispersal; Fig. 1b), followed by isolation of eastern Atlantic populations and a recovery from an ancestral population crash. Although this history is consistent with a population polytomy ($\mathrm{H}_{\mathrm{META}}$), it would also be consistent with $\mathrm{H}_{\mathrm{WAO}}$ if isolation events were temporally staggered.

Regardless of which nuanced interpretation is correct, the fast-flowing Gulf Stream is likely to have been a major force in the diversification of the Atlantic *H. erectus* complex, and acts as a transporter of both early life history stages (i.e. larvae and young) and abundant aggregations of marine organisms associated with *Sargassum* rafts (Casazza & Ross, 2008) that can survive for generations as free-floating habitats (Theil & Gutow, 2005). The Gulf Stream has been proposed as the primary source of the ichthyofauna of Bermuda (Smith-Vaniz *et al.*, 1999), the Caribbean mesopelagic fauna in the northern Sargasso Sea (Jahn, 1976) and some western Atlantic groups in the eastern Atlantic Azores archipelago (Ávila *et al.*, 2009). In addition, the Gulf Stream shapes the contemporary dispersal of numerous species, including sea turtles (Blumenthal *et al.*, 2009) and American and European eels (Kleckner & McCleave, 1982), and may be responsible for the dispersal of the western Atlantic *H. erectus*, which was recently found in the Azores (Woodall *et al.*, 2009).

Regarding European and West African populations, our findings indicate that these *H. hippocampus* populations diverged around 484 ka (95% CI: 268 ka to 1.39 Ma). Estimates of N_e of these descendant populations suggest that both contemporary populations are large and have undergone significant expansions since the original founder colonization (Fig. 4, Table 1). These findings are consistent with Woodall *et al.* (2011), who concluded that these expansions were probably affected by interglacial cycles in the late Pleistocene (190 to 21 ka). In addition, the Cape Verde frontal zone, where the southward-flowing Canary Current begins to shift westwards, has been observed as a barrier to gene flow in several eastern Atlantic species, and may have contributed to the observed divergence (Vangriesheim *et al.*, 2003; Woodall *et al.*, 2011).

Our estimates of trans-Atlantic and European/West African divergence times will be biased downwards if gene flow occurred after more ancient isolation events, a condition that is common in trans-Pacific ichthyofauna (Lessios & Robertson, 2006). Indeed, given the strong possibility that the Gulf Stream currents involved cyclical acceleration and deceleration, a more complex history involving demographic booms and busts and episodic periods of high gene flow should be considered an alternative hypothesis to be tested with population genomic-scale sampling. However, this sort of complex scenario is still consistent with isolation after trans-Atlantic dispersal.

Geographical patterns of Atlantic seahorse diversity

As the first reported study to include population-level sampling throughout the range of *H. erectus* and *H. patagonicus*, our reconstruction of species trees shows a clear monophyletic placement of *H. patagonicus* within the *H. erectus* complex and lineage independence from *H. erectus* (Figs 3 & 4). These results support the range restriction of *H. erectus* to an area north of the Orinoco outflow, and indicate that the species description of *H. patagonicus* (Argentina) (Piacentino & Luzzatto, 2004) may need to be extended to Brazilian populations.

Although both species have been shown to be under considerable fishing pressure (Dias *et al.*, 2002; Vincent *et al.*, 2011), our estimates of long-term effective population sizes indicate that *H. erectus* was historically around 10 times more numerous than the South American species

H. patagonicus. Currently, millions of seahorses are traded globally each year. Being Brazil's sixth most economically important ornamental export (Vincent *et al.*, 2011), the demographic and evolutionary independence of *H. patagonicus* should qualify it as a unique genetic unit in need of further study and protection (Moritz, 2002). In addition, our results also support those of Woodall *et al.* (2011), who concluded that the West African population of *H. hippocampus* is genetically distinct from the European population. Like *H. patagonicus*, the West African population of *H. hippocampus* should also be considered a priority for conservation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 (a) PCR and sequencing methods, (b) samples, collection locations, collectors, and GenBank accession numbers.

Appendix S2 (a) Principal components analysis, (b) model selection, and (c) gene trees.

Appendix S3 (a) Intraspecific population statistics, and (b) observed summary statistics.

BIOSKETCH

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