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1 Original Article

2 **Phylogeography of the snake pipefish, *Entelurus aequoreus* (Family:**
3 **Syngnathidae) in the northeastern Atlantic Ocean**

4

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18 Running title: Phylogeography of the snake pipefish

19 **ABSTRACT**

20 The snake pipefish, *Entelurus aequoreus*, is a widespread marine species occurring in pelagic
21 and coastal environments in the northeastern Atlantic Ocean. Recently, the snake pipefish
22 underwent a short-lived, yet substantial, increase in abundance and range expansion into
23 arctic waters. However, little is known about the species' population structure or if different
24 ecotypes contributed to this outbreak. Specimens (n=178) were sampled from 25 locations
25 from six regions spanning 1.9 million km². A fragment of the mitochondrial cytochrome *b*
26 gene and control region was used to assess population structure and genetic diversity. Both
27 loci showed high haplotype diversity (H_d) and low nucleotide diversity (π) over all sampled
28 locations. A genetic signature of population expansion was evident through mismatch
29 distributions and tests for recent population expansion (Fu's F_s , Tajima's D , and R_2).
30 Effective population size analyses (Bayesian Skyline Plot) suggest an ancient expansion (50-
31 100 thousand years ago). However, we found neither significant population differentiation
32 (AMOVA) among regions, nor evidence of genetically distinct ecotypes. This lack of
33 structure is likely due to a pelagic life style, fast development and long distance dispersal
34 aided by ocean currents. Our work highlights the need for further research to better
35 understand the recent outbreak and how this species may respond to future environmental
36 challenges.

37 **Keywords:** Bayesian Skyline Plot, control region, cytochrome *b*, fish, life history,
38 mitochondrial DNA, pelagic, population increase, population structure, range expansion

39 **Introduction**

40 The introduction of molecular techniques to the study of species distributions has
41 greatly improved our knowledge regarding species' evolutionary history. Current genetic
42 tools allow scientists to assess historical species distribution patterns, gene flow between
43 populations, the identification of source populations, routes and patterns of invasion, and
44 timing of range shifts, expansions as well as contractions (Hewitt, 1999; Schmitt, 2007;
45 Moran & Alexander, 2014). For example, the assessment of levels of gene flow and genetic
46 diversity between geographically separate populations is of particular interest for
47 conservation practises because it can facilitate the identification of genetically independent
48 populations or units, and guide conservation efforts (e.g., Bernard, Feldheim, Heithaus *et al.*,
49 2016). These studies highlight the importance of linking genetic data with species historical
50 and geographical information to make biologically relevant interpretations. The discipline of
51 phylogeography does exactly that by looking at geographical patterns of genetic diversity
52 across populations or species over time (Avice, 2000).

53 In this study, we investigate the phylogeography of the snake pipefish, *Entelurus*
54 *aequoreus* L. 1758. This species is a member of the family Syngnathidae, the pipefishes,
55 seahorses and seadragons, a group characterized by its unique form of reproduction, male
56 pregnancy (Dawson, 1986). Life history traits appear to shape the past and present
57 geographical distributions of syngnathids (Mobley, Small & Jones, 2011). Syngnathids tend
58 to be poor swimmers with small fins and armoured bodies, and most are strict habitat
59 specialists, relying heavily on crypsis for survival (Vincent, Berglund & Ahnesjö, 1995). All
60 species in this family produce free-living juveniles (Hasse, 1974; Mi, Kornienko & Drozdov,
61 1998; Monteiro, Almada & Vieira, 2003; Wetzel & Wourms, 2004) with short (Wilson &
62 Vincent, 1998; Planas, Blanco, Chamorro *et al.*, 2012) or entirely absent pelagic dispersal
63 phases (Silva, Monteiro, Almada *et al.*, 2006; Silva, Monteiro, Vieira *et al.*, 2006). Having a

64 short pelagic dispersal phase is known to significantly limit dispersal potential (Grantham,
65 Eckert & Shanks, 2003). Among adults, limited seasonal vertical migrations can occur, with
66 individuals of some species coming into warmer shallow waters for mating, and returning to
67 deeper waters at the end of breeding season or during brooding (Vincent *et al.*, 1995;
68 Monteiro, Berglund, Vieira *et al.*, 2006).

69 Previous studies on syngnathids have generally revealed relatively high genetic
70 diversity and high population structuring, indicative of large effective population sizes and
71 low dispersal ability (reviewed in Mobley *et al.*, 2011). Most northern-hemisphere temperate
72 syngnathid species show evidence of population expansions towards northern regions after
73 the end of the last glaciation period (circa 20 thousand years before present (ka); Woodall,
74 Koldewey, Santos *et al.*, 2009; Mobley, Small, Jue *et al.*, 2010; Wilson & Eigenmann
75 Veraguth, 2010; Woodall, Koldewey, Boehm *et al.*, 2015) following similar northerly
76 expansion of suitable habitat, i.e., seagrass meadows (Olsen, Stam, Coyer *et al.*, 2004). Yet,
77 their strong habitat dependency and limited dispersal capability is also reflected in their
78 current geographical distributions that generally show high levels of population
79 differentiation on relatively small geographical scales (e.g. Lourie, Green & Vincent, 2005;
80 Wilson, Stiller & Rouse, 2016; Stiller, Wilson, Donnellan *et al.*, 2017) and strong indications
81 of limited dispersal (e.g. Chenoweth, Hughes & Connolly, 2002; reviewed in Mobley *et al.*,
82 2011; Wilson & Orr, 2011). Exceptions to these patterns have been interpreted as a result of
83 recent colonization events (Nickel & Cursons, 2012), assumed to occur via rafting, where
84 individuals drift with floating marine vegetation on ocean currents (Teske, Hamilton, Palsbøll
85 *et al.*, 2005; Fedrizzi, Stiassny, Boehm *et al.*, 2015).

86 From a phylogeographical perspective, the snake pipefish is an interesting species to
87 study because of its wide geographic distribution, recent range expansion into polar waters,
88 and dramatic fluctuations in abundance. Historically, this species inhabits a vast geographical

89 range in the temperate northeastern Atlantic, spanning from the European continental shelf to
90 the west, southern Norway and Iceland in the north, to the Azores in the south and the Baltic
91 Sea to the east (Dawson, 1986). Normally, the species is encountered throughout its range at
92 low densities (Harris, Beare, Toresen *et al.*, 2007). However, between 2003 and 2007 the
93 snake pipefish reappeared in large numbers in coastal areas where it had for decades been
94 considered rare (e.g. northern Wadden Sea, Polte & Buschbaum, 2008), including brackish
95 estuarine waters (e.g. the Severn Estuary, Henderson & Bird, 2010). During this time, the
96 snake pipefish was caught in numbers several orders of magnitude higher than in catches
97 prior to 2003 (Kloppmann & Ulleweit, 2007; van Damme & Couperus, 2008). The snake
98 pipefish also underwent a geographical range expansion into the Barents and Greenland Seas
99 by 2005 (Harris *et al.*, 2007; Rusyaev, Dolgov & Karamushko, 2007) and the first ever
100 records of occurrence in Svalbard were reported by August 2006, representing a 15°
101 latitudinal expansion northwards (approximately 1650km, Fleischer, Schaber & Piepenburg,
102 2007). After 2007, populations of the snake pipefish declined dramatically and returned to
103 low levels of abundance throughout its geographic range (Heath, Neat, Pinnegar *et al.*, 2012).
104 There is anecdotal evidence that this species has undergone mass mortality events off the
105 European continental shelf in the Atlantic Ocean in 1885, 1887, and in the North Sea in 1911,
106 although no satisfactory explanation for these events exists (Brongersma-Sanders, 1957). It is
107 possible that these mass mortality events are an indication of previous population increases in
108 snake pipefish abundance although data to corroborate this link are currently lacking.

109 The snake pipefish is also an interesting species to investigate phylogeographically
110 because of its unique life history traits. Unlike most other syngnathids that are predominantly
111 associated with benthic habitats, the snake pipefish is described primarily as an oceanic
112 species displaying a pelagic lifestyle and is found in both coastal and oceanic waters to
113 depths up to 100m (Dawson, 1986; Kloppmann & Ulleweit, 2007). Because this species does

114 not require benthic habitats to reproduce they may breed in open waters and offspring as well
115 as adults may be transported and mixed by ocean currents.

116 Finally, the potential for cryptic species to contribute to the temporary population
117 increase and range expansion of the snake pipefish has not yet been addressed. Previously,
118 two species have been proposed for *E. aequoreus* over the years based on differences in body
119 size, colour, position of the dorsal fin and in number of rays (Yarrel, 1839; Moreau, 1881;
120 Fries, Ekström & Sundevall, 1895; Holt & Byrne, 1906; Dunker, 1915). However, these two
121 species are currently not recognized (Dawson, 1986). Additional lines of investigation have
122 suggested coastal and oceanic habitat-specific phenotypes, or ‘ecotypes’, based on
123 morphology (van Damme & Couperus, 2008) or timing of breeding between coastal benthic
124 populations (summer) and oceanic pelagic populations (spring) (Kloppmann & Ulleweit,
125 2007). Thus, the potential for ecotypes or cryptic species to exist within *E. aequoreus* needs
126 to be resolved with molecular markers.

127 In this study, we investigate phylogeographic patterns in the snake pipefish
128 throughout its contemporary distribution. Our specific goals are to assess population structure
129 and historical patterns of population expansion over the geographical range of the snake
130 pipefish. Further, we investigate whether molecular data support proposed coastal benthic
131 and pelagic ecotypes or cryptic species. To achieve these goals, we used mitochondrial DNA
132 (mtDNA, cytochrome *b* and control region) markers to investigate genetic differentiation and
133 genetic variation in snake pipefish from 25 locations among six geographical regions
134 spanning over 1.9 million km² of their range in the Northeastern Atlantic Ocean.

135 **Materials and methods**

136 **Collections**

137 A total of 237 snake pipefish were collected from 25 locations in the Northeastern
138 Atlantic Ocean between 2003 and 2010 using a variety of capture methods (Table 1, Fig. 1,
139 Supplementary file S1). We assigned samples to regions based on conventional naming
140 schemes of local water bodies or coastal areas. These regions correspond to regions defined
141 by the OSPAR Commission (Region I: Arctic Waters = Norwegian Sea; Region II: Greater
142 North Sea = North Sea; Region III Celtic Seas = Continental Shelf; Region IV Bay of Biscay
143 and Iberian Coast = Spanish Coast) with the exception that Skagerrak/Kattegat were analysed
144 separately from the North Sea due to the potential influence of the Baltic Sea, and the French
145 Coast was analysed separately as it lies on the boundary of North Sea and Celtic Seas
146 regions.

147 DNA was extracted from a small piece of tail tissue (~1cm) using a Qiagen DNeasy
148 kit from live, frozen or EtOH preserved fish. A portion of the mitochondrial cytochrome *b*
149 gene and control region was amplified. We used primers L14725 (Pääbo, Thomas, Whitfield
150 *et al.*, 1991) and H15926 (Wilson, Vincent, Ahnesjö *et al.*, 2001) to amplify the cytochrome
151 *b* locus, and primers L15926 (Kocher, K., Meyer *et al.*, 1989) and H16498 (Meyer, Kocher,
152 Basasibwaki *et al.*, 1990) to amplify the control region. Fragments were amplified via
153 polymerase chain reaction (PCR) in 30µl volumes containing 3µl of 10X buffer, 1.8 µl of
154 dNTPs (10µM of each dNTP), 3 µl of MgCl₂ (50mM), 1.5 µl of each primer (10µM), and 0.5
155 µl of Taq (5 units/µl; InviTaq, Stratech Biomedical, Birkenfeld, DE). The PCR thermal
156 profile consisted of an initial denaturation at 95°C (2 min), followed by 35 cycles of 94°C (30
157 sec), reannealing temperature (30 sec), 72°C (90 sec), and a final extension at 72°C for 10
158 min. Reannealing temperature for cytochrome *b* was 48°C and control region was 56°C. PCR
159 products were purified before sequencing with Illustra™ ExoStar (GE Healthcare,
160 Buckinghamshire, UK) using 5µl of PCR product and 2µl of ExoStar. PCR products were

161 sequenced with the forward and reverse primers using an ABI 3100 DNA Analyzer (Applied
162 Biosystems, Foster City, CA, USA).

163 Contigs were created using forward and reverse sequences using CODONCODE
164 ALIGNER v. 5.1.5 (Codon Code Corporation, Centerville, MA, USA) for each individual
165 and aligned for each locus using MUSCLE (Edgar, 2004). Sequences were verified by eye
166 and trimmed. Unique haplotype sequences were identified using ‘DNA to haplotype collapser
167 and converter’ in FaBox v. 1.41 (<http://birc.au.dk/fabox>) and deposited in GenBank
168 (accession numbers #: cytochrome *b*, KY857646 - KY857823; control region, KY965149 -
169 KY965308).

170 Genetic analyses

171 Relationships between mitochondrial haplotypes were analysed for each locus
172 independently to assess whether population structuring exists among the six regions
173 combined over all time periods. Standard haplotype (h) and nucleotide (π) diversity statistics
174 (Nei, 1987) were calculated for each region using DNASP 5.10.01 (Librado & Rozas, 2009).
175 Mismatch distributions were investigated in both loci independently, and evidence for recent
176 population expansion was tested using Tajima’s D test (Tajima, 1989), Fu’s F_s test (Fu, 1997)
177 and the Ramos-Onsins and Rozas’s R_2 statistic (Ramos-Onsins & Rozas, 2002),
178 recommended for small sample sizes (Ramírez-Soriano, Ramos-Onsins, Rozas *et al.*, 2008).
179 Tajima’s D , F_s and R_2 were calculated using the total number of mutations, excluding sites
180 with alignment gaps or missing data, and significance was ascertained using coalescent
181 simulations with 1000 replicates as implemented by DNASP. Population expansion under the
182 constant size growth model was used to estimate R_2 .

183 To visualize the relationship between mitochondrial haplotypes from different
184 regions, a haplotype network was constructed using HAPLOVIEWER (Salzburger, Ewing &

185 von Haeseler, 2011) based on maximum likelihood trees drawn in DNAML in PHYLIP
186 v3.695 (Felsenstein, 1989) for both loci independently. To test for significant population
187 subdivision among individual collections, we conducted pairwise ϕ_{ST} tests for cytochrome *b*
188 and control region sequences using ARLEQUIN v3.5.2.1 (Excoffier, Smouse & Quattro,
189 1992; Excoffier, Laval & Schneider, 2005). Significance for pairwise differences was
190 ascertained using an exact test with 100,000 permutations. An Analysis of Molecular
191 Variance (AMOVA) was used to test the proportion of genetic differentiation within and
192 between regions using ARLEQUIN for both loci independently pooled across years.
193 AMOVA was also used to test whether there is support for genetic differentiation between
194 pelagic and coastal benthic populations. Only locations with a minimum of five sequenced
195 individuals were included in pairwise ϕ_{ST} and AMOVA analyses, except for the three
196 Norwegian Sea individuals that were included in the cytochrome *b* dataset since they
197 represented a unique location. Significance of AMOVAs was determined using 99,999
198 permutations as implemented in ARLEQUIN.

199 We analysed the potential for fluctuations in effective population size using Bayesian
200 Skyline Plot (BSP, Drummond, Rambaut, Shapiro *et al.*, 2005), a coalescent-based method
201 implemented in BEAST 1.8.4 (Drummond & Rambaut, 2007). We first estimated the best-fit
202 model of nucleotide substitution for each gene using JModelTest 2.0 (Darriba, Taboada,
203 Doallo *et al.*, 2012) on all samples. We selected the best-fit models according to the Bayesian
204 Information Criterion, which were HKY+I+G and HKY+I for cytochrome *b* and the control
205 region, respectively. We then ran the BSP analysis on the concatenation of the two
206 mitochondrial loci using samples that successfully amplified both cytochrome *b* and the
207 control region (n=140) using specific substitution models and mutation rates for each
208 partition. We chose a mutation rate of 1×10^{-8} substitutions per nucleotide site per year for
209 cytochrome *b* and 5×10^{-8} substitutions per nucleotide site per year for the control region

210 based on recommendations by Bowen, Muss, Rocha *et al.* (2006) and Mobley *et al.* (2010)
211 assuming a constant mutation rate. We assumed a generation time of one generation per year
212 based on unimodal distributions of body size in juveniles caught in plankton tows (Kirby,
213 Johns & Lindley, 2006). Adults collected within a year show a bimodal distribution in body
214 size but this may be accounted for by sexual dimorphism in body length (van Damme &
215 Couperus, 2008) also suggesting one reproductive cycle per year.

216 The BSP analysis consisted of 1×10^8 generations; parameters were sampled every
217 10,000 generations of which 10% was discarded as burn-in. In order to check the analysis
218 performance (i.e., the convergence of parameters by visually checking the effective sample
219 size ($ESS > 200$) values), we used TRACER 1.6 (Rambaut, Suchard, Xie *et al.*, 2014).

220 **Ethical statement**

221 The present study was conducted in accordance to local and European Union law and no
222 permit was needed for collecting fish. The study did not involve any endangered or protected
223 species.

224

225 **Results**

226 **Molecular diversity**

227 We resolved 900 bp of the cytochrome *b* gene from 178 snake pipefish from a large
228 portion of their range. Cytochrome *b* yielded 94 unique haplotypes ($H_d = 0.967 \pm 0.006$ S.D.)
229 and 82 polymorphic sites ($\pi = 0.0050 \pm 0.0002$ S.D., Table 2).

230 Sequence analyses of 385 bp of the control region locus in 160 snake pipefish
231 revealed variation in a dinucleotide microsatellite repeat in the control region at site 287.

232 Most sequences contained [TA]₁₀ but [TA]₉ occurred at a low frequency (0.16) and was
233 present in all geographical regions analysed except the Norwegian Sea samples that failed to
234 amplify at this locus (Table 1). Inclusion of a mitochondrial microsatellite is problematic for
235 several reasons (Lunt, Whipple & Hyman, 1998). For example, microsatellites generally have
236 much higher mutation rates (in terms of the gain or loss of a repeat) than nucleotide
237 substitutions, and mutation rates in general are unknown for mitochondrial microsatellites
238 (Sia, Butler, Dominska *et al.*, 2000) and for this microsatellite in particular. Moreover, we did
239 not determine if heteroplasmy, or the coexistence of nonidentical mtDNA molecules in the
240 same individual, is occurring in this species. Heteroplasmy is common in species that have
241 microsatellites in mtDNA in the AT-rich or control region causing difficulties for population
242 genetics analyses (Lunt *et al.*, 1998; Mayer & Kerth, 2005). Additionally, length variation in
243 the microsatellite can be considered a gap or missing data and thus may be inappropriate for
244 some analyses (Yang & Rannala, 2012). Therefore, we excluded the variable repeat from all
245 analyses, and we resolved 383 bps of the control region resulting in 63 unique haplotypes (H_d
246 = 0.893 ± 0.015 S.D.) and 43 polymorphic sites ($\pi = 0.0054 \pm 0.0003$ S.D., Table 2).

247 **Population structure**

248 A maximum likelihood haplotype network constructed for cytochrome *b* showed four
249 major ($n \geq 10$) haplotypes (Fig. 2a). However, these haplotypes were comprised of
250 representative individuals from most regions such that no genetic clustering could be
251 discerned. For the control region, a maximum likelihood haplotype network also showed four
252 major haplotypes and individuals from all populations were represented in these major
253 haplotypes (Fig. 3). Overall, haplotype networks showed a star-like topology with high
254 numbers of low-frequency mutations representative of a recent population expansion.

255 Pairwise ϕ_{ST} values ranged from -0.119 to 0.212 with a mean of -0.009 for
256 cytochrome *b*, and ranged from -0.290 to 0.242 with a mean of -0.030 for the control region
257 (Appendix S1). No significant differences between pairs of collections were detected after
258 Bonferroni adjustment to correct for multiple comparisons (Rice, 1989). An AMOVA among
259 collections within regions pooled across years indicated no significant population structuring
260 (ϕ_{ST}) for either cytochrome *b* or the control region (Table 3, Appendix S1). Finally, no
261 evidence for genetically distinct ecotypes or cryptic species was found when investigating
262 individuals captured in nearshore benthic habitats versus pelagic captures (Table 3). In all
263 AMOVA comparisons, the variance explained by among groups and among collections
264 within groups was negligible in comparison to the variance within collections (Table 3).

265 **Population expansion**

266 Significant values of Fu's F_s , Tajima's D , and Ramos-Onsins and Rozas's R_2 found in
267 cytochrome *b* sequences, with the exception of the Spanish coast collection, support a
268 scenario of recent population expansion when samples were pooled within locations and
269 sampling times (Table 2). The control region, on the other hand, showed significant F_s
270 values, but only Continental shelf and French Coast collections showed significant departures
271 from neutrality in Tajima's D and R_2 tests (Table 2). When collections were pooled across all
272 location and sampling times, both the cytochrome *b* and control region showed highly
273 significant F_s , D and R_2 values indicating recent population expansion was apparent (Table
274 2). Mismatch distributions were unimodal and failed to reject the hypothesis of the sudden
275 expansion model (Appendix S1).

276 Bayesian Skyline Plot analysis with concatenated cytochrome *b* and control region
277 sequences revealed that snake pipefish experienced a historical effective population size
278 expansion starting about 100ka and obtaining current effective population sizes around 50ka

279 (Fig. 3). Individual effective population size analyses showed expansions approximately
280 125ka for cytochrome *b* and 40ka for control region sequences (Appendix S1).

281 **Discussion**

282 Our study provides insight into the phylogeographic history of the snake pipefish, a
283 widely distributed syngnathid species in the northeastern Atlantic with a pelagic lifestyle.
284 Results from molecular analyses did not reveal any clear patterns in population structure by
285 regions, despite relatively high haplotype diversity estimates. However, we did uncover a
286 signature of a Pleistocene population expansion in the northeastern Atlantic approximately
287 50-100ka. We also did not find any evidence for genetically distinct coastal or pelagic
288 ecotypes or cryptic species, indicating that differences in phenotype are likely due to
289 differences in ecological conditions. Taken together, our results point to a large population of
290 snake pipefish throughout its contemporary northeastern Atlantic distribution and that such a
291 high degree of homogenization is likely the result of a combination of specialized/unique life
292 history traits and mixing by oceanic currents.

293 ***Historical phylogeography***

294 The history of the northeastern Atlantic is one of extreme climatic changes, with
295 multiple glaciation cycles until the late Pleistocene (last interglacial period ~125 ka, last
296 glacial maximum ~20ka, Mokeddem, McManus & Oppo, 2014). During this period, many
297 marine species were deeply impacted by glacial activity, which caused large reductions
298 and/or shifts in suitable habitat (Mäkinen & Merilä, 2008) occasionally leading to precipitous
299 population declines (Almada, Pereira, Robalo *et al.*, 2008; Boehme, Thompson, Fedak *et al.*,
300 2012). The subsequent recolonization of the northeastern Atlantic oft times leads to complex
301 genetic signatures of glacial refugia, range expansions and bottlenecks (e.g. Coyer, Peters,
302 Stam *et al.*, 2003; Gysels, Hellemans, Pampoulie *et al.*, 2004; Luttikhuizen, Campos, van

303 Bleijswijk *et al.*, 2008; Maggs, Castilho, Foltz *et al.*, 2008; Robalo, Castilho, Francisco *et al.*,
304 2012).

305 The current study suggests that the snake pipefish underwent a population expansion
306 in the northeastern Atlantic Ocean approximately 50-100ka during the Pleistocene. This
307 scenario is supported by a star-like network topology, mismatch distribution analyses and an
308 increase in effective population size indicating a recent expansion and/or a short evolutionary
309 history of the species in the northeastern Atlantic Ocean (Grant & Bowen, 1998). Several
310 other species show limited geographic partitioning and a sudden population expansion much
311 earlier than the last glacial maximum similar to the snake pipefish. For example, the time of
312 population expansion in the snake pipefish as estimated by BSP is similar to that for Atlantic
313 Cod (*Gadus morhua*) which shows population expansion ~60ka in the northeastern Atlantic
314 based on mismatch distributions of the mitogenome (Carr & Marshall, 2008). Other species
315 that show signatures of population expansion predating the last glacial maximum include
316 pelagic migrating species such as Atlantic bluefin tuna *Thunnus thynnus* (Bremer, Viñas,
317 Mejuto *et al.*, 2005) and the European anchovy, *Engraulis encrasicolus* (Silva, Horne &
318 Castilho, 2014). In contrast, other sympatric nearshore species of syngnathids closely
319 associated with seagrass habitats show more recent recolonization of the northeastern
320 Atlantic (15-36ka) based on coalescence analysis (Wilson & Eigenmann Veraguth, 2010).
321 Thus, it appears that pelagic species show a reduced influence of Pleistocene glacial cycles in
322 the northeastern Atlantic.

323 Our estimates of expansion time are likely conservative, given that mutation rates are
324 time dependent and are higher in younger lineages (Ho, Phillips, Cooper *et al.*, 2005; Ho,
325 Lanfear, Bromham *et al.*, 2011; Crandall, Sbrocco, DeBoer *et al.*, 2012; Grant, 2015). It is
326 therefore possible that time since expansion is more recent than estimated from BSP. It is also
327 probable that the differences in mutation rates chosen for cytochrome *b* and the control region

328 contribute to the discrepancy between individual locus estimates of expansion time and may
329 have led to a more gradual expansion time using the concatenated loci as compared to
330 individual loci (Appendix S1). Fluctuations in drift, in population size, selection, and
331 reproductive skew may also influence divergence times (Burrige, Craw, Fletcher *et al.*,
332 2008; Grant, 2015; Niwa, Nashida & Yanagimoto, 2016). Finally, results of the BSP do not
333 show recent (i.e., 10ka) fluctuations in effective population size although the probability of
334 mtDNA markers capturing these phenomena is extremely unlikely.

335 ***Population structure***

336 Population structuring over a large geographical range was not evident in this species
337 from ϕ_{ST} and AMOVA results. These results mirror those reported for several Atlantic fish
338 species where high mobility and/or high dispersal potential are highlighted as causes for little
339 or no population sub-structuring (Nesbo, Rueness, Iversen *et al.*, 2000; Dannewitz, Maes,
340 Johansson *et al.*, 2005; Carr & Marshall, 2008; Limborg, Hanel, Debes *et al.*, 2012). Our
341 results suggest that despite having relatively low swimming ability, the snake pipefish is
342 capable of dispersing over long distances, likely aided by a pelagic lifestyle and faster
343 development rates relative to other northeastern Atlantic syngnathid species (Braga
344 Goncalves, Ahnesjö & Kvarnemo, 2016). A similar pattern has been described in the non-
345 migratory, demersal marine fish, *Sebastes schegelii*, where the association of larvae and
346 juveniles with rafting precludes population genetic differentiation throughout its geographical
347 range (Zhang, Yanagimoto, Zhang *et al.*, 2016). These results provide a stark contrast with
348 several species of syngnathid that show population substructure over large spatial scales
349 using mitochondrial markers (Lourie & Vincent, 2004; Lourie *et al.*, 2005; Teske *et al.*, 2005;
350 Mobley *et al.*, 2010; Wilson & Eigenmann Veraguth, 2010; Wilson *et al.*, 2016).

351 Greater resolution of population sub-structuring in snake pipefish could potentially be
352 provided by additional nuclear markers. However, attempts to design microsatellite markers

353 for this species have not yet yielded sufficient numbers of polymorphic microsatellites to
354 conduct such analyses (KB Mobley, I Braga Goncalves, unpublished data). Future studies
355 that benefit from combined mtDNA and nuclear markers may reveal additional insights into
356 the phylogeography of this species including resolution of the time since population
357 expansion and estimate gene flow within the species.

358 ***Range expansion***

359 Although a significant number of snake pipefish were caught in polar regions during
360 the population increase in 2003-2007, they may have originated in the southern Norwegian
361 Sea and drifted to these locations in ocean currents (Nesbo *et al.*, 2000; Luttkhuizen *et al.*,
362 2008). In our study, we obtained only a few samples in polar waters from the Norwegian Sea
363 in 2008. Haplotypes obtained from these samples were not distinct from southern populations
364 suggesting that they are derived from the same large Atlantic gene pool. Due to the low
365 abundance of snake pipefish after the population increase in north Atlantic waters (Heath *et*
366 *al.*, 2012), future surveys should investigate whether or not these fish continue to inhabit
367 polar regions representing a true range expansion or whether these were just transient
368 individuals that rafted northward on ocean currents during the 2003-2007 outbreak.

369 ***Cryptic species***

370 Previous studies have documented phenotypic differences between snake pipefish
371 collected in coastal and oceanic areas (Holt & Byrne, 1906; Zhang *et al.*, 2016). Based on
372 these differences, two species have been proposed previously, *E. aequoreus* found in oceanic
373 waters and *E. anguineus* found in inshore habitats (Yarrel, 1839; Moreau, 1881), although *E.*
374 *anguineus* is not currently recognized as a valid species (Dawson, 1985). These oceanic and
375 coastal ‘ecotypes’ were recently described and a third potential intermediate ecotype that
376 shares phenotypic similarities with both coastal and oceanic forms has been proposed (van
377 Damme & Couperus, 2008). Despite differences in coloration and body condition, van

378 Damme and Couperus (2008) found no differences in ring and fin ray counts between
379 perceived ecotypes in either sex and therefore concluded that all specimens belong to a single
380 species. Based on mitochondrial haplotype frequencies, we also find no support that oceanic
381 pelagic and benthic coastal ecotypes of snake pipefish collected in these habitats are different
382 species. Instead, the snake pipefish appears to form a single species in the northeastern
383 Atlantic and phenotypic plasticity in response to local ecological conditions encountered is
384 the most probable explanation for the presence of multiple ecotypes.

385

386 *Conservation concerns*

387 According to the International Union for Conservation and Nature red list, *E.*
388 *aequoreus* is evaluated as least concern (IUCN, 2017). Despite this listing, the sudden and
389 substantial population increase in snake pipefish, although short lived, had a dramatic effect
390 on the ecology of the northeastern Atlantic during the outbreak, and therefore warrants
391 special mention. The significant increase in the numbers of snake pipefish in European waters
392 was paralleled by a similar increase in the number of pipefish fed by parents to seabird
393 nestlings in several species around the UK, Norway, Iceland, and the Faroe Islands
394 (Luttikhuizen *et al.*, 2008; Anderson, Evans, Potts *et al.*, 2014). The hard exoskeleton and
395 relatively low nutritional content of the snake pipefish (Harris, Newell, Daunt *et al.*, 2008)
396 make them unsuitable for consumption by nestlings and adults alike, and their increased use
397 as food items was associated with seabird breeding failures in the UK (Mavor, Parsons,
398 Heubeck *et al.*, 2005; Mavor, Parsons, Heubeck *et al.*, 2006; Luttikhuizen *et al.*, 2008).

399 Despite the ecological significance of this species, the cause of the recent population
400 increase of the snake pipefish still remains unknown, as does its subsequent decline (Heath *et*
401 *al.*, 2012). Several non-mutually exclusive hypotheses have been put forward to explain its

402 population increase and expansion, namely: 1) a rise in surface seawater temperatures
403 promoting longer breeding seasons and higher recruitment (Kirby *et al.*, 2006; Gremillet &
404 Boulinier, 2009; Neumann, Ehrich & Kroncke, 2009; Anderson *et al.*, 2014), 2) a by-product
405 of tracking changing and/or shifting plankton communities (van Damme & Couperus, 2008),
406 3) promoted by the establishment of invasive algal species such as the Japanese seaweed,
407 *Sargassum muticum*, that increased the amount of suitable habitat for successful reproduction
408 in the coastal regions (Gysels *et al.*, 2004) and, 4) a result of decreasing population size of
409 interspecific competitors such as Lesser Sandeels (*Ammodytes marinus*) due to fishing and
410 climate change (Heath *et al.*, 2012; Anderson *et al.*, 2014). Yet, there is no conclusive
411 evidence to explain the sudden expansion.

412 The increase in snake pipefish potentially started in one source spot on the continental
413 shelf and dispersed over the entire northeastern Atlantic. Interestingly, the increase and
414 expansion in the pelagic environment itself can be seen as a strategy of the species to
415 colonize new suitable habitats. However, expansions of the same magnitude appear to be
416 very rare: there are indications that a similar population increase took place at the end of the
417 nineteenth century (Brongersma-Sanders, 1957; van Damme & Couperus, 2008). Climate
418 change is expected to change ocean currents which, together with higher sea surface
419 temperatures, will affect larval import, export and recruitment, leading to faster development,
420 shorter larval stages and dispersal into new habitats (Kendall, Poti & Karnauskas, 2016).
421 Improving our understanding of snake pipefish reproductive biology, habitat, feeding
422 ecology, and dispersal potential is a critical next step to help pinpoint how current and future
423 changes in climate and in prey distributions in the northeastern Atlantic may potentiate
424 further population increases, which in turn may affect community structure.

425 **Conclusions**

426 Understanding species' current distribution patterns and historical demography is a
427 fundamental goal in evolutionary biology. Our study contributes to this goal by investigating
428 phylogeographic patterns in a species that has undergone a sudden population increase and
429 range expansion that had a negative impact on the ecosystem of the northeastern Atlantic.
430 Although the cause for the recent population increase and range expansion and contraction
431 are still unknown, the phylogeographic patterns uncovered in our study demonstrate that the
432 snake pipefish represents a single large population with no evidence of population sub-
433 structuring. This result, in contrast to all other syngnathids studied to date, may be explained
434 by the pelagic lifestyle and poor swimming capabilities of the species, allowing individuals to
435 be transported long distances by ocean currents. Our study adds to the understanding of this
436 ecologically important species and future studies should incorporate a wider range of genetic
437 markers to investigate population demographics, particularly concerning the recent
438 population increase.

439

440 **Supplementary Material**

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

442 Appendix S1: Population pairwise Φ_{ST} estimates, mismatch distributions, and individual

443 Bayesian skyline plots for cytochrome *b* and control region loci.

444

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458

459 **Data Accessibility**

460 All cytochrome *b* and control region sequences will be available in Genbank and supporting
461 sample information and haplotype lists will be deposited in Dryad.

462

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750 Table 1. *Entelurus aequoreus* collections. Region of collection, number of individuals collected (n), year collected, location, collection
 751 abbreviation (AB), habitat, latitude, longitude, the number of individuals amplifiable at the cytochrome *b* locus (n_{cytb}) and control region locus
 752 (n_{CR}), and presence of the microsatellite repeat [TA]₉ in the control region are listed.

Region	n	Year	Location	AB	Habitat	Latitude	Longitude	n_{cytb}	n_{CR}	[TA] ₉
Continental Shelf	5	2005	Atlantic Ocean	CS1	pelagic	51°45'0.61"N	11°45'0.61"W	3	5	0
	2	2005	Atlantic Ocean	CS2	pelagic	56°10'2.39"N	9°47'4.81"W	2	1	0
	9	2005	Atlantic Ocean	CS3	pelagic	50°45'0.00"N	11°7'0.01"W	8	7	2
	15	2005	Atlantic Ocean	CS4	pelagic	51°15'1.80"N	13°42'1.80"W	14	13	3
	10	2007	Atlantic Ocean	CS5	pelagic	49°8'15.99"N	10°22'48"W	10	5	1
	23	2010	Atlantic Ocean	CS6	pelagic	51°52'42.60"N	13°8'3.01"W	22	21	3
Spanish Coast	10	2007	Galacia, ES	SC	coastal	42°15'N	8°52'W	10	8	1
North Sea	3	2005	North Sea	NS01	pelagic	57°48'34.99"N	0°52'50.70"W	3	1	0
	5	2005	North Sea	NS02	pelagic	57°52'7.14"N	3°14'54.06"W	5	2	2
	5	2005	North Sea	NS03	pelagic	57°44'55.03"N	1°21'28.26"W	5	2	0
	1	2005	North Sea	NS04	pelagic	58°10'10.99"N	0°33'42.23"W	1	1	0
	10	2005	North Sea	NS05	pelagic	58°10'37.27"N	3°10'12.00"W	10	7	5
	8	2007	North Sea	NS06	pelagic	56°21'38.99"N	2°4'58.19"W	8	8	1
	2	2007	North Sea	NS07	pelagic	56°7'34.21"N	3°28'3.00"E	2	2	0
	7	2007	North Sea	NS08	pelagic	53°28'53.40"N	0°54'54.00"E	7	7	1
	1	2007	North Sea	NS09	pelagic	55°36'16.20"N	2°46'31.19"E	1	1	0
	1	2007	North Sea	NS10	pelagic	55°53'26.41"N	4°15'45.61"E	1	1	0
	6	2007	North Sea	NS11	pelagic	56°45'29.41"N	1°33'30.60"W	6	5	0
	6	2007	North Sea	NS12	pelagic	55°23'28.79"N	1°34'34.79"E	6	6	0
Skagerrak/Kattegat	2	2003	Kølpn/Deget, DK	SK1	coastal	57°27'17.66"N	10°35'53.61"E	2	2	0
	1	2004	Dronningmølle, DK	SK2	coastal	56°6'6.07"N	12°24'34.77"E	1	1	0
	9	2005	Gåsö, SE	SK3	coastal	58°14'23.32"N	11°22'44.86"E	7	0	--

	35 ¹	2006	Gåsö, SE	SK4	coastal	58°14'23.32"N	11°22'44.86"E	20	19	2
Norwegian Sea	1	2008	Norwegian Sea	NOR1	pelagic	68°15'40.02"N	4° 7'45.00"E	0	0	--
	5	2008	Norwegian Sea	NOR2	pelagic	68°15'24.66"N	0°31'45.36"W	3	0	--
French Coast	55 ²	2010	Bay de Roscoff, FR	FC	coastal	48°42'13.75"N	3°55'11.67"W	21	35	4
Total	23							178	160	25
	7									

753 ¹A subset of 20 individuals was extracted. ²A subset of 35 individuals was extracted.

754 Table 2. Genetic diversity indices and tests of neutrality pooled across years in regions for Cytochrome *b* (900 bp) and control region (383 bp
 755 with microsatellite removed) loci. Number of individuals sequenced (n), number of unique haplotypes (H), haplotype diversity (H_d , \pm S.D.),
 756 nucleotide diversity (π , \pm S.D.), Tajima's D (D), Fu's F_s (F_s), and Ramos-Onsins and Rozas's R_2 (R_2) test are given. Asterisks denote significant
 757 departures from neutrality: $P < 0.05 = *$, $P < 0.01 = **$, $P < 0.001 = ***$).

Region	Cytochrome <i>b</i>							Control region						
	n	H	H_d	π	D	F_s	R_2	n	H	H_d	π	D	F_s	R_2
Continental Shelf	59	41	0.972 (0.011)	0.0049 (0.0004)	-1.97**	-41.267***	0.038***	52	25	0.919 (0.022)	0.0052 (0.0004)	-2.04***	-76.50***	0.037***
Spanish Coast	10	7	0.911 (0.077)	0.0059 (0.0008)	-0.53	-0.89	0.127	8	6	0.929 (0.084)	0.0053 (0.0011)	0.25	-2.58*	0.023
North Sea	55	36	0.962 (0.015)	0.0048 (0.0004)	-1.82**	-31.99***	0.045***	43	24	0.942 (0.020)	0.0072 (0.0006)	-1.31	-19.64***	0.068
Skagarrak/Kattegat	30	22	0.968 (0.019)	0.0049 (0.0005)	-1.50*	-14.72***	0.063**	22	12	0.905 (0.044)	0.0064 (0.0009)	-1.27	-5.76***	0.079
Norwegian Sea	3	3	1.000 (0.272)	0.0030 (0.0009)	--	--	--	0	--	--	--	--	--	--
French Coast	21	19	0.986 (0.022)	0.0055 (0.0006)	-1.48*	-14.32***	0.069**	35	21	0.943 (0.024)	0.0074 (0.0009)	-1.70*	-16.11***	0.062*
Pooled	178	94	0.967 (0.006)	0.0050 (0.0002)	-2.22***	-133.75***	0.025***	160	63	0.893 (0.015)	0.0054 (0.0003)	-2.29***	-76.50***	0.025**

758 Table 3. Analysis of molecular variance (AMOVA) of *Entelurus aequoreus* based on 900 bp of mtDNA cytochrome *b* (Cytb) sequence and 383
 759 bp of mtDNA control region (CR) sequence (microsatellite at position 287 excluded). The percent variation (% var), and P value are listed for
 760 among groups (Φ_{CT}), among collections within groups (Φ_{SC}) and within collections Φ_{ST} . AMOVAs were conducted with all collections within
 761 regions (regions) and all collections pooled within habitat (coastal vs. pelagic).

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763

		Among groups			Among collections within groups			Within collections	
		% var	Φ_{CT}	P value	% var	Φ_{SC}	P value	Φ_{ST}	P value
All populations	Cytb	0.11	0.0011	0.4534	-0.62	-0.0062	0.5535	-0.0051	0.6189
	CR	3.76	0.0376	0.0603	-3.11	-0.0324	0.9151	0.0065	0.4495
Habitat	Cytb	-0.52	-0.0052	0.7534	-0.28	-0.0028	0.4951	-0.0080	0.6165
	CR	2.82	0.0282	0.0937	-1.48	-0.0152	0.8135	0.0135	0.4472

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766 **Figure Legends**

767 Fig. 1. Sampling locations for *Entelurus aequoreus* in the northeastern Atlantic Ocean.
768 Predominant ocean currents are shown in grey (after OSPAR, 2010). See Table 1 for sample
769 information and abbreviations.

770 Fig. 2. Maximum likelihood networks for *Entelurus aequoreus* pooled over years for a)
771 mtDNA *cytochrome b* sequences (900bp) and b) mtDNA control region sequences with
772 microsatellite at position 287 removed (383bp). Each circle represents a haplotype and its
773 size is proportional to its total frequency. Colors correspond to regions (see Table 1 and text
774 for descriptions). Black crossbars represent a single nucleotide mutation and filled in circles
775 represent reconstructed haplotypes not sampled.

776 Fig. 3. Bayesian Skyline Plot (BSP) showing changes in effective population size through
777 time (thousand years before present, ka). Dashed line represents the median posterior
778 estimate of the effective population size. The grey area delimited by continuous black lines
779 shows the 95% highest posterior density limits.