Phylogenetic relationships, origin and historical biogeography of the genus *Sprattus* (Clupeiformes: Clupeidae)

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The genus *Sprattus* comprises five species of marine pelagic fishes distributed worldwide in antitropical, temperate waters. Their distribution suggests an ancient origin during a cold period of the earth's history. In this study, we evaluated this hypothesis and corroborated the non-monophyly of the genus *Sprattus*, using a phylogenetic approach based on DNA sequences of five mitochondrial genome regions. *Sprattus sprattus* is more closely related to members of the genus *Clupea* than to other *Sprattus* species. We also investigated the historical biogeography of the genus, with the phylogenetic tree showing two well-supported clades corresponding to the species distribution in each hemisphere. Time-calibrated phylogenetic analyses showed that an ancient divergence between Northern and Southern Hemispheres occurred at 55.8 MYBP, followed by a diversification in the Oligocene epoch in the Northern Hemisphere clade (33.8 MYBP) and a more recent diversification in the Southern Hemisphere clade (34.2 MYBP). Historical biogeography analyses indicated that the most recent common ancestor (MRCA) likely inhabited the Atlantic Ocean in the Southern Hemisphere. These results suggest that the ancestral population of the MRCA diverged in two populations, one was dispersed to the Northern Hemisphere and the other across the Southern Hemisphere. Given that the Eocene was the warmest epoch since the Paleogene, the ancestral populations would have crossed the tropics through deeper cooler waters, as proposed by the isothermal submergence

hypothesis. The non-monophyly confirmed for the genus *Sprattus* indicates that its systematics should be re-evaluated.

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Abstract

The genus *Sprattus* comprises five species of marine pelagic fishes distributed worldwide in antitropical, temperate waters. Their distribution suggests an ancient origin during a cold period of the earth's history. In this study, we evaluated this hypothesis and corroborated the non-monophyly of the genus *Sprattus*, using a phylogenetic approach based on DNA sequences of five mitochondrial genome regions. *Sprattus sprattus* is more closely related to members of the genus *Clupea* than to other *Sprattus* species. We also investigated the historical biogeography of the genus, with the phylogenetic tree showing two well-supported clades corresponding to the species distribution in each hemisphere. Time-calibrated phylogenetic analyses showed that an ancient divergence between Northern and Southern Hemispheres occurred at 55.8 MYBP, followed by a diversification in the Oligocene epoch in the Northern Hemisphere clade (33.8 MYBP) and a more recent diversification in the Southern Hemisphere clade (34.2 MYBP). Historical biogeography analyses indicated that the most recent common ancestor (MRCA) likely inhabited the Atlantic Ocean in the Southern Hemisphere. These results suggest that the ancestral population of the MRCA diverged in two populations, one was dispersed to the Northern Hemisphere and the other across the Southern Hemisphere. Given that the Eocene was the warmest epoch since the Paleogene, the ancestral populations would have crossed the tropics through deeper cooler waters, as proposed by the isothermal submergence hypothesis. The non-monophyly confirmed for the genus *Sprattus* indicates that its systematics should be re-evaluated.

Keywords: BEAST, antitropical distribution, sprat, molecular clock, *Clupea*.

Introduction

Antitropical distribution patterns—when closely related taxa have geographic distributions to the north and south of the tropics, but not within—are an active line of research in evolutionary biogeography that can benefit greatly from using congeneric species in phylogenetic context. Congeneric species share a common history from their ancestral population, and several studies have shown that the combined analyses of biogeographic history and time-calibrated phylogenies in congeneric species provide a greater insight into the evolutionary processes involved (e.g., Lavoué et al., 2013). There are still important ecological and commercial fish genera with antitropical distribution patterns that remain to be studied, such as the genus *Sprattus*.

The five extant species currently assigned to the genus *Sprattus* (Figure 1; Clupeiformes, Clupeidae, Clupeinae) are small marine pelagic fishes that inhabit coastal areas and are well known for their schooling behavior (Whitehead, 1988; Fricke, Eschmeyer & Van der Laan, 2021). They are important components of several food webs and some species are commercially important (Frederiksen et al., 2006). These species occur in cooler waters and have an antitropical distribution (Whitehead, 1988; Figure 1). *Sprattus sprattus* (Linnaeus, 1758) is the most widely distributed species, and it is the only species in the genus found in the Northern Hemisphere, mainly around the coasts of Europe (Whitehead, 1988; Fricke, Eschmeyer & Van der Laan, 2021). *Sprattus fuegensis* (Jenyns, 1842) is found on the South American coast, mainly in the Patagonian shelf from the Pacific and Atlantic Oceans (Whitehead, 1988; Aranis et al., 2007; Canales-Aguirre et al., 2016; Fricke, Eschmeyer & Van der Laan, 2021). The other three species are found in Oceania: *S. novaehollandiae* (Valenciennes, 1847) in south-eastern Australia, and *S. antipodum* (Hector, 1872) and *S. muelleri* (Klunzinger, 1879) on the coast of New Zealand (Whitehead, Smith & Robertson, 1985; Whitehead, 1988; Fricke, Eschmeyer & Van der Laan, 2021).

Phylogenetic analyses have shown that the genus *Sprattus* is sister to the genus *Clupea* (Lavoué et al., 2007; Li & Ortí, 2007), and it has been suggested that they diversified between 2.66–6.75 MYBP (Jérôme et al., 2003; Cheng & Lu, 2006), which is consistent with the Miocene record of *Clupea*. Moreover, the extant *Clupea* species are thought to have radiated during the Pliocene (3.3–3.5 MYBP; Grant, 1986; Wilson, Teugels & Meyer, 2008), which is when the genus *Sprattus* is thought to have diverged. More recent studies based on large fossil-calibrated phylogenies suggested that the genus *Sprattus* is a paraphyletic group, and *S. sprattus* is more

closely related to *Clupea* spp. than to its relatives in the Southern Hemisphere (Lavoué et al., 2013; Bloom & Lovejoy, 2014; Egan et al., 2018).

No study has examined the biogeographic origin of the genus *Sprattus*; though, information of species with similar antitropical distribution pattern have been conducted. For example, studies of extant populations of *Sardinops* species showed a recent diversification event between 0.2–2 MYBP (Grant & Leslie, 1996; Bowen & Grant, 1997; Grant & Bowen, 1998), whereas species included in the genus *Engraulis* diversified between 5–10 MYBP (Grant, Leslie & Bowen, 2005). When considering marine species that have an antitropical distribution, the tropical zone appears to act as a barrier to long-distance dispersal, restricting gene flow between the Northern and Southern Hemispheres (Grant, Lecomte & Bowen, 2010). Experimental studies aiming to evaluate the thermal tolerance of two temperate species of Clupeidae (e.g., *Clupea harengus* and *Sardinops sagax*) evidenced their low tolerance for warm (tropical) waters (Martínez-Porchas, Hernández-Rodríguez & Bückle-Ramírez, 2009; Peck et al., 2012). These results reinforce the hypothesis that warm waters act as a dispersal barrier.

Considering the current antitropical distribution pattern of the genus *Sprattus*, we hypothesize that the lower sea temperatures of the tropics during the cooler glacial periods between the Miocene and Pliocene might have provided a window of opportunity for the most recent common ancestor of *Sprattus* to disperse to the other hemisphere. In this study we test the origin and the monophyly of the genus *Sprattus* using a phylogenetic approach based on DNA sequences from five mitochondrial genome regions (mtDNA). We also examine the historical biogeography of the group, and we used a molecular clock to determine the pattern and timing of species diversification.

Materials and Methods

Taxon sampling

Sprattus species have a least concern status for the IUCN Red List and are not listed under CITES. We did not kill fishes for the purpose of this study; instead, tissue samples were provided by researchers worldwide. Unfortunately, samples for *Sprattus novaehollandiae* were impossible to obtain, therefore we used only three *Sprattus* species from the Southern Hemisphere. All tissue samples arrived fixed in ethanol 90%, and their general capture locations were *S. fuegensis*

(n = 7) from Chilean fjords in the Southeast Pacific Ocean, *S. sprattus* (n = 5) from Norwegian fjords in the Northeast Atlantic Ocean, *S. muelleri* (n = 4) from Auckland Harbour, and *S. antipodum* (n = 1) from Wellington Harbour (New Zealand).

DNA extraction, PCR and DNA sequencing

Total genomic DNA was dissolved in a buffer containing proteinase K and SDS detergent, and then extracted using a standard phenol-chloroform protocol (Sambrook et al., 1989). DNA was precipitated in 70% ethanol and resuspended in 50 µL of TE buffer. DNA was quantified using a NanoDrop ND-1000 spectrophotometer and diluted to a concentration of 20 ng/µL.

Five mitochondrial fragments were amplified using genus-specific primers (762 bp for Cytochrome b, CytB; 857 bp for Cytochrome Oxidase subunit I, COI; 827 bp for NADH dehydrogenase subunit 2, ND2; and 348 bp for NADH dehydrogenase subunit 3, ND3) designed in this study, and one primer pair described previously (1107 bp for Control Region, CR; Palumbi et al., 1991; Bernatchez, Guyomard & Bonhomme, 1992; see Supporting Information Table S1). The genus-specific primers were designed from the complete mitochondrial genomes sequences deposited in GenBank: *Sprattus sprattus* (NC009593), *S. muelleri* (NC16669) and *S. antipodum* (NC16673). For CytB, COI, ND2, and ND3 fragments, the PCRs were conducted in 30 µL volumes containing 1X PCR Buffer (Invitrogen®; Tris-HCl 200 mM, pH 8,4, KCl, 500 mM), 3 mM $MgCl_2$, 0.2 mM of each dNTP's, 0.2 µM of each primer, 0.4 mg/mL of BSA, 1.5 units of Taq DNA polymerase (Invitrogen®), and 2 ng of genomic DNA. Thermal cycling was performed in an MJ Research PTC-200 Thermal Cycler with the following parameters: 95° C for 180 s, followed by 35 cycles of 94° C for 30 s, 55° C for 30 s, 74° C for 60 s, and a final extension at 74° C for 300 s. For CR the PCR was amplified using 2 mM $MgCl_2$ and the thermo cycling parameters: 94° C for 300 s, followed by 35 cycles of 94° C for 30 s, 54° C for 60 s, 74° C for 90 s, and a final extension at 74° C for 600 s. PCR products were purified with ExoSAP-IT® following manufacturer's guidelines and sequenced in both directions using an ABI 3730xl Genetic Analyzer (Massey University Genome Sequencing Service). Sequences were deposited in GenBank database under the accession numbers MW075156-MW075219. Additional sequences to genus *Sprattus* were included in the ingroup for further analyses: i) *Clupea harengus* (KC193777) and *Clupea pallasii* (AP009134), including two herring subspecies from *C. pallasii* (*C. p. marisalbi* and *C. p. suworowi*), given their close relatedness to the genus

153 *Sprattus*; ii) *Ethmidium maculatum* (AP011602), *Ramnogaster melanostoma* (GQ890211-
154 GQ890214, KU288994- KU288995), and *Strangomera bentincki* (GenBank accession numbers
155 solicited), given their close relatedness with the *Sprattus-Clupea* clade; iii) *Potamalosa*
156 *richmondia* (AP011594) and *Hyperlophus vittatus* (AP011593), because they are more distantly
157 related genera to the *Sprattus-Clupea* clade; and iv) *Sprattus sprattus* (AP009234), *Sprattus*
158 *muelleri* (AP011607), and *Sprattus antipodum* (AP011608) to increase the number of sequences
159 of our target genus. As outgroups, we included *Gilchristella aestuaria* (AP011606) and *Ehirava*
160 *fluviatilis* (AP011588), two species of the subfamily Ehiravinae used for rooting and time
161 calibration purposes.
162 Initial alignment was performed in Geneious® 6.0.5 (Kearse et al., 2012), and the final
163 alignment was adjusted by eye. Phylogenetic analyses were conducted separately on each gene
164 (to compare each gene tree) and concatenated fragments (because mitochondrial DNA
165 constitutes a single heritable unit). Divergence time and historical biogeography analyses were
166 conducted using a concatenated alignment of the five mitochondrial fragments. Our concatenated
167 data matrix included 13 sequences (one taxa each) and 3228 characters.
168
169 **Phylogenetic analyses and divergence time**
170 Before conducting the phylogenetic analyses, we performed Xia's test implemented in DAMBE
171 v5 (Xia et al., 2003; Xia, 2013) to evaluate whether the DNA sequences we used showed
172 evidence of saturation by substitution (i.e., back mutations), which would need to be corrected
173 using a model of sequence evolution during the phylogenetic analyses. We estimated and
174 compared a substitution saturation index with a critical substitution saturation index (Xia et al.,
175 2003; Xia, 2013) to test that the data set is informative for performing phylogenetic analyses.
176 The results of Xia's test suggest that there is a low level of saturation in our data set, where the
177 critical index of substitution saturation values was significantly higher than the observed index of
178 substitution saturation values (Supporting Information Table S2).
179 We ran a Bayesian Markov Chain Monte Carlo (BMCMC) phylogenetic analysis that
180 included a general likelihood-based mixture model of gene-sequence evolution and a Reversible-
181 Jump Markov Chain Monte Carlo procedure (Pagel & Meade, 2004, 2006, 2008; Gascuel, 2005).
182 This phylogenetic reconstruction was implemented in BayesPhylogenies v1.1 software (Pagel &
183 Meade, 2004). This approach enables possible models and parameters to be explored, converging

184 towards the model that best fits the data in the sample of posterior trees (Pagel & Meade, 2008).
185 We ran five independent chains using 10^6 generations, sampling every 10,000th tree sample, and
186 burning the first 25% of the trees. Finally, we obtained the phylogenetic consensus tree using
187 750 tree samples.
188 Approximate divergence times among *Sprattus* species were estimated using a Bayesian
189 approach implemented in the BEAST v2 software (Heled & Drummond, 2008; Drummond et al.,
190 2012; Bouckaert et al., 2014). To obtain divergence times, we used the Log-Normal Relaxed
191 Clock Model (LNCM; Drummond et al., 2006; Drummond & Suchard, 2010). We ran this model
192 five times using the most complex sequence evolution model, GTR+I+G, with 10,000,000
193 generations sampling each 10,000 generations. The outputs of each run were combined in
194 LogCombiner software to increase the Effective Sample Size (ESS) to be at least > 200. The ESS
195 of a parameter sampled from an MCMC is the number of effectively independent draws from the
196 posterior distribution of the Markov Chain.
197 To obtain the posterior distribution of the estimated divergence time, the age of a fossil,
198 †*Lecceclupea ehiravaensis*, dated during the late Campanian in the Late Cretaceous epoch at
199 about 74 MYBP was used. (Taverne, 2011 interpreted this age as part of the Campanian-
200 Maastrichtian; however, 74 MYBP is currently considered within the Campanian according to
201 the ICS International Chronostratigraphic Chart, 2021; www.stratigraphy.org.) This age was
202 used as a calibration point to constrain the age in the *Gilchristella aestuaria* and *Ehirava*
203 *fluviatilis* node. †*Lecceclupea ehiravaensis* has been shown to be a crown member of the clade
204 (*Ehirava*, *Gilchristella*; see Taverne, 2011). Prior age distribution of this clade follows a
205 lognormal distribution using the age boundaries of the geological stage from which the fossil was
206 excavated (i.e., 95% credibility interval). An offset of 74 MYBP was applied to the model.
207 Subsequently, we used the Log-Normal Relaxed Clock Model and previous set parameters to run
208 10 independent Markov Chain Monte Carlo (MCMC) simulations with a chain length of 10^7
209 generations. Sampling was conducted every 10,000 generations and we used as prior
210 distributions the following parameters: the base frequency, proportion invariant sites, and
211 proportions of each transition and transversion, all of those to increase the effective sample size.
212 The individual runs were combined using LogCombiner burning 250 trees per each sample.
213 Finally, a maximum clade credibility tree was created in TreeAnnotator, which enable a
214 summary tree to be visualized in FigTree v1.4 (https://github.com/rambaut/figtree/releases).

215

216 **Historical biogeography**

217 We inferred the historical distribution of the genus *Sprattus* and its close relatives using their
218 current distribution (i.e., longitude and latitude as continuous traits). This approach was chosen
219 over the multistate discrete data for the following reasons: i) discrete data could bias the
220 ancestral state of a descendant species distributed in the same geographical region; ii) continuous
221 data permit identifying dispersal trends; and iii) classical discrete multistate estimation does not
222 consider the spherical nature of the earth (O'Donovan, Meade & Venditti, 2018; Gardner, Surya
223 & Organ, 2019; Avaria-Llautureo et al., 2020). For these, we used the current geolocation to
224 infer the ancestral distribution for each node of the phylogenetic tree. To reconstruct the
225 distribution, we used the Geo Model (O'Donovan, Meade & Venditti, 2018) and implemented
226 BayesTraits v3.0 (Pagel & Meade, 2004). The Geo Model estimates the posterior distribution of
227 their geo-position across phylogenetic nodes. We used tree samples obtained in BMCMC
228 phylogenetic analyses and a trait matrix. We ran 10^6 generations sampled every 10,000
229 generations to obtain a parameters sample. Posteriorly, a 25% burned-in was used to avoid
230 including parameters sampled before the convergence of the Markov Chain, and a final sample
231 of 750 parameters was obtained. The ancestral distribution of each node was plotted on a
232 paleogeographical perspective using mapast v0.1 R package (Varela & Rothkugel, 2018). We
233 combine paleomaps from 10, 30, 50, 90, 110 MYBP using SETON2012 as a global plate motion
234 model (Seton et al., 2012).

235

236

237 ## Results

238 Phylogenetic tree reconstructions using the concatenated fragments (Figure 2) and each
239 mitochondrial fragment independently showed a similar pattern (Supporting Information Figure
240 S1). Each extant *Sprattus* species forms a monophyletic group. The *Sprattus* species were
241 distributed in the phylogenetic tree in two main clades that matched their antitropical
242 distribution, each in one hemisphere. The Northern Hemisphere clade included *Sprattus sprattus*
243 and the species *Clupea harengus* and *C. pallasii* (including their subspecies); the Southern
244 Hemisphere clade included *Sprattus fuegensis*, *S. antipodum*, *S. muelleri*, *Ramnogaster*
245 *melanostoma,* and *Strangomera bentincki*. However, overall, the genus *Sprattus* is polyphyletic,

because *S. sprattus* is closely related to *Clupea* and *S. fuegensis*, whereas *S. antipodum* and *S. muelleri* are closely related to *Ramnogaster* and *Strangomera*.

The time-calibrated phylogenetic analyses showed a divergence between Northern and Southern Hemispheres that was dated at 55.8 MYBP (early Eocene; Figure 3A). There was also another diversification event among the Northern Hemisphere clade at 33.8 MYBP (boundary between Eocene and Oligocene), splitting *Sprattus sprattus* from *Clupea* species. Current species of *Clupea* diverged about 8.5 million years ago (late Miocene). For the Southern Hemisphere clade, species diverged at 33.2 MYBP (early Oligocene). Among the species of the Southern Hemisphere clade, *Strangomera bentincki* split from other *Sprattus* species around 22.6 MYBP (early Miocene), *Sprattus fuegensis* split at 13.3 MYBP (middle Miocene) from their New Zealand relatives, and the most common recent ancestor of *S. antipodum* and *S. muelleri* diverged around 5.6 MYBP (boundary between Miocene and Pliocene). Ancestral distributions (Figure 3B–G) show that the MRCA of the Northern and Southern clades likely inhabited the Southern Hemisphere in the Atlantic Ocean (Figure 3D).

Discussion

Non-monophyletic genus *Sprattus*

We confirmed that the genus *Sprattus* is a polyphyletic group with an antitropical distribution, challenging the taxonomic status of the *Sprattus* species. Considering the two geographic clades in opposing hemispheres, the Northern clade closely relates *S. sprattus* with the genus *Clupea*, and the Southern clade closely relates the rest of *Sprattus* members with *Strangomera bentincki* and *Ramnogaster melanostoma*. The relationship among species from the Southern clade has not been described before. This taxonomic incongruence in the genus *Sprattus* has also been identified in studies that use large phylogenies in Clupeiformes and have focused in the identification of the biogeographic or diadromy origin, body size, dispersal pattern, or trophic niche evolution of the group (Lavoué et al., 2013; Bloom & Lovejoy, 2014; Egan et al., 2018; Bloom, Burns & Schriever, 2018; Avaria-Llautureo et al., 2020). Although some of these studies are based on DNA of different types (i.e., mt or nDNA) or taxa (i.e., *Sprattus* members and its close relatives), they support the polyphyly of the genus *Sprattus*. Therefore, our results provide

277 further support for *Sprattus* being polyphyletic and add *S. fuegensis* and *Strangomera bentincki*
278 as pieces of the puzzle to understand the evolution in the Southern clade.
279 Taxonomic classification and phylogenetic relationships among the genera *Sprattus*,
280 *Ramnogaster*, *Strangomera,* and *Clupea* are unclear if they are only based on morphological and
281 meristic traits. All these taxa resemble the *Clupea* type and were first classified as species of
282 *Clupea* (Linnaeus 1758; Whitehead, Smith & Robertson, 1985; Whitehead, 1988). The genus
283 *Sprattus* was erected by Girgensohn (1846) based on *S. haleciformis*, which was later
284 synonymized with *S. sprattus* (Whitehead, 1988), defining the absence of a pterotic bullae as the
285 key diagnostic feature (Mathews, 1884; Whitehead, 1964, 1988; Whitehead, Smith & Robertson,
286 1985). However, fewer pelvic rays and an anteriorly placed pelvic fin (Whitehead, 1988) have
287 also been used to differentiate *Sprattus* from *Clupea*. The two genera also differentiate in key
288 reproductive traits, whereas *Sprattus* produces pelagic eggs, *Clupea* produces demersal eggs that
289 attach to the seabed or vegetation (Haegele & Schweigert, 1985; Whitehead, 1988). Finally, the
290 genera *Sprattus* and *Ramnogaster* share the absence of a pterotic bullae, but differ in fin-ray
291 numbers (Whitehead, 1988), whereas *Sprattus* differs from *Strangomera* on having more gill
292 rakers (Whitehead, 1988).
293 Incomplete sorting lineage, introgression, or convergence of morphological traits could
294 be plausible explanations for the current *Sprattus* taxonomic classification and our gene tree. The
295 first two can be ruled out, because none of the species of this study shared or had similar
296 haplotypes. Introgression may also be ruled out, because the fishes have different reproductive
297 strategies: pelagic or demersal eggs (Haegele & Schweigert, 1985; Whitehead, 1988), so there is
298 little opportunity for cross-fertilization. However, introgression could be true if divergence in
299 reproductive ecology occurred at an initial stage older than 33.8 MYBP between *S. sprattus* and
300 *Clupea* species. Introgression and ancient hybridization events could be identified by comparing
301 mtDNA and nDNA (Saitoh et al., 2011), however, this has not been detected in clupeid
302 phylogenies (Bloom & Lovejoy, 2014). We cannot discard the convergence of morphological
303 traits explanation given that there are traits that look similar and others that support the
304 separation of *Sprattus* and *Clupea* (Mathews, 1884; Whitehead, 1964, 1988; Whitehead, Smith &
305 Robertson, 1985).
306 For *Sprattus* species from the Southern Hemisphere, we found that *S. fuegensis* from South
307 America is the sister to New Zealand's sympatric *S. antipodum* and *S. muelleri*. Nonetheless, we

need to keep in mind that we could not include *S. novaehollandiae*, hence further studies should include this species. For New Zealand sprats, it only has been suggested that these species might have different ecological requirements considering their sympatry (Whitehead, Smith & Robertson, 1985). We suggest that further investigations be done to disentangle the mechanisms that promoted sympatric speciation for *S. antipodum* and *S. muelleri*.

Divergence time and historical biogeography

The results based on a fossil calibration showed that the two antitropical clades diverged in the Eocene (55.8 MYBP; older than we hypothesized), with a likely origin in the Atlantic Ocean in the Southern Hemisphere. The species within the Northern Hemisphere clade diverged at 33.8 and in the Southern Hemisphere at 33.4 MYBP, during the early Oligocene. Cheng et al. (2006) and Jérôme et al. (2003) estimated that the divergence event of the two genera occurred between 6.75–2.66 MYBP (late Neogene-early Quaternary). This estimation disagrees with the older divergence time found in our study, which could be explained by the calibrating method used by the authors. Different calibrating methods typically yield different results, and each method has its own particular challenges. In previous studies the authors used a standard nucleotide substitution rate for fish, which is a method that depends on the timescale over which those rates are measured (Hipsley & Müller, 2014) and could generate an overestimation of divergence times (Phillips, 2009; Ho et al., 2011; Hipsley & Müller, 2014). Fossil calibrations do not produce this problem, although the uncertainty in age and phylogenetic position present a different challenge (Hipsley & Müller, 2014). To address this and avoid the overestimation of the divergence time, we ran our analysis based on the fossilized birth-death process calibration method and a Bayesian framework, which included the uncertainty of dating species divergences and yield with more accurate node age estimates (Heath, Huelsenbeck & Stadler, 2014; Bouckaert et al., 2014; Gavryushkina et al., 2017).

The Eocene was the warmest geological epoch of the last 65 million years (Zachos et al., 2001), where sea surface temperatures in the Atlantic tropical areas may have been up to 38 ºC (Cramwinckel et al., 2018). The ancestor of Clupeoidei originated and diversified in the tropical Indo-West Pacific region during the Lower Cretaceous (119 MYBP, Lavoué et al., 2013), and it would have been adapted to warm, marine temperatures (i.e., > 25º C; Lavoué et al., 2013; Bloom & Lovejoy, 2014). Considering this, our analyses show that the Clupeidae lineage spread

to the Southern Hemisphere earlier than the clades that included *Sprattus*, *Clupea* and close relatives. Similarly, the species of *Potamalosa*, *Hyperlophus* and *Ethmidium* also inhabit temperate waters in the Southern Hemisphere, suggesting that this old south-distributed group of fishes was able to cross the tropics but not to adapt to the warmer environment. Nonetheless, extant members of the genera *Sprattus* and *Clupea* are now distributed antitropically in much colder temperate waters (Whitehead, Smith & Robertson, 1985; Lavoué et al., 2013), and although they mainly inhabit marine environments (Bloom & Lovejoy, 2014), they can also inhabit areas with highly variable environments, such as fjords (e.g., Glover et al., 2011; Canales-Aguirre et al., 2016, 2018).

Antitropical distribution patterns are traditionally explained by dispersal and vicariance mechanisms (Stepien & Rosenblatt, 1996; Grant & Bowen, 1998; Burridge, 2002; Le Port, Pawley & Lavery, 2013). Dispersalists have proposed several hypotheses to explain dispersal across the tropics: island integration (Rotondo et al., 1981), dispersal at shallow depths during glaciations (Lindberg, 1991), and isothermal submergence (Hubbs, 1952). Island integration refers to the formation of endemic biotas through the movement of individuals using islands or seamounts (Rotondo et al., 1981). In our case, we can discard this explanation, because clupeids are typically marine and inhabit productive coastal areas (Whitehead, 1988). Dispersal at shallow depths during glaciations is a well-recognized dispersal mechanism for several pelagic fishes during the Pleistocene (Burridge & White, 2000; Burridge, 2002; Grant, Leslie & Bowen, 2005). The isothermal submergence hypothesis refers to the possibility that marine organisms adapted to cool or temperate areas are able to disperse across the tropical region through deeper, colder tropical waters (Hubbs, 1952). Taking into account that the MRCA of these two clades diversified in the warm Eocene, and then each clade diversified between the late Eocene and early Oligocene epochs the isothermal submergence hypothesis seems to be the most plausible explanation. This later because the temperatures begin to decrease until initiation of Antarctic glaciation (Zachos et al., 2001) and some clupeoids, such as herrings, may dive as much as 200 m (Blaxter, Denton & Gray, 1981). Vicariant mechanisms such as plate tectonic, relictual distribution, and equatorial isolation by climatic change or biological interactions have been advocated by others studies (Stepien & Rosenblatt, 1996; Saitoh et al., 2011). However, mechanisms associated with plate tectonics are not supported by our results, because the divergence time among nominal species of *Sprattus* and *Clupea* would have occurred during the

Eocene, and the present continental configuration closely resembles the configuration of the continents during that time. Studies, such as those by Grant & Bowen (1998) and Grant, Leslie & Bowen (2005) on marine pelagic fishes have supported a dispersalist mechanism to explain the antitropical distribution and exclude vicariant explanations as well.

Dispersion from their ancestral habitat involved adaptation to colder waters, while simultaneously expanding their tolerance to fluctuations in salinity, allowing them to also colonize low saline habitats. The warmer equatorial waters have remained as a key barrier to dispersal between hemispheres, which has only been crossed when windows of colder environments appeared across the tropics or, more plausibly, by using deeper, colder tropical waters as proposed by the isothermal submergence hypothesis.

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Captions for Figures

Figure 1. Distributional map of extant *Sprattus* and closely related species used in this study. Red dashed line represents *Sprattus fuegensis*; brown is S. *novaehollandiae*; orange is *S. muelleri*; light blue is *S. antipodum*; and green is *S. sprattus*. Yellow solid line represents *Clupea harengus*; purple is *C. pallasii*; gray is *Ramnogaster melanostoma*; and blue is *Strangomera bentincki*.

Figure 2. Bayesian consensus tree concatenating of mitochondrial genes from 750 more likely trees. Branch lengths are proportional to the number of substitutions per nucleotide position. Numbers at nodes are posterior probabilities from Bayesian analyses. Grey rectangles indicate current hemisphere distribution. Red branches for *Sprattus fuegensis*, orange for S. *muelleri*, light blue for *S. antipodum*, and green for *S. sprattus*.

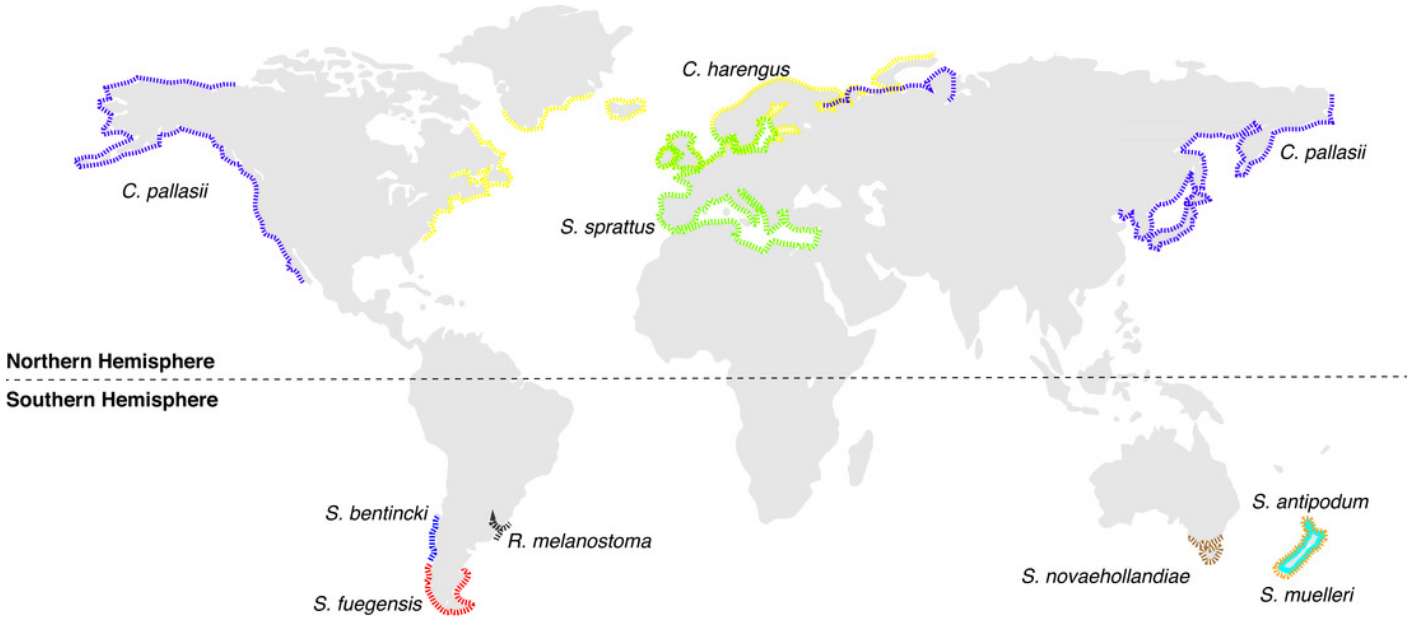
Figure 3. Time-calibrated phylogenetic tree based on Bayesian relaxed-clock analyses (A) and reconstruction of the ancestral geopositioned nodes (B – G). Numbers at nodes are divergence time since the root of each species. Horizontal colored bars indicate the 95% HPD of divergence

587 times, and the scale axis shows divergence times as millions of years ago (MYBP). Analyses
588 based on the topology and branch lengths of the Bayesian phylogenetic trees. Colored dots in B –
589 G correspond to posterior distribution of ancestral locations measured in longitude and latitude.
590 Colors are associated to horizontal-colored bars in (A). Paleomap reconstructions from 10, 30,
591 50, 90, 110 MYBP were obtained using SETON2012 global plate motion models.
592
593
594

Figure 1

Distributional map of extant *Sprattus* and closely related species used in this study.

Red dashed line represents *Sprattus fuegensis*; brown is S. *novaehollandiae*; orange is *S*. *muelleri*; light blue is *S*. *antipodum*; and green is *S*. *sprattus*. Yellow solid line represents *Clupea harengus*; purple is *C*. *pallasii*; gray is *Ramnogaster melanostoma*; and blue is *Strangomera bentincki*



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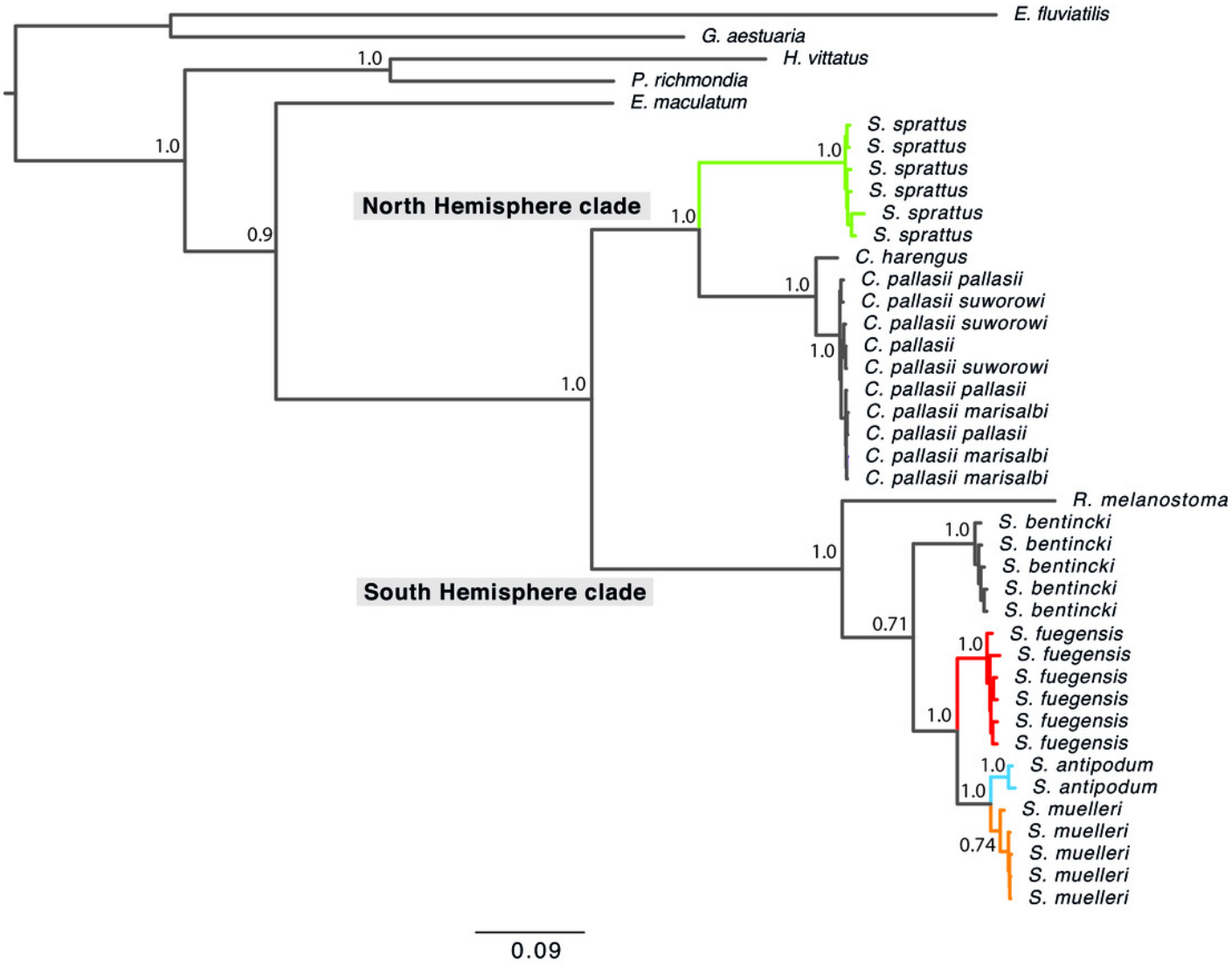

Figure 3

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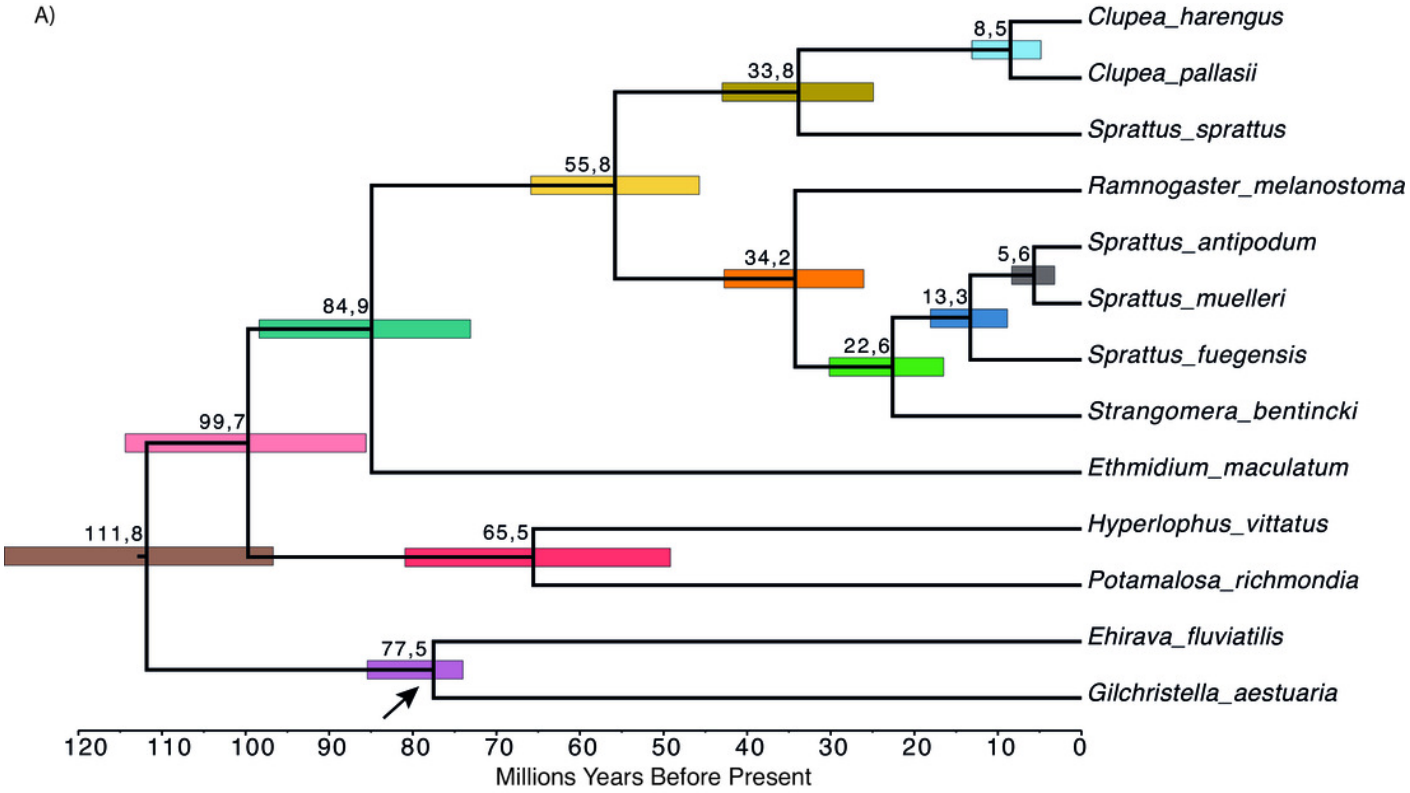
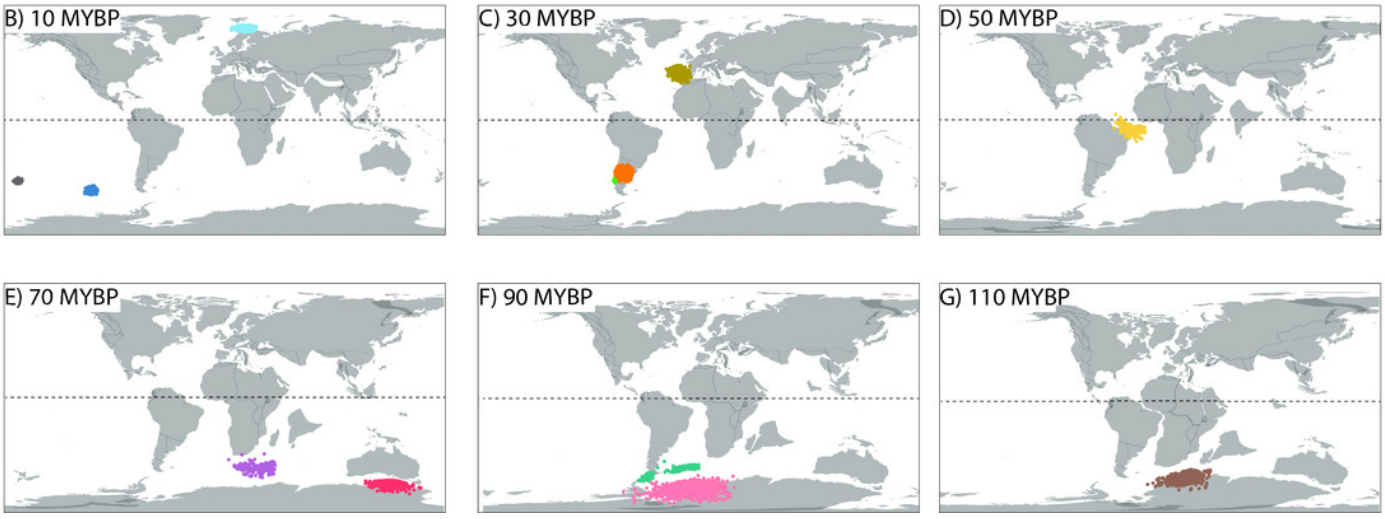
Numbers at nodes are divergence time since the root of each species. Horizontal colored bars indicate the 95% HPD of divergence times, and the scale axis shows divergence times as millions of years ago (MYBP). Analyses based on the topology and branch lengths of the Bayesian phylogenetic trees. Colored dots in B – G correspond to posterior distribution of ancestral locations measured in longitude and latitude. Colors are associated to horizontal-colored bars in (A). Paleomap reconstructions from 10, 30, 50, 90, 110 MYBP were obtained using SETON2012 global plate motion models.


A)
8,5
33,8
55,8
34,2
5,6
13,3
84,9
22,6
99,7
111,8
65,5
77,5
Clupea_harengus
Clupea_pallasii
Sprattus_sprattus
Ramnogaster_melanostoma
Sprattus_antipodum
Sprattus_muelleri
Sprattus_fuegensis
Strangomera_bentincki
Ethmidium_maculatum
Hyperlophus_vittatus
Potamalosa_richmondia
Ehirava_fluviatilis
Gilchristella_aestuaria
120 110 100 90 80 70 60 50 40 30 20 10 0
Millions Years Before Present


B) 10 MYBP
C) 30 MYBP
D) 50 MYBP
E) 70 MYBP
F) 90 MYBP
G) 110 MYBP