# Evidence of stock connectivity, hybridization and misidentification in white anglerfish support the need of a genetics-informed fisheries management framework

**Running title: White anglerfish genetics and management**

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**Abstract**

Understanding population connectivity within a species as well as potential interactions with its close relatives is crucial to define management units and to derive efficient management actions. However, although genetics can reveal mismatches between biological and management units and other relevant but hidden information such as species misidentification or hybridization, the uptake of genetic methods by the fisheries management process is far from having been consolidated. Here, we have assessed the power of genetics to better understand the population connectivity of white angelfish (*Lophius piscatorius*) and its interaction with its sister species, the black anglerfish (*L. budegassa*). Our analyses, based on thousands of genome-wide single nucleotide polymorphisms, show three findings that are crucial for white anglerfish management. We found i) that white anglerfish is likely composed of a single panmictic population throughout the Northeast Atlantic, challenging the three-stock based management, ii) that a fraction of specimens classified as white anglerfish using morphological characteristics are genetically identified as black anglerfish (*L. budegassa*) and iii) that the two *Lophius* species naturally hybridize leading to a population of hybrids of up to 20% in certain areas. Our results set the basics for a genetics-informed white anglerfish assessment framework that accounts for stock connectivity, revises and establishes new diagnostic characters for *Lophius* species identification and evaluates the effect of hybrids in the current and future assessments of the white anglerfish. Furthermore, our study contributes to provide additional evidence of the potentially negative consequences of ignoring genetic data for assessing fisheries resources.

**Key-words:** *Lophius piscatorius, Lophius budegassa*, genetic connectivity, hybridization, misidentification, SNPs, stock delimitation

## Introduction

Sustainable exploitation of fisheries resources relies on effective fisheries management actions, which in turn rely on accurate fisheries assessment, that is, the process that includes the synthesis of information on life history, fishery monitoring, and resource surveys for estimating stock size and harvest rate relative to sustainable reference points (Methot & Wetzel, 2013). The process of fisheries assessment is usually applied independently to pre-established management units (so-called stocks), of which parameters such as growth, recruitment, and natural and fishing mortality are assumed to be intrinsic and not dependent on emigration or immigration rates (Cadrin, 2020). Genetic data has demonstrated ability to delineate populations within a species, that is, to identify groups of sexually interbreeding individuals which possess a common gene pool, but has also revealed hidden phenomena such as species misidentification (Garcia-Vazquez, Machado-Schiaffino, Campo, & Juanes, 2012) or hybridization (Pampoulie et al., 2020).

Fisheries stocks are often defined upon political and administrative considerations (Stephenson, 2002); yet, failure to align stocks with natural populations results in unfeasibility to establish an accurate relationship between productivity and harvest rates and can result in local reduction of populations and, in extreme cases, to local population collapse (Bonfil, 2005). Misidentification can be a common phenomenon when morphologically similar species are caught simultaneously, and results in misled exploitation rate estimations when those are based on reported catches (Marko et al., 2004). Hybridization, the successful reproduction between different species (Arnold,

72 1997; Stronen & Paquet, 2013), has been reported in teleost fishes (Schwenke, 2013;
73 Yaakub, Bellwood, van Herwerden, & Walsh, 2006), but incidence and associated
74 consequences in commercial fisheries has been scarcely explored (Epifanio & Nielsen,
75 2000); yet, hybridization could play a key role in diversity loss and even parental
76 species extinction, which has important consequences for management and
77 conservation (Allendorf, Leary, Spruell, & Wenburg, 2001). An example of species
78 whose management could largely benefit from genetic-derived information is the
79 white anglerfish (*Lophius piscatorius*, Linnaeus, 1758), a bottom-living fish that moved
80 from being a bycatch species in the last century (Hislop et al., 2001) to become one of
81 the most valuable demersal species in southern and western Europe (ICES, 2010).
82 The white anglerfish inhabits the Northeast Atlantic and Mediterranean Sea, where it
83 is assessed by the International Council for the Exploration of the Sea (ICES) and the
84 General Fisheries Commission for the Mediterranean (GFCM), respectively. Within the
85 Northeast Atlantic, the white anglerfish is managed as three stocks (Figure 1): the
86 Northern Shelf stock (Skagerrak, Kattegat, North Sea, West of Scotland and Rockall),
87 the Northern stock (Celtic Seas and Northern Bay of Biscay) and the Southern Stock
88 (Atlantic and Iberian waters) (ICES, 2019a, 2019b); yet, the few studies assessing the
89 population structure, based on otolith shape analysis (Cañás, Stransky, Schlickeisen,
90 Sampedro, & Fariña, 2012), tagging surveys (J. Landa, Quincoces, Duarte, Fariña, &
91 Dupouy, 2008; Laurenson, Johnson, & Priede, 2005) and molecular markers, including
92 allozymes (Crozier, 1987), mitochondrial DNA (Charrier et al., 2006) and microsatellites
93 (Blanco, Borrell, Cagigas, Vázquez, & Prado, 2008), did not find differences between
94 stocks. However, this needs to be confirmed with the analysis of a large number of
95 genomic markers, which has been effective in resolving the population structure of

96 marine fish when other markers failed (Leone, Álvarez, García, Saborido-Rey, &
97 Rodriguez-Ezpeleta, 2019; Rodríguez-Ezpeleta et al., 2016; Rodríguez-Ezpeleta et al.,
98 2019). The white anglerfish coexists with its sympatric sister species, the black bellied
99 anglerfish (*Lophius budegassa*), which has a more southern distribution (Relini, 1999;
100 Ungaro et al., 2002). The outer morphology of both species is similar which is why they
101 are often confused, although the color of the epithelium that covers the abdominal
102 cavity, called peritoneum (white in *L. piscatorius* and black in *L. budegassa*) is
103 considered a unequivocal diagnostic character for individuals larger than 15 cm
104 (Caruso, 1986). Yet, genetic analyses based on polymerase chain reaction amplification
105 of restriction fragment length polymorphisms (PCR-RFLP) used for species
106 identification have revealed mislabeling among the two species (Armani et al., 2012).
107 There is thus a need for resolving the population structure of white anglerfish within
108 the Northeast Atlantic, including its relationship with Mediterranean populations, and
109 for understanding its interaction with its sister species, the black anglerfish. For that
110 aim, we have used thousands of genome-wide distributed SNP markers discovered and
111 genotyped through restriction site associated DNA sequencing (RAD-seq) and found i)
112 that white anglerfish from the three stocks within the Northeast Atlantic are
113 genetically connected, ii) first evidence of hybridization between white and black
114 anglerfish and iii) records of black anglerfish misidentified as white anglerfish due to a
115 lack of black peritoneum. These findings have important implications for white
116 anglerfish management and conservation, while revealing the significant advantage of
117 including genomics in fisheries assessment in general.
118
119 **Material and methods**

120 *Tissue sampling, DNA extraction, and species identification*

121 *Lophius piscatorius* (n=693) and *Lophius budegassa* (n=31) samples were collected
122 from Northeast Atlantic Ocean and Mediterranean Sea locations using scientific
123 surveys and commercial fisheries (Table S1; Figure 1). Sampling of *L. piscatorius* was
124 carried out so as to cover a large part of the geographic range of this species within the
125 Atlantic, whereas samples of *L. budegassa* were collected opportunistically as they
126 were only used for comparison purposes. Each individual was assigned to either
127 species by the color of the peritoneum, white for *L. piscatorius* and black for *L.*
128 *budegassa* (Caruso, 1986). Maturity was assigned following the 5 stages key (ICES,
129 2007). From each individual, a ~1 $cm^3$ muscle tissue sample was excised and
130 immediately stored in 96% molecular grade ethanol at -20 °C until DNA extraction.
131 Genomic DNA was extracted from about 20 mg of muscle tissue using the Wizard®
132 Genomic DNA Purification kit (Promega, WI, USA) following the manufacturer's
133 instructions. Extracted DNA was suspended in Milli-Q water and concentration was
134 determined with the Quant-iT dsDNA HS assay kit using a Qubit® 2.0 Fluorometer (Life
135 Technologies). DNA integrity was assessed by electrophoresis, migrating about 100 ng
136 of Sybr™ Safe-stained DNA on an agarose 1.0% gel. A polymerase chain reaction
137 restriction fragment length polymorphism (PCR-RFLP) method based on mitochondrial
138 DNA (Armani et al., 2012) was used for authentication of all specimens collected. In
139 order to further validate the PCR-RFLP results, for 122 of the samples, a fragment of
140 the mitochondrial cytochrome b (cytb) gene was amplified with the primers GluFish-F
141 and THR-Fish-R (Sevilla et al., 2007) using the following amplification conditions:
142 denaturation at 95 °C for 3 min; 35 cycles at 95 °C for 30 s, 57 °C for 30 s, 72 °C for 60 s;

143 and final extension at 72 °C for 10 min. The PCR products were purified and sequenced
144 using Sanger.

145 *RAD-seq library preparation and sequencing*

146 Restriction-site-associated DNA libraries were prepared for 306 *L. piscatorius* and 27 *L.*
147 *budegassa* individuals (Table S1) following the methods of Etter, Bassham, Hohenlohe,
148 Johnson, and Cresko (2012). Five individuals were run by duplicate starting from the
149 tissue sample to check for replicability. Between 300 and 600 ng of starting DNA
150 (depending on integrity) was digested with the *SbfI* restriction enzyme and ligated to
151 modified Illumina P1 adapters containing 5bp sample-specific barcodes. Pools of 32
152 individuals were sheared using the Covaris® M220 Focused-ultrasonicator™ Instrument
153 (Life Technologies) and size selected to 300-500 bp with magnetic beads. Following the
154 Illumina P2 adaptor ligation, each library was amplified using 12 PCR cycles and
155 batches of three pools were paired-end sequenced (100 bp) on an Illumina HiSeq4000.

156 *SNP genotype table generation*

157 Generated RAD-tags were analyzed using *Stacks* version 2.4 (Catchen, Hohenlohe,
158 Bassham, Amores, & Cresko, 2013). Quality filtering and demultiplexing were per-
159 formed using the *Stacks* module *process_radtags* removing reads with ambiguous ba-
160 ses and with an average quality score lower than 20 in at least one stretch of 15 nucle-
161 otides and using a maximum of 1 mismatch when rescuing single-end barcodes. Only
162 reads whose forward and reverse pair passed quality filtering were kept and the mod-
163 ule *clone_filter* was applied to remove PCR duplicates. *De novo* and reference-based
164 assemblies were performed for consistency since each approach has disadvantages:
165 the genome used for the reference-based approach is highly fragmented (Dubin,
166 Jørgensen, Moum, Johansen, & Jakt, 2019) and represents only one of the species in-

167 cluded in this study, and the *de novo* approach could be affected by assembly parame-
168 ters choice. For the *de novo* assembly, the module *ustacks* was used to assemble fil-
169 tered reads into putative orthologous loci per individual, with a minimum coverage
170 depth required to create a stack (parameter -m) of 3, and a maximum nucleotide mis-
171 matches allowed between stacks (parameter -M) of 4. RAD-loci were then assembled
172 using the module *cstacks* with a maximum of 6 allowed mismatches between sample
173 loci when generating the catalogue (parameter -n). Matches to the catalogue for each
174 sample were searched using *sstacks* and transposed using *tsv2bam*. For the reference
175 mapped assembly, the filtered reads were mapped against the available draft white
176 anglerfish genome (Dubin et al., 2019) using the BWA-MEM algorithm (Li, 2013), and
177 the resulting SAM files converted to BAM format, sorted and indexed using SAMTOOLS
178 (Li et al., 2009). Mapped reads were filtered to include only primary alignments and
179 correctly mate mapped reads. The following steps were applied to both the *de novo*
180 and reference mapped catalogs including only samples with a minimum of 30,000
181 RAD-loci. Paired-end reads were assembled into contigs and SNPs derived were identi-
182 fied and genotyped using the module *gstacks.* In order to avoid ascertainment bias
183 (Rodríguez-Ezpeleta et al., 2016), SNP selection was performed separately on each
184 subset of individuals to be analyzed (Table S2). For that aim, the module *populations*
185 was used to select the tags present in RAD-loci found in at least 90% of the individuals,
186 and *PLINK* version 1.07 (Purcell et al., 2007) was used to select samples with a mini-
187 mum of 0.85 genotyping rate, and SNPs with a minimum of 0.95 genotyping rate and a
188 minimum allele frequency (MAF) bigger than 0.05. Tags on which the selected SNPs
189 were located were mapped against the complete mitochondrial genome of *Lophius*

190 *piscatorius* (NC_036988.1) with the BWA-MEM algorithm (Li, 2013) and it was con-
191 firmed that the final genotype dataset did not contain mitochondrial SNPs.

192 *Genetic diversity, population structure and hybridization analyses*

193 The following analyses were performed using only the first SNP per tag. Expected (He)
194 and observed (Ho) heterozygosity and average pairwise $F_{ST}$ values were computed with
195 PLINK (Purcell, 2009) and GENEPOP (Rousset, 2014) respectively. Principal component
196 analysis (PCA) was performed without any *a priori* assignment of individuals to
197 populations using the package *adegenet* (Jombart & Ahmed, 2011) in R version 3.5.0
198 (Team, 2018). The genetic ancestry of each individual was estimated using the model-
199 based clustering method implemented in ADMIXTURE (Alexander, Shringarpure,
200 Novembre, & Lange, 2015) assuming from 2 to 5 ancestral populations (K) and setting
201 1000 bootstrap runs. The value of K with the lowest associated error value was
202 identified using ADMIXTURE's cross-validation procedure assuming from 1 to 5
203 ancestral populations. Using *NewHybrids* (E. Anderson & Thompson, 2002), posterior
204 probabilities of each individual´s membership as a pure parent, first (F1) or second (F2)
205 generation hybrids or backcrosses were calculated applying default parameters to the
206 200 SNPs with less than 1% of missing data that presented the highest Fst between
207 genetically assigned *L. piscatorious* and *L. budegassa* samples.

208

209 **Results**

210 *Genetic species identification based on mitochondrial markers*

211 In all samples for which it was conducted, cytb gene sequencing confirmed PCR-RFLP
212 results (Table S1). Species authentication was positive for all 31 specimens identified as
213 *Lophius budegassa*. However, from the 693 specimens identified as *L. piscatorius*

214 based on the colour of their peritoneum, 76 were genetically identified as *L.*
215 *budegassa*. This incongruency between the colour of the peritoneum and the
216 mitochondrial based genetic identification (Figure 2) suggests that species
217 identification based on the colour of the peritoneum results in erroneous taxonomic
218 assignment or that mitochondrial markers are ambiguous for species identification,
219 which needs to be evaluated using nuclear markers.

220 *Population structure based on nuclear markers*

221 The genotype table including individuals from both species resulted in 323 and 326
222 individuals (of which 27 were *L. budegassa*) and 16712 or 23126 SNPs after filtering
223 when using a *de novo* or reference mapped assembly respectively (Table S2).
224 Replicates resulted in 0.9947% identical genotypes. The PCA and ADMIXTURE analyses
225 based on these datasets grouped most samples in three clearly distinguishable groups,
226 and results were virtually identical among the *de novo* and reference assembly
227 datasets (Figure 3; Figure S1) and subsequent analyses were based on the former. In
228 the PCA (Figure 3A), samples are disposed along the first principal component
229 (accounting for 80.82% of the total variance) into three main groups. The first group
230 includes individuals provided, and genetically confirmed, as *L. piscatorius*. The second
231 group (in the middle) includes 25 individuals initially identified as *L. piscatorius*,
232 although 22 of them are assigned to *L. budegassa* according to mitochondrial DNA. The
233 third group includes individuals initially identified as *L. piscatorius* and *L. budegassa*,
234 but all of them assigned to *L. budegassa* according to mitochondrial DNA. Additionally,
235 there are five individuals (all with *L. budegassa* mitochondrial DNA) located in between
236 the main three clusters. This sample grouping is coherent with the different types of

237 genetic admixture patterns observed according to the presence of two ancestral
238 populations, best K=2 (Figure 3B): samples included in the first and third groups in the
239 PCA are not admixed and belong to different ancestral populations, and samples
240 included in the middle group in the PCA are admixed with equal contribution from
241 both ancestral populations. Samples located between groups in the PCA show
242 consistent patterns in admixture analyses with about 75% or 25% of contribution from
243 the first and third groups, respectively.

244 *Misidentification and hybridization*

245 Together, these results indicate that some of the samples morphologically identified as
246 *L. piscatorius* are *L. budegassa* despite presenting a white peritoneum (Figure 3C).
247 Samples in the middle group and adjacent ones have a significantly higher observed
248 average heterozygosity (0.77) than samples in first and third groups (0.05 and 0.03,
249 respectively), which could indicate that these individuals are hybrids. This was con-
250 firmed by NewHybrids, which assigned all individuals in the middle group as new gen-
251 eration hybrids between the two species (F1), and the five remaining individuals as
252 backcrosses between F1 hybrids and white or black anglerfish (Figure 3C). Mitochon-
253 drial DNA from all hybrids except for 3 F1 was of *L. budegassa.* Considering the propor-
254 tion of hybrid individuals within the ones bearing *L. piscatorius* or *L. budegassa* mito-
255 chondrial DNA as estimated from RAD-sequencing data, we calculated the percentage
256 of hybrid and misidentified individuals among those provided as *L. piscatorius*. Overall,
257 we found about 4 and 10% misidentified and hybrid individuals, respectively. However,
258 these were not distributed homogeneously across stocks and areas within the same
259 stock (Figure 4). Misidentified individuals were more present in the most southern re-

260 gion of the Southern stock (Portuguese coast) and Mediterranean Sea, with 1/3 of the
261 individuals identified as *L. piscatorius* being *L. budegassa,* less frequent in the Northern
262 stock (2%), and absent in Northern Shelf stock. Hybrid individuals were absent in the
263 Southern stock and North Sea (belonging to the Northern Shelf stock) and were most
264 frequent in the northern Bay of Biscay and Celtic seas (13%).

265 *Connectivity of white anglerfish within the Atlantic*

266 The genotype table including only those individuals genetically identified as *L.*
267 *piscatorius* include 238 or 232 individuals and 6233 or 6246 SNPs when including or
268 not Mediterranean samples, respectively. PCA and ADMIXTURE analyses based on
269 those datasets (Figure 5) reveal strong differentiation between Mediterranean and
270 Atlantic samples ($F_{ST}$ = 0.057) and no genetic differentiation within the Atlantic ($F_{ST}$ =
271 0), as also suggested by ADMIXTURE (best K=1).

272

273 **Discussion**

274 *What´s in a white anglerfish sample?*

275 We found that several of the samples provided as white anglerfish (*Lophius*
276 *piscatorius*) by expert scientists involved in surveys targeting anglerfish were indeed
277 black anglerfish or hybrids. This implies that the commonly used diagnostic character
278 for species identification, the color of the peritoneum, is not discriminative as all the
279 black anglerfish and hybrid individuals provided as white anglerfish had white
280 peritoneum. Indeed, it has already been suggested that some young individuals cannot
281 be distinguished by the color of the peritoneum (*e.g.* some that show a white
282 peritoneum with small black dots can be assigned to either black or white anglerfish

283 when using another diagnostic character such as the number of dorsal fin rays (J.
284 Landa and A. Antolínez, unpublished data). Here, we found misidentified individuals
285 that are large (up to 75cm), suggesting that the reason for misidentification is not
286 related to the size of the specimens. In light of these findings, alternative
287 characteristics for species identification are needed (e.g. dorsal and anal fin ray counts
288 or length of the cephalic dorsal fin spines (Caruso, 1986)), so that misidentification
289 does not affect data collection. Additionally, a way of identifying hybrids would be
290 needed. Notably, in our dataset, the hybrid size distributions was significantly smaller
291 than that of the *L. piscatorius* individuals ($p<0.05$ in the Mann-Whitney test), although
292 the small sample size of hybrids and confounding factors (e.g. different proportion of
293 hybrids in the different stocks, age classes) prevent drawing conclusions from this fact
294 at this stage.

295 *Causes and consequences of interspecific hybridization*

296 We provide, to the best of our knowledge, the first evidence of natural hybridization
297 between *Lophius piscatorius* and *L. budegassa*, a phenomenon that can occur between
298 closely related species sharing morphological, ecological and reproductive
299 compatibilities (Montanari, Hobbs, Pratchett, Bay, & Van Herwerden, 2014). *Lophius*
300 *spp.* hybrids were predominant in Northern Bay of Biscay and Celtic seas, where both
301 species´ distribution range overlap and have been historically abundant (Figure S2).
302 There is also overlap in both species´ bathymetric distribution (20-1000m and 100-
303 500m depth respectively for white and black anglerfish)(Azevedo, 1995; Caruso, 1986;
304 García-Rodríguez, Pereda, Landa, & Esteban, 2005; Quincoces, Santurtún, & Lucio,
305 1998), and in spawning periods, both spawning in winter and spring (Ofstad, Angus,
306 Pedersen, & Steingrund, 2013; Quincoces, 2002.). This overlap in space, depth and

307 time could have even been increased by recent changes of geographical and
308 bathymetric distributions of both species, potentially as a consequence of climate
309 change (Maltby, Rutterford, Tinker, Genner, & Simpson, 2020). According to
310 mitochondrial DNA, which is maternally inherited in most animals (Moritz, Dowling, &
311 Brown, 1987), we found that most hybrids (27 out of 30) resulted from a black
312 anglerfish mother and a white anglerfish father. We found a few backcrosses (hybrids
313 crossed with parental species) and no crosses involving two hybrids. This could be due
314 the presence of a stable hybrid zone with hybrids being less able to produce offspring
315 (Hayden et al., 2010; Mirimin et al., 2014) or to recent changes that have induced
316 hybridization between both species so newly that they did not have time to produce a
317 hybrid-derived population. The presence of a stable hybrid zone between black and
318 white anglerfish does not forcedly imply changes in the evolution of the parental
319 species but could imply management uncertainties. However, if hybridization is recent,
320 we cannot discard a process of evolutionary novelty (Budd & Pandolfi, 2010), through
321 parental species acquiring new functions (T. M. Anderson et al., 2009) and even
322 producing new species (Verheyen, Salzburger, Snoeks, & Meyer, 2003), or of
323 biodiversity loss, through extinction of parental species (Seehausen, 2006). Thus,
324 monitoring the hybrid zone and adjacent areas is crucial to understand the role of
325 hybridization in management and conservation of white and black anglerfish.

326 *Management implications of stock connectivity, hybridization and misidentification*

327 Our results show that the Northeast Atlantic and Mediterranean white anglerfish
328 populations are genetically isolated, challenging previous findings based on genetic
329 data (Charrier et al., 2006), and that the white anglerfish within the Northeast Atlantic
330 Ocean constitutes a genetically homogeneous population, shedding light into previous

331 unconclusive results based on genetic and non-genetic data (Blanco et al., 2008; Cañás
332 et al., 2012; Crozier, 1987; Charrier et al., 2006; J. Landa et al., 2008; Laurenson et al.,
333 2005). These results point towards the necessity to harmonize current stock definitions
334 within the Northeast Atlantic. Morphometric characteristics such as length of maturity
335 (Duarte, Azevedo, Landa, & Pereda, 2001) or weight-length relationships (Jorge Landa
336 & Antolínez, 2018) differ between stocks. These differences are potentially due to
337 sampling biases, differences in scales and/or measurement methods or interannual
338 differences among studies, as well as the possible influence of different fishing
339 pressure or environmental conditions between areas. However, they could also be due
340 to the inclusion of different proportions of misidentified and hybrid individuals in each
341 stock.
342 Although in the Northern and Southern stocks the assessment is done separately for
343 white and black anglerfish, they are combined for management, being Total Allowable
344 Catches (TAC) for *Lophius spp*. In the Northern Shelf stock, the two species are
345 assessed and managed as one assuming that black anglerfish in this area are rare.
346 However, the obvious increase of black anglerfish in this stock (Figure S2) has created
347 concern and suggestions to separate species have been made (ICES, 2018). The
348 deliberate (in the North Shelf stock) or non-deliberate (in the Northern and Southern
349 stocks) inclusion of an unknown (and perhaps variable) proportion of black anglerfish
350 in the white anglerfish assessment will likely lead to some bias. Therefore, some
351 corrections could be applied to the total catches and the length composition data.
352 However, the proportions of black anglerfish included in these assessments seem
353 relatively small (<10%) and would likely not have a major impact on the assessments.
354 The implications of including hybrids in the stock assessment are more difficult to

355 anticipate as biological characteristics of hybrids are unknown. The main concern is
356 that inclusion of hybrids would lead to erroneous inferences of reproductive potential
357 because this feature is inferred from the biomass of mature fish and the reproductive
358 output of anglerfish hybrids is unknown. If, as in many other species (Mallet, 2007),
359 they have no or neglectable offspring, they should not be included in the spawning
360 biomass as otherwise they could lead to an overestimation of productivity, even to a
361 level below of required to support a sustainable fishery (Morgan et al., 2012). On the
362 other hand, if, as the presence of backcrossed individuals suggest, they are
363 reproductively viable, further studies are required to assess hybrid fitness compared to
364 pure individuals.
365 Despite supporting stock merging, the analyses presented here suggest a series of
366 concerns that should be considered for white anglerfish management and which affect
367 differently each of the stocks. The southernmost locations of the Southern Stock and
368 the Mediterranean Sea are more affected by misidentification, the northernmost
369 locations of the Northern stock are more affected by hybridization, and the North Shelf
370 stock does not seem to be affected by either. The over and under representation of
371 misidentified individuals (black anglerfish with white peritoneum) in the south and
372 north respectively could be simply explained by differences in black anglerfish
373 abundance. However, both species are moving northwards, as shown in the species
374 distribution maps (FigureS2), and so the proportion of misidentified individuals could
375 increase in northern areas as black anglerfish becomes more abundant. This northward
376 movement of both species could also contribute to enlarge the hybrid zone towards
377 northern areas. If the proportion of hybridization and mislabeling varies significantly
378 over time, then this will have implications for the robustness of the assessment for

these stocks. Thus, genetic studies monitoring white and black anglerfish populations across the Northeast Atlantic and Mediterranean Sea through time are needed.

*Outlook*

Our study shows that genetic analyses can be used to confirm or reject existing hypothesis about, for example, stock connectivity but, most importantly, that they can reveal hidden phenomena that were not foreseen, such as the thus far unknown hybridization between white and black anglerfish. Yet, despite the power of genetics to provide fisheries assessment relevant information, there are still barriers for the uptake of genetic data by fisheries management, which can be due to a variety of factors (Bernatchez et al., 2017; Ovenden, Berry, Welch, Buckworth, & Dichmont, 2015), such as lack of clear communication of genetic concepts by geneticists to end-users and reluctance to change or adapt established assessment and management procedures. The white anglerfish is a highly valuable species (around 30K tons with a corresponding value of around 142 million euro; data extracted from https://stecf.jrc.ec.europa.eu/dd/fdi) with a well-established data collection, and assessment and management frameworks. Thus, we anticipate that these results will set the basics for an imminent genetics informed fisheries management for this species. Indeed, a genetics informed fisheries management is essential to ensure the basics of fisheries science, whereby maximum sustainable yield can only be reached by efficient management of distinct populations.

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**Data availability statement**

Demultiplexed and quality-filtered RAD-tag reads are available at the NCBI Sequence Read Archive (SRA) under accession number SUB9963797 and final datasets and scripts utilized for this project are available at https://github.com/rodriguez-ezpeleta/Lophius_PopGen

**Author Contributions**

NRE, JTM, AZ, MS, IQ and IC designed the study. HG, FB, IH and JL provided samples. IM generated data. IAS, NDA, IPA and NRE analyzed data. IAS, NRE, AU, HDG, FB, IH, JL, IQ interpreted data. IAS and NRE wrote the first draft of the manuscript and integrated input from all authors.

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609 **Figure legends**

610

611 Figure 1. Samples included in this study, and in parentheses those genotyped with RAD-seq,
612 where black triangles indicate Lophius budegassa samples and coloured circles, Mediterranean
613 (red), Atlantic southern stock (purple), Atlantic northern stock (green) or Atlantic northern
614 shelf stock (blue) Lophius piscatorius samples.

615

616 Figure 2. A. Specimen of anglerfish captured in the northern coast of Scotland (65 cm and 4.4
617 kg) with white peritoneum, emerald green eyes, and stippled skin, genetically identified as
618 black anglerfish. B. Agarose gel electrophoresis of the resulting PCR-RFLP band for white (L.
619 pis) and black anglerfish (L. bud) showing that this specimen (Sample) is genetically identified
620 as black anglerfish.

621

622 Figure 3. A. Principal Component Analysis (PC1=80.82%; PC2=0.26%) where main
623 differentiated groups are circled. B. Individual ancestry proportions estimated by ADMIXTURE
624 when assuming two ancestral populations. C. For each group identified int the PCA, whether
625 individuals composing it are assigned to L. piscatorius (white fish or mitochondrion), L.
626 budegassa (black fish or mitochondrion) or both according to visual or mitochondrial DNA
627 based species identification, admixture pattern and assignment according to NewHybrids.

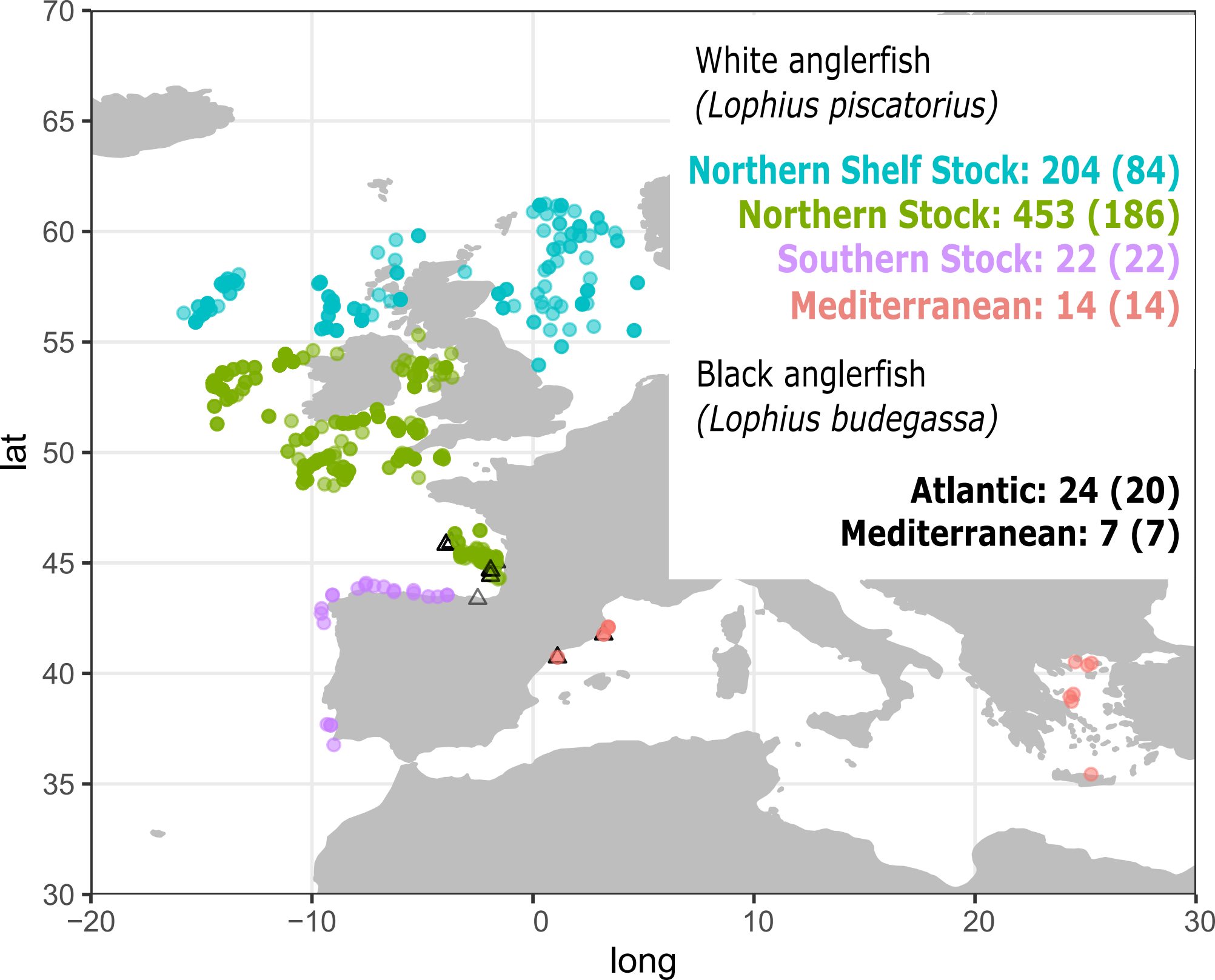
628

629 Figure 4. Proportion of hybrid (grey areas and numbers) and *L. budegassa* (black areas and
630 numbers) individuals among those morphologically identified as *L. piscatorius* for each stock
631 (large circles) and, within stocks, for smaller areas (small circles indicating number of sampled
632 individuals per area). Colours refer to stocks as in Figure 1.

633

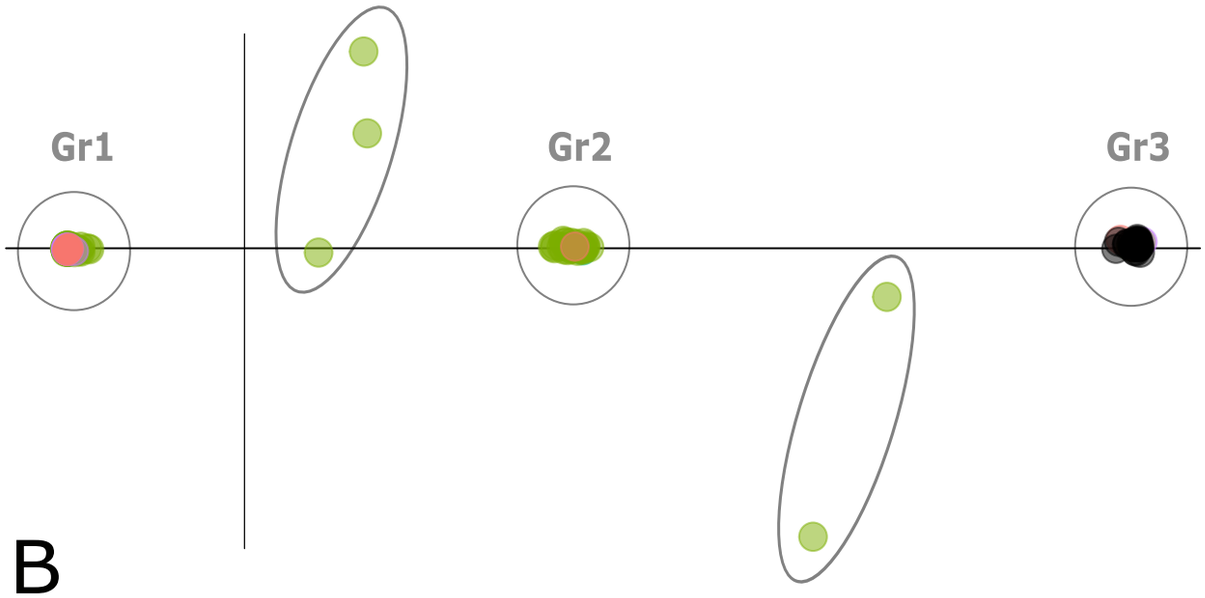
634 Figure 5. A. Principal Component Analysis (PCA) of samples genetically identified as white
635 anglerfish when including (left) or not (right) Mediterranean samples. B. Individual ancestry
636 proportions of samples genetically identified as white anglerfish estimated by ADMIXTURE
637 when assuming two ancestral populations including (above) or not (below) Mediterranean
638 samples.

639


White anglerfish
(Lophius piscatorius)
Northern Shelf Stock: 204 (84)
Northern Stock: 453 (186)
Southern Stock: 22 (22)
Mediterranean: 14 (14)
Black anglerfish
(Lophius budegassa)
Atlantic: 24 (20)
Mediterranean: 7 (7)
lat
long
70
65
60
55
50
45
40
35
30
−20
−10
0
10
20
30

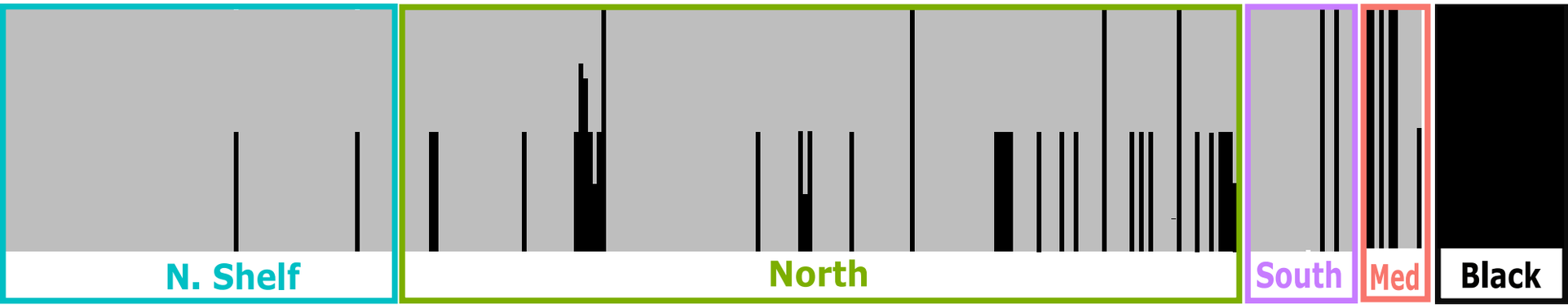
A
B
L. pis
Sample
L. bud

A

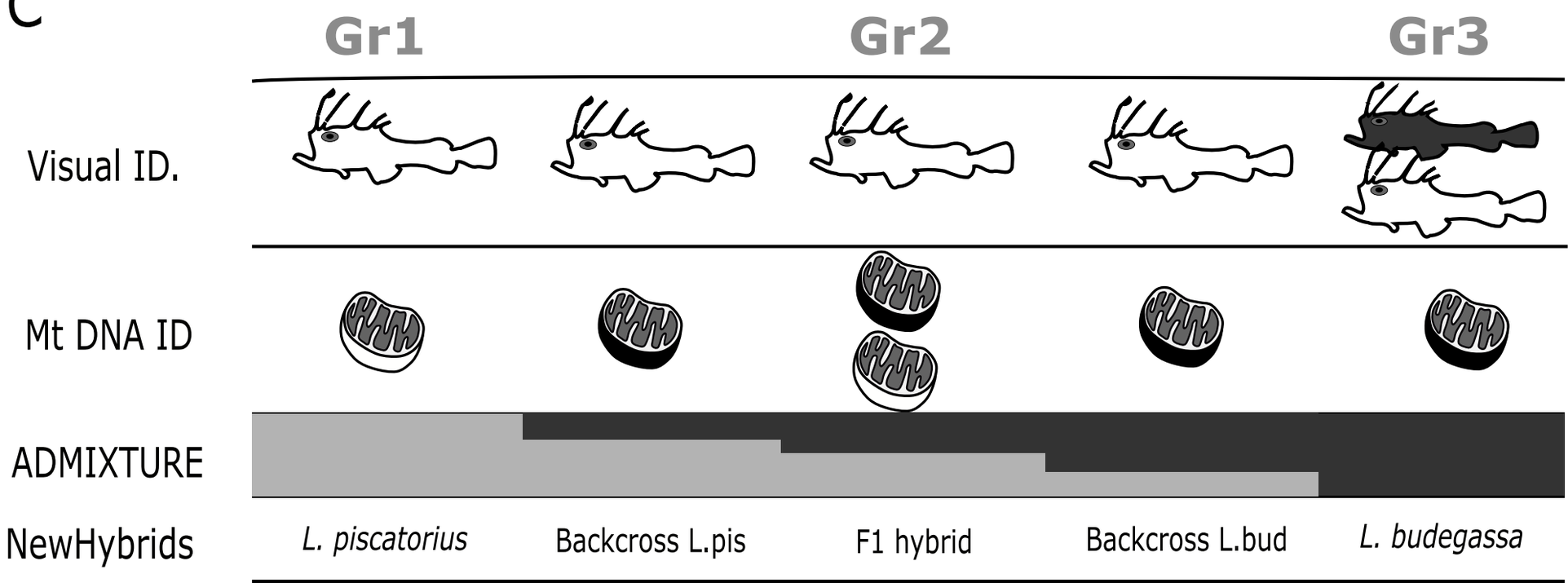


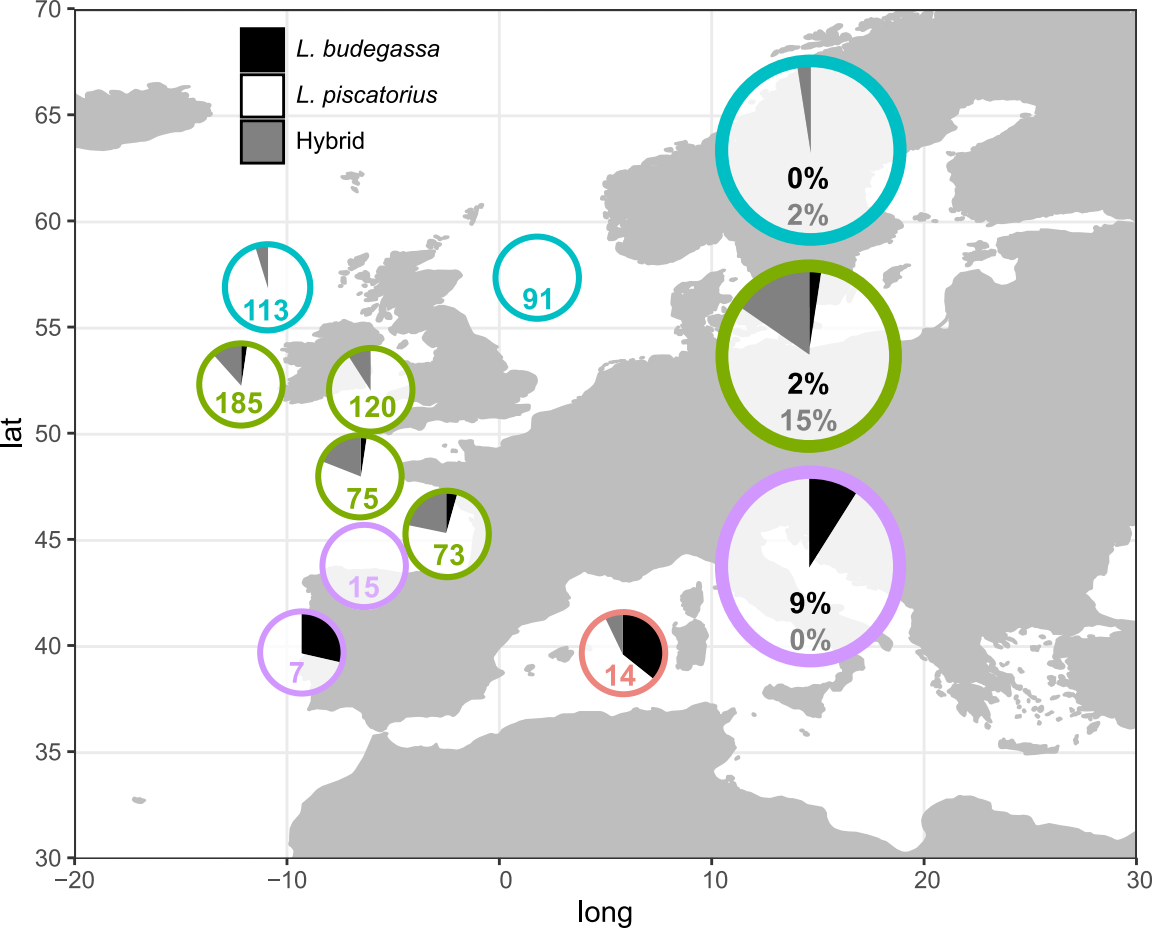

| | Gr1 | Gr2 | Gr3 |
|---|---|---|---|
| N. Plat | 82 | 2 | 0 |
| North | 144 | 25 | 4 |
| South | 20 | 0 | 2 |
| Med | 6 | 1 | 5 |
| Black | 0 | 0 | 27 |

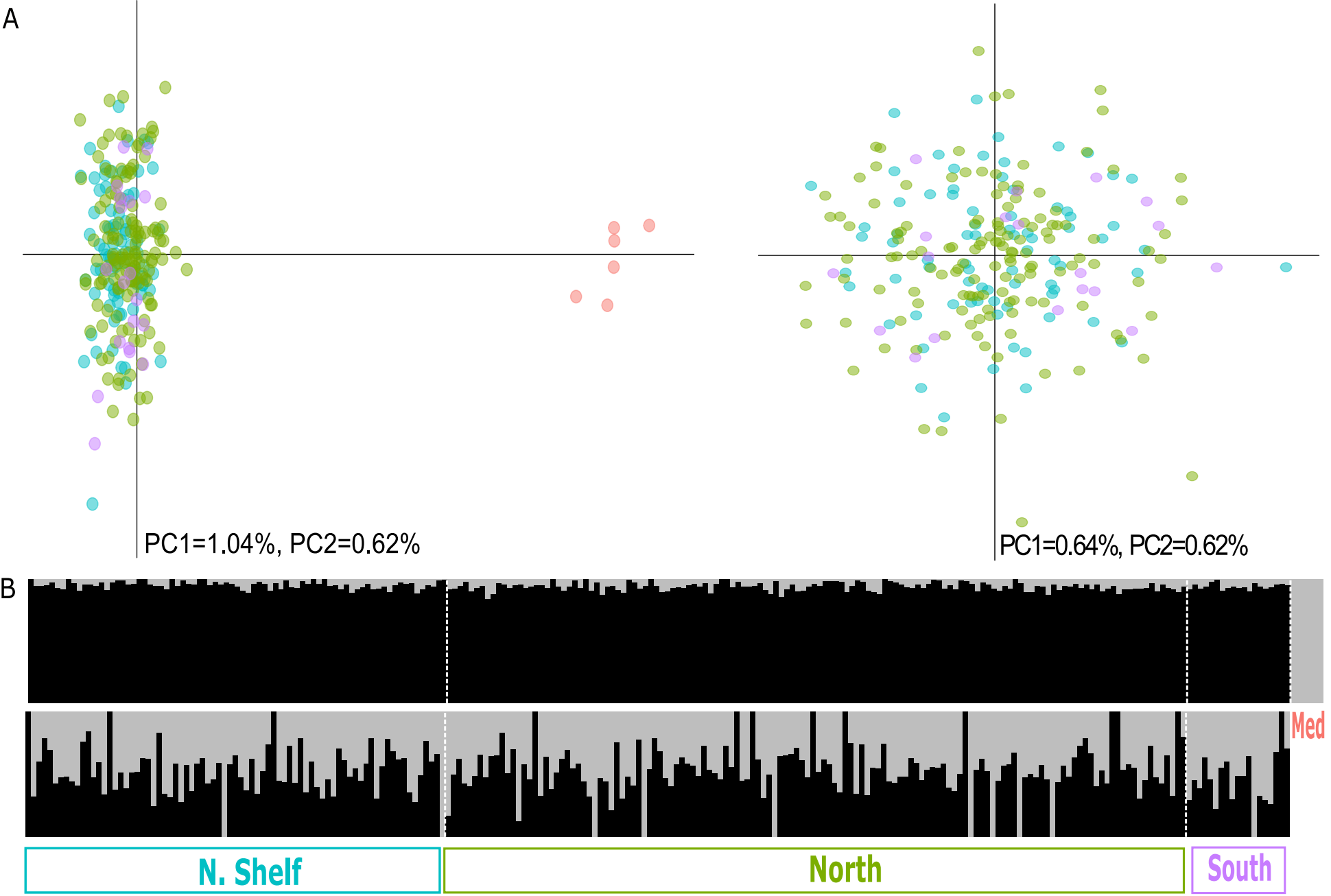
B




C




L. budegassa
L. piscatorius
Hybrid
0%
2%
113
91
185
120
2%
15%
75
73
15
9%
0%
7
14
lat
long
70
65
60
55
50
45
40
35
30
−20
−10
0
10
20
30


A
PC1=1.04%, PC2=0.62%
PC1=0.64%, PC2=0.62%
B
Med
N. Shelf
North
South