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*Genetic analysis reveals historical and contemporary population dynamics in the longfin squid *Doryteuthis gahi**

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1 **Original article**

2 **Genetic analysis reveals historical and contemporary population dynamics in the**
3 **longfin squid *Doryteuthis gahi*: implications for cephalopod management and**
4 **conservation.**

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21 **ABSTRACT**

22 Cephalopod population sustainability in the face of intensifying harvesting pressure and
23 climate change requires knowledge of connectivity, plasticity and adaptation. Population
24 genetic structure of the Patagonian longfin squid *Doryteuthis gahi* was assessed across
25 centres of abundance around the Falkland Islands, central-southern Chile and Peru.
26 Microsatellite and mtDNA data partitioned samples into two groups; one consisting of the
27 Peruvian samples, the other comprised the Chilean and Falklands samples, with no sub-
28 structuring within either group. Recurrent demographic independence between groups can be
29 linked to abrupt changes in continental shelf features between the Peruvian and Chilean sites
30 restricting adult dispersal. Phylogeographic analyses indicate a prolonged period of isolation
31 between the Peruvian and Chilean-Falkland populations which may have diverged in
32 allopatric glacial refugia. Both groups have experienced dissimilar historical population size
33 dynamics with the Peruvian population exhibiting signals of size fluctuations that align with
34 similar responses in other species to postglacial changes in the productivity of the Humboldt
35 Current system. Genetic homogeneity among Chilean and Falklands samples indicates
36 connectivity across current management boundaries and adds to evidence that squid typically
37 display connected populations over large geographical areas unless specific oceanographic
38 features restrict gene flow. High gene flow among Chilean and Falkland samples points to
39 environmental heterogeneity and phenotypic plasticity underpinning morphological
40 differences between *D. gahi* from both areas. Recognition of the Peruvian and Chilean-
41 Falklands groups as distinct evolutionary significant units is recommended on account of
42 existing and incipient genetic, adaptive and ecological divergence.

43 **Keywords:** squid; phylogeography; dispersal; plasticity; sustainability; Pleistocene

44

45 **Introduction**

46 Cephalopods have become an increasingly important fishery resource (Arkhipkin *et al.*, 2015;
47 van der Kooij *et al.*, 2016) and are viewed as alternatives to many dwindling traditional
48 finfish fisheries (Caddy and Rodhouse, 1998). Cephalopods are considered highly susceptible
49 to overfishing as their annual or sub-annual life histories mean there is little opportunity to
50 adjust fishing strategies during the lifetime of a single cohort (Pierce *et al.*, 2008). Some
51 cephalopod fisheries have already declined with such declines expected to have wide ranging
52 impacts on the marine ecosystems within which the species occupy important roles as
53 predators and prey (Xavier *et al.*, 2014). A fundamental requirement for the sustainable
54 management of commercially harvested taxa is an accurate understanding of patterns and
55 processes of population connectivity. Traditional methods of population estimation are often
56 unsuitable for cephalopods (Young *et al.*, 2006) because many species are not amenable to
57 standard ontogenetic tagging methods (Arkhipkin, 2005) and because of extensive
58 phenotypic plasticity (van der Vyver *et al.*, 2016) that may compromise estimates of
59 population cohesion and independence. Population genetic approaches have therefore
60 emerged as powerful tools to understand cephalopod population dynamics. Population
61 genetic structure can provide insight into the interplay of connectivity, adaptation and
62 plasticity, and their roles underpinning biogeography, population dynamics and phenotypic
63 variation and accordingly contribute to improve predictions of how populations will respond
64 to harvesting and environmental change (King *et al.*, 2017).

65 The Patagonian longfin squid *Doryteuthis gahi* (Loliginidae; formerly *Loligo gahi*,
66 Roa-Ureta and Arkhipkin, 2007) is a small (<50 cm total length) neritic squid distributed
67 along the continental shelves of the southeastern Pacific (SEP) and southwestern Atlantic
68 (SWA) from Peru to northern Argentina and across the Patagonian Shelf around the Falkland
69 Islands (Roper *et al.*, 1984). It is most abundant in waters around the Falkland Islands

70 supporting an important directed fishery in the Falkland Islands Interim conservation zone
71 (FICZ). The species' SEP distribution spans the Humboldt Current system, recognised as the
72 most productive marine ecosystem on earth and it is harvested off Peru and south-central
73 Chile. *D. gahi* has an annual life cycle and is semelparous. It is restricted to the continental
74 shelf, spawning inshore, with juveniles then moving to deeper waters of the shelf edge and
75 continental slope to feed and grow (Arkhipkin *et al.*, 2000) where they are targeted by the
76 commercial fishery (Arkhipkin *et al.*, 2008). Adults produce capsules with hundreds of eggs
77 that adhere to seaweeds or soft corals (Arkhipkin *et al.*, 2000), a life history trait that may limit
78 its dispersal potential in comparison to pelagic spawning squid (Ibanez and Poulin, 2014).

79 The species is one of the most intensively studied squids from a fishery biology
80 perspective, and there has been considerable research into environmental associations with
81 recruitment patterns in SWA and SEP waters. However, underlying spatial connectivity
82 patterns among the main centres of abundance (Falklands, Chile and Peru) remain unresolved
83 as population genetic studies to date have used different markers and non-overlapping
84 sampling. Shaw *et al.* (2004) reported no differentiation among sites and cohorts sampled
85 around the Falkland Islands. They also found a high level of differentiation between the
86 Falklands samples and a single sample from Peru; however, they did not include Chilean
87 sites. Ibanez *et al.* (2012) reported clear mitochondrial DNA (mtDNA) COI haplotype-
88 frequency differences between samples collected from Peru and central Chile, but did not
89 analyse samples from southern Chile or the SWA. The authors also highlighted that the
90 sampling range did not permit firm conclusions as to the role of geographical distance as an
91 isolating factor. Morphological patterns add an intriguing context to these genetic studies
92 with Vega *et al.* (2002) describing significant morphological differences among samples from
93 Peru, Chile and the Falklands, with the Falklands squid being the most distinct. Vega *et al.*
94 (2002) proposed that connectivity would be greater between populations in southern Chile

95 and Peru than between populations in southern Chile and the Falklands or other SWA
96 locations, based on the morphological patterns and predictions of oceanographic influences
97 on dispersal, specifically the splitting of the Sub-Antarctic Current on the Chilean coast (~47°
98 S) into a southward flowing Cape Horn Current and northward flowing Humboldt current.
99 Resolution of even greater divergence between the SEP and SWA than that reported within
100 the SEP (Ibanez *et al.*, 2012; Ibanez and Poulin, 2014) could support the alternative
101 taxonomic classification suggested by Nesis (1987), wherein Atlantic *D. gahi* represent a
102 separate species previously known as *L. patagonica*.

103 The objective of this study was to assess genetic diversity among spatial and temporal
104 samples collected from Peruvian, Chilean and Atlantic (Falklands) waters using
105 microsatellite and mtDNA markers to permit integration of previous studies and address
106 several questions relevant to fishery management, evolution and systematics, specifically:

- 107 1. Are Atlantic and Chilean populations of squid genetically differentiated?
- 108 2. Do Peruvian and Chilean samples exhibit nuclear differentiation, and if so how do
109 macrogeographic patterns inform our understanding of historical and current stock
110 isolating mechanisms?
- 111 3. Do neutral genetic relationships among samples align with morphological patterns,
112 and what information does this provide as to the roles of genetics and plasticity in the
113 contemporary ecology and evolution of these populations?
- 114 4. Do available data support taxonomic amendments?

115

116 **Materials & Methods**

117 **Sample collection and molecular analyses**

118 Adults were collected in 2009 and 2010 during June and July at several sites off the Peruvian
119 and Chilean coasts, as well as a single sample around the Falklands (Table 1, Figure 1) using
120 research and commercial vessels. Tissue biopsies were preserved in ethanol. Total DNA was
121 extracted using a CTAB-chloroform/IAA method (Winnepenninckx *et al.*, 1993). Nuclear
122 genetic variation was assessed by genotyping samples at the same six microsatellite loci
123 (Lgah3, Lgah8, Lgah10, Lgah11, Lfor3, LodrP19) as in Shaw *et al.* (2004). Genotypic data
124 for the same microsatellite loci for the Nov-LE, Nov-sE and Jul-sNN (Falklands) and
125 Peruvian samples from Shaw *et al.* (2004) were included to broaden the geographical and
126 temporal range. Nov-LE and Nov-sE were collected from the most important fishery area east
127 of the Falklands and comprised long- and short-size classes respectively, whereas Jul-sNN a
128 sample from an unfished area north of the Falklands.

129 A 582 bp portion of the mtDNA COI gene was amplified by PCR in a subset of
130 randomly selected individuals using the primers: forward 5-
131 ACTGGGAAAACCTGGTTCCT-3 and reverse 5-AAATGTTGATAAAGAATAGGG-3.
132 PCRs were performed in 10ul volumes containing ~50ng template DNA, 5ul Biomix (Bioline
133 UK), 0.025UM of each primer and using a thermoprofile consisting of an initial denaturation
134 step (95°C for 3 min) followed by 35 cycles of 95°C for 30 s, 50°C for 30 s and 72°C for 30 s,
135 and a final cool down step (4°C for 60 s). Amplicons were sequenced with the forward primer
136 using BigDye technology on an ABI 3500 system (Applied Biosystems) following
137 manufacturer's recommendations, and sequences were edited and aligned using BIOEDIT
138 (Hall, 1999).

139

140 **Statistical analysis of microsatellite data**

141 Genetic variation within samples was characterised using the number of alleles (N_A), allelic
142 richness (A_R), observed heterozygosity (H_O), and expected heterozygosity (H_E), all calculated
143 using GENALEX 6.2 (Peakall and Smouse, 2006). Genotype frequency conformance to
144 Hardy-Weinberg expectations (HWE) and genotypic linkage equilibrium between pairs of
145 loci were tested using exact tests (10 000 batches, 5000 iterations) in GENEPOP 3.3
146 (Rousset, 2008).

147 Genetic structure was investigated using the Bayesian clustering method implemented
148 in the program STRUCTURE (Pritchard *et al.*, 2000) to identify the most probable number of
149 genetic clusters (K) (from a range of 1–5) within the data. The analysis was performed both
150 with and without prior sample information (as recommended by Hubisz *et al.*, 2009) and with
151 multiple parameter permutations (admixture and correlated allele frequencies, as
152 recommended by Pritchard *et al.*, 2000). Each run consisted of a burn-in of 10^6 steps
153 followed by 5×10^6 steps with three runs performed for each K model tested. Optimal models
154 were assessed using $L(K)$ and where there was support for $K > 1$, ΔK (Evanno *et al.*, 2005)
155 was also assessed. Genetic differentiation among samples was quantified using global and
156 pairwise F_{ST} values with significance assessed with P values following 10 000 permutations
157 in FSTAT (Goudet, 1995). F_{ST} values were also estimated using the null allele correction
158 method in FreeNA (Chapuis and Estoup, 2007). F_{ST} matrices were visualised using principal
159 coordinate analysis in GENALEX. Mantel tests, implemented in GENALEX, were used to
160 test for isolation by distance using the correlation between pairwise F_{ST} and geographical
161 (shortest sea distances) distances between sample sites. Hierarchical analysis of molecular
162 variance (AMOVA; Excoffier *et al.*, 1992) was performed in ARLEQUIN (Excoffier and
163 Lischer, 2010) to partition genetic variance among groups of samples (F_{CT}) and among
164 samples within groups (F_{SC}) with significances determined with 1000 permutations.
165 Grouping of samples was defined according to STRUCTURE results. Randomisation

166 procedures in FSTAT were used to detect significant differences in heterozygosity, A_R , F_{IS} ,
167 F_{ST} and relatedness among user-defined groups of samples following 10 000 permutations.

168

169 **Statistical analysis of mtDNA data**

170 Analyses were performed using ARLEQUIN unless stated otherwise. Genetic diversity was
171 estimated using haplotype (h) and nucleotide (π) diversity. A minimum spanning network
172 was constructed in NETWORK (www.fluxus-engineering.com/sharenet.htm). Differentiation
173 between pairs of samples was quantified using pairwise Φ_{ST} with significances assessed by
174 10 000 permutations. Fu's F_s (Fu, 1997) and Tajima's D (Tajima, 1989) were used to test for
175 deviations from mutation-drift equilibrium. Mismatch distributions, the frequency
176 distributions of pairwise differences between haplotypes within a sample and simulated
177 distributions under a model of demographic expansion, were compared using the sum of
178 squared deviations (SSD) as a test statistic with significance assessed after 10 000 bootstrap
179 replications. The timing of expansions (T) was estimated from $T = \tau/2u$ (Rogers and
180 Harpending, 1992). IMA2 (Hey, 2010) was used to estimate divergence times between groups
181 with 1 000 000 burn-in generations and > 5 000 000 sampling generations so that the
182 minimum ESS across parameters was > 50 (Hey and Nielsen, 2004). For both mismatch and
183 IMA2 analyses a mutation rate (u) of 2% per million years was used to compare with the
184 results of Ibanez *et al.* (2012).

185

186 **Results**

187 **Microsatellite genetic variation**

188 The total number of alleles per locus ranged from 12 to 27 (average = 21.8). Multilocus
189 levels of variability were similar across samples (Table 1). No significant linkage
190 disequilibrium between loci was detected across all samples pooled or within individual
191 samples. Single locus tests for HWE for each of the 14 samples revealed 29 significant
192 deviations, which in all cases were due to heterozygote deficits. Heterozygote deficits were
193 most common for the microsatellite loci Lgah3 (8 deficits), Lgah10 (7 deficits) and Lgah11
194 (6 deficits). No other loci exhibited significant deviations for more than three samples.

195 STRUCTURE analyses unanimously supported a model of $K = 2$, regardless of the
196 settings used, indicating two groups, one containing the Peruvian samples and the other
197 containing the Chilean and Falklands samples (Figure 2). In all cases, individuals were
198 assigned to one of the groups with high probabilities with no evidence of between group
199 migrants or admixed genotypes. Repeated analysis for these groups separately provided no
200 support for substructuring ($K = 1$ had a probability of > 0.99 with all other K values assayed
201 having a probability of ~ 0). This spatial pattern of differentiation of the Peru from the
202 reciprocally cohesive Chilean and Falklands samples was also evident from F_{ST} based
203 analyses (Figure 3A). While there was a significant correlation between F_{ST} and geographical
204 distance (Figure 3B; Mantel; $R^2 = 0.336$, $P < 0.001$), the pattern of pairwise tests, as well as
205 the STRUCTURE clustering, supported a non-clinal genetic break between Algarrobo
206 (northern-most Chilean sample) and the southern-most Peruvian sample. Comparisons
207 between samples from Chile-Falklands vs Peru yielded significant pairwise F_{ST} ($P < 0.05$) in
208 46 of 48 cases (Table 2) with 44 remaining significant after Bonferroni correction (Rice,
209 1989). Among the Chilean and Falklands samples only one of 28 comparisons yielded
210 significant F_{ST} (corresponding global $F_{ST} = 0.001$; $P = 0.17$), whereas among the Peruvian
211 samples only four of 15 pairwise F_{ST} values were significant (corresponding global $F_{ST} =$
212 0.004 ; $P = 0.0008$). In line with this, the AMOVA showed a greater proportion of variation

213 between these geographical groups (0.91%; $F_{CT} = 0.009$; 95% CI = 0.002–0.015) than within
214 groups (0.19%; $F_{SC} = 0.002$; 95% CI = -0.0001–0.004). The pattern of global and pairwise
215 F_{ST} was unchanged after correction for putative null-allele effects. Two-tailed randomisation
216 tests revealed no differences between the regional groups using various indices of genetic
217 variability ($A_R P = 0.2$; $H_O P = 0.8$; $H_S P = 0.9$; $F_{IS} P = 0.8$; $F_{ST} P = 0.4$).

218 **mtDNA variation**

219 Pruning of mtDNA COI sequences permitted the comparison of 515 bp across 206
220 individuals revealing 18 haplotypes (Table 3; GenBank accession numbers MK253056–
221 253073). Overall divergence was shallow with a maximum of 3 mutations between adjacent
222 haplotypes, and most adjacent haplotypes were separated by a single mutation (Figure 4).
223 MtDNA patterns aligned with the divergence between the Peruvian and Chilean-Falklands
224 groups indicated by microsatellites with only two haplotypes shared between these groups, 6
225 haplotypes found only among the Chilean-Falklands samples and 10 haplotypes found only
226 among Peruvian samples. Pairwise Φ_{ST} were significant between these groups but non-
227 significant between samples within groups (Table 4). Despite the larger number of haplotypes
228 in the Peruvian samples, these samples exhibited lower haplotype diversities (Table 3). The
229 Peruvian samples also exhibited departures from neutrality with significantly negative values
230 of Fu's F_S and Tajima's D , and no significant deviations of observed mismatch distributions
231 from expectations under an expansion model (Table 3). In contrast, the Chilean-Falkland
232 samples exhibited non-significant Fu's F_S and Tajima's D test results, but significant
233 deviations from mismatch expansion models (Table 3). Global τ for the Peruvian samples
234 was 0.9 (range 0.5–1.9), which resulted in a mean expansion time estimate of 43 689 (range
235 24 272–92 233) years ago. IMa2 analysis produced a mean estimate of the divergence time
236 between the Peruvian and the Chilean-Falklands mtDNA populations of 325 408 years
237 (confidence interval, 80 500–957 500). IMa2 also reported reciprocally low mtDNA

238 migration rates (per thousand generations) from the Chilean-Falkland group into the Peru
239 group ($m = 0.017$) and in the other direction ($m = 0.013$).

240

241 **Discussion**

242 This is the first genetic study to include samples from the species' three key centres of
243 abundance, Peru and central-southern Chile in the SEP and the Falkland Islands in the SWA,
244 and to employ mtDNA and nuclear markers. The salient result was the spatially coherent
245 partitioning of samples into two divergent groups, one consisting of the Peruvian samples,
246 with the other consisting of the Chilean and Falklands samples. Differentiation between these
247 groups was evident from mtDNA and nuclear allele frequency analysis, mtDNA
248 phylogeographic structure and individual based clustering analysis, collectively confirming a
249 high degree of reciprocal demographic independence over various temporal scales. A gap in
250 the distribution of samples between southern Peru and Algarobbo (central Chile) sites was
251 due to the absence, or at least low abundance, of individuals in this area. This distribution
252 break has been noted for other cephalopods (Ibanez *et al.*, 2009) and is discussed below.
253 There was also a spatial gap in samples within the Chilean-Falkland group between the
254 Puerta Navarino and Puerto Montt sites. However, the lack of differentiation between these
255 sites and overall spatio-temporal homogeneity among Chilean-Falkland samples indicates
256 that cryptic structuring is unlikely. The results corroborate and provide a range-wide context
257 to previous studies as well as information on connectivity patterns relevant to fishery
258 management and to eco-evolutionary responses to historical climate change.

259 The differentiation between the Peruvian and Chilean-Falkland groups aligns with
260 previous studies that reported mtDNA differentiation between Peruvian and central-Chilean
261 samples (Ibanez *et al.*, 2012; Ibanez and Poulin, 2014). This study reveals *D. gahi* from such

262 central-Chilean locations to be genetically cohesive with samples from southern Chile and the
263 SWA. This geographically extensive connectivity among Chilean-Falkland samples indicates
264 that that the differentiation of the Peruvian samples is not due to a range wide isolation-by-
265 distance effect *per se* but rather a breakdown in connectivity occurring between the southern
266 Peru and Algarobbo sites. Accordingly the significant Mantel test results can be attributed to
267 a non-clinal barrier effect as described in other studies (e.g. Plouviez *et al.*, 2013).

268 Connectivity among populations is a complex process influenced by intrinsic factors
269 such as dispersal ability and extrinsic factors such as environmental heterogeneity. The area
270 between the southern Peru and Algarobbo sites is associated with three distinct biogeographic
271 regions that have been shaped by historical and contemporary processes (Camus, 2001; Thiel
272 *et al.*, 2007) and harbour a suite of properties that may restrict connectivity (Thiel *et al.*,
273 2007). Although more sampling is required to define the boundaries and drivers of this
274 structuring, the abrupt changes in the extent and depth of the continental shelf (Morales,
275 1984) are obvious candidate isolating factors. The continental shelf off the Chilean coast is
276 narrow and practically absent in northern areas to 28° S (Morales, 1984). This shelf-depth
277 heterogeneity likely separates not only Peruvian and central Chilean spawning grounds but
278 also likely represents a barrier to adult dispersal given the species' restriction to continental
279 shelf waters. The absence of fisheries landings between 20° S and 34° S confirms low
280 abundances of *D. gahi* adults in that area and is compatible with a dispersal barrier. Similar
281 oceanographic barrier effects have been reported for other squid species (Shaw *et al.*, 1999;
282 Triantafillos and Adams, 2001; Herke and Foltz, 2002). For *D. gahi*, both the absence of a
283 pelagic egg stage and the strong swimming behaviour of its paralarvae, expected to actively
284 promote retention in inshore spawning areas, are likely to also limit dispersal (Ibanez *et al.*,
285 2012; Ibanez and Poulin, 2014). Overall, the results suggest that both physical structuring by

286 the environment and the species' life history are contributing to contemporary demographic
287 independence between the Peruvian and Chilean-Falkland populations.

288 Pleistocene glaciations have also shaped the genetic structure of several species
289 throughout the Humboldt Current system (HCS) and have imprinted signatures of northern
290 range contractions during glacials and southern expansions during interglacials in a number
291 of species (Cardenas *et al.*, 2009; Haye *et al.*, 2014; Pardo-Gandarillas *et al.*, 2018) including
292 the cephalopods *Dosidiscus gigas* (Ibanez and Poulin, 2014) and *Octopus mimus* (Pardo-
293 Gandarillas *et al.*, 2018). In contrast to results for *Doryteuthis gahi*, genetic studies for *D.*
294 *gigas* and *O. mimus* report no differentiation between Peruvian and Chilean sites and larger
295 haplotype diversities among Peruvian sites than southern sites (Ibanez and Poulin, 2014;
296 Pardo-Gandarillas *et al.*, 2018). These differences between species have been interpreted to
297 reflect a distinct glacial history for *D. gahi* involving persistence during the LGM in a
298 southern refuge, with the Peruvian populations founded by post-glacial colonists from this
299 southern ancestral population (Ibanez *et al.*, 2012; Ibanez and Poulin, 2014). However, allele-
300 haplotype number is a better indicator of 'refugial richness' than gene diversity (Widmer and
301 Lexer, 2001). Hence, the considerably larger number of haplotypes in the Peruvian samples
302 than in the Chile-Falkland samples indicates occupancy of a northern refuge similar to other
303 cephalopods. This does not rule out a southern glacial refuge for *D. gahi*, given the cold
304 tolerance of the species. Time-since-divergence estimates here predate the LGM and support
305 a hypothesis of vicariance in separate glacial refugia. However, an important consideration is
306 the suitability of the divergence rates used in these analyses. We used the same 'species' level
307 divergence rates previously used for *D. gahi* and other population level studies; however,
308 these may underestimate intraspecific divergence rates and potentially overestimate the age
309 of demographic events (Ho *et al.*, 2005; Ho and Shapiro, 2011; Grant, 2015; Hoareau *et al.*,
310 2016). A ten-fold rate correction following Pardo-Gandarillas *et al.* (2018) aligns the

311 timeframe of Peruvian and Chilean-Falkland divergence more closely within the postglacial
312 period (Rabassa *et al.*, 2011). Regardless of the exact date of divergence, gene-flow estimates
313 support a prolonged period of isolation between the Peruvian and Chilean-Falkland groups.

314 Demographic and neutrality tests indicate different past population size dynamics for
315 the Peruvian and Chilean-Falkland groups, consistent with prolonged independence. In line
316 with previous studies, the Chilean-Falkland population conformed to equilibrium
317 expectations of a stable population while the Peruvian population exhibited non-equilibrium
318 signatures compatible with population size changes (Ibanez *et al.*, 2012; Ibanez and Poulin,
319 2014). While non-equilibrium signatures may also be due to locus-specific selection (Grant *et*
320 *al.*, 2016), similar results for Peruvian samples of *D. gigas* and *O. mimas* point to concordant
321 population size changes across species within the Peruvian waters. The timing of these
322 changes can be placed within the post-glacial period which has encompassed considerable
323 changes in HCS productivity (Pardo-Gandarillas *et al.*, 2018). Overall, the intraspecific
324 variability for *D. gahi* and comparative patterns for other cephalopods in the region highlight
325 a complex interplay between species ecology, distribution and local environmental conditions
326 that shape responses to environmental events.

327 Based on morphological differences and assumed oceanographic impacts on dispersal,
328 Vega *et al.* (2002) predicted greater connectivity between *D. gahi* from Peru and Chile than
329 between Chile and the SWA. Both the mtDNA and microsatellite results contradict this
330 prediction with the lack of genetic differentiation among the Chilean and Falklands samples
331 compatible with derivation from a single genetically panmictic population. This also fits with
332 a broader pattern of geographically extensive gene flow reported for the highly mobile neritic
333 loliginid squids *Loligo forbesi* (Shaw *et al.*, 1999), *L. opalscens* (Reichow and Smith,
334 2001), *L. reynaudi* (Shaw *et al.*, 2010) and *Doryteuthis pealeii* (Shaw *et al.*, 2010). Genetic
335 homogeneity among populations within the Chilean-Falkland group supports genetic

336 connectivity across current geopolitical stock boundaries in the region. Such a mismatch
337 between biological and management units has important implications for sustainable
338 management, as management units representing only a portion of a connected population can
339 present problems with understanding population-stock dynamics and their environmental
340 linkages (Frisk *et al.*, 2008). The present results direct an appreciation of the connected
341 nature of *D. gahi* across Chilean, Argentinean and Falkland waters and international
342 cooperation in its management.

343 Despite the differing demographic histories and levels of mtDNA diversity between
344 the Peruvian and Chilean-Falklands populations, levels of nuclear (microsatellite) variability
345 were similar across samples, including those from fished and unfished (Jul-sNN) areas.
346 Differences between markers may be due to the typically larger mutation rates of
347 microsatellite loci and lower levels of genetic drift compared to mtDNA. Levels of nuclear
348 variation are also similar to those in other squids (Shaw *et al.*, 2010). The results indicate that
349 despite the pronounced recruitment-abundance fluctuations and harvesting intensity, these
350 populations have retained high levels of genetic variation and if current spawning stock sizes
351 and management practices are maintained, genetic drift is not strong enough to reduce neutral
352 genetic diversity. Such genetic variability is recognised as fundamental for sustainable yields
353 and adaptability of populations (Kenchington *et al.*, 2003).

354 Patterns of variation in morphological and genetic diversity can provide insights into
355 the interplay between genetic and environmental factors. The high gene flow among the
356 Chilean and Falklands *D. gahi* supports environmental heterogeneity and not genetic drift as
357 the driver of the pronounced phenotypic differentiation of *D. gahi* in Falkland waters (Vega
358 *et al.*, 2002). Van der Vyver *et al.* (2016) suggest that temperature is a driver of phenotypic
359 divergence despite high levels of gene flow in the ecologically similar *L. reynaudii* around
360 the southern African coast. It is perhaps noteworthy that *D. gahi* around the Falklands (~5° C)

361 experience considerably lower temperatures than Chilean (11–13° C) and Peruvian (18–28°
362 C) populations. Regardless of the environmental driver, the pattern indicates that the
363 morphological differences between Falkland and Chilean *D. gahi* are largely due to plasticity,
364 with genetic adaptation involved only if selection on specific loci is sufficiently intense to
365 mitigate the effects of genome-wide gene flow. In contrast, the prolonged genetic isolation of
366 the Peruvian population indicates that divergence of these samples likely reflects the interplay
367 between neutral (genetic drift) and non-neutral (local adaptation) genetic processes and
368 phenotypic plasticity.

369 The systematic position of *D. gahi* has been the subject of conjecture with suggestions
370 that SWA and SEP populations are distinct species, *L. patagonica* and *L. gahi* (Nesis, 1987),
371 respectively. The lack of genetic structuring within the Chilean-Falkland group does not
372 support divergence delineated by a SWA-SEP boundary *per se*. It could be argued that the
373 Peruvian and Chilean-Falkland populations reflect distinct “species-like units” (*sensu* Collins
374 and Cruickshank, 2013) because of the high level of genetic divergence, their phenotypic
375 diagnosability and allopatric nature. However, in the absence of mtDNA reciprocal
376 monophyly a much less ambiguous case can be made that the Peruvian and Chilean-Falkland
377 groups are distinct evolutionary significant units (ESU’s). The estimated time since
378 divergence supports independence for at least for 30 000 years while the sharing of only two
379 central haplotypes between the groups can be attributed to retention of ancestral
380 polymorphism. As such, these criteria satisfy the ESU concept of Fraser and Bernatchez
381 (2001), wherein ESU’s are groups that “have followed independent evolutionary trajectories
382 for variable periods of time” and have “limited or no impact on the evolution, genetic
383 variance and demography of other such lineages”. Although ecological exchangeability
384 (Crandall *et al.*, 2000) cannot be tested, the different oceanic environments that these groups
385 inhabit and the likelihood for divergent adaptations support the view these groups might not

386 be adaptively exchangeable. Each group thus represents a substantial portion of the
387 evolutionary history and future potential of the species that merit formal recognition.

388 Overall, the results of this study add to evidence that neritic squid typically exhibit
389 highly connected populations over large geographical areas of continuous habitat but that
390 localised oceanographic features, in this case a habitat break, can be strong isolating factors.
391 The results importantly provide a neutral genetic framework to understand connectivity in a
392 commercially important species but also suggest the occurrence of environmental plasticity
393 and potential for local adaptation. Given that plasticity and adaptation may influence
394 population responses to harvesting and climate change in different ways, disentangling such
395 processes using genomic methods (King *et al.*, 2017) will complement knowledge of
396 population demographics and contribute toward both population and ecosystem
397 sustainability.

398 **Author contributions**

399 NMK performed the analysis and drafted the manuscript. All authors contributed critically to
400 drafts and have given final approval.

401 **Data accessibility**

402 Data will be deposited on Dryad.

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581 Figure 1. Distribution of *D. gahi* sample sites across the SE Pacific and SW Atlantic Oceans.
582 Numbers correspond to sample information in Table 1.

583 Figure 2. Bar graph showing the Bayesian clustering of individuals under the optimal model
584 of $K=2$ (Admixture assumed and NO LOCPRIOR used). Numbers designate sample sites in
585 Table 1 and Figure 1.

586 Figure 3. A- Principal Coordinate analysis of pairwise F_{ST} values between samples (numbers
587 correspond to sample codes in Table 1, and colour coded according to region: green = Peru;
588 blue = Chile; red = Falklands). B – Graph showing the positive correlation between F_{ST}
589 values and geographical distance between sample sites.

590 Figure 4. Phylogenetic relationships among *D. gahi* mtDNA COI haplotypes. Adjacent
591 haplotypes are all separated by a single substitution except H16 and H3 which are separated
592 by 3 substitutions. Haplotype disc sized by proportional abundance and colour coded by
593 occurrence (green = common to both Peru and Chile-Falklands; red = Peru only; yellow =
594 Chile-Falkland only).

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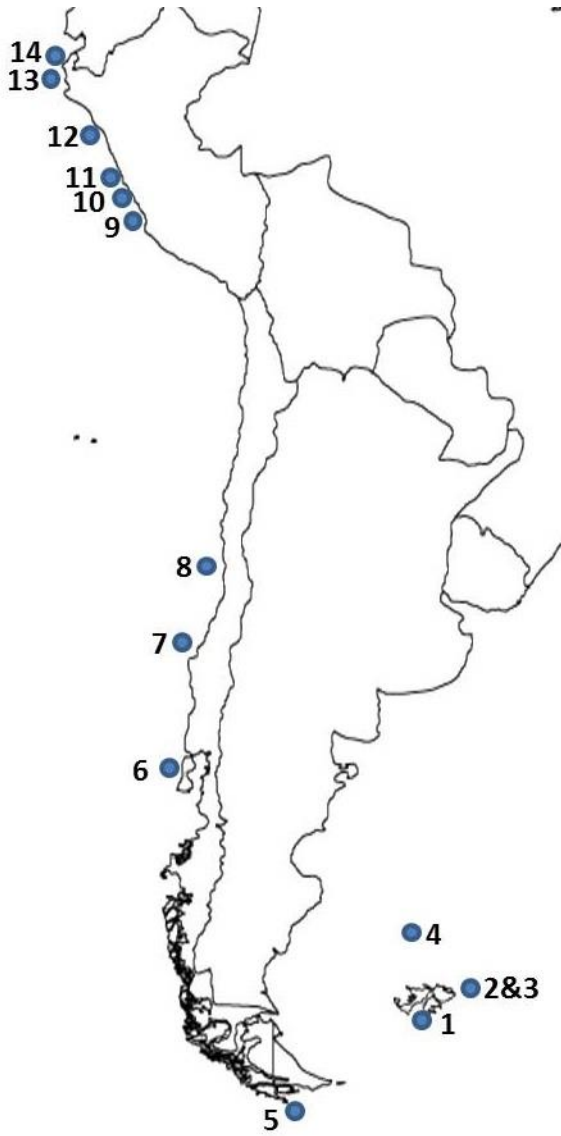
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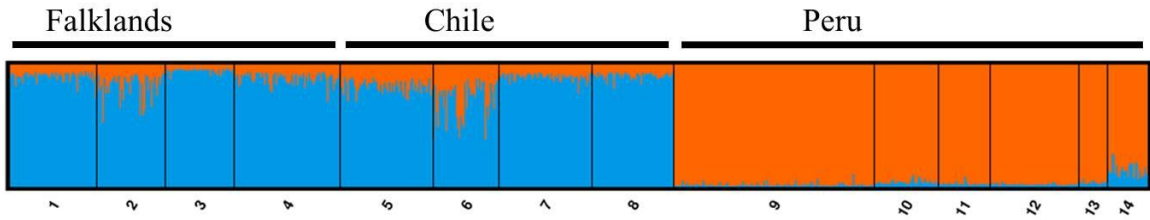
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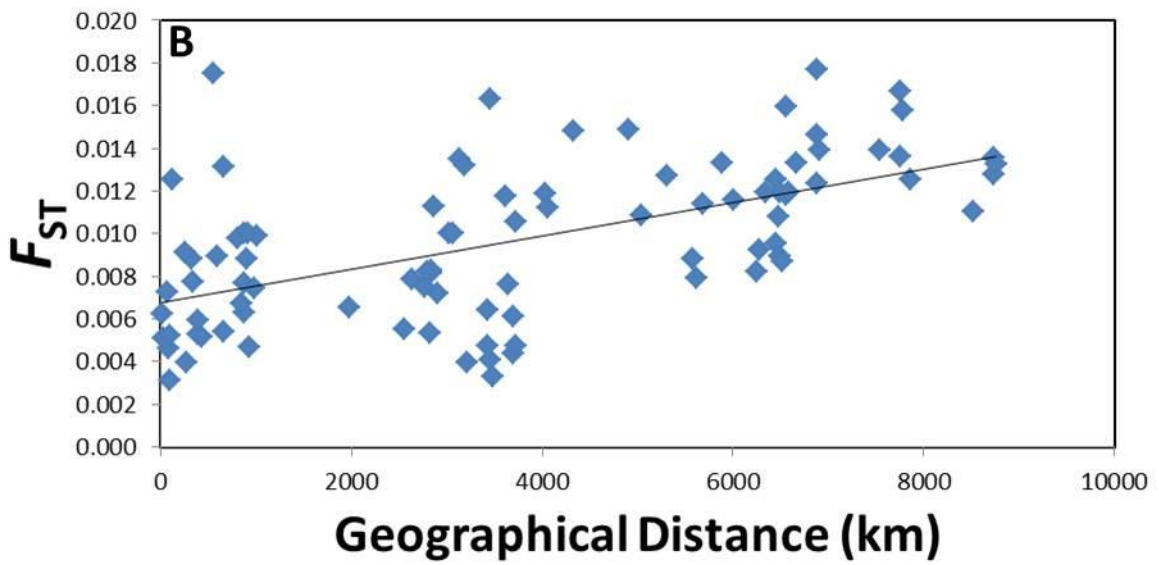
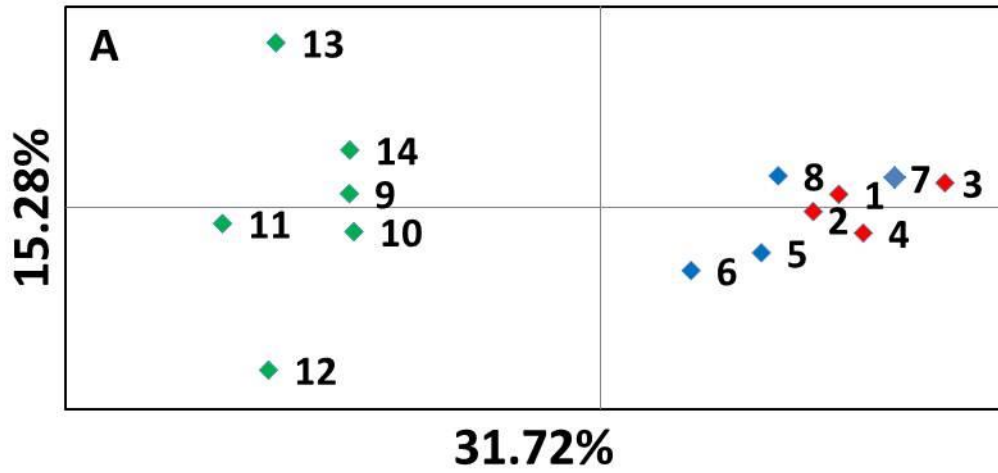
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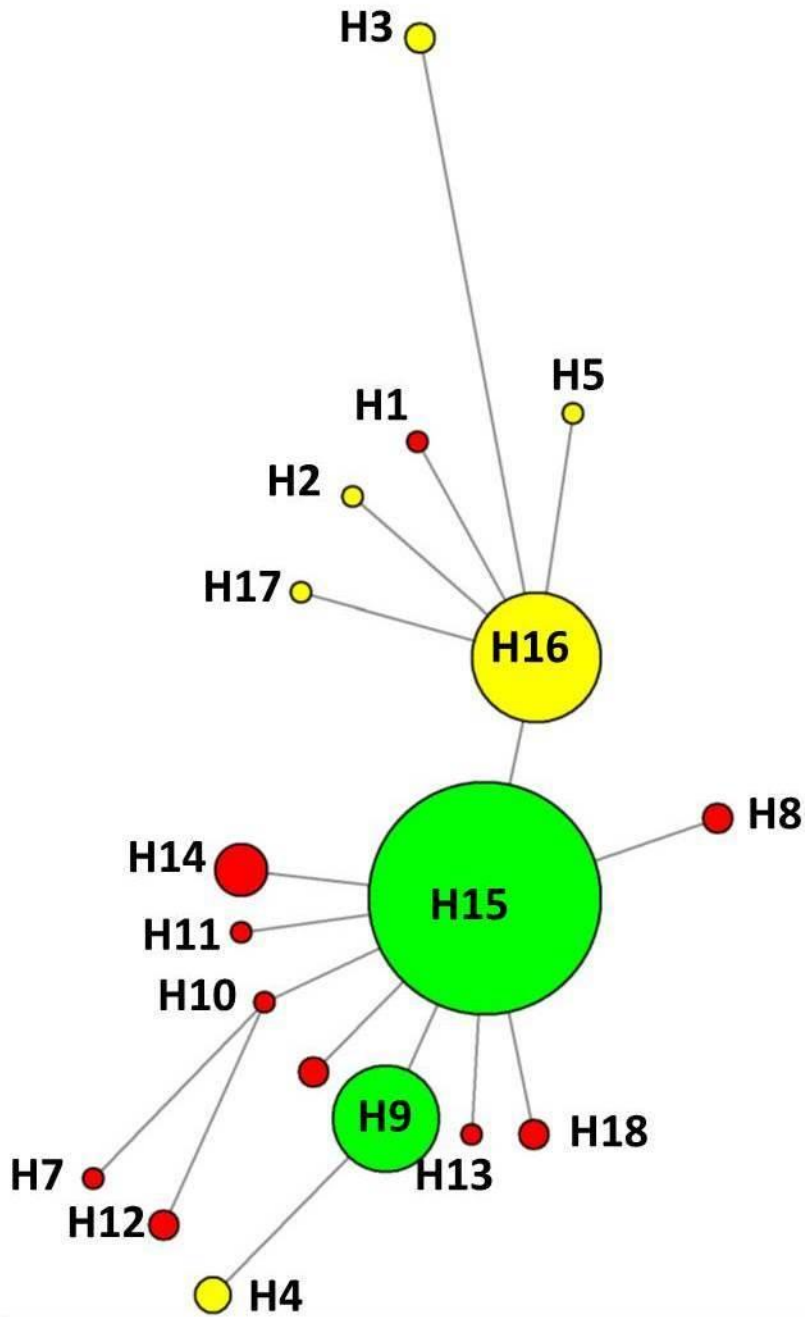


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635 Table 1. Sample information and basic indices of genetic variation in *D. gahi*, including mean
 636 (across loci) allele number (N_a), allelic richness (A_r) and observed (H_O) and expected (H_E)
 637 heterozygosities

Sample area	Sample code	Coordinates	Year collected	Sample size	N_a	A_r	H_O	H_E
Falklands								
Falklands South	1	52°54'S, 60°16'W	2009	61	13.8	11.1	0.71	0.84
Falklands Nov-LE	2	51°41'S, 57°33'W	1999	48	14	11.3	0.77	0.84
Falklands Nov-SE	3	51°48'S, 57°43'W	1999	48	15.2	11.9	0.74	0.84
Falklands Jul-SNN	4	48°39'S, 60°18'W	2000	74	15.3	11.6	0.75	0.86
Chile								
Puerta Navarino	5	56°20'S, 67°W	2009	65	15	11.3	0.71	0.85
Puerto Montt	6	41°36'S, 73°W	2009	46	14	11.8	0.76	0.88
Concepcion	7	36°33'S, 72°W	2009	55	15.5	11.5	0.76	0.85
Algarrobo	8	33°01'S, 71°W	2010	67	15	10.9	0.77	0.85
Peru								
South Peru	9	12°06'S, 77°11'W	2000	140	17.7	11.3	0.76	0.87
Callao Ventanilla	10	11°50'S, 77°05'W	2010	45	12.7	11.8	0.73	0.84
Huacho Tartacay	11	11°24'S, 77°35'W	2010	36	13.5	11.2	0.75	0.85
Chimbote	12	9°20'S, 78°30'W	2010	62	14.7	11.6	0.75	0.84
Paita	13	5°00'S, 81°03'W	2010	20	11.8	10.8	0.73	0.84
Olaya	14	3°55'S, 81°00'W	2010	28	12.2	11.9	0.71	0.84

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639 Table 2. Pairwise F_{ST} values between *D. gahi* samples, with estimates differing significantly
 640 from zero in bold. Shaded cells are estimates between the Falkland-Chile and Peru groups.
 641 Numbers before sample names and on top row correspond to Table 1 and Figure 1.
 642 Underlined values denote F_{ST} that became non-significant after Bonferroni correction.

	1	2	3	4	5	6	7	8	9	10	11	12
1.Falk_South	-											
2.Falk_East_L	0.003	-										
3.Falk_East_S	0.005	0.006	-									
4.Falk_north	0.005	0.005	0.006	-								
5. Puerta Navarino	0.005	0.006	0.008	0.005	-							
6.Puerto Montt	0.008	0.008	0.008	0.007	0.007	-						
7.Concepcion	0.004	0.005	0.006	0.004	0.006	0.009	-					
8.Algarrobo	0.003	0.004	0.006	0.005	0.005	0.007	0.004	-				
9.South Peru	0.009	0.009	0.012	0.009	0.008	0.008	0.010	0.009	-			
10.Callao Ventanilla	0.008	0.010	0.013	0.011	0.009	0.012	0.010	0.007	0.005	-		
11.Huacho Tartacay	0.012	0.012	0.016	0.012	0.011	0.011	0.014	0.011	0.005	0.007	-	
12. Chimbote	0.013	0.012	0.018	0.014	0.012	0.012	0.016	0.013	0.008	0.009	0.009	-
13. Paita	0.014	0.014	0.017	0.016	0.015	0.015	0.015	0.011	0.009	0.010	0.010	0.017
14. Olaya	0.011	0.013	0.014	0.013	0.013	0.013	0.013	0.011	0.010	0.007	0.010	0.013

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647 Table 3. Summary of mtDNA variability (Nseq = number of individuals sequenced; Nhap =
648 number of haplotypes; h = haplotype diversity, π = nucleotide diversity), results of
649 demographic tests (Fu's F_s , Tajima's D , Mismatch distribution SSD – in all cases significant
650 values in bold) and haplotype abundance in *D. gahi* samples from the Falkland Islands, Chile
651 and Peru.

Sample (Nseq)	Falklands South (32)	Puerto Montt (31)	Algarrobo (31)	South Peru (30)	Chimbote (36)	Paita (20)	Olaya (26)
Nhap	4	7	6	6	8	4	6
h (π)	0.68 (0.002)	0.77 (0.002)	0.72 (0.002)	0.36 (0.001)	0.35(0.0001)	0.28 (0.001)	0.43 (0.001)
Fu's F_s (P)	0.68 (NS)	-1.78 (NS)	-1.17 (NS)	-4.24 (P<0.001)	-7.07 (P<0.001)	-2.07 (P< 0.001)	-4.12 (P< 0.001)
Tajima's D (P)	-0.32 (NS)	-1.06 (NS)	-0.99 (NS)	-1.74 (P= 0.03)	-2.01 (P =0.01)	-1.87 (P= 0.019)	-1.72 (P= 0.03)
P Mismatch SSD	< 0.001	< 0.001	< 0.001	0.77	0.49	0.5	0.45
Hap1	0	0	0	1	0	0	0
Hap2	0	1	1	0	0	0	0
Hap3	1	1	1	0	0	0	0
Hap4	0	0	1	0	0	0	0
Hap5	0	1	0	0	0	0	0
Hap6	0	0	0	0	1	0	1
Hap7	0	0	0	0	1	0	0
Hap8	0	0	0	1	0	0	1
Hap9	8	8	5	1	1	1	1
Hap10	0	0	0	0	1	0	0
Hap11	0	0	0	0	1	0	0
Hap12	0	0	0	0	1	1	0
Ha[13	0	0	0	1	0	0	0
Hap14	0	0	0	2	1	1	2
Hap15	8	9	11	24	29	17	21
Hap16	15	10	12	0	0	0	0
Hap17	0	1	0	0	0	0	0
Hap18	0	0	0	0	0	0	2

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653 Table 4. Pairwise Φ_{ST} values between *D. gahi* samples (values significantly different from
654 zero are in bold). Shaded cells are estimates between the Falkland-Chile and Peru groups.

	Falklands South	Puerto Montt	Algarrobo	South Peru	Chimbote	Paita
Falklands South						
Puerto Montt	- 0.01					
Algarrobo	- 0.01	- 0.01				
South Peru	0.34	0.25	0.24			
Chimbote	0.35	0.27	0.24	- 0.01		
Paita	0.35	0.36	0.25	- 0.02	- 0.02	
Olaya	0.30	0.21	0.21	- 0.02	- 0.01	- 0.02

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