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Published in:
ICES Journal of Marine Science

DOI:
10.1093/icesjms/fsz009

Publication date:
2019

Citation for published version (APA):
McKeown, N., Arkhipkin, AI. I., & Shaw, P. (2019). Genetic analysis reveals historical and contemporary population dynamics in the longfin squid *Doryteuthis gahi*: implications for cephalopod management and conservation. *ICES Journal of Marine Science*, *76*(4), 1019-1027. [fsz009].
https://doi.org/10.1093/icesjms/fsz009

Original article

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

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ABSTRACT

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

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

Introduction

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

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

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

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

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

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

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

Materials & Methods

Sample collection and molecular analyses

Adults were collected in 2009 and 2010 during June and July at several sites off the Peruvian and Chilean coasts, as well as a single sample around the Falklands (Table 1, Figure 1) using research and commercial vessels. Tissue biopsies were preserved in ethanol. Total DNA was extracted using a CTAB-chloroform/IAA method (Winnepenninckx *et al*., 1993). Nuclear genetic variation was assessed by genotyping samples at the same six microsatellite loci (Lgah3, Lagh8, Lgah10, Lgah11, Lfor3, LodrP19) as in Shaw *et al.* (2004). Genotypic data for the same microsatellite loci for the Nov-LE, Nov-sE and Jul-sNN (Falklands) and Peruvian samples from Shaw *et al*. (2004) were included to broaden the geographical and temporal range. Nov-LE and Nov-sE were collected from the most important fishery area east of the Falklands and comprised long- and short-size classes respectively, whereas Jul-sNN a sample from an unfished area north of the Falklands.

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

Statistical analysis of microsatellite data

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

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

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

Statistical analysis of mtDNA data

Analyses were performed using ARLEQUIN unless stated otherwise. Genetic diversity was estimated using haplotype (h) and nucleotide (π) diversity. A minimum spanning network was constructed in NETWORK (www.fluxus-engineering.com/sharenet.htm). Differentiation between pairs of samples was quantified using pairwise Φ_{ST} with significances assessed by 10 000 permutations. Fu's Fs (Fu, 1997) and Tajima's D (Tajima, 1989) were used to test for deviations from mutation-drift equilibrium. Mismatch distributions, the frequency distributions of pairwise differences between haplotypes within a sample and simulated distributions under a model of demographic expansion, were compared using the sum of squared deviations (SSD) as a test statistic with significance assessed after 10 000 bootstrap replications. The timing of expansions (T) was estimated from $T = \tau/2u$ (Rogers and Harpending, 1992). IMa2 (Hey, 2010) was used to estimate divergence times between groups with 1 000 000 burn-in generations and > 5 000 000 sampling generations so that the minimum ESS across parameters was > 50 (Hey and Nielsen, 2004). For both mismatch and IMa2 analyses a mutation rate (u) of 2% per million years was used to compare with the results of Ibanez *et al*. (2012).

Results

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

between these geographical groups (0.91%; F_{CT} = 0.009; 95% CI = 0.002–0.015) than within groups (0.19%; F_{SC} = 0.002; 95% CI = -0.0001–0.004). The pattern of global and pairwise F_{ST} was unchanged after correction for putative null-allele effects. Two-tailed randomisation tests revealed no differences between the regional groups using various indices of genetic 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).

mtDNA variation

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

361 experience considerably lower temperatures than Chilean (11–13^{o} C) and Peruvian (18–28^{o}
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

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

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

Author contributions

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

Data accessibility

Data will be deposited on Dryad.

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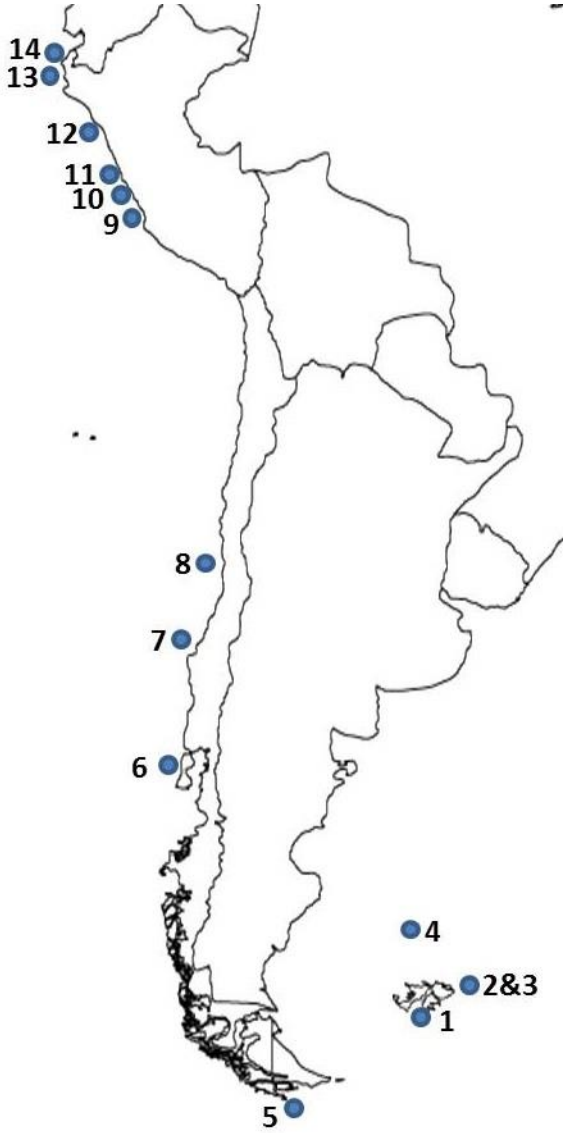
Figure 1. Distribution of *D. gahi* sample sites across the SE Pacific and SW Atlantic Oceans. Numbers correspond to sample information in Table 1.

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

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

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



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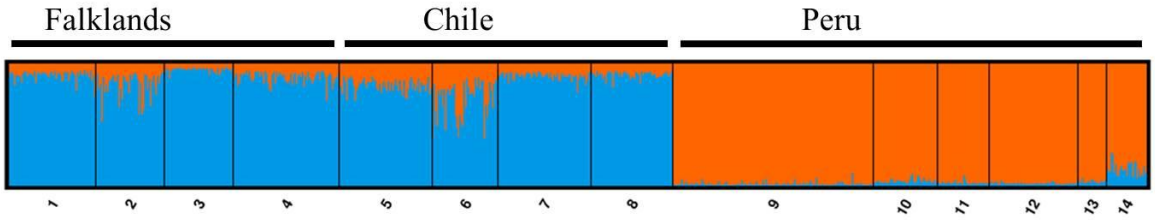

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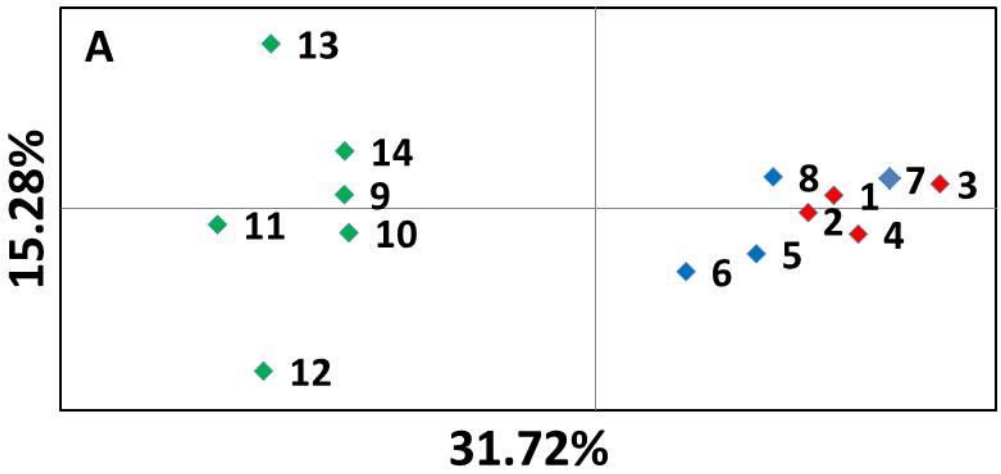

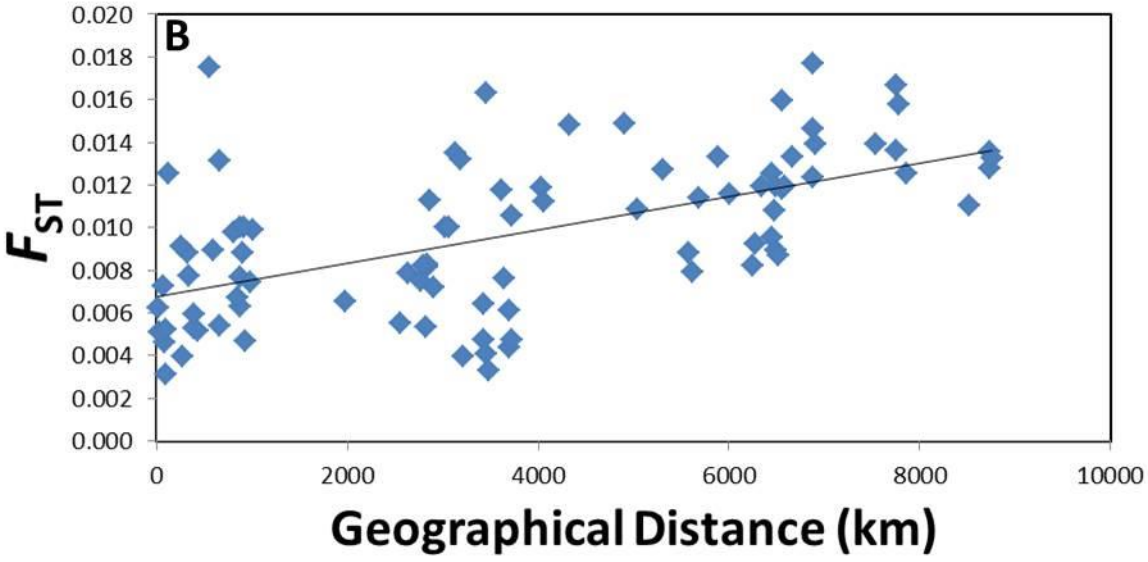

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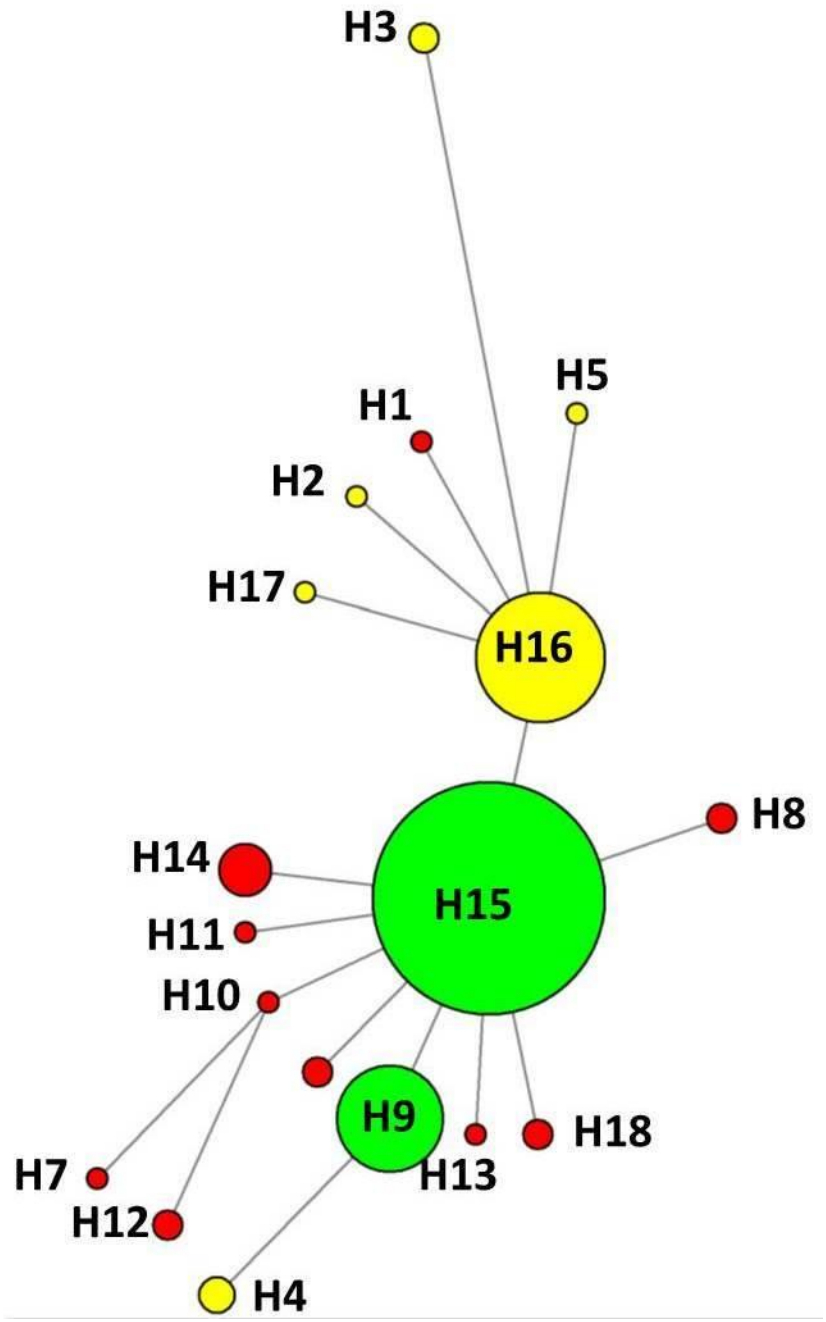

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635 Table 1. Sample information and basic indices of genetic variation in *D. gahi*, including mean
636 (across loci) allele number (N_a), allelic richness (A_r) and observed (H_O) and expected (H_E)
637 heterozygosities

Sample area	Sample code	Coordinates	Year collected	Sample size	N_a	A_r	H_O	H_E
Falklands								
Falklands South	1	52º54'S, 60º16'W	2009	61	13.8	11.1	0.71	0.84
Falklands Nov-LE	2	51º41'S, 57º33'W	1999	48	14	11.3	0.77	0.84
Falklands Nov-SE	3	51º48'S, 57º43'W	1999	48	15.2	11.9	0.74	0.84
Falklands Jul-SNN	4	48º39'S. 60º18'W	2000	74	15.3	11.6	0.75	0.86
Chile								
Puerta Navarino	5	56º20'S, 67ºW	2009	65	15	11.3	0.71	0.85
Puerto Montt	6	41º36'S, 73ºW	2009	46	14	11.8	0.76	0.88
Concepcion	7	36º33'S, 72ºW	2009	55	15.5	11.5	0.76	0.85
Algarrobo	8	33º01'S, 71ºW	2010	67	15	10.9	0.77	0.85
Peru								
South Peru	9	$12^0$06'S, 77º11'W	2000	140	17.7	11.3	0.76	0.87
Callao Ventanilla	10	11º50'S, 77º05'W	2010	45	12.7	11.8	0.73	0.84
Huacho Tartacay	11	11º24'S, 77º35'W	2010	36	13.5	11.2	0.75	0.85
Chimbote	12	9º20'S, 78º30'W	2010	62	14.7	11.6	0.75	0.84
Paita	13	5º00'S, 81º03'W	2010	20	11.8	10.8	0.73	0.84
Olaya	14	3º55'S, 81º00'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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Table 3. Summary of mtDNA variability (Nseq = number of individuals sequenced; Nhap = number of haplotypes; *h* = haplotype diversity, π = nucleotide diversity), results of demographic tests (Fu's *F*s, Tajima's *D*, Mismatch distribution SSD – in all cases significant values in bold) and haplotype abundance in *D. gahi* samples from the Falkland Islands, Chile 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 (0.00
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

Table 4. Pairwise Φ_{ST} values between *D. gahi* samples (values significantly different from 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