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Mitochondrial genome diversity and population structure of the giant squid *Architeuthis*: genetics sheds new light on one of the most enigmatic marine species

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Despite its charismatic appeal to both scientists and the general public, remarkably little is known about the giant squid *Architeuthis*, one of the largest of the invertebrates. Although specimens of *Architeuthis* are becoming more readily available owing to the advancement of deep-sea fishing techniques, considerable controversy exists with regard to topics as varied as their taxonomy, biology and even behaviour. In this study, we have characterized the mitochondrial genome (mitogenome) diversity of 43 *Architeuthis* samples collected from across the range of the species, in order to use genetic information to provide new and otherwise difficult to obtain insights into the life of this animal. The results show no detectable phylogenetic structure at the mitochondrial level and, furthermore, that the level of nucleotide diversity is exceptionally low. These observations are consistent with the hypotheses that there is only one global species of giant squid, *Architeuthis dux* (Steenstrup, 1857), and that it is highly vagile, possibly dispersing through both a drifting paralarval stage and migration of larger individuals. Demographic history analyses of the genetic data suggest that there has been a recent population expansion or selective sweep, which may explain the low level of genetic diversity.

1. Introduction

The giant squid, *Architeuthis* spp., is one of the largest invertebrates. Described primarily from remains in sperm whale stomachs, from carcasses of dead or moribund animals found floating on the ocean surface or washed up on beaches, and, rarely, from fresh specimens caught by deep-sea trawling activity [1], it was not until 2004 that a live specimen was observed in its natural habitat [2] and this year that the first video footage was published [3]. Because of their huge size and elusive nature, many myths and legendary sea monsters have

been based on them, including the fabled sinker of ships, the Kraken. They have also been of considerable interest and speculation both in the scientific community and in popular literature, inspiring authors such as Jules Verne and Herman Melville. However, despite this, the scarcity of preserved specimens means knowledge about their biology, distribution and taxonomy is very limited, and, indeed, often confused with fiction. For example, while claims have been made of individuals measuring up to 50 m in total length [4], a more realistic estimate is a maximum total length of 18 m for females [5], with males reaching slightly smaller sizes [4]. What is more certain is that, with the exception of the polar regions, *Architeuthis* are globally distributed [6,7]. Furthermore, they are probably voracious carnivores, preying upon fish, but also smaller cephalopods [1], including other giant squid [8]. Carbon and nitrogen isotope profiles obtained from analysing upper beaks suggest *Architeuthis* undergoes an ontogenetic diet shift early in life, abandoning smaller prey of relatively low trophic status in favour of larger prey of higher status. Isotope profiles also indicate that adult giant squid inhabit relatively small, well-defined and productive areas, where food resources have a constant carbon isotope composition [9]. It is also clear that juvenile giant squid are hunted by many other animals, including dolphins, fish and sea birds [1,10], and the adults are consumed in large quantities by whales, especially the sperm whale, *Physeter macrocephalus* [11]. As such it is likely (although unproved) that the giant squid population must be large, in order to sustain such levels of predation by whales. A number of other characters are more uncertain. There has been debate, for example, about the activity level and metabolism of *Architeuthis*. Although many scientists have concluded that giant squid must be sluggish ambush-predators, which wait quietly in the water column for their prey to stumble into them [12,13], more recent data [14] based on a comparison of citrate synthase activity between cephalopods appears to support quite the opposite, consistent with recent photographic evidence [2]; the giant squid portrayed in these images seems to be a highly active predator with considerable strength [15].

The taxonomy of the giant squid remains controversial. Since the first description as *Architeuthis dux* by the Danish naturalist Japetus Steenstrup in 1857, as many as 21 nominal species of *Architeuthis* have been described [6,16]. Given the fact that many of these were described based exclusively on location, and in some cases on incomplete remains such as single beaks, suckers or arms regurgitated by sperm whales, the majority of these have been considered likely to be synonymous, and today different opinions suggest there may be as many as eight or as few as one species (with three subspecies) [1,4,17–21]. In addition to this uncertainty, the life cycle and life history of the giant squid also remain enigmatic, as do growth rate, time of sexual maturity and longevity, with the many estimates of maximum age ranging from 1 to 46 years [9,22–24].

Given the obvious difficulty of providing answers to some of the earlier-mentioned questions using conventional monitoring/observational techniques, alternative approaches are required if more is to be understood about the giant squid. Recent advances in DNA sequencing techniques have made the sequencing of large stretches of DNA (e.g. mitochondrial genomes) both quick and economical, and mitochondrial genome-based analyses are playing an increasingly important

role in phylogenetic and population genetic studies [25,26]. In order to both reassess the estimated number of species and describe the global population structure of the giant squid, we have generated a dataset of 37 complete and six partial mitochondrial genomes (mitogenomes) using samples collected across its known range.

2. Methods

(a) Samples

Forty-three *Architeuthis* soft tissue samples were analysed in this study. Detailed information about the tissue samples can be found in the electronic supplementary material, table S1. *Architeuthis* are rarely caught alive, and many tissue samples were derived from the carcasses of dead animals found stranded on beaches or floating moribund on the ocean surface, although several tissue samples were derived from animals taken as accidental by-catch on deep-sea trawlers and frozen specimens subsequently donated to local scientific institutions. Many of the tissue samples had undergone some degree of decay (or digestion), owing to the quality of the source specimens (see the electronic supplementary material, table S1).

(b) DNA extraction

DNA extraction from cephalopods is challenging owing to high concentrations of mucopolysaccharides found in their soft tissues, which tend to co-separate with DNA during extraction, and subsequently can inhibit many downstream enzymatic processes. Furthermore, the muscular tissues of *Architeuthis* (as well as squid belonging to 15 other families of the order Oegopsida) naturally contain high levels of ammonia, conferring additional complications to DNA extraction [27]. Tissue samples were initially digested at 56°C with agitation, in a cetyltrimethylammonium bromide (CTAB) containing buffer (100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 20 mM EDTA; 2% w/v CTAB; 0.2% w/v dithiothreitol; 500 µg ml⁻¹ proteinase K). Subsequently, the digests underwent three purifications, each of one volume of 25:24:1 phenol:chloroform:isoamyl alcohol saturated with 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. One volume of ddH₂O was subsequently added to the aqueous phase in order to dilute the salt concentration, prior to precipitation of the DNA by adding one volume of 100 per cent isopropanol and centrifugation at 20 000 r.p.m., followed by a 70 per cent ethanol wash step. The extracted DNA was eluted in 1× TE buffer pH 8.0 (10 mM Tris-HCl, pH 8.0; 1 mM EDTA). The quality of all extracts was assessed by electrophoresis on 0.7 per cent agarose gels, as well as by fluorometric quantitation on the Qubit1.0 fluorometer (Invitrogen, Carlsbad, CA).

(c) Mitogenome amplification and sequencing

An unpublished *Architeuthis* mitogenome sequence present in GenBank (NC_011581, hereafter referred to as the reference sequence) indicates that the mitogenome contains two duplicated regions (see the electronic supplementary material, figure S1) that show a remarkably high degree of sequence similarity of approximately 99.9 per cent [28]. To verify the accuracy of this phenomenon, we designed four pairs of primers (see the electronic supplementary material, table S2) to independently PCR amplify each duplicated region in four individuals chosen to span the geographical distribution of *Architeuthis* (caught in the proximity of the Falkland Islands, Florida, South Africa and New Zealand). Reactions were undertaken with Platinum Taq DNA polymerase high fidelity (Invitrogen), in 25 µl volumes containing 1× high-fidelity PCR buffer, 0.2 mM dNTPs, 2–3 mM magnesium sulphate (primer pair-specific; see electronic

supplementary material, table S2), 0.4 μM each primer and 10 units of polymerase per reaction. The thermal cycling conditions were as follows: 5 min at 94°C, 40 cycles of (30 s at 94°C, 30 s at 63°C, 1 min kb^{-1} at 68°C), final extension of 5 min at 68°C. Sanger sequencing of these initial amplicons, using both the original PCR primers and nested sequencing primers (see the electronic supplementary material, table S2), confirmed that the conserved duplications are a general feature of the *Architeuthis* mitogenome, thus subsequent mitogenome amplification was performed in such a way that the two copies of each duplicate region could be kept separate.

The mitogenome dataset was generated using several strategies, depending on the quality of the DNA in each sample. For samples where DNA preservation was sufficient to allow large (more than 4.7 kb) PCR amplicons to be generated ($n = 37$), mitogenomes were generated using long-range PCR coupled to Roche GS FLX or Illumina HiSeq2000 sequencing [26,29]. Briefly, each mitogenome sequenced in this way was PCR amplified using a combination of primer sets, tailored to the DNA quality of the sample, which yielded amplicons spanning the entire length of the mitogenome (see the electronic supplementary material, table S2). Following PCR amplification, amplicons for each individual were purified, quantified and pooled at equimolar concentration in two pools per individual so as to keep copies of duplicated genes in separate pools. Post pooling, the DNA was sheared to approximately 200–600 bp in size using a Bioruptor (Diagenode, Liege, Belgium), then converted into either Illumina or GS FLX libraries following the manufacturers' guidelines. GS FLX libraries incorporated MID identifiers, and Illumina libraries were indexed through library PCR. The libraries were sequenced at the Danish National High-throughput DNA Sequencing Centre.

The quality of the DNA in the remaining five samples was not sufficient for mitogenome generation using the above method. Therefore, we additionally used the 'target capture' principle described by Maricic *et al.* [30] to generate data for these samples. In this method, the DNA extracts were directly converted into indexed Illumina sequencing libraries, then denatured and incubated with biotinylated 'bait' generated from long-range *Architeuthis* mtDNA amplicons derived from the higher-quality material. Subsequent streptavidin-based capture of the biotinylated bait, now hybridized to the mitochondrial sequences from the library, enabled target sequencing of the mitogenomes on a HiSeq2000 platform. For full details on the method, see Maricic *et al.* [30].

(d) Assembly and sequence verification

After sequencing, the different datasets (amplicon-FLX, amplicon-HiSeq, bait-captured) were processed, using three different approaches relevant to the technology. For amplicon-FLX mitogenomes ($n = 34$), sequence reads were initially sorted by MID, then assembled into contigs with the publicly available *Architeuthis* reference sequence (NC_011581), using the software GsMAPPER (Roche). These contigs were aligned manually in MEGA v. 5.04 [31] and then imported into GENEIOUS PRO v. 5.3.6 [32] for further inspection. Because minor sequencing and assembly errors are common, especially with regard to mononucleotide repeats, all sequence differences in the alignment were verified by manually inspecting the assembly of reads for the individual sequences and making sure the contig was correct. In the cases of variable monomer length, the number was standardized according to the reference sequence, to account for the commonly accepted limitation of the GS-FLX platform with regard to accurately sequencing monomers of five or more bases in length. In this regard, we note that, as all monomers are located in protein-coding genes, if real, any length fluctuation with regard to the reference sequence would lead to frame shifts, which thus suggested they were unlikely to be real. Once the contig sequences were verified, the matching halves of

each individual mitogenome were joined together to construct the complete mitogenome sequence.

For amplicon-HiSeq ($n = 4$) and bait-captured mitogenomes ($n = 5$), raw sequence reads were trimmed to remove sequencing adapters as well as adapter dimmers, using the ADAPTERREMOVAL tool [33]. Sequences with average- to low-quality stretches were also filtered out. Trimmed reads were mapped to references using BWA v. 0.5.9-r26-dev [34], and duplicate reads, as well as reads with multiple hits were removed, using SAMTOOLS v. 0.1.18 (r982:295) [35]. Alignments were then visualized using the GENEIOUS DNA sequence analysis package. For the five bait-captured mitogenomes, the duplicated regions were excluded, because during assembly of the sequence it was not possible to assign reads to the appropriate copy with certainty. Thus, the length of these sequences was reduced to 11 655 bp in length.

(e) Population genetic analyses

Initial data analysis indicated that the level of nucleotide variation in the alignment was too low to allow generation of a well-supported phylogenetic tree of the mitogenomes. Therefore, to visualize the relationship between the samples, a haplotype network was generated, based on the concatenated protein-coding genes from the 38 complete mitogenomes, using the median-joining method implemented in NETWORK v. 4.61 [36]. Descriptive statistics (π , h and H_d ; the neutrality index Tajima's D and the dN/dS ratio) were calculated for the whole genomes, for all the protein-coding genes combined and for each gene separately (including both copies of the duplicated genes), using DNASP v. 5.1 [37]. A small number of the mitogenomes were incomplete, thus some of the single gene statistics were calculated based on alignments of a subset of the 44 sequences, as in each case incomplete sequences were removed. F_{ST} was also computed with DNASP. The calculation of F_{ST} values between different locations was only possible between locations that were represented by more than one individual (Florida, Japan, Australia and New Zealand), as the calculation requires a sequence diversity index.

DnaSP was also used to generate a pairwise mismatch distribution on the data, as well as the pairwise difference plot parameter τ , and the raggedness index. To reduce the amount of noise that might result from including regions with pronounced differences in their substitution patterns, the analysis was restricted to the concatenated protein-coding genes, thus excluding the tRNAs, rRNAs and the two control regions, but including both copies of duplicated genes. The haplotype network was drawn on the same alignment for the sake of consistency.

The extremely limited fossil record of coleoid cephalopods provides a significant challenge for studies that aim to use molecular clock analysis to date past events, thus resulting in the absence of a mutation rate estimate for any species closely related to *Architeuthis*. Therefore, we used four different mutation rates to tentatively estimate a time of expansion, based on the pairwise difference plot parameter τ (the median) [38]. Estimates of the first two mutation rates were obtained using MEGA v. 5.04, as well as the Jukes and Cantor model of substitution, based on (i) the corrected distances between sequences, (ii) estimates for the divergence time of families within Oegopsida as calculated by Strugnell *et al.* [39], which provide an upper and lower bound for the time of divergence of Architeuthidae from other oegopsid families, and (iii) the phylogenetic position of *A. dux* between *Watasenia scintillans* (Family Enoploteuthidae) and *Sthenoteuthis oualaniensis* (Family Ommastrephidae) as determined from the mitochondrial genome tree (see the electronic supplementary material, figure S2) [40].

The time-dependency hypothesis of mutation rates [41] suggests that rates estimated from relatively ancient fossil calibrations are likely to significantly underestimate the mutation rate as

Table 1. Summary genetic diversity statistics. π , nucleotide diversity; H_d , haplotype diversity.

region	length	π	s.d.	H_d	s.d.	Tajima's D	stat. sig.
whole mitogenomes	20 331	0.00066	0.00005	1	0.006	-2.51828	$p < 0.001$
all genes	14 976	0.00068	0.00005	1	0.006	-2.49263	$p < 0.01$
control region A	554	0.00166	0.00034	0.613	0.087	-1.66738	$0.10 > p > 0.05$

relevant to questions of recent demographic history. Therefore, additional mutation rates from published studies of gastropod evolution were also used. These estimates of gastropod divergence rates range from 0.67 to 2.4 per cent per million years [42], and despite deriving from a different class of molluscs, these may provide a better estimate of the *Architeuthis* mutation rate than those derived from very ancient divergences.

3. Results

Thirty-seven complete and six partial mitogenome sequences were generated, representing individuals from across the range of *Architeuthis* (see the electronic supplementary material, table S1). Sample average sequencing depth varied from 22- to 1051-fold, and successful application of the bait-capture method developed by Maricic *et al.* [30] confirmed the use of this method when working with challenging degraded materials. An image showing a visualization of the alignment of all the obtained sequences as well as the reference sequence is given in electronic supplementary material, figure S3. The mitogenome data are deposited in NCBI GenBank under accession numbers KC701722–KC701764 (see the electronic supplementary material, table S1).

The level of nucleotide diversity in the sequences was extremely low, with only 181 segregating sites of the 20 331 bp-long sequence alignment. Values of the nucleotide diversity index (π) and the haplotype diversity (H_d) are given in table 1 for the alignment of entire mitogenomes, for the concatenated protein-coding genes and for the first of the two control regions (CR-A). The dN/dS value for the alignment of 38 concatenated protein-coding gene sequences (including both copies of duplicated genes) was 0.0540. A complete set of genetic diversity and neutrality statistics for all individual genes is given in the electronic supplementary material, table S3.

The observed haplotype diversity was high at the mitogenome level, with each specimen containing a unique haplotype, as can be seen in the median-joining haplotype network (figure 1). Despite this high haplotype diversity, the nucleotide diversity statistic is very low in comparison with estimates from other marine dwelling species (table 2).

The dataset surprisingly exhibits no evidence of any phylogeographic structure (figure 1). This observation is supported by the low F_{ST} estimates calculated, using the whole mitogenomes for samples from Australia, Spain, Florida, New Zealand and Japan (table 3; negative F_{ST} values are likely to reflect the imprecision of the algorithm used by the software to estimate this value, and thus should be considered zero). A limitation of F_{ST} worth taking into consideration here is when it is used with mtDNA of highly variable species, as the high variability cuts down the number of shared haplotypes between any two

populations. The F_{ST} values presented here should therefore not be over-interpreted, but rather seen in the context of the other analyses.

The results of a pairwise-differences analysis using the 38 complete mitogenomes can be seen in figure 2. Both the shape of the distribution and the low raggedness index ($r = 0.0075$) provide strong evidence that *Architeuthis* has undergone a population expansion. By using τ , the estimated mode of the mismatch distribution, and an estimated generation time of 3 years, we estimated the time of expansion by the relationship $\tau = 2ut$, where $u = 2\mu k$ (t is the generation time, μ the mutation rate and k is the length of the DNA fragment used for the analysis; in this case 14 976 bp). A generation time of 3 years was chosen as it lies in the mid-range of suggested maximum ages of giant squid [9,22,23]. As mentioned earlier, three potential mutation rates were explored. The corrected distance between *A. dux* and *Sthenoteuthis oualaniensis* calculated with the Jukes and Cantor substitution model was 0.235. The upper bound for the Architeuthidae split, based on the Ommastrephidae split, was 33 Ma, and the lower bound, based on the enoploteuthid family split, was 220 Ma. Thus, the two new mutation rate estimates were calculated as 0.356 per cent Myr^{-1} and 0.053 per cent Myr^{-1} , respectively. These provided a time estimate of *Architeuthis* population expansion of between 109 248 and 728 318 years ago.

The calculations based on previously published gastropod mutation rates, ranging from 0.335 to 1.2 per cent Myr^{-1} (mutation rate is half the divergence rate) [42], yielded a more recent range for expansion time of 115 977 to 32 377 years ago.

Exploring a range of the *Architeuthis* generation time (which is unknown) to between 1 and 10 years, instead of 3 years, in combination with the fastest and slowest mutation rate estimates (1.2% and 0.053%, respectively), results in a wider estimated time range with an upper bound of 10 792 and a lower bound of 2 427 727 years ago.

4. Discussion

(a) Low genetic variability

The *Architeuthis* mitogenomes exhibit remarkably low nucleotide diversity (π), despite deriving from across its global distribution. In contrast to published records for other marine species (table 2), the diversity is lower than all except one, the basking shark *Cetorhinus maximus*, whose low diversity is believed due to a recent bottleneck [38]. It is notable, however, that the basking shark and coelacanth have a long generation time [50,51] and presumed small global population size, whereas *Architeuthis* populations are believed to be large. *Architeuthis* nucleotide diversity is also far lower than other squid (table 2), including 44 times lower than that of another oegopsid squid, the Humboldt squid *Dosidicus gigas* (based



Figure 1. Median-joining haplotype network of 38 complete giant squid mitogenomes. Geographical origin is coded by colour, matching those on the map in the electronic supplementary material, figure S6.

Table 2. Nucleotide diversity of the mitochondrial control region and cytochrome b.

species	common name	π	region	distribution	source
<i>Architeuthis dux</i>	giant squid	0.0004	cytochrome b	global	—
<i>Dosidicus gigas</i>	Humboldt squid	0.0177	cytochrome b	eastern Pacific	[43]
<i>Cetorhinus maximus</i>	basking shark	0.0013	control region	global	[38]
<i>Architeuthis dux</i>	giant squid	0.0017	control region	global	—
<i>Latimeria chalumnae</i>	coelacanth	0.0027	control region	east Africa	[44]
<i>Coregonus</i> spp.	European whitefish	0.0042	control region	northern Europe	[25]
<i>Orcinus orca</i>	killer whale	0.0052	control region	global	[45]
<i>Sepioteuthis lessoniana</i>	oval squid	0.0124	control region	Taiwan	[46]
<i>Xiphias gladius</i>	swordfish	0.0148	control region	global	[47]
<i>Carcharodon carcharias</i>	great white shark	0.0203	control region	global	[48]
<i>Thunnus obesus</i>	bigeye tuna	0.0540	control region	global	[49]

on the cytochrome b gene) [43], and seven times lower than a restricted population of oval squid *Sepioteuthis lessoniana* (based on the control region).

An additional curious feature of *Architeuthis* is that the relationship between nucleotide and haplotype diversity falls outside the 95th percentile of the positively correlated relationship between population-level estimates of haplotype (H_d) and nucleotide (π) diversity, which is reported for Cox1 by

Goodall-Copestake *et al.* [52], with $\pi = 0.00121$ and $H_d = 0.864$ for one copy of Cox1, and $\pi = 0.00073$ and $H_d = 0.622$ for the other copy. It is interesting that even in the context of such a diverse set of animals (the study spans several phyla, including Chordata, Mollusca, Crustacea, Arachnida, Hexapoda and Porifera) *Architeuthis* remains an odd one out.

It is difficult to reconcile this low genetic diversity with the reasonable assumption that *Architeuthis* are globally

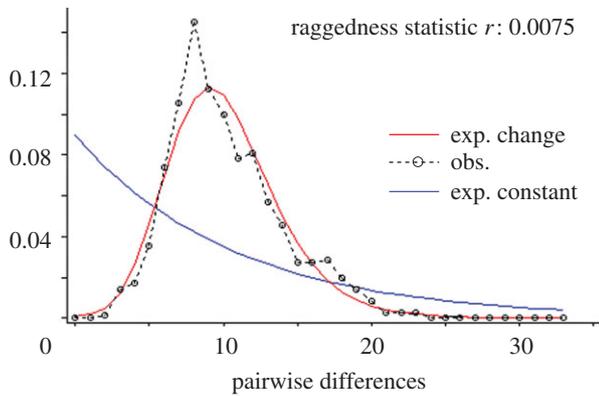


Figure 2. A mismatch distribution constructed from an alignment of 38 concatenated protein-coding gene sequences (including both copies of duplicated genes). The red and blue lines indicate expected shapes of the distribution after a rapid expansion and constant population size, respectively. A plot based on whole mitogenomes can be found in the electronic supplementary material, figure S4.

Table 3. Pairwise F_{ST} results for population comparisons. We only considered populations of $n > 2$ for the analysis.

population 1	n	population 2	n	F_{ST}
Australia	5	Florida	3	-0.011
Australia	5	Japan	6	-0.006
Australia	5	New Zealand	15	-0.015
Australia	5	Spain	3	-0.025
Spain	3	Florida	3	-0.017
Spain	3	Japan	6	0.006
Spain	3	New Zealand	15	-0.031
Florida	3	Japan	6	-0.012
Florida	3	New Zealand	15	0.01
Japan	6	New Zealand	15	0.001

distributed with relatively large population size. One explanation might be a low rate of mitochondrial DNA evolution, something observed among other marine organisms, in particular within the cnidarian class Anthozoa (sea anemones, corals and sea pens) [53], which are an order of magnitude slower than the rate observed among most other marine invertebrate species. Given that physiological and life-history traits such as metabolic rate and generation time can influence mutation rates [54], one might question whether such factors affect *Architeuthis*. Low metabolic rate seems unlikely, as recent data [14] demonstrate little difference between the metabolic activity of *Architeuthis* and that of highly active smaller squid, some of which show considerably more diversity (table 2). Long generation time is also an unlikely cause. While *Architeuthis* generation time remains uncertain, most published estimates based on statolith growth rings, isotope analyses and growth models suggest 1–6 years [9,22,24,55,56], and the FAO species catalogue suggests a maximum of 14 years [19], far below that needed to significantly decrease the mutation rate.

We alternatively considered whether low mutation rate might relate to the *Architeuthis* mitogenome duplications. Each duplication maintains near 100 per cent identity, despite their presence in all sequenced oegopsid squid mitogenomes [39], plus a non-oegopsid species *Bathyteuthis abyssicola* (NC_016423; indicating an origin at least 100 Ma). A low mutation rate in itself cannot explain this intraspecific sequence conservation between duplicates, as interoegopsid comparison shows that the duplicated genes in different species are indeed diverging, while somehow remaining identical within each individual—presumably by some form of gene conversion, resulting in concerted evolution of the two regions. As far as the mechanism of this concerted evolution is concerned, this study cannot present a satisfactory explanation, but it is possible that it happens via occasional recombination [57], or perhaps the duplicated sequences form stable secondary structures, which are somehow selectively beneficial, thereby causing mutations to be under negative selection. The latter, however, would be expected to lead to a decreased rate of divergence in the duplicated regions relative to the rest of the genome, which does not appear to be the case.

Alternative explanations to the idea of a low mutation rate keeping the nucleotide diversity low include a recent selective sweep, or a bottleneck coupled with expansion. While positive selection analysis using TreeSAAP [58,59] (data not shown) provides no evidence to support the former hypothesis, the ‘star-like’ shape of the network, Tajima’s D , the mismatch distribution and a Bayesian skyline plot analysis (see the electronic supplementary material, figure S5) support the latter. Although application of the alternative mutation rates and a generation time of 3 years estimates the time of expansion to roughly 32 000–730 000 years ago, the validity of the mutation rates used is questionable, given the uncertainty around the parameters on which they were estimated. Nevertheless, we believe the most accurate estimate will fall closest to those calculated using the gastropod rates, owing to the effects of time-dependency rendering the other mutation rates inappropriate for such shallow-time investigations [41]. These rates also yield a younger date of expansion, fitting well with the extremely low diversity and suggesting that the causal event was quite recent.

Unfortunately, genetic data alone cannot provide a plausible explanation, whether climatic or biological, as to why this might have happened. Given that *Architeuthis* are cosmopolitan, with (assumed) considerable dispersal abilities, the event must have had the potential to affect the entire global population simultaneously. Given this, alternative explanations can be suggested. One such explanation, which is consistent with expansion, would be a sudden inflation of a population that was historically smaller. If the population size was originally restrained by predation and/or competition, then the cause of the inflation could have been related to a decline in the number of predator or competitor species. This would be comparable with the effect of the 1700 to late 1800s industrialized whaling, which removed almost all of their main natural predators, though this event in particular is almost certainly too recent to be the explanation. A mechanism more plausible than fisheries could be climatic effects, such as the last ice age changing the abundance or distribution of competitors such as predatory fish. The role of cephalopods in the ecosystem tends to be that of subdominant predators, which often increase radically in biomass when other species (particularly their predators and direct

competitors) become depleted as a result of heavy fishing [5]. This has been observed, for example, in the spard fishery off Sahara [5,60], the gadoid fishery in the northwest Atlantic and the trawl fishery in the Gulf of Thailand [5]. Furthermore, it has been estimated that the documented increase in tuna catches has resulted in an extra 20 million tonnes of squid in the world ocean [61]. Thus, a recent inflation is a possibility, but equally difficult to explain in terms of concrete events.

(b) Biological and taxonomic implications of the data

If the low mitochondrial diversity is indicative of variation at the nuclear genome level in *Architeuthis*, then the data strongly suggest that globally only a single species of *Architeuthis* exists, namely *Architeuthis dux* (Steenstrup, 1857), consistent with the suggestion of a previous study on *Architeuthis* beak morphometrics [17]. The genetic data also demonstrate that *Architeuthis* lack phylogeographic structure. This observation is consistent with several hypotheses. On the one hand, it is possible that until recently *Architeuthis* existed as a single, small, geographically restricted population, which has subsequently expanded globally in a non-ordered fashion (implying capability for long-distance dispersal), distributing their variation among new regional populations. This, in turn, implies *either* adults are nomadic (an observation inconsistent with (i) beak isotope profile data suggesting adults inhabit relatively confined areas [9] and (ii) their flexible diet [62] negating the need for adults to disperse in search of food), *or* dispersal happens via migrations of juveniles [63] and small pelagic paralarvae. These are capable of dispersing over long distances via currents, as is the case for many cephalopod species, as well as deep-sea foraminiferans, in which genetic analysis demonstrates similarity between Arctic and Antarctic populations of three common deep-sea species separated by distances of up to 17 000 km [64]. Other examples include surgeonfish (which show no population structure between Brazil and mid-Atlantic populations, concluded to be the result of a drifting larval stage of roughly 50–70 days duration [65]) and other cephalopods, generally large species of oceanic ommastrephids [66]. It is even hypothesized that the large size of these squids is an adaptation to living in such large current systems [67].

Ultimately, however, if *Architeuthis* disperse over such large areas, then it is difficult to explain why they may have been restricted to one area in the first place. Given this, we find neither scenario likely, and believe an alternative explanation is warranted. We hypothesize that a panmictic global population of *Architeuthis* has existed for a considerable time. This would adequately explain the lack of population structure, as it is estimated that an average of just one individual exchanged between two populations per generation will be enough to prevent genetic differentiation between them [68] and broad dispersers are, indeed, generally genetically homogeneous over large spatial scales [69]. Admittedly, this raises additional questions, including how a relatively old panmictic population has maintained such low nucleotide diversity, how the reproductive system and life cycle of this species could sustain such panmixia, and what might be the primary mode of dispersal. As discussed previously, we cannot offer a satisfactory explanation for the low diversity, and this requires future study to resolve. With regard to the biology of *Architeuthis*, the panmictic distribution again seems best explainable through pan-oceanic dispersal of paralarvae and juveniles, with assistance from the currents in the upper

layers of the oceans. Under such a model, we hypothesize that they would prey on pelagic zooplankton and smaller animals, until reaching a sufficiently large size, after which they would descend to their closest nutrient-rich deep habitat (e.g. a continental upwelling zone [9,70]), where they would remain until maturation. The global ocean currents (see the electronic supplementary material, figure S6) powered by the thermohaline circulation, also known as the Great Ocean Conveyor, could drive the continual homogenization of the giant squid global population by transporting the juveniles.

This type of life history would be consistent with the findings from a study on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope profiles along giant squid beaks [9] that demonstrate ontogenetic shift in diet early in life from smaller prey of relatively low trophic status to larger prey of higher status, and fluctuations in $\delta^{13}\text{C}$ values observed near the rostral tip, which may be associated with a greater intrinsic variability in the carbon isotope composition of relatively small prey and/or transient migratory behaviour early in life [63].

5. Conclusion

Although our results are based on a single marker, mtDNA, the sampling encompasses the known range of *Architeuthis* and strongly suggests that the family Architeuthidae consists of a single species of giant squid, namely *Architeuthis dux* (Steenstrup, 1857). If so, this species is cosmopolitan and probably has a substantial population size, yet shows unusually low levels of mitochondrial nucleotide diversity. This may be due, at least in part, to a population expansion. *Architeuthis* also lack discernible population structure, with no genetic differentiation between samples from regions as far apart as Florida and Japan. This suggests *Architeuthis* are highly migratory, probably through a pelagic paralarval stage, dispersed via the global thermohaline circulation. Full validation of our findings and interpretations will first and foremost require analyses of nuclear sequences. This would allow for distinction between demographic expansion and selective sweep, as a nuclear DNA study could incorporate several unlinked loci in the analyses. A further investigative avenue of potential interest, which arose from analysis of the selection test performed within Oegopsida, is to explore whether there are traces of positive selection between oegopsid and non-oegopsid squids in the particular genes that have been duplicated. This could account for the high degree of protein sequence similarity observed between all five oegopsid species, and possibly help explain the concerted evolution of the duplications.

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