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Abstract

Cephalopod fisheries are increasing, but little is known about the cryptic diversity of some key commercial species. Recent studies have shown that cryptic speciation is common in cephalopods, including several oceanic squids formerly considered 'cosmopolitan species.' Further efforts are needed to investigate the cryptic diversity of commercial species, to inform management and support sustainable fisheries practices. *Thysanoteuthis rhombus* is an oceanic squid, currently recognized as the single species of the family Thysanoteuthidae. *T. rhombus* has a global distribution in tropical and subtropical waters and is an economically important species, with the highest catches occurring off Okinawa in Japan and of potential fishery resource for other countries due to their high abundance and large size. Here, we used sequences from 12S rRNA, 16S rRNA, and cytochrome *c* oxidase I to characterize its cryptic diversity using samples collected throughout most of its known geographic range. We identified three different putative species whose distributions are concordant with main ocean basins: *Thysanoteuthis major*, the most abundant species, is widely distributed in the North Pacific Ocean, North Indian Ocean, and limits of the South Atlantic Ocean; *Thysanoteuthis rhombus*, is distributed in the North and South Atlantic Ocean and Mediterranean Sea; and *Thysanoteuthis* cf. *filiferum*, likely the least sampled to date, is found in the southwestern Pacific Ocean. A sister relationship was observed between *T. rhombus* and *T. major*, and *T.* cf. *filiferum* was found to be the most divergent species. Based on our divergence estimation, we hypothesize that the closure of the Isthmus of Panama during the early Pliocene played a significant role in the split of *T. rhombus* and *T. major*, while the split of their ancestor with *T.* cf. *filiferum* coincided with an increase in the Pacific Walker Circulation and the longitudinal gradient of surface temperatures in the Pacific Ocean during the Late Oligocene and Early Miocene. Our work identifies three different putative species within *Thysanoteuthis* and has potential use for improving fishery management and conserving the diversity in these species.

Keywords (separated by '-') Cephalopod - Oegopsida - Thysanoteuthidae - Oceanic squid - Species delimitation

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ORIGINAL RESEARCH

Cryptic biodiversity in the commercial diamondback squid Thysanoteuthis rhombus Troschel 1857

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Abstract Cephalopod fisheries are increasing, but little is known about the cryptic diversity of some key commercial species. Recent studies have shown that cryptic speciation is common in cephalopods, including several oceanic squids formerly considered 'cosmopolitan species.' Further efforts are needed to investigate the cryptic diversity of commercial species, to inform management and support sustainable fisheries practices. *Thysanoteuthis rhombus* is an oceanic squid, currently recognized as the single species of the family Thysanoteuthidae. *T. rhombus* has a global distribution in tropical and subtropical waters and is an economically important species, with the highest catches occurring off Okinawa in Japan and of potential fishery resource for other countries due to their high abundance and large size. Here, we used sequences from 12S rRNA, 16S rRNA, and cytochrome *c* oxidase I to characterize its cryptic diversity using samples collected throughout most of its known geographic range. We identified three

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different putative species whose distributions are concordant with main ocean basins: *Thysanoteuthis major*, the most abundant species, is widely distributed in the North Pacific Ocean, North Indian Ocean, and limits of the South Atlantic Ocean; *Thysanoteuthis rhombus*, is distributed in the North and South Atlantic Ocean and Mediterranean Sea; and *Thysanoteuthis* cf. *filiferum*, likely the least sampled to date, is found in the southwestern Pacific Ocean. A sister relationship was observed between *T. rhombus* and *T. major*, and *T.* cf. *filiferum* was found to be the most divergent species. Based on our divergence estimation, we hypothesize that the closure of the Isthmus of Panama during the early Pliocene played a significant role in the split of *T. rhombus* and *T. major*, while the split of their ancestor with *T.* cf. *filiferum* coincided with an increase in the Pacific Walker Circulation and the longitudinal gradient of surface temperatures in the Pacific Ocean during the Late Oligocene and Early Miocene. Our work identifies three different putative species within *Thysanoteuthis* and has potential use for improving fishery management and conserving the diversity in these species.

Keywords Cephalopod · Oegopsida · Thysanoteuthidae · Oceanic squid · Species delimitation

Introduction

Defining adequate the limits of stock, or a group of individuals of a single species sharing the same life history parameters and occupying a specific geographic area (Secor 2014), is crucial for the preservation and sustainable exploitation of fishery resources in the short and long term (Carvalho and Hauser 1994). While defining the limits between species with clearly different morphologies might be relatively simple, delimitation becomes more challenging when sibling species undergo subtle morphological differentiation. This happens in cryptic species (Bickford et al. 2007), in which species present indistinguishable morphology but whose populations are reproductively isolated.

Large genetic divergences in cryptic species occurring sympatrically provide strong evidence that these species do not interbreed, while in allopatric species, large genetic divergence can be associated with local adaptation, genetic drift, or biogeographic barriers (Yoder et al. 2002). The detection of allopatric speciation in commercially important marine species has a direct consequence for management policies. The spatially based management policies for the originally recognized species must be changed by reducing the geographic area they apply to and by creating new policies for each cryptic species (Bickford et al. 2007; Fernández-Álvarez et al. 2020). These new policies must also consider possible differences in demographic parameters and responses to external disturbances, being only applied once the new cryptic species are properly characterized (von der Heyden et al. 2014). Identifying genetic divergences is the first critical step towards a more comprehensive biological characterization of the cryptic species, providing valuable information to support the necessary changes in the former management policies of these species. Genetic divergences can be estimated with traditional DNA markers such as mitochondrial genes (*e.g.*, Neves et al. 2020). Such characterization is readily possible even in poorly studied groups given the availability of universal primers. These molecular characterizations have been useful to identify cryptic species complexes in harvested marine groups that inhabit distinct ecological environments or geographic areas (Amor et al. 2014; Cheng and Sha 2017; Nieuwenhove et al. 219; Fernández-Álvarez et al. 2020).

Abundance data indicate that many cephalopod populations are increasing, and their fisheries are likely to continue growing given the ongoing decline of other harvested marine animals (Arkhipkin et al. 2015; Doubleday et al. 2016; Sauer et al. 2019). In 2020, cephalopods contributed 4.7% (3.7 million tonnes) of the total worldwide landings of marine species (FAO 2022). Hence, it will be crucial to establish a clear understanding of commercial cephalopod diversity and zoogeography to manage these resources sustainably in the coming years.

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Squids of the monotypic family Thysanoteuthidae Keferstein 1866, are large, muscular, oceanic oegopsids, globally distributed in tropical and subtropical oceanic waters, and of commercial importance for many countries. *Thysanoteuthis rhombus* Troschel 1857b, a has a short lifespan of around one year, maturing by about seven months and reaching up to one meter of dorsal mantle length and up to 30 kg of weight (Nigmatullin and Arkhipkin 1998). While paralarvae and juveniles of this squid occur in the epipelagic zone (Nigmatullin and Arkhipkin 1998), adults are found in mesopelagic waters during the day and near-surface waters at night (Nigmatullin et al. 1995). *T. rhombus* is an economically important resource in Japan, where it reaches the highest fishery landings worldwide with an annual average of 4,900 tonnes between 1998 and 2003 (Nigmatullin et al. 1995; Bower and Miyahara 2005). The main fishery grounds of this squid are in the Sea of Japan off Hyogo Prefecture and in waters off the Ryukyu archipelago (Nigmatullin et al. 1995; Bower and Miyahara 2005). Between 1990 to 2012, annual fishery landings ranging from 10 to 1179 tonnes, and 800 to 2600 tonnes were registered in the ports of Hyogo and Okinawa, respectively (Arkhipkin et al. 2015). Estimated stock abundances ranged from 2×10^5 to 6×10^5 individuals in Japan from 1999 to 2004 (Miyahara and Gorie 2005).

Moreover, the fishery of this animal has begun in the Atlantic Ocean, specifically off Caribbean (*e.g.*, Jamaica, Dominican Republic, and Santa Lucia) and the Canary Islands (Escánez-Pérez et al. 2012). In other countries such as the Philippines (Dickson et al. 2000), Vanuatu (Nimoho et al. 2014), Ecuador (Loor-Andrade et al. 2017), Mexico (Alejo Plata and Urbano-Alonso 2018), the Maldives (MASPLAN 2018), India (Sajikumar et al. 2020), and Peru (Roque-Sánchez and Donayre Salazar 2021), *T. rhombus* is being considered a potential exploitable resource because of its incident presence in fishing activities targeting other oceanic squids of similar size. For example, in the Eastern Pacific Ocean, this animal has been incidentally fished while targeting the Humboldt squid *Dosidicus gigas* (d'Orbigny 1835[in 1834–1847]) (Arkhipkin et al. 2015).

Oceanic barriers within the wide geographic range of the nominal *T. rhombus* are believed to have influenced the cryptic speciation in several other large oceanic cephalopods that show subtle or no morphological differences. Recent studies have found large genetic divergences in these oceanic cephalopods, indicating cryptic speciation and prompting the split of nominal classification (Fernández-Álvarez et al. 2020, 2021). For example, the monotypic genus *Ommastrephes* d'Orbigny 1834 [in 1834–1847] was split into 4 cryptic species inhabiting distinct geographic areas (Fernández-Álvarez et al. 2020). Large genetic divergences among these cryptic species appear to be related to restricted dispersal by main oceanic gyres and currents during early stages despite the large-scale horizontal migrations performed by subadults and adults to find suitable feeding and mating grounds (Bower and Ichii 2005; Fernández-Álvarez et al. 2020). *T. rhombus* has similar life-history traits in early stages, horizontal dispersal associated with ocean drift currents, but does not perform active swimming for feeding and spawning (Miyahara et al. 2008; Onitsuka et al. 2010). Such characteristics made us hypothesize that ocean currents and other biogeographic barriers in tropical and subtropical regions might have affected gene flow among populations of *Thysanoteuthis*, causing cryptic speciation.

This study aims to determine whether *Thysanoteuthis* Troschel 1857b, a is indeed a monotypic genus or species complex. To achieve this, we assessed the global biodiversity of *Thysanoteuthis* by analyzing mitochondrial sequences of the cytochrome *c* oxidase I (COX1), and the large 16S) and small (12S) ribosomal subunits (rrRNAs)from 41 individuals collected across all the ocean basins where this animal is commonly found.

Materials and methods

Sample collection

We collected 37 specimens in 13 different locations across different ocean basins (Fig. 1, Table 1). The specimens were collected through jigging, trawl net, gill net, recreational spear fishing and fish markets. After collection, a small piece of muscle mantle was kept in 98% ethanol for molecular procedures.

DNA isolation and Sanger sequencing

Genomic DNA was isolated using the TNES-urea buffer and the phenol–chloroform protocol (Asahida

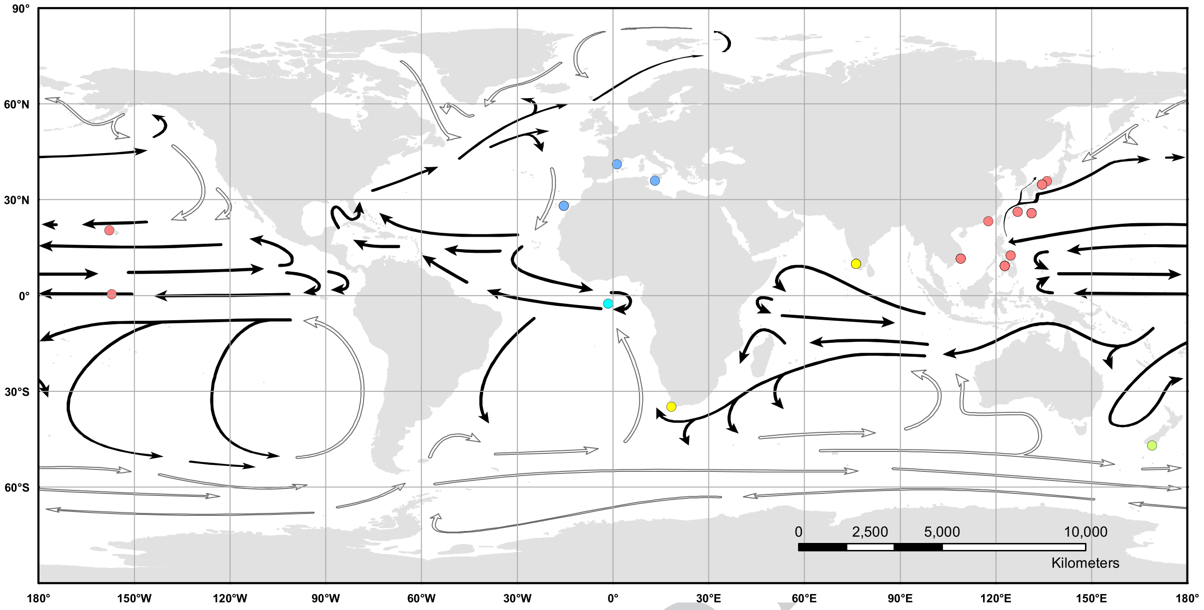

Fig. 1 Locations of samples used in this study. In red the location of individuals from the North Pacific Ocean; in yellow the location of individuals from the Arabian Sea in the North Indian Ocean and its limits near the borders of the South Atlantic Ocean; in blue the location of individuals from the North Atlantic Ocean and the Mediterranean Sea; cyan, individual sampled in the Gulf of Guinea; and in green, the single individual collected in the South Pacific Ocean. Black and white arrows indicate the main warm and cold ocean currents. The layer of ocean currents was downloaded from AmeriGEO (https://www.amerigeo.org/). This website compiled data on ocean currents from the NOAA National Weather Service and the United States Army. The layer was edited to include the main branches of the Tsushima and Kuroshio currents following reference maps constructed by Park et al. (2013, Fig. 19) and Lee et al. (2019, Fig. 1b), who collected information from oceanographic surveys

et al. 1996). Short regions of the COX1, and 16S and 12S rRNAs genes were amplified. We used the universal primers LCO1490 and HCO2198 (Folmer et al. 1994) for COX1, 16Sar and 16Sbr (Palumbi 1996) for 16S, and 12Sai and 12Sbi (Simon et al. 1994) for 12S. The master mix for PCR consisted of a total volume of 10 µl, containing 1ul of 10X Ex Taq Buffer (ThermoFisher Scientific), 0.8 µl of 10 mM dNTPs, 0.2 µl of each 10 µM primer, 0.05 µl of 5U/µl TaKaRa Ex TaqTM DNA (Takara Bio Inc., Shiga, Japan), 4.75 µl of ddH2O, and 3 µl of 20 ng/µl DNA. PCR was conducted using a Mastercycler Gradient 96-well system (Eppendorf, Hamburg, Germany) with the following general conditions: initial denaturation 94 °C for 2 min, 35 cycles of 94 °C for 30 s, gene-specific annealing temperature (Ta) for 40 s, 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. The Ta for COX1 was 45.5 °C, for 16S ranged from 42–57 °C, and for 12S was 52 °C. PCR products were cleaned with ExoSAP-IT (Affymetrix/USB Corporation, Cleveland, OH, USA) and sequenced using the forward primer and the BigDye v3.1 Terminator Sequencing Kit (Applied Biosystems, CA, USA) on the genetic analyzer (ABI 3130xI, Applied Biosystems, CA, USA).

Data analyses

Sequences were manually curated, pooled with other publicly available sequences (Table 1, Supplementary Table S1), and aligned using the default settings of MUSCLE (Edgar 2004) implemented in the software Aliview v.1.6 (Larsson 2014). The resulting alignments of COX1 encompassed a total of 41 sequences, while those of 16S and 12S contained 38 sequences (Table 1). To generate an initial overview of the relationship between our samples, we collapsed only *Thysanoteuthis* sequences into haplotypes and generated haplotype networks per gene using the TCS statistical parsimony method implemented in PopArt (Leigh and Bryant 2015). These networks preliminary subdivided our samples in three groups. These groups were

Table 1 *Thysanoteuthis* individuals used for this study (N = 37) including their GenBank accession numbers and reference sequences downloaded from public databases or reconstructed from previous publications (N = 4)

Species and group	Locality	N	GenBank accession number			References		
			COX1	12S	16S	COX1	12S	16S
	Hyogo, Japan (NW Pacific Ocean)	4	OP970837–OP970840	OP971222–OP971225	OP970878–OP970881	This study	This study	This study
	Fukui, Japan (NW Pacific Ocean)	1	OP970836	OP971221	OP970877	This study	This study	This study
	Kumejima, Okinawa, Japan (NW Pacific Ocean)	3	OP970849–OP970851	OP971234–OP971236	OP970890–OP970892	This study	This study	This study
Thysanoteuthis rhombus Group 1	Minamid-aitojima, Okinawa, Japan (NW Pacific Ocean)	8	OP970841–OP970848	OP971226–OP971233	OP970882–OP970889	This study	This study	This study
	Catarman, Philippines (NW Pacific Ocean)	2	OP970858–OP970859	OP971243–OP971244	OP970899–OP970900	This study	This study	This study
	Bayawan, Philippines (NW Pacific Ocean)	6	OP970852–OP970857	OP971237–OP971242	OP970893–OP970898	This study	This study	This study
	Ninh Thuan, Vietnam (11°34’N 109°02’E, NW Pacific Ocean)	4	OP970860–OP970863	OP971245–OP971248	OP970901–OP970903	This study	This study	This study
	Kochi, India (Arabian Sea)	3	OP970864–OP970866	OP971249–OP971251	OP970904–OP970906	This study	This study	This study
	Capetown, South Africa (South Indian Ocean*)	1	OP970867	OP971252	OP970907	This study	This study	This study
	South China Sea (23°17′N 117°37′E, NW Pacific Ocean)	1	MT733875	MT733875	MT733875	(Tang et al. 2021)	(Tang et al. 2021)	(Tang et al. 2021)
	Hawaii (20°29′N 157°45′W, NE Tropical Pacific Ocean)	1	EU735371	–	EU735236	(Lindgren 2010)	–	(Lindgren 2010)
	Line Islands (0°23′N, 157°01′W	1	GU188439	–	–	–	–	–
	Central Tropical Pacific Ocean, William F. Gilly, pers. comm.)	1	GU188438	–	–	–	–	–

Table 1 (continued)

Species and group	Locality	N	GenBank accession number			References		
			COX1	12S	16S	COX1	12S	16S
Thysanoteuthis rhombus Group 2	Lampedusa Island, Italy (Mediterranean Sea)	1	OP970869	OP971254	OP970908	This study	This study	This study
	Tarragona, Spain (Mediterranean Sea)	1	OP970868	OP971253	OP970911	This study	This study	This study
	Canary Islands, Spain (NE Atlantic Ocean)	1	OP970870	OP971255	OP970909	This study	This study	This study
	Gulf of Guinea (South Atlantic Ocean)	1	OP970871	OP971256	OP970910	This study	This study	This study
Thysanoteuthis rhombus Group 3	New Zealand (46°54'S 169°00'E, SW Pacific Ocean)	1	OP970872	OP971257	OP970912	This study	This study	This study

NW North West, *SW* South West, *NE* North East

(*) Sample location is technically within the borders of the Southern Atlantic Ocean but overlaps with the boundaries of the warm Agulhas current in the South Indian Ocean

used to estimate intra- and intergroup uncorrected p-distances in MEGA X (Kumar et al. 2018) and as an initial reference for other species delimitation approaches.

Molecular species delimitation

We delimited cryptic species by using the distance-based method Automatic Barcode Gap Discovery (ABGD, Puillandre et al. 2012) and three different phylogenetic-based methods: the Bayesian Poisson Tree Process (bPTP, (Zhang et al. 2013), the single-threshold Generalized Mixed Yule Coalescent (GMYC, Pons et al. 2006; Monaghan et al. 2009), and the Bayesian Multispecies Coalescence (MSC, Rannala and Yang 2003; Yang and Rannala 2010).

Given that the GMYC, bPTP, and MSC require a phylogenetic tree to be performed, we constructed phylogenetic trees using a concatenated alignment of all genes. These trees were built through maximum likelihood (ML) and Bayesian approaches in the IQ-TREE v.2.1.1 (Minh et al. 2020) and BEAST v.2.6 (Bouckaert et al. 2019), respectively. We included various species from the family Ommastrephidae Steenstrup 1857 as outgroups (Supplementary Table S1) since they are the closest known relative of Thysanoteuthidae (Fernández-Álvarez et al. 2022). For the ML analysis, we used the best substitution model selected for each gene by ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE. The support of the clades in the ML tree was assessed using 1,000 replicates of the ultrafast likelihood bootstrap (Hoang et al. 2017). For the Bayesian approach, we obtained an ultrametric tree for each gene and for the concatenated alignment using the HKY + I substitution model, a clockwise rate, and a Markov chain Monte Carlo (MCMC) of 10^5 steps. We used FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) to visualize the ML and Bayesian trees.

The ABGD method delimits species by establishing a genetic distance threshold, also known as barcoding gap, which assumes that the divergence of sequences within hypothetical species is less than the divergence between species. We run the ABGD method on their web server https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html with the default parameters under the p-distance, the simplest distance model.

The GMYC method assigns branches of an ultrametric tree to the speciation or coalescence within species by maximizing the likelihood of the GMYC model. In the GMYC model, branches of different species are modelled in a Pure-birth or Yule process (Udny Yule 1925), and branches within species are modelled in a neutral coalescences process (Hudson and Others 1990). The PTP approach models speciation using the number of substitutions (provided through a phylogenetic tree) instead of the modelled time. The PTP assumes that the number of substitutions between species is larger than within species. The GMYC and the MCMC sampler implementation of the PTP method (also known as bPTP) were run in their web interface available at https://species.h-its.org/, using the gene trees generated in BEAST.

We also employed the multispecies coalescence model implemented in the software Bayesian Phylogenetics and Phylogeography (BPP) v.4.2 (Yang 2015). We used the analysis A11 for joint species delimitation and species tree inference that incorporates phylogenetic uncertainty and thus does not require a fix guided tree (Yang and Rannala 2014). Under this analysis, the program changes the species tree and tries to merge putative species but never splits the species into novel species. Since many individuals distributed in the North Pacific Ocean or the Atlantic Ocean plus Mediterranean Sea share haplotypes or are very closely related (as displayed in our TCS network), we reasoned that the species&tree parameter is best represented as (((Group1, Group2), Group3), outgroup). In addition, we used $\Theta = 0.0025$ (which represents the average of the maximum and minimum K2P genetic distance between individuals of the North Pacific Ocean) and $\tau = 0.02$ (assuming a mutation rate of 4.07×10^{-9} and a divergence time between *Thysanoteuthis* and Ommastrephidae of 51 Mya, Supplementary Figure S4). Since our input species tree consisted of at least four different species, we decided to run all the species model priors available in BPP (prior 0, 1, 2, and 3). For each of the species model priors, we also used the algorithm 0 and 1 of species delimitation reported in Yang and Rannala (2010). Our runs included 3 loci, a burn-in of 8,000, sample frequency of 2 and a total of 200,000 samples. The number of species detected was evaluated as the one resulting in the highest posterior probability.

Additional sequences

We also included six COX1 haplotypes collected off Galapagos Island (Kitaura et al. 1998). These sequences were generated using Polymerase Chain Reaction Single-Strand Conformation Polymorphism and are shorter than sequences generated using Sanger sequencing. We reconstructed these sequences following Table 1 in Kitaura et al. (1998). Moreover, we also included two publicly available COX1 sequences collected off Line Islands (Central Pacific) from the National Centre for Biotechnology Information. These sequences were pooled with our newly generated COX1 sequences and analysed through a ML tree using IQ-TREE v.2.1.1.

Literature review

To categorize the hidden species found using molecular sequences into previously assigned taxonomic species, we reviewed the taxonomic literature of the genus *Thysanoteuthis*. The compilation of Sweeney and Young (2003) was used as the initial reference literature of *T. rhombus* synonyms, followed by other bibliographic reviews.

Divergence time

The complete mitochondrial genome of the individuals from Tarragona (Fernández-Álvarez et al. 2022) and the South China Sea (Tang et al. 2021) were used to produce robust estimations on the divergence times of cryptic *Thysanoteuthis* species using BEAST, in combination with the mitochondrial gene fragments obtained in this work. At present, there is no complete mitogenome available for South Pacific *Thysanoteuthis*, For this analysis, we also used complete mitochondrial genomes from representative Ommastrephidae, Spirulida Owen 1836, Loliginidae Lesueur 1821, Sepiidae Leach 1817, and two Octopodiformes to include lineages that present fossil calibration data (Supplementary Table S1). Nucleotides from protein-coding sequences were aligned based on their amino acid alignment using MUSCLE in Aliview v.1.6 and concatenated into a single matrix. Sequences of 12S and 16S rRNAs were also aligned using MUSCLE in Aliview. Gaps were manually deleted from the borders of the 12S and 16S rRNA matrix. The partitioned analysis was run using the best substitution model selected by bmodeltest (Bouckaert and Drummond 2017) under all the reversible model search implemented in BEAST. The run was also performed under the Birth–Death process as tree prior (Kendall 1948; Rannala and Yang 1996; Gernhard 2008), and the Fast Relaxed Clock Log-Normal (Zhang and Drummond 2020). We used two fossil calibrations, the first was for the stem lineage of *Doryteuthis* Naef 1912 to 48 million of years ago (Mya), the minimum age of a statolith fossil from the middle Eocene (Neige et al. 2016). This age was initially reported as a fossil of the Myopsida group, but it was later corrected as a fossil of *Doryteuthis* because the location of the fossil corresponded to the North American lineage (Pardo-Gandarillas et al. 2018; Anderson and Marian 2020). The node was calibrated with a shape of 2, a scale of 1, and off-set of 48, with the option "use originate" marked. These parameters place the 97.5% quantile distribution at 54.8 Mya, similar as in Anderson and Marian (2020). The second calibration included *Spirula* and its descendants to 75 Mya reported in Fuchs et al. (2013). The node was calibrated with a shape of 2, a scale of 1, and off-set of 75, with the option "use originate" marked. These parameters ensure that the 97.5% quantile distribution falls in the ranges estimated by Tanner et al. (2017). The analysis was run for 100 million generations, saving trees every 1000 generations. Convergence was evaluated by an ESS of more than 300 in Tracer 1.7.1 (Rambaut et al. 2018).

Results

Species delimitation

The maximum likelihood tree and the configurations of the haplotype networks clustered our samples in three groups that correspond to distinct ocean basins: group 1 from the North Pacific Ocean and Indian Ocean; group 2, North- and South-East Atlantic Ocean and Mediterranean Sea; group 3, the single individual from the South Pacific Ocean (Fig. 2, Supplementary Note 1, and Supplementary Figure S1). The individual from the South Pacific Ocean is separated from the others for more than 20 mutational steps in the TCS haplotype network (Fig. 2), representing the largest divergence between the three groups. We also found a clear gap between the intra- and inter-group mean p-distances, consistent with the configuration of the TCS haplotype networks. The p-distances within groups ranged from 0 to 1.2% for both the 12S and 16S rRNA genes, and 0.2–1.7% for the COX1 gene (Table 2), while the distances between groups ranged from 1.86–5.13%, 3.04–6.93%, and 6.26–16.43% for the 12S rRNA, 16S rRNA and COX1 genes, respectively (Table 3).

The ABGD method also identified three groups in our samples, which are concordant with the clustering of the haplotype networks and the differences among intra- and intergroup p-distances (Figs. 2, 3). Using 16S rRNA, ABGD detected a primary partition that split *Thysanoteuthis* into these three groups, while the analysis with 12S rRNA and COX1 resulted in only two groups (Supplementary Table S2). However, recursive partitions, which work better when more than a single gap distance can be present between samples, were able to identify three groups with the 12S rRNA and COX1 genes.

The GMYC analysis using 16SrRNA and COX1 also identified three groups. The same three groups were recovered through the bPTP analysis using COX1 sequences. However, the GMYC analysis using 12SrRNA delimited only two groups: (1) North Pacific, North Indian, Atlantic, and Mediterranean waters, and (2) South Pacific waters, while bPTP analyses with 12SrRNA and 16SrRNA defined six non-consistent groups (Fig. 2). The three additional groups included a few individuals from the North Pacific, North Indian Ocean, or the Mediterranean Atlantic group. These groups, however, might be a bias of the programs related to the higher abundance of samples and haplotypes from the North Pacific Ocean, North Indian Ocean, and the Mediterranean-Atlantic group in relation to the southern Pacific Ocean sample, or a consequence of the uncertainty of the phylogenetic tree used given the close relationship and low node support between some haplotypes within these groups (Fig. 4).

Using different species delimitation algorithms and species model priors, BPP analysis provided the highest posterior probability for three *Thysanoteuthis* species (group 1, group 2, and group 3). All the runs resulted in at least a posterior probability of 0.999 supporting three species with over 60% of the running samples supporting the same species tree topology proposed by the ML and Bayesian trees.

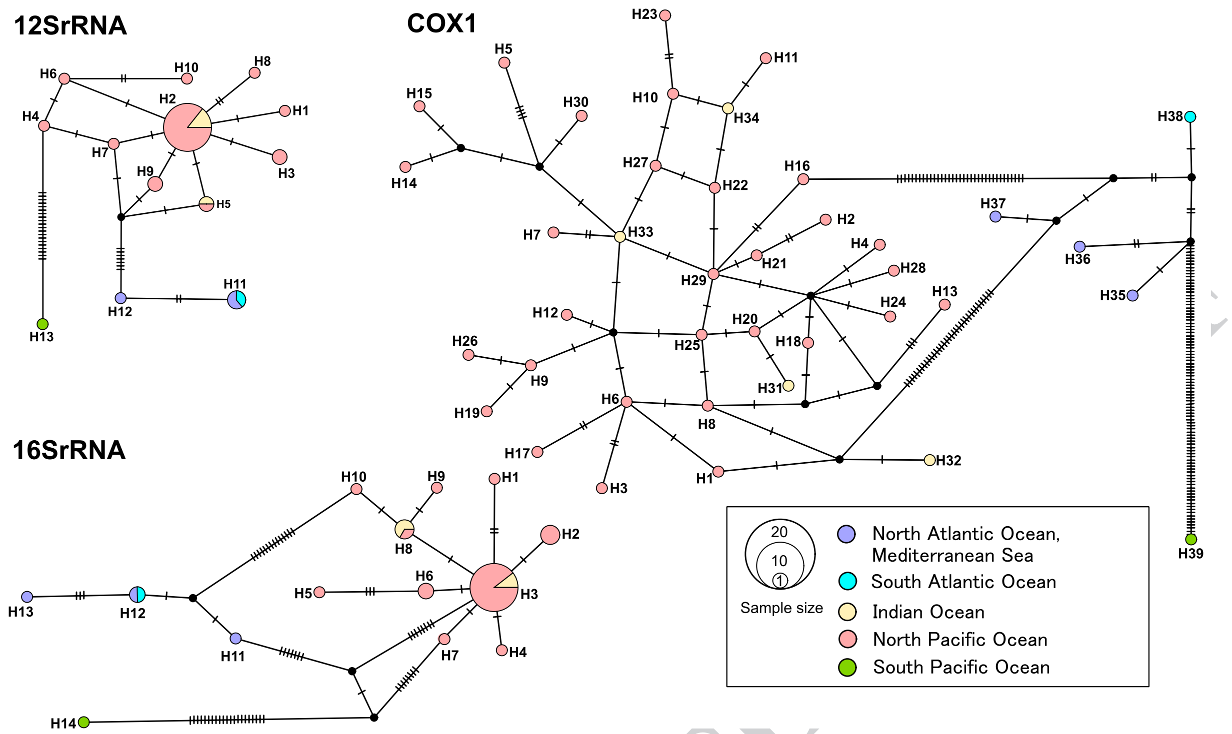

Fig. 2 TCS haplotype networks for each mitochondrial gene. The sizes of the circles in the legend are proportional to the number of individuals sharing the same haplotype. A single mutational step is represented as a small tick between haplotypes. Abbreviations: 12S rRNA, 12S ribosomal RNA; 16S rRNA, 16S ribosomal RNA; COX1, cytochrome oxidase *c* subunit I

Table 2 Estimates of intra-group p-distances based on three mitochondrial genes

	Gene	12S rRNA	16S rRNA	COX1
Thysanoteuthis Group 1	n	33	33	34
	Mean	0.2	0.2	0.8
	Range	0–1.2	0–1.2	0.2–1.7
Thysanoteuthis Group 2	n	4	4	4
	Mean	0.2	0.6	1.1
	Range	0–0.5	0–1.0	0.5–1.6
Thysanoteuthis Group 3*	n	1	1	1

n the number of individuals, *12S rRNA* 12S ribosomal RNA, *16S rRNA* 16S ribosomal RNA, *COX1* cytochrome oxidase *c* subunit I,

(*) Mean and range of intragroup *p*-distances were not calculated because this group only contains a single known individual

The additional six COX1 haplotypes reported in Kitaura et al. (1998) and two public available COX1 sequences from off Line Island allowed us to increase our geographic coverage in the North Pacific Ocean. A ML tree that includes these sequences placed them as part of *Thysanoteuthis* Group 1 from the North Pacific Ocean, suggesting a wide distribution of this group, from the Northwest Pacific to the Northeast Pacific off Galapagos Island (Supplementary Note 1 and 2, Supplementary Figure S2 and S3).

Proposed nomenclature assignment

Based on the evidence from the molecular results, we propose to recategorize the previous nominal classification of *T. rhombus* into three species. Unfortunately, we could not characterize these cryptic species by morphology due to the logistic challenges of collecting whole individuals. Neither we did not perform genetic analyses using nuclear markers. In addition, the current literature does not list regional

Table 3 Estimates of intergroup p-distances based on three mitochondrial genes

12S rRNA	*Thysanoteuthis* Group 1	*Thysanoteuthis* Group 2
Thysanoteuthis Group 2	2.43 (1.86–3.02)	
Thysanoteuthis Group 3	5.09 (4.66–5.59)	5.13 (5.13–5.13)
16S rRNA	*Thysanoteuthis* Group 1	*Thysanoteuthis* Group 2
Thysanoteuthis Group 2	3.65 (3.04–4.67)	
Thysanoteuthis Group 3	6.19 (5.89–6.71)	6.37 (5.91–6.93)
COX1	*Thysanoteuthis* Group 1	*Thysanoteuthis* Group 2
Thysanoteuthis Group 2	6.90 (6.26–7.51)	
Thysanoteuthis Group 3	16.09 (15.65–16.43)	15.53 (15.18–15.96)

12S rRNA 12S ribosomal RNA, *16S rRNA* 16S ribosomal RNA, *COX1* cytochrome oxidase *c* subunit I

Values indicate the mean and range (in parentheses) of percentage intergroup distances

morphological differences with diagnostic characters. We included a single morphological diagnosis for our collections to identify them as part of the *Thysanoteuthis* genus following Fernández-Álvarez et al. (2022).

Genus *Thysanoteuthis* Troschel 1857b, a

Thysanoteuthis Troschel 1857b, a:40–76 [69].

Synonyms:

Cirrobrachium Hoyle 1904

Type species: *Thysanoteuthis rhombus* Troschel 1857b, a

Group 1

Thysanoteuthis major (Gray 1828).

Sepioteuthis major Gray 1828:3, Pl. 4 Fig. 1.

Type repository: not resolved according to Sweeney and Young (2003).

Type locality: Cape of Good Hope, South Africa.

Synonyms:

Thysanoteuthis nuchalis Pfeffer 1912:531, according to Sasaki (1929:301).

Type material: ZMB Holotype Moll.-62,339, in accordance with Glaubrecht and Salcedo-Vargas (2000:277).

Type locality: Ningpo (Yellow Sea, China), see Pfeffer (1912:534).

Distribution: The North Pacific Ocean, Indian Ocean and its western limits within the warm Indian Ocean current. Reconstructed COX1 sequences collected off Galapagos Island suggest that the distribution of this species also includes the Northeast Pacific Ocean (Supplementary Note 2, Supplementary Figure S3).

Remarks: The complete mitogenome of this species was described by Tang et al. (2021) under the name of *T. rhombus*.

Group 2

Thysanoteuthis rhombus Troschel 1857b, a

Thysanoteuthis rhombus Troschel 1857b, a:70, Pl. 4 Fig. 12, Pl 5 Figs. 1, 2, 3 and 4.

[See Okutani et al. (1987:149)].

Type material: unresolved, according to Sweeney and Young (2003).

Type locality: Messina, Sicily (Mediterranean Sea).

Synonyms:

Thysanoteuthis elegans Troschel 1857b, a:74, Pl 4 Fig. 10, 11. [fide Sasaki (1929:301)].

Type material: unresolved [fide Sweeney and Young 2003].

Type locality: Messina, Sicily (Mediterranean Sea).

? *Cirrobranchium danae* Joubin 1933:7, Figs. 3, 4, 5, 6, 7, 8 and 9. [fide Nesis (1987:237)].

Type material: ZMUC Holotype; specimen not extant [fide Kristensen and Knudsen (1983:221)].

Type locality: North central Atlantic (12.97ºN, 32.82ºW).

Distribution: Mediterranean Sea and the North and Equatorial Atlantic Ocean at least until the Gulf of Guinea (Supplementary Note 2, Supplementary Figure S3).

Remarks: The complete mitogenome of this species was described by Fernández-Álvarez et al. (2022).

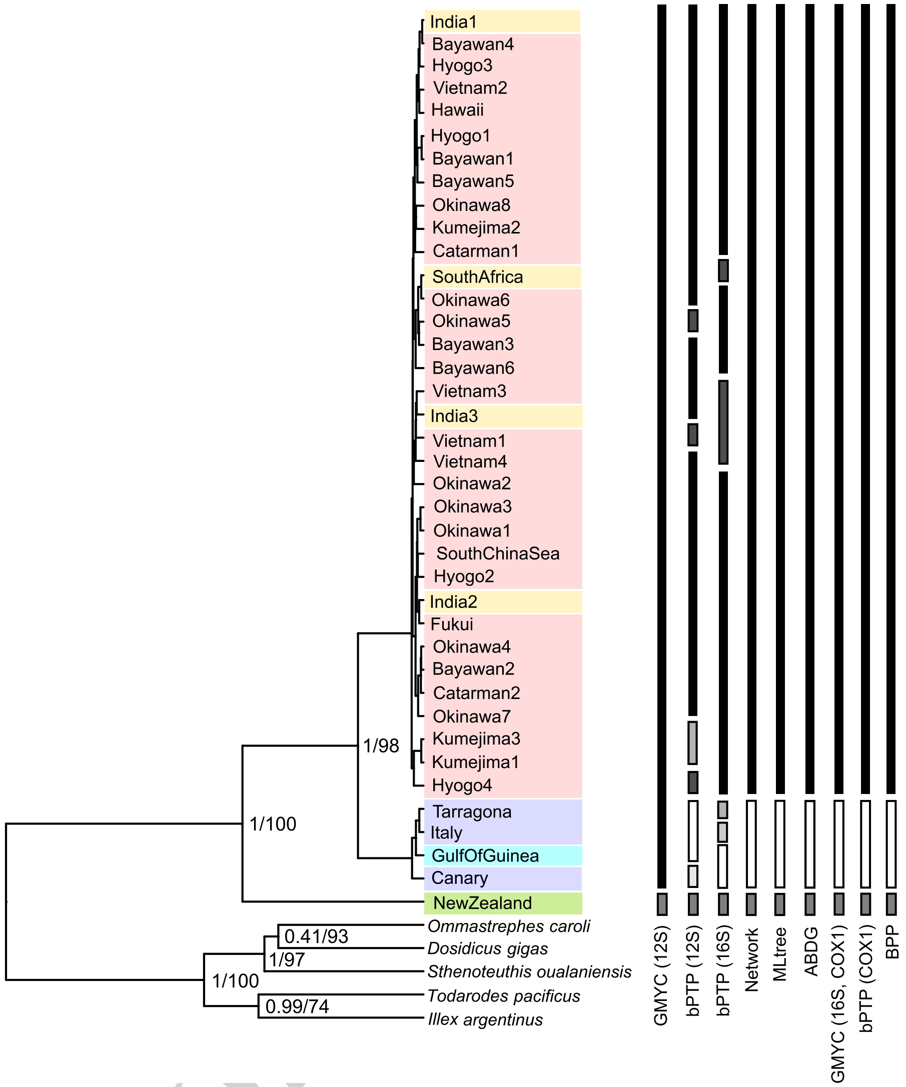

Fig. 3 Consensus clustering of cryptic *Thysanoteuthis* species. The consensus is based on all the approaches used in this work. The results are plotted in a Bayesian phylogenetic tree generated in BEAST v.2.6 (Bouckaert et al. 2019). Annotations in each node indicate posterior probability from Bayesian analysis (left) and bootstrap value from maximum likelihood analysis (right) carried out in BEAST v.2.6 and IQ-TREE v.2.1.1 (Minh et al. 2020), respectively. Absence of a gene name for a delimitation approach indicates that the same results were obtained with each of the three mitochondrial genes. ABGD consensus represents the results using the recursive partition. Different gray-scaled columns within one delimitation method indicate different putative species. Color shades in the tips designate ocean basins of sampling: light red, north-western Pacific Ocean; yellow, Indian Ocean; light blue, Mediterranean Sea and North Atlantic Ocean; cyan; South Atlantic Ocean; and green, South Pacific. Abbreviations12S: 12S ribosomal RNA, 16S: 16S, ribosomal RNA, COX1: cytochrome oxidase c subunit I

Group 3

Thysanoteuthis cf. *filiferum* (Hoyle 1904).

? *Cirrobrachium filiferum* Hoyle 1904:28, Fig E, according to Nesis (1987:237).

Type material: unresolved, according to Sweeney and Young, (2003).

Type locality: Marquesas Islands, Equatorial central Pacific (0.83ºN, 137.9ºW).

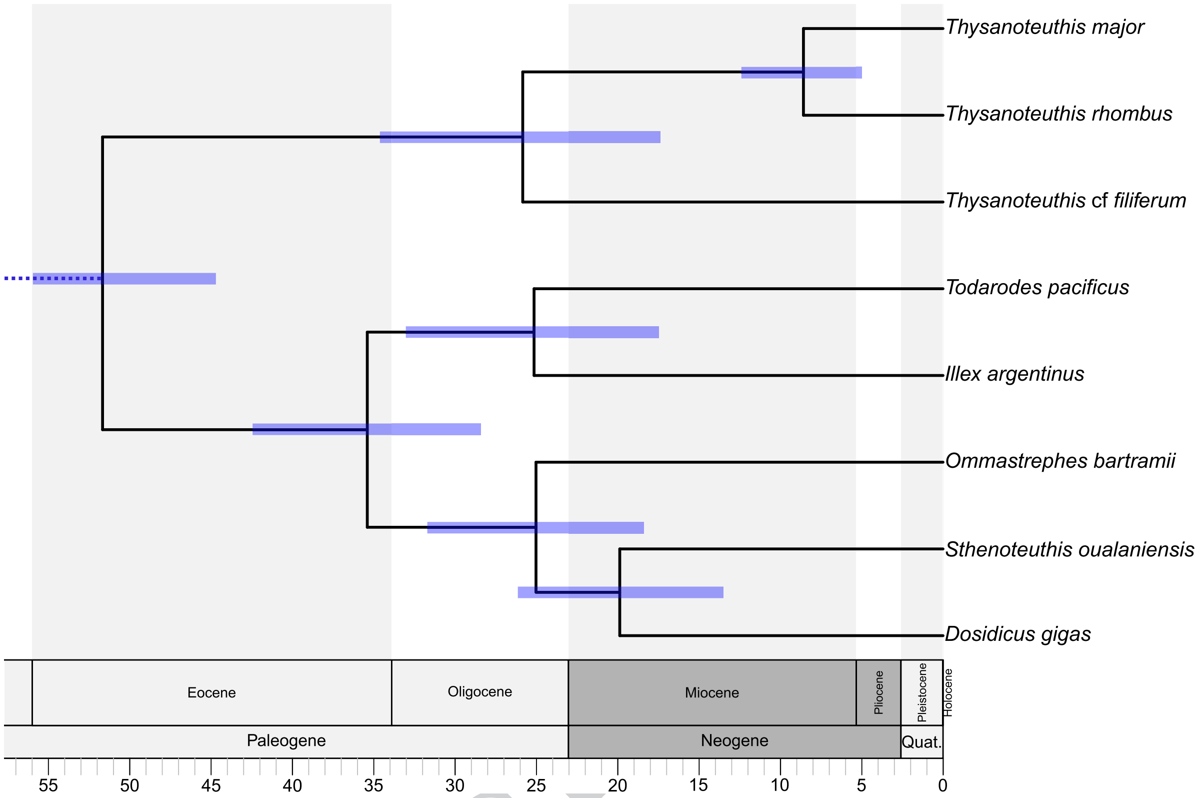

Fig. 4 Divergence times of cryptic *Thysanoteuthis* species. The tree was generated in BEAST v2.6 (Bouckaert et al. 2019) based on the mitochondrial genomes and 12S rRNA, 16S rRNA, and COX1 sequences (*T.* cf. *filiferum*). X-axis indicates the time in millions of years. Outgroups and calibration points were removed to improve the visualization of the chronogram. Confidence intervals of divergence times are represented using blue-shaded rectangles; dashed lines within the rectangles indicate continuity of the interval. A complete chromogram that includes the outgroups, and the calibration point can be found in Supplementary Figure S4

Distribution: The South Pacific Ocean, off New Zealand (Supplementary Note 2, Supplementary Figure S3).

Remarks: *Thysanoteuthis* group 3 is the most divergent species of the species complex. Our molecular characterization is based only on a single individual sequenced. The identification of this species as *T.* cf. *filiferum* is based on the only *Thysanoteuthis* species described from the South Pacific Ocean from a location different from the type locality of the nominal species. We recommend using this identification with caution at present, to be confirmed in future through morphological and molecular comparison with *Thysanoteuthis* from the Marquesas Islands (the type locality of *T. filiferum*).

Divergence time

We found that the earliest split occurred between *T.* cf. *filiferum* from the South Pacific Ocean and the ancestor of *T. major* and *T. rhombus*, at around 25.84 Mya (High Posterior Density, HPD, 95%, 17.37–34.73 Mya) during the Late Oligocene and Early Miocene. The last common ancestor between *T. major* and *T. rhombus* was present at around 8.58 Mya (HPD 95%: 5.07–12.37 Mya), overlapping with the end of the Miocene and the Early Pliocene (Fig. 4).

Discussion

We report the presence of three cryptic putative species within the widely distributed and large muscular squid formerly attributed to *Thysanoteuthis rhombus* Troschel 1857b, a. We characterize these three cryptic putative species with TCS haplotype networks, phylogenetic relationships, and several species delimitation approaches using three mitochondrial genes. These three cryptic species occur allopatrically, being found in different ocean basins with a clear dominant presence in warm oceanic currents (Fig. 1, Supplementary Figure S3). The first species, *Thysanoteuthis major*, is found predominantly in the eastern and western North Pacific Ocean, North Indian Ocean, and the limits of the warm current of the Indian Ocean close to the South Atlantic Ocean; the second, *Thysanoteuthis rhombus*, is distributed in the North- and South-East Atlantic Ocean and Mediterranean Sea; and the third, *Thysanoteuthis* cf. *filiferum*, is presently known in the south-western Pacific Ocean. While the geographic coverage of our sampling is very extensive, some areas such as the southern Equatorial and north-western Atlantic, and the southeastern Pacific are currently unexplored.

Thysanoteuthis rhombus *is a species complex*

The successful delimitation of three species included the haplotype network configurations for each gene, barcoding gaps, ABGD, BPP with different species model and species delimitation priors, GMYC for all genes except the 12S rRNA gene, and bPTP for the COX1 gene (Table 3, Figs. 2 and 3).

The mean values of barcoding gaps exceeded the intermediate, so-called 'grey zone' of animal speciation, of 0.5% to 2%, a range estimated across many organisms with different life histories and ecology (Roux et al. 2016). The mean interspecific distances between these species also exceed around 10 times the mean intraspecific distances (Tables 2 and 3), in agreement with the threshold value proposed in Hebert et al. (2004). To avoid relying only on the mean values of the barcoding gap, we also compared the smallest interspecific and largest intraspecific distances (Meier et al. 2008). These values do not overlap, supporting the presence of three *Thysanoteuthis* species (Tables 2 and 3).

The ABGD approach corroborated the presence of three species. Although only 16S rRNA with the primary partition was able to discriminate the three species, the recursive partitions of both 12S rRNA and COX1 were also able to discriminate three species as well (Supplementary Table S2). We anticipated recursive partitions to provide better estimates in our study because they assume the presence of more than a single barcoding gap (Puillandre et al. 2012). In our case, the ABGD primary partition may have assumed a single gap distance between group 3 and group 1 plus 2, since their divergence of the group 3 is very large. However, recursive partitions were able to recognise the genetic divergence between group 1 and 2 by introducing a second gap distance that discriminates these two groups.

The results that indicate either fewer or more than three species may be due to the low sample size, mutation rate of the gene, and the characteristics of the models used. For example, the GMYC method using a phylogenetic tree constructed from 12S rRNA sequences only identified two putative species, one including *T.* cf. *filiferum* from off New Zealand, and the remaining individuals clustered into another species (Fig. 3). This may be due to recent and rapid radiation across cryptic species and ancestral high polymorphism relative to the other nodes, which can introduce uncertainty in the GMYC method (Esselstyn et al. 2012; Reid and Carstens 2012). The deep ancestral split between the single individual collected from off New Zealand and the rest of the individuals, along with the relatively faster radiation of the latter group, may explain the underestimation of the number of species in the GMYC approach. In addition to GMYC, the bPTP analyses using the 12S rRNA and 16S rRNA genes inferred six putative species: *T.* cf. *filiferum* and three extra species composed of one to five individuals, with the individuals from North Pacific Ocean representing more than one species. Such a result suggests that unbalance sampling can affect the correct discrimination of species with this method.

While our study included samples covering a wide geographic range, it is possible that additional unrecognized species within the family Thysanoteuthidae may exist. We particularly lacked samples from the South Indian Ocean, where some other oceanic 'species' are hypothesized to have cryptic diversity (*e.g.*, *Spirula spirula* Linnaeus 1758, based on morphology in Hoffmann et al. 2021).

Mode of speciation in Thysanoteuthidae

Our results suggest that the speciation of *Thysanoteuthis* was not driven by strong ecological adaptations. Sajikumar et al. (2020) and Nigmatullin et al. (1995) identified two groups of *Thysanoteuthis* based on distribution, growth, and spawning time. They recognized one group distributed in tropical waters (*e.g.*, southeastern Arabian Sea) that grows fast and spawns throughout the year; and another group in subtropical waters (in Mediterranean, near southern Japan, and the Southeast Pacific) that grows slowly and spawns mainly in warm seasons. However, these two groups do not correspond to the species we found here: their 'subtropical group' includes individuals from all three of the species delimited here. Subtropical *Thysanoteuthis* of all known species appear to share similarities in growth rates and spawning, despite the large genetic divergences among them, indicating a convergent adaptation of these species to their environment. These similarities together with relatively slow, inactive swimming (Miyahara et al. 2008), patchy distribution of individuals (Nigmatullin et al. 1995), and allopatric distribution of *Thysanoteuthis* species, are typical characteristics of allopatric non-ecological speciation in divergent lineages (Czekanski-Moir and Rundell 2019). Under this mode of speciation, minimal ecological divergence is often observed, since species face similar environmental conditions within their respective geographic ranges (Czekanski-Moir and Rundell 2019).

Divergence in **Thysanoteuthis** *cryptic species*

The cryptic *Thysanoteuthis* species recognized here likely diverged during strong historical oceanographic changes and physical barriers. The midpoint split of *T. cf. filiferum* with the ancestor of *T. major* plus *T. rhombus* was around 25.84 Mya (HDP 95%: 17.37–34.73 Mya), and coincides with changes in the intensity of the Pacific Walker Circulation and an increase of the sea surface temperature gradient in the Pacific Ocean, during the Late Oligocene and Early Miocene (23–27 Mya) (Yan et al. 2021: Figs. 1, 3 d). These changes in ocean circulation and latitudinal gradient of temperatures joined with the absence of a strong anticlockwise warm current off the eastern of New Zealand (Fig. 1) might have enhanced isolation of *T.* cf. *filiferum*.

Our estimates also show that *T. major* and *T. rhombus* split around 8.58 Mya (HPD 95%: 5.07–12.37 Mya), the upper HPD coinciding with the time of collision of Central America with South America around 12 Mya, (Hou and Li 2018) and the lower HPD with the final closing of the Isthmus of Panama around 3.5 Mya (Leigh et al. 2014). These events may have acted as a physical barrier promoting the speciation of these two taxa, similar to what has been observed in the loliginid squids *Sepioteuthis* Blainville 1824 (Ulloa et al. 2017) and *Doryteuthis* (Sales et al. 2017), the odd bobtail *Heteroteuthis* Gray 1849 (Sanchez et al. 2021), and several octopuses genera such as *Muusoctopus* Gleadall 2004 (Gleadall 2013), *Octopus* Cuvier 1798 and *Paroctopus* Naef 1923 (Lima et al. 2020).

Similar patterns of cryptic allopatric speciation have also been reported in other oceanic squids, which may have been similarly influenced by the events described above (Supplementary Table S3). One such is the recently resolved *Ommastrephes* species complex, whose family Ommastrephidae is sister to the Thysanoteuthidae (Fernández-Álvarez et al. 2022). The distributions of *T. major* and T. cf. *filiferum* appear to correspond with those of *Ommastrephes bartramii* Lesueur 1821 (*Ommastrephes* Group 4) and *Ommastrephes brevimanus* (Gould 1852) (*Ommastrephes* Group 3) in the North and South Pacific Ocean (Fernández-Álvarez et al. 2020), respectively. In addition, the distribution of *Ommastrephes caroli* (Furtado 1887) (*Ommastrephes* Group 1) and *T. rhombus* appear to be similar in the northeast Atlantic Ocean.

The parallel divergences in cryptic *Ommastrephes* and *Thysanoteuthis* species may be related to similar life-history traits in early stages. Early ontogenetic stages were also proposed to be a main factor in speciation in *Ommastrephes* (Fernández-Álvarez et al. 2020). The buoyant egg masses of Ommastrephidae and Thysanoteuthidae drift over the pycnocline in the upper layers of the water column (Escánez-Pérez et al. 2012; Puneeta et al. 2015). Small paralarvae of 1.1–2.2 mm of mantle length hatch from these egg masses and drift with the ocean currents for weeks (Villanueva et al. 2016). These paralarvae are characterized by underdeveloped hunting features:

underdeveloped arms in Ommastrephidae, and arms mostly lacking suckers in *Thysanoteuthis* (see Fernández-Álvarez et al. 2017 and Wakabayashi et al. 2005, respectively). Both also lose the buccal papillae and develop stronger beaks and other prey-hunting characters, such as stronger arms, at similar sizes of 3–5 mm of mantle length. While in Ommastrephidae, these changes correspond to a transition from passive feeding on detritus to a more active predatory strategy (Fernández-Álvarez et al. 2018), the diets of paralarval *Thysanoteuthis* across these life stages remain unknown (Miyahara et al. 2006).

The cryptic lineages of *Thysanoteuthis* do appear to show one marked difference from those of *Ommastrephes*: *T. major* ranges from the North Pacific to the North Indian Ocean and its borders in the warm Indian current next to the South Atlantic Ocean, while *Ommastrephes* across this range are split into two different species: *O. bartrami* (North Pacific only) and *Ommastrephes cylindraceus* (d'Orbigny 1835 [in 1834–1847]) in the South Indian and South and Equatorial Atlantic Ocean (Fernández-Álvarez et al. 2020). Similarly, *O. caroli* distributes until the Canary Current and the North Atlantic Equatorial Current, while *T. rhombus* can cross these oceanographic barriers at least until the Gulf of Guinea. Differences in horizontal migration of adults might explain the wide geographic range of *T. major* and the speciation observed in *Ommastrephe*s. Horizontal dispersal is high in *Thysanoteuthis* paralarvae, young and adults forms, because it is associated with ocean drift currents in tropical and subtropical regions (Nishimura 1966; Bower and Miyahara 2005); while in *Ommastrephes*, adult dispersal is associated with suitable spawning grounds in subtropical regions and feeding grounds near subarctic boundaries (Bower and Ichii 2005).

Conservation of **Thysanoteuthis** *cryptic species*

Thysanoteuthis currently has a high commercial value in Japan (Arkhipkin et al. 2015; Bower and Miyahara 2005; Miyahara et al. 2008) and its harvesting is increasing in other locations such as The Philippines (Dickson et al. 2000). In addition, successfully exploratory fisheries targeting *Thysanoteuthis* have been conducted in several regions, such as New Caledonia, Fiji, Vanuatu, Cook Islands, Martinique Island, Dominican Republic, Santa Lucia Island, Jamaica, Maldives, and Canary Islands, suggesting a potential increase for worldwide fisheries of *Thysanoteuthis* in the coming years (Aiken et al. 2007; Herrera-Moreno et al. 2011; CRFM 2012; Escánez-Pérez et al. 2012; Nimoho et al. 2014; MASPLAN 2018). This reinforces the necessity for proper species and stock delimitations to ensure the sustainable harvesting of these species and their conservation.

While our analysis is limited to mitochondrial genes, it is clear that *Thysanoteuthis* species have large genetic divergences and should be considered and managed as different species. Thus, our study provides initial, genetic-based evidence to inform the development of regional management and conservation plans for *Thysanoteuthis*. The allopatric occurrence of *Thysanoteuthis* species should make it a relatively straightforward recognition and delimitation of separate stocks in the North Pacific Ocean (*T. major* in Japan and The Philippines as the main fisheries ground) and the North Atlantic Ocean (*T. rhombus*). In the latter ocean basin, the major fishery grounds are in the Caribbean islands where an emerging artisanal fishery has developed since the 2000s (Herrera-Moreno et al. 2011). In contrast, in the eastern Atlantic *T. rhombus* is only sporadically caught mainly in small fishing villages in the Canary Islands mainly as an accessory species of *Loligo forbesii* Steenstrup 1856 fishery (Escánez-Pérez et al. 2012). Thus, the species delimitation of *Thysanoteuthis* reported here will benefit its fisheries management in these locations and the new emerging fishing grounds.

Recent international initiatives for sustainable harvesting of large oceanic squids with wide distribution are limited to the Humboldt squid *Dosidicus gigas* (https://www.sprfmo.int/science/species-profiles/). For example, the Regional Fisheries Management Organizations (RFMOs) (*i.e.*, inter-governmental organization engaged in long-term conservation and sustainable harvesting of fisheries resources) have established management measures and responsibilities for vessels and countries targeting *D. gigas* that include capture quotas, restriction of fishing grounds, and satellite monitoring of fishing activities. In contrast, current management policies for *Thysanoteuthis* spp. have been only established in Japan (Okinawa), the Maldives, and waters off Hawaii and the United State (U.S.) Pacific Islands. In Okinawa, the place with the highest annual fishery landings in Japan, closed seasons and the prohibition and restriction for specific fishing methods are established as management measures (https://www.pref.okinawa.jp/site/norin/suisan/kaiku/sodeikagyoki.html). In the Maldives, the management plan includes the obtention of licenses for harvesting and processing, restriction of fishing areas and gears, and provision of information about geographic location of fishing areas and landings (https://www.gov.mv/en/files/draft-diamondback-squid-management-plan-2020.pdf--1). In the Pacific Islands of the U.S., permits are emitted for harvesting activities only within the exclusive economic zone of the Western Pacific and by boarding vessels greater than 50 ft in length (https://www.wpcouncil.org/). However, a broader approach involving international cooperation might be an option for a harvest of *Thysanoteuthis* species since a single is distributed across different ocean basins.

Applying our current understanding of these three species' distribution, 55.52%, 37.30%, and 4.30% of the historical records retrieved from the Global Biodiversity Information Facility (GBIF) (GBIF 2022) and the Ocean Biodiversity Information System (OBIS) (https://www.OBIS.org) since 1950 and sampled individuals in Nigmatullin et al. (1995) and Ikeda et al. (2003) likely correspond to *T. rhombus*, *T. major,* and *T.* cf. *filiferum* respectively. Of these, *T.* cf. *fileferum* clearly remains the least-known species, although it is not yet clear whether its small proportion of *Thysanoteuthis* records reflects lower population densities or simply less sampling effort (Supplementary Note 2, Supplementary Figure S3). Pending further samples from the South Pacific, a useful follow-up step would be estimating the genetic diversity of these cryptic species to evaluate potential population sizes and gain a clearer picture of their geographic range.

Establishing robust policies that can effectively manage sustainable fisheries requires understanding the biology of the intraspecific groups with temporal and spatial integrity randomly mating (*i.e.*, stocks, Ihssen et al. 2011). Thus, another important next step for *Thysanoteuthis* in regions where it is or may soon be fished is characterizing these species inter- and intra-specifically. Estimating variation at these levels would support the development of policies capable of preserving the short- and long-term integrity of stocks within each respective species (Carvalho and Hauser 1994). In addition, future efforts should be made to collect and analyse more samples from no surveyed areas in this study, such as the tropical western and eastern Atlantic Ocean, southern Indian Ocean, and southern Pacific Ocean regions, in search of additional cryptic species in this genus.

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Author contributions DD generated and analysed the data and wrote the manuscript under the supervision of GS. SM and KK executed the experiments and generated data. AE, AM, FL, HB, JGM, KKS, KSohamed, KM, ChS, FÁFÁ, and GS provided samples and edited the manuscript. GS and FÁFÁ supervised the study, designed the research, and provided funding. All the authors reviewed and approved the final manuscript.

Data availability Alignments and raw data generated can be found in FigShare (https://doi.org/10.6084/m9.figshare.22559374). All the new sequences generated here are in the National Center for Biotechnology Information (NCBI) database under the GenBank accession numbers OP970836-OP970872 (cytochrome oxidase *c* subunit I.), OP970877-OP970912 (16S ribosomal RNA), and OP971221-OP971257 (12S ribosomal RNA).

Declarations

Conflict of interest All the authors declared that they have no conflict of interest.

References

Aiken K, Kumagai N, Yasuda T, Jones I (2007) The egg trace method of identifying diamondback squid fishing grounds in Jamaican waters.

Alejo-Plata MDC, Urbano-Alonso B (2018) The finding of diamond squid *Thysanoteuthis rhombus* in the Gulf of Tehuantepec Northeastern Tropical Pacific. Hidrobiológica 28(1):147–150

Amor MD, Norman MD, Cameron HE, Strugnell JM (2014) Allopatric speciation within a cryptic species complex of Australasian Octopuses. PLoS ONE 9:e98982. https://doi.org/10.1371/journal.pone.0098982

Anderson FE, Marian JEAR (2020) The grass squid *Pickfordiateuthis pulchella* is a paedomorphic loliginid. Mol Phylogenet Evol 147:106801. https://doi.org/10.1016/j.ympev.2020.106801

Arkhipkin AI, Rodhouse PGK, Pierce GJ et al (2015) World squid fisheries. Rev Fish Sci Aquacult 23:92–252. https://doi.org/10.1080/23308249.2015.1026226

Asahida T, Kobayashi T, Saitoh K, Nakayama I (1996) Tissue preservation and total DNA extraction form fish stored at ambient temperature using buffers containing high concentration of urea. Fish Sci 62:727–730

Bickford D, Lohman DJ, Sodhi NS et al (2007) Cryptic species as a window on diversity and conservation. Trends Ecol Evol 22:148–155. https://doi.org/10.1016/j.tree.2006.11.004

Blainville HMD de (1824) Mollusques, Mollusca (Malacoz.), 1–392. In: Dictionnaire des Sciences Naturelles (F. Cuvier, ed.), 32. Levrault, Strasbourg et Paris, & Le Normant, Paris.

Bouckaert RR, Drummond AJ (2017) bModelTest: bayesian phylogenetic site model averaging and model comparison. BMC Evol Biol 17:42. https://doi.org/10.1186/s12862-017-0890-6

Bouckaert R, Vaughan TG, Barido-Sottani J et al (2019) BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. PLoS Comput Biol 15:e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Bower JR, Ichii T (2005) The red flying squid (*Ommastrephes bartramii*): a review of recent research and the fishery in Japan. Fish Res 76:39–55. https://doi.org/10.1016/j.fishres.2005.05.009

Bower JR, Miyahara K (2005) The diamond squid (*Thysanoteuthis rhombus*): a review of the fishery and recent research in Japan. Fish Res 73:1–11. https://doi.org/10.1016/j.fishres.2005.01.020

Carvalho GR, Hauser L (1994) Molecular genetics and the stock concept in fisheries. Rev Fish Biol Fish 4:326–350. https://doi.org/10.1007/BF00042908

Cheng J, Sha Z (2017) Cryptic diversity in the Japanese mantis shrimp *Oratosquilla oratoria* (Crustacea: Squillidae): allopatric diversification, secondary contact and hybridization. Sci Rep 7:1972. https://doi.org/10.1038/s41598-017-02059-7

CRFM (2012) Report of Eighth Annual Scientific Meeting – Kingstown, St. Vincent & the Grenadines, National Reports. CRFM Fishery Report, 1(1): 54.

Cuvier G (1798) Tableau élémentaire de l'histoire naturelle des animaux. Baudoin, Paris.: i-xvi, 1–710, Pl. 1–14.

Czekanski-Moir JE, Rundell RJ (2019) The ecology of non ecological speciation and nonadaptive radiations. Trends Ecol Evol 34:400–415. https://doi.org/10.1016/j.tree.2019.01.012

d'Orbigny A (1834) Mollusques. Voyage Dans L'amérique Meridionale 5:1–758

Dickson JO, Ramiscal RV, Magno B (2000) Diamondback squid (*Tysanoteuthis rhombus*) exploration in the South China Sea, Area III: Western Philippines. In: Proceedings of the Third Technical Seminar on Marine Fishery Resources Survey in the South China Sea, Area III: Western Philippines, 1999. Secretariat, Southeast Asian Fisheries Development Center, 32–38.

Doubleday ZA, Prowse TAA, Arkhipkin A et al (2016) Global proliferation of cephalopods. Curr Biol 26:R406–R407. https://doi.org/10.1016/j.cub.2016.04.002

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797. https://doi.org/10.1093/nar/gkh340

Escánez-Pérez AE, Elena RR, González AFG, Sierra AG (2012) On the occurrence of egg masses of the diamond-shaped squid *Thysanoteuthis rhombus* Troschel, 1857 in the subtropical eastern Atlantic (Canary Islands). A potential commercial species? Zookeys 222:69–76. https://doi.org/10.3897/zookeys.222.2835

Esselstyn JA, Evans BJ, Sedlock JL et al (2012) Single-locus species delimitation: a test of the mixed Yule–coalescent model, with an empirical application to Philippine round-leaf bats. Proc Royal Soc b: Biol Sci 279:3678–3686. https://doi.org/10.1098/rspb.2012.0705

FAO (2022) The state of world fisheries and aquaculture 2022. Rome, FAO, Towards Blue Transformation. https://doi.org/10.4060/cc0461en

Fernández-Álvarez FÁ, Martins CPP, Vidal EAG, Villanueva R (2017) Towards the identification of the ommastrephid squid paralarvae (*Mollusca*: *Cephalopoda*): morphological description of three species and a key to the north-east Atlantic species. Zool J Linn Soc 180:268–287. https://doi.org/10.1111/zoj.12496

Fernández-Álvarez FÁ, Machordom A, García-Jiménez R et al (2018) Predatory flying squids are detritivores during their early planktonic life. Sci Rep 8:3440. https://doi.org/10.1038/s41598-018-21501-y

Fernández-Álvarez FÁ, Braid HE, Nigmatullin CM et al (2020) Global biodiversity of the genus *Ommastrephes* (*Ommastrephidae*: *Cephalopoda*): an allopatric cryptic species complex. Zool J Linn Soc 190:460–482. https://doi.org/10.1093/zoolinnean/zlaa014

Fernández-Álvarez FÁ, Sánchez P, Villanueva R (2021) Morphological and molecular assessments of bobtail squids (*Cephalopoda*: *Sepiolidae*) reveal a hidden history of biodiversity. Front in Mar Sci 7:1233. https://doi.org/10.3389/fmars.2020.632261

Fernández-Álvarez FÁ, Taite M, Vecchione M et al (2022) A phylogenomic look into the systematics of oceanic squids (order Oegopsida). Zool J Linn Soc 194:1212–1235. https://doi.org/10.1093/zoolinnean/zlab069

Folmer O, Black M, Hoeh W et al (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Fuchs D, Iba Y, Ifrim C et al (2013) *Longibelus* gen. nov., a new Cretaceous coleoid genus linking Belemnoidea and early Decabrachia. Palaeontology 56:1081–1106. https://doi.org/10.1111/pala.12036

Furtado A (1887) Sur une nouvelle espèce de céphalopode appartement an genre Ommatostrephes. Memorias Da Academia Real Das Sciencas De Lisboa 6(2):3–16

GBIF (2022) GBIF Occurrence Download https://doi.org/10.15468/dl.yrvgrv. Accessed 3 Jul 2022.

Gernhard T (2008) The conditioned reconstructed process. J Theor Biol 253:769–778. https://doi.org/10.1016/j.jtbi.2008.04.005

Glaubrecht M, Salcedo-Vargas MA (2000) Annotated type catalogue of the *Cephalopoda* (Mollusca) in the Museum für Naturkunde, Humboldt University of Berlin. Mitteilungen Aus Dem Museum Für Naturkunde Berlin, Zoologischen 76(2):269–282

Gleadall IG (2004) Some old and new genera of octopus. Interdisciplin Inform Sci 10(2):99–112

Gleadall IG (2013) A molecular sequence proxy for *Muusoctopus januarii* and calibration of recent divergence among a group of mesobenthic octopuses. J Exp Mar Biol Ecol 447:106–122. https://doi.org/10.1016/j.jembe.2013.02.017

Gould AA (1852) Mollusca and shells. In: United States exploring expedition during the years 1838, 1839, 1840, 1841, 1842 under the command of Charles Wilkes. Boston. 12: 1–510; atlas 1856: 1–16.

Gray JE (1828) Spicilegia Zoologica; or original figures and short systematic descriptions of new andunfigured animals. Treuttel, Wurtz and Co., London, Part I

Gray JE (1849) Catalogue of the Mollusca in the collection of the British Museum I: Cephalopoda antepedia. British Museum, London, p 164

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM (2004) Identification of birds through DNA Barcodes. PLoS Biol 2:e312. https://doi.org/10.1371/journal.pbio.0020312

Herrera-Moreno A, Betancourt Fernández L, Silva M, et al (2011) Coastal fisheries of the Dominican Republic. Rome: Food and Agriculture Organization of the United Nations (FAO).

Hoang DT, Chernomor O, von Haeseler A et al (2017) UFBoot2: improving the ultrafast bootstrap approximation. Mol Biol Evol. https://doi.org/10.1093/molbev/msx281

Hoffmann R, Weinkauf MFG, Fuchs D, Lukeneder A (2021) Is there more than one species in the genus Spirula (*Cephalopoda*: *Decabrachia*): evidence for an Atlantic-Pacific divide. J Molluscan Stud. https://doi.org/10.1093/mollus/eyab001

Hou Z, Li S (2018) Tethyan changes shaped aquatic diversification. Biol Rev Camb Philos Soc 93:874–896. https://doi.org/10.1111/brv.12376

Hoyle WE (1904) Reports on the Cephalopoda. Bull Museum Comp Zool at Harvard College Cambridge 43(1):1–72

Hudson RR (1990) Gene genealogies and the coalescent process. Oxford Surv Evolut Biol 7:44

Ihssen PE, Booke HE, Casselman JM et al (2011) Stock identification: materials and methods. Can J Fish Aquat Sci. https://doi.org/10.1139/f81-230

Ikeda Y, Onaka S, Kidokoro H, Sakamoto W (2003) Notes on an unsusual catch of the juvenile diamond-shaped squid *Thysanoteuthis rhombus* in Pacific waters off southern Hokkaido. Fish Sci 69:660–662. https://doi.org/10.1046/j.1444-2906.2003.00671.x

Joubin L (1933) Notes préliminaires sur les Céphalopodes des croisières du DANA (1921–1922), 4e Partie. Annals De L'institut Océanographiques 13(1):1–49

Kalyaanamoorthy S, Minh BQ, Wong TKF et al (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods 14:587–589. https://doi.org/10.1038/nmeth.4285

Keferstein W (1866) Kopffusser: Cephalopoda Cuv., 1307–1464, plates 110–136. In H.G. Bronn, Die Klassen und Ordnungen des Thier-reiches: Weichthiere (Malacozoa). 1500 pages, 136 plates. Leipzig und Heidelberg, 1862–1866.

Kendall DG (1948) On the generalized "birth-and-death" process. Annal Math Stat 19:1–15

Kitaura J, Yamamoto G, Nishida M (1998) Genetic Variation in populations of the diamond-shaped squid *Thysanoteuthis rhombus* as examined by mitochondrial DNA sequence analysis. Fish Sci 64:538–542. https://doi.org/10.2331/fishsci.64.538

Kristensen TK, Knudsen J (1983) A catalogue of the type specimens of *Cephalopoda* (Mollusca) in the zoological museum. Univ Copenhagen Steenstrupia 9(10):217–227

Kumar S, Stecher G, Li M et al (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics 30:3276–3278. https://doi.org/10.1093/bioinformatics/btu531

Leach WE (1817) Synopsis of the orders, families and genera of the class Cephalopoda. Zool Misc Being Descript Interest Anim 3(30):137–141

Lee E-Y, Park K-A (2019) Change in the recent warming trend of sea surface temperature in the East Sea (Sea of Japan) over decades (1982–2018). Remote Sens 11:2613. https://doi.org/10.3390/rs11222613

Leigh JW, Bryant D (2015) popart: full-feature software for haplotype network construction. Methods Ecol Evol 6:1110–1116. https://doi.org/10.1111/2041-210X.12410

Leigh EG, O'Dea A, Vermeij GJ (2014) Historical biogeography of the Isthmus of Panama. Biol Rev Camb Philos Soc 89:148–172. https://doi.org/10.1111/brv.12048

Lesueur CA (1821) Descriptions of several new species of cuttlefish. J Acad Nat Sci Philadelphia 2(1):86–101

Lima FD, Strugnell JM, Leite TS, Lima SMQ (2020) A biogeographic framework of octopod species diversification: the role of the Isthmus of Panama. PeerJ 8:e8691. https://doi.org/10.7717/peerj.8691

Lindgren AR (2010) Molecular inference of phylogenetic relationships among *Decapodiformes* (*Mollusca*: *Cephalopoda*) with special focus on the squid order Oegopsida.

Mol Phylogenet Evol 56:77–90. https://doi.org/10.1016/j.ympev.2010.03.025

Linnaeus C (1758). Systema Naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Editio decima, reformata, 1: 824

Loor-Andrade P, Pincay-Espinoza J, Carrera-Fernández M, Rosas-Luis R (2017) Feeding habits of billfishes (Carangaria: Istiophoriformes) in the Ecuadorian Pacific Ocean. Neotrop Ichthyol. https://doi.org/10.1590/1982-0224-20160162

MASPLAN (2018) Republic of Maldives Project for the formulation of Master plan for sustainable Fisheries (MASPLAN). Technical report of the Republic of Maldives Ministry of Fisheries and Agriculture.

Meier R, Zhang G, Ali F (2008) The use of mean instead of smallest interspecific distances exaggerates the size of the "barcoding gap" and leads to misidentification. Syst Biol 57:809–813

Minh BQ, Schmidt HA, Chernomor O et al (2020) IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol 37:1530–1534. https://doi.org/10.1093/molbev/msaa015

Miyahara K, Gorie S (2005) Comparison of CPUEs in the diamond squid angling fishery off Hyogo prefecture, the Sea of Japan. Bull. Hyogo Pref. Tech Cent Agri Forest Fish (fish Sect) 38:15–23 ((**in Japanese with English abstract**))

Miyahara K, Fukui K, Ota T et al (2006) Laboratory observations on the early life stages of the diamond squid *Thysanoteuthis rhombus*. J Molluscan Stud 72:199–205

Miyahara K, Ota T, Hatayama J et al (2008) Tagging studies on the diamond squid (*Thysanoteuthis rhombus)* in the western Sea of Japan. Bull Jap Soc Fish Oceanogr 72:30–39

Monaghan MT, Wild R, Elliot M et al (2009) Accelerated species inventory on Madagascar using coalescent-based models of species delineation. Syst Biol 58:298–311. https://doi.org/10.1093/sysbio/syp027

Naef A (1912) Teuthologische Notizen. 4 Die Gattungen der Loliginidae. Zool Anz 39:741–745

Naef A (1923) Cephalopoda Part III. Fauna Flora Gulf of Naples Monograph 35(2):313–917

Neige P, Lapierre H, Merle D (2016) New Eocene coleoid (Cephalopoda) diversity from statolith remains: Taxonomic assignation, fossil record analysis, and new data for calibrating molecular phylogenies. PLoS ONE 11:e0154062. https://doi.org/10.1371/journal.pone.0154062

Nesis KN (1987) Cephalopods of the World; Squids, cuttlefishes, Octopuses, and Allies. Publications, Neptune City, NJ, USA, T.F.H

Neves JMM, Almeida JPFA, Sturaro MJ et al (2020) Deep genetic divergence and paraphyly in cryptic species of Mugil fishes (Actinopterygii: Mugilidae). Syst Biodiv 18:116–128. https://doi.org/10.1080/14772000.2020.1729892

Nieuwenhove AHMV, Ratsimbazafy HA, Kochzius M (2019) Cryptic diversity and limited connectivity in octopuses: recommendations for fisheries management. PLoS ONE 14:e0214748. https://doi.org/10.1371/journal.pone.0214748

Nigmatullin CM, Arkhipkin AI, Sabirov RM (1995) Age, growth and reproductive biology of diamond-shaped squid *Thysanoteuthis rhombus* (Oegopsida:Thysanoteuthidae). Marine Ecol Prog Series 124:73–87

Nigmatullin CM, Arkhipkin AI (1998) A review of the biology of the diamondback squid, *Thysanoteuthis rhombus* (Oegopsida: Thysanoteuthidae). In: Okutani T (ed) Contributed papers to International Symposium on Large Pelagic Squids : July 18–19, 1996, for JAMARC's 25th anniversary of its foundation. Japan Marine Fishery Resources Research Center, Tokyo, 155–181.

Nimoho G, Amos G, Fujii M et al (2014) Diamondback squid and egg mass record in Vanuatu. SPC Fish Newslett 144:48–52

Nishimura S (1966) Notes on the occurrence and biology of the oceanic squid, *Thysanoteuthis rhombus* troschel, in japan. Publ Seto Mar Biol Lab 14:327–349. https://doi.org/10.5134/175443

Okutani T, Tagawa M, and Horikawa H (1987) Cephalopods from continental shelf and slope around Japan. 194 pages. Tokyo: Japan Fisheries Resource Conservation Association.

Onitsuka G, Hirose N, Miyahara K et al (2010) Numerical simulation of the migration and distribution of diamond squid (Thysanoteuthis rhombus) in the southwest Sea of Japan. Fish Oceanogr 19:63–75. https://doi.org/10.1111/j.1365-2419.2009.00528.x

Owen R (1836) Descriptions of some new and rare Cephalopoda, collected by Mr. George Bennet, Corr. Memb. Z.S. Proc Zool Soc London 37:19–24

Palumbi (1996) Nucleic acids II : the polymerase chain reaction. Molecular systematics 205–247.

Pardo-Gandarillas MC, Torres FI, Fuchs D, Ibáñez CM (2018) Updated molecular phylogeny of the squid family Ommastrephidae: Insights into the evolution of spawning strategies. Mol Phylogenet Evol 120:212–217. https://doi.org/10.1016/j.ympev.2017.12.014

Park K-A, Park J-E, Choi B-J et al (2013) An oceanic current map of the east sea for science textbooks based on scientific knowledge acquired from oceanic measurements. Sea: J Korean Soc Oceanograph 18:234–265. https://doi.org/10.7850/jkso.2013.18.4.234

Pfeffer G (1912) Die Cephalopoden der Plankton-Expedition. Zugleich eine monographische übersicht der Oegopsiden Cephalopoden. Ergebnisse Der Plankton-Expedition Der Humboldt-Stiftung 2:1–815

Pons J, Barraclough TG, Gomez-Zurita J et al (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst Biol 55:595–609. https://doi.org/10.1080/10635150600852011

Puillandre N, Lambert A, Brouillet S, Achaz G (2012) ABGD, automatic barcode gap discovery for primary species delimitation. Mol Ecol 21:1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x

Puneeta P, Vijai D, Yoo H-K et al (2015) Observations on the spawning behavior, egg masses and paralarval development of the ommastrephid squid *Todarodes pacificus* in a laboratory mesocosm. J Exp Biol 218:3825–3835. https://doi.org/10.1242/jeb.127670

Rambaut A, Drummond AJ, Xie D et al (2018) Posterior summarization in bayesian phylogenetics using tracer 1.7. Syst Biol 67:901–904. https://doi.org/10.1093/sysbio/syy032

Rannala B, Yang Z (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. J Mol Evol 43:304–311. https://doi.org/10.1007/BF02338839

Rannala B, Yang Z (2003) Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. Genetics 164:1645–1656. https://doi.org/10.1093/genetics/164.4.1645

Reid NM, Carstens BC (2012) Phylogenetic estimation error can decrease the accuracy of species delimitation: a Bayesian implementation of the general mixed Yule-coalescent model. BMC Evol Biol 12:196. https://doi.org/10.1186/1471-2148-12-196

Roux C, Fraïsse C, Romiguier J et al (2016) Shedding light on the grey zone of speciation along a continuum of genomic divergence. PLoS Biol 14:e2000234. https://doi.org/10.1371/journal.pbio.2000234

Sajikumar KK, Sasikumar G, Venkatesan V et al (2020) Distribution, age and growth of the diamondback squid, *Thysanoteuthis rhombus* (Cephalopoda: Thysanoteuthidae) from the tropical Arabian Sea. Fish Res 224:105478. https://doi.org/10.1016/j.fishres.2019.105478

Sales JBDL, Rodrigues-Filho LFDS, Ferreira YDS, Carneiro J, Asp NE, Shaw PW, Sampaio I (2017) Divergence of cryptic species of *Doryteuthis plei* Blainville, 1823 (Loliginidae, Cephalopoda) in the Western Atlantic Ocean is associated with the formation of the Caribbean Sea. Mol Phylogene Evol 106:44–54. https://doi.org/10.1016/j.ympev.2016.09.014

Sanchez G, Fernández-Álvarez FÁ, Taite M et al (2021) Phylogenomics illuminates the evolution of bobtail and bottletail squid (order Sepiolida). Commun Biol 4:1–9. https://doi.org/10.1038/s42003-021-02348-y

Sasaki M (1929) A monograph of the dibranchiate cephalopods of the Japanese and adjacent waters. Journal of the College of Agriculture, Hokkaido Imperial University 20(supplement):1–357

Sauer WHH, Gleadall IG, Downey-Breedt N et al (2019) World octopus fisheries. Rev Fish Sci Aquacult. https://doi.org/10.1080/23308249.2019.1680603

Secor DH (2014) The Unit Stock Concept: Bounded Fish and Fisheries. In: Stock Identification Methods. Academic Press, https://doi.org/10.1016/B978-0-12-397003-9.00002-3

Simon C, Frati F, Beckenbach A et al (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomol Soc Am 87:651–701. https://doi.org/10.1093/aesa/87.6.651

Steenstrup J (1857) Oplysning om en ny Art af Blaekspruetter. Oversigt over Det Kongelige Danske Videnskabernes Selskabs Forhandlinger 1857(1/2):11–14

Sweeney MJ and Young RE (2003) Taxa associated with the Family Thysanoteuthidae Keferstein, 1866. http://tolweb.org/accessory/Thysanoteuthidae_Taxa?acc_id=2333 [29–01–2021]

Tang Y, Zhang X, Ma Y, Zheng X (2021) Descriptive study of the mitogenome of the diamondback squid (*Thysanoteuthis rhombus* Troschel, 1857) and the evolution of mitogenome arrangement in oceanic squids. J Zoolog Syst Evol Res. https://doi.org/10.1111/jzs.12478

Tanner AR, Fuchs D, Winkelmann IE et al (2017) Molecular clocks indicate turnover and diversification of modern coleoid cephalopods during the Mesozoic marine revolution. Proc Royal Soc b: Biol Sci 284:20162818. https://doi.org/10.1098/rspb.2016.2818

Troschel (1857a) (Teuthida: Thysanoteuthidae) calamar diamante, registro en la zona costera de Ica, Perú. Rev Peru Biol 28:e18055–e18098

Troschel FH (1857b) Bermerkungen uber die Cephalopoden von Messina. Archiv Fur Naturgeschichte 23(1):40–76

Udny Yule G (1925) A mathematical theory of evolution, based on the conclusions of Dr. JC Willis, FRS. Philos Trans R Soc Lond B Biol Sci 213:21–87

Ulloa PM, Hernández CE, Rivera RJ, Ibáñez CM (2017) Biogeografía histórica de los calamares de la familia Loliginidae (Teuthoidea: Myopsida). Latin American J Aquat Res 45:113–129. https://doi.org/10.3856/vol45-issue1-fulltext-11

Villanueva R, Vidal EAG, Fernández-Álvarez FÁ, Nabhitabhata J (2016) Early mode of life and hatchling size in cephalopod molluscs: influence on the species distributional ranges. PLoS ONE 11:e0165334. https://doi.org/10.1371/journal.pone.0165334

von Der Heyden S, Beger M, Toonen RJ, van Herwerden L, Juinio-Meñez MA, Ravago-Gotanco R, Bernardi G (2014) The application of genetics to marine management and conservation: examples from the Indo-Pacific. Bull Marine Sci 90(1):123–158. https://doi.org/10.5343/bms.2012.1079

Wakabayashi T, Tsuchiya K, Segawa S (2005) Morphological changes with growth in the paralarvae of the diamondback squid *Thysanoteuthis rhombus* Troschel, 1857. Phuket Marine Biol Center Res Bull 66:167–174

Yan Q, Korty R, Zhang Z et al (2021) Large shift of the Pacific walker circulation across the Cenozoic. Natl Sci Rev 8:nwaa101. https://doi.org/10.1093/nsr/nwaa101

Yang Z (2015) The BPP program for species tree estimation and species delimitation. Curr Zool 61:854–865. https://doi.org/10.1093/czoolo/61.5.854

Yang Z, Rannala B (2010) Bayesian species delimitation using multilocus sequence data. Proc Natl Acad Sci U S A 107:9264–9269. https://doi.org/10.1073/pnas.0913022107

Yang Z, Rannala B (2014) Unguided species delimitation using DNA sequence data from multiple Loci. Mol Biol Evol 31:3125–3135. https://doi.org/10.1093/molbev/msu279

Yoder AD, Burns MM, Génin F (2002) Molecular evidence of reproductive isolation in sympatric sibling species of mouse lemurs. Int J Primatol 23:1335–1343. https://doi.org/10.1023/A:1021187106641

Zhang R, Drummond A (2020) Improving the performance of Bayesian phylogenetic inference under relaxed clock models. BMC Evol Biol 20:1–28. https://doi.org/10.1186/s12862-020-01609-4

Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29:2869–2876. https://doi.org/10.1093/bioinformatics/btt499

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