Taxonomy is destiny: resolving the systematics of unstable squid families using integrative taxonomy to aid cephalopod conservation

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A thesis submitted through the
Institute for Applied Ecology New Zealand,
Auckland University of Technology

in fulfilment of the requirements for the degree of
Doctor of Philosophy

supervised by Dr Kat S. R. Bolstad

2018

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material that, to a substantial extent, has been submitted for the award of any other degree or diploma from a university or institute of higher learning.

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Co-Authored Work

Chapter 3 of this thesis was co-authored with Tsunemi Kubodera and Kathrin S. R. Bolstad. The author of this thesis contributed 80% to this manuscript (designed study, identified samples with morphology, performed genetic analyses, and wrote the manuscript), T. Kubodera contributed 10% (collected tissue samples, reviewed the manuscript) and K. S. R. Bolstad contributed 10% (helped design study, and proofread and reviewed the manuscript).

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Foreward

The new nomenclature in this thesis is not issued for public and permanent scientific record, or for purposes of zoological nomenclature, and is not published within the meaning of the International Code of Zoological Nomenclature (ICZN).

Dedication

This thesis is for the squids.

Acknowledgements

Above all, I would like to thank Kat Bolstad—the best Doktorvater I could have hoped for—for her fantastic proofreading skills, endless patience, and constant reassurance; without her, this thesis would never have been completed. Many thanks to my kaupapa whānau in the AUT Lab for Cephalopod Ecology & Systematics (ALCES): Alex Lischka for moral support, listening and understanding throughout this process; Aaron Boyd Evans for going on amazing squid adventures in the world as well as in taxonomy; and Jesse Kelly, for encouraging me to be independent and for being fantastic company during many late nights in the lab. Thanks to past members of ALCES: Steve O'Shea for the most intact *Asperoteuthis lui* specimen available from New Zealand waters for morphological examination, octopus advice, and for being the reason I started on the path of squid taxonomy; and Jens Horstkotte for his work on the New Zealand histioteuthids.

Thanks to my whānau in Canada: Mum and Dad, Wendy and Andy Braid, for their support; my sister Debbie Harn for being there to talk to when I needed someone, at all hours; my brother-in-law Bryan Harn and my nephew Tobin Harn for being there and listening to my squid stories; Katie Arnup, for visiting me and understanding; Kareen Drot, for always listening; and my Granny and Granddad, Audrey and Philip Braid, for always encouraging my pursuit of the science I love. Thanks to Natasha Serrao for always being available to talk to; John James Wilson and Ariel Levitsky for always catching up when we can; Tim Bartley for meaningful talks and life advice; Chris Ho for always making time (and tea) for me.

Thanks to my whānau in New Zealand: Gary Peebles, for helping me get through the last several years—we named *Fragariateuthis* after you; Hazel Raeside for listening to my stories about squids and phylogenies; Zeke Raeside for making my life better and more balanced; Anne Lloyd-Jones for help with squid dissections; Brenda Lloyd for good conversations; Ryan Foote for being good company; J. Berit Bolstad Anderson for hugs; Keegan Lloyd-Jones for keeping me company close to the end; and my fellow Green Ninjas, especially Luke Sullivan and Lilli Matthews, for meaningful conversations and support.

I would like to thank members of the Hanner Lab at the University of Guelph, both past and present for their help through my PhD journey, especially Bob Hanner, for reagents, lab space, abundant stories, encouragement, and for helping me to keep fighting the good fight. Thanks to Jeff Gross at the University of Guelph Genomics Facility for beautiful sequences, insightful advice, and reassurance.

Thanks to other people at the Auckland University of Technology: Dave Towns, my secondary supervisor, for helping me find the story in thesis; Chris Pook, for being there when I needed him, so we could make cool things like a laser-cut 96-well gel comb and tray; Ruth Cink, for being an honorary ALCES member; Rachel Boyle, for advice in the lab; Paul McBride for assistance with phylogenies; Kevin Lee for advice on phylogeny building; Colleen Higgins for advice on DNA extractions; and Fabrice Merien from the Roche Lab for advice in the lab.

Thanks to Annie Lindgren at the Center for Life in Extreme Environments at Portland State University for her advice on phylogeny building and different programs. Thanks to Jan Strugnell at the Centre for Sustainable Tropical Fisheries and Aquaculture at James Cook University, for letting me come and work in her lab. Thanks to Mandy Reid at the Australia Museum (AM) and to Mike Vecchione at the Smithsonian National Museum of Natural History (NMNH) for tissue used in Chapter 4. Thanks to Saji Kumar at the Central Marine Fisheries Research Institute (CMFRI) for tissue of *Histiothauma* aff. *miranda* used in Chapter 4. Thanks to Alejandro Salcedo-Vargas for his insight into the chiroteuthid families and for sharing his experiences with me.

I am grateful to the Royal Society of New Zealand's Hutton Fund, which supported the genetic analysis for Chapters 2 and 3. Thanks to AUT for the Vice Chancellor scholarship, which supported me during my studies. Thanks to the Roche Lab at the Auckland University of Technology for consumables and lab space for the genetic analysis for funding part of the sequencing for Chapters 4 and 5. Thanks to NIWA for funding part of the sequencing for Chapter 5.

I would like to thank the Museum of New Zealand Te Papa Tongarewa, particularly Bruce Marshall for his taxonomic insight and help accessing specimens in the collection, and the National Institute of Water and Atmospheric Research, Ltd. (NIWA); thanks to Sadie Mills and Diana MacPherson for indefatigable on-site help and support in Wellington, and especially to Darren Stevens for his beak identifications, access to his beak collection, and photographs of *Asperoteuthis lui*. Thanks to the National Museum of Nature and Science (NSMT) for allowing access to the collections,

particularly Mrs. Yatabe and to Tsunemi Kubodera, for his amazing collection of tissue that was used in Chapters 3 and 4. Thanks to Adrian Turner at the Auckland University for assistance with critical-point drying, and Patrick Conor at AUT for platinum plating, and imaging assistance. I would like to thank the Field Museum of National History (FMNH), particularly Janet Voight, Jochen Gerber, and Martin Pryzdia for tissue samples of chiroteuthids and histioteuthids from the eastern Pacific Ocean.

Many thanks the scientists and crew of the RV *Tangaroa* for specimens that were used in Chapters 2, 3, 4 and 5 and for always checking carefully for cephalopods (especially Ian "Hearn" Smith, for finding the most complete *Asperoteuthis lui* specimen available for genetic analysis). Special thanks to Neil Bagley for being an amazing shift leader; Derrick Parkinson for helping me adjust to life on a boat and for saving baby sharks; Dan 'Safety Dan' MacGibbon for specimen collection and special annotations; Bryce Bennett for assistance with specimen collection and helping me learn how to tie knots; Lucy Van Oosterom for helping me every day; Brit Finucci for going through the experience of TAN1401 with me; Bex Gibson for excellent company; Adèle Dutilloy for amazing company, support, and creativity; and Blair Lovell-Gadd for helping me through over a month at sea.

Specimens from Chapter 5 were collected as part of the project "Biodiversity of the Kermadec Islands and offshore waters of the Kermadec Ridge—a coastal, marine mammal and deep-sea survey (TAN1612)". Support for the survey came from Marine Funding Advisory Research Group, the National Institute of Water and Atmospheric Research Ltd (NIWA), Te Ohu Kai Moana, Ngati Kuri and Te Aupouri. The following institutions funded staff to participate in the voyage: University of Auckland, Auckland War Memorial Museum, Department of Conservation, Massey University, Museum of New Zealand Te Papa Tongarewa, NIWA, and The Pew Charitable Trust; with additional budget and gear support from Auckland War Memorial Museum, Ministry for the Environment, NIWA (project COBR1705), Te Papa, and The Pew Charitable Trust.

I would like to thank the band Frightened Rabbit, for their beautiful music that helped me get through this entire process. Especially Scott, who is deeply missed.

And thanks to the squids who were used in this study.

Abstract

The waters around New Zealand host one of the highest biodiversities of squid and octopus species in the world, yet until the late 1990s, little taxonomic work was published on this unique assemblage. While some groups have recently been (or are in the process of being) resolved, others remain poorly understood, and certain regions also remain insufficiently studied. Accurate portraits of these regions' teuthofauna have historically been precluded both by limited sample availability, and by lack of taxonomic clarity in the locally occurring groups. The infrequent capture and fragmentary nature of deep-sea squid specimens have also contributed to systematic confusion, but the field of integrative taxonomy (using molecular data in combination with traditional morphological characters) shows great promise as a tool for resolving cephalopod taxonomy at both the species and higher levels. This thesis uses integrative taxonomy to remedy some of the greatest knowledge gaps in our understanding of the New Zealand (and wider Pacific) teuthofauna, by assessing the cephalopod diversity of the Kermadec Islands region, following much-needed studies on the ecologically important (but taxonomically problematic) deep-sea squid clades Histioteuthidae and the chiroteuthid families.

The Kermadec Islands in northern New Zealand waters are in a near-pristine environment that has barely begun to be explored. In order to protect the biodiversity of this area, the Kermadec–Rangitāhua Ocean Sanctuary has been proposed, to extend the area of protection offered by the existing Kermadec Marine Reserve. However, the cephalopod biodiversity of this area—especially the deep-sea taxa—remains poorly known, and a better understanding is required for the establishment of the Sanctuary. Specimens collected on the recent expedition titled 'Biodiversity of the Kermadec Islands and offshore waters of the Kermadec Ridge—a coastal, marine mammal and deep-sea survey (TAN1612)' enabled an integrative taxonomic approach to critically appraise the region's cephalopod fauna. However, several groups considered likely to occur in the region were known to require taxonomic attention; thus, studies were first undertaken to improve the systematic resolution in the taxonomically unstable 'chiroteuthid families' and the histioteuthids, in order to increase the accuracy of this appraisal.

The deep-sea chiroteuthid families represent a clade of taxonomically problematic squids, united by their tentacle-club morphology, and include the Chiroteuthidae, Mastigoteuthidae, Joubiniteuthidae, Promachoteuthidae, Batoteuthidae, and Magnapinnidae. These families are all poorly known, but following a recent review

of the Mastigoteuthidae, the Chiroteuthidae is the most speciose family and the one most in need of taxonomic attention. In order to test whether oegopsid species-level systematic resolution could be improved using a combination of three mitochondrial genes (cytochrome *c* oxidase subunit I [COI], 16S rRNA, and 12S rRNA) and morphological characters, *Asperoteuthis lui* was used as a case study. This large, enigmatic chiroteuthid was previously only known from its holotype (a partial specimen taken from a fish stomach). This species now appears to have a circumglobal austral distribution rather than being a New Zealand endemic, as was previously reported, and is the most commonly consumed asperoteuthid in the diets of several apex predators. Results from a wider study of the chiroteuthids in the Pacific Ocean, using morphology in combination with the same three mitochondrial genes, reveal that two genera in the Chiroteuthidae, *Chiroteuthis* and *Asperoteuthis*, appear polyphyletic and are in need of further systematic attention. A potentially endemic *Chiroteuthis* species that appears new to science (*C.* aff. *veranyi*) is now known from New Zealand waters. This study also nearly doubles the number of publicly available sequences for this clade. Out of the three mitochondrial genes, 12S rRNA showed the least interspecific variation; in combination with morphology, COI and 16S rRNA are therefore likely to suffice for both systematic and ecological applications.

Squids in the family Histioteuthidae represent a substantial biomass in the deep sea, and play an important role in marine food webs as prey for apex predators. This family was last reviewed nearly 20 years ago, based solely on morphology, and unnamed species are known to exist; within the New Zealand region, these animals are the primary prey of sperm whales, and have high importance for a variety of other vertebrate predators. Herein, a global integrative taxonomic analysis of the Histioteuthidae was undertaken, using 16S rRNA and the COI to test the hypothesis of morphological species groups as genera. This analysis recognises *Calliteuthis*, *Stigmatoteuthis*, *Histioteuthis*, *Histiothauma*, *Fragariateuthis* gen. nov., and *Navia* gen. nov. The number of species in this family has increased from 19 to 25–29, with at least nine now known to occur in the New Zealand region. Both 16S rRNA and COI are useful for ecological studies and taxonomy (especially since many gut content analyses of squid predators use 16S rRNA), but given the high congruence observed between Barcode Index Numbers (BINs) and morphological species distinctions, basic species identification can be likely also be achieved using just COI and morphology.

Following these focused taxonomic studies, the cephalopod biodiversity of the Kermadec Islands region was catalogued using morphology and COI, and critically

compared with the few existing earlier reports. Results indicate that the cephalopod diversity in the region is nearly double what was previously believed, raising the known total from 42 to at least 70 species. In addition, 28 species are reported for the first time from the Kermadec region, 13 of which represent new records for the entire New Zealand EEZ, and five of which are potentially new to science. Thirty-four species found in the Kermadecs have not been reported anywhere else in New Zealand's EEZ; thus, the proposed Kermadec–Rangitāhua Ocean Sanctuary would protect habitat utilised by over 50% of New Zealand's known cephalopod diversity, including 17 possibly endemic species.

This thesis has provided new insight into two of the most abundant and ecologically important squid clades (both in New Zealand and in the wider Pacific), and also the most accurate review of the cephalopod diversity in the Kermadec Islands region, supporting the establishment of the Kermadec–Rangitāhua Ocean Sanctuary. DNA barcode reference libraries have been established for the Histioteuthidae and the chiroteuthid families. The histioteuthid species complexes and the Chiroteuthidae are still in need of full revision. For this to be possible, continued collections for new specimens will be required to enable further integrative taxonomic analyses. Future studies should investigate the dietary habits of these clades to fully understand their role in the ecosystem.

Chapter 1: General Introduction

The waters around New Zealand host one of the highest biodiversities of squid and octopus species in the world, yet until the late 1990s, little taxonomic work was published on this unique assemblage. The first studies in the region were conducted by Berry (1913, 1916), who described cephalopods from the Kermadec Islands (far northeastern New Zealand). These studies described new squid species and still represent the most thorough direct review of the Kermadec cephalopods to date. A review of the cephalopod fauna in the waters around New Zealand was undertaken by Dell (1952), who later (1959) added several new local cephalopod records. After this time, our knowledge of cephalopod biodiversity was gained from a few focused studies on specific groups, such as Riddell's (1985) complete morphological review of the local Enoploteuthidae (which remains the best reference work for this family in New Zealand waters). Even the common arrow squids (*Nototodarus sloanii* [Gray, 1849] and *N. gouldi* [McCoy, 1888]), which have been commercially fished since the 1970s, were understudied and it was not until nearly two decades after the start of the fishery that the two separate species were distinguished (Smith, Mattlin, Roeleveld, & Okutani, 1987). Focused efforts to systematically establish accurate accounts of New Zealand's local cephalopod biodiversity did not begin until O'Shea (1999) reviewed the octopuses present in our waters.

While some groups have recently been (or are in the process of being) resolved, others remain poorly understood, and certain regions also remain insufficiently studied. Following O'Shea's (1999) New Zealand octopus monograph, projects aiming to improve the understanding of local squid fauna have been undertaken by a group of taxonomists in the AUT Lab for Cephalopod Ecology & Systematics (ALCES) (see Table 1). Hoving et al. (2014) identified a number of additional oegopsid families as being greatly in need of global review; four of these are now being examined by ALCES members A. Evans and J. Kelly (Table 1). Two of the remaining families considered most in need of systematic review—the Chiroteuthidae Gray, 1849, and the Histioteuthidae Verrill, 1881 (which are among the most abundant and speciose deep-sea squids worldwide)—are addressed in this thesis. Although recent local morphological revisions were undertaken for the genera *Chiroteuthis* d'Orbigny [in Férussac & d'Orbigny], 1841 (Mensch, 2010) and *Histioteuthis* d'Orbigny [in Férussac & d'Orbigny], 1841 (Horstkotte, 2008), a phylogenetic analysis of both families is still needed. But even well-understood cephalopod groups remain poorly reported in some

Table 1—Taxonomic status of deep-sea oegopsid squids worldwide, and in New Zealand waters. Families identified as most in need of systematic attention in grey (based on Hoving et al., 2014). Number of genera and species based on Hoving et al. (2014) and the work of ALCES, and number from New Zealand (NZ) waters based on Spencer et al. (2017) and references listed in 'systematic status'.

Oegopsid (deep sea) squid taxa	Known genera (# known in NZ)	Known species (# known in NZ)	Systematic status
Architeuthid families			
Architeuthidae	1(1)	1(1)	Global genetic analysis (mitochondrial) showed a single cosmopolitan species, *Architeuthis dux* (Winkelmann et al., 2013).
Neoteuthidae	4(2)	4(2)	Four poorly known monotypic genera, all known from very few specimens (and no early life stages). Global review and phylogenetic analysis needed. Single specimen of *Nototeuthis dimegacotyle* Nesis & Nikitina, 1986, reported in NZ waters from stomach content of orange roughy (*Hoplostethus atlanticus* Collett, 1889) (D. Stevens, pers. comm.).
Brachioteuthidae	2(1)	5–7?(≥ 3)	Unstable; never globally reviewed. Phylogenetic and global revision needed. NZ species presently under review (Bolstad, in prep.).
Chiroteuthid families			
Chiroteuthidae	4(2)	19(4)	Unstable; never globally reviewed. Revision needed. NZ *Chiroteuthis* species reviewed (based on morphology) by Mensch (2010). Phylogenetic analysis needed.
Batoteuthidae	1(0)	1(0)	One known species (but at least one additional species suspected, see Guerra et al., 2012). Phylogenetic analysis needed.
Joubiniteuthidae	1(0)	1(0)	Atlantic review by Young & Roper (1969a). Phylogenetic analysis and global revision needed.
Magnapinnidae	1(0)	3(0)	Atlantic review by Vecchione & Young (2006), with additional species believed to exist. Phylogenetic analysis and global revision needed, pending the collection of new material.
Mastigoteuthidae	6(4)	~17(5)	Beta taxonomy revised with integrative taxonomy (Braid et al., 2014); alpha taxonomy of NZ species reviewed by Braid & Bolstad (2015). Additional species suspected (pers. obs.); global revision needed.
Promachoteuthidae	1(0)	3(0)	Three named species with additional species believed to exist (Young & Vecchione, 2016a). Family status review by Roper & Young (1968). Phylogenetic analysis and global species-level revision needed, pending the collection of new material.

Table 1—Continued.

Oegopsid (deep sea) squid taxa	Known genera (# known in NZ)	Known species (# known in NZ)	Systematic status
Cranchiidae	13(11)	60(15)	Beta taxonomy stabilised by Voss (1980); Pacfic and Atlantic/Gulf of Mexico (Judkins, in prep.) fauna presently under review (Evans, in prep.); phylogenetic review in prep.
Cycloteuthidae	2(2)	4(2)	Atlantic review by Young & Roper (1969b); additional taxa known to exist (pers. obs.). Phylogenetic analysis and global revision needed.
Enoploteuthid families			
Ancistrocheiridae	1(1)	1(1)	Globally reviewed by Young et al. (1998) based on morphology. One known species, but others suspected based on COI divergences (pers. obs.). Global morphological and phylogenetic revision needed.
Enoploteuthidae	4(3)	43(8)	Globally reviewed by Young et al. (1998) based on morphology. NZ taxa reviewed by Riddell (1985). Phylogenetic analysis needed.
Lycoteuthidae	4(2)	6(2)	Globally reviewed by Young & Harman (1998) based on morphology. Phylogenetic analysis needed.
Pyroteuthidae	2(2)	6(5)	Globally reviewed by Young et al. (1998) based on morphology. NZ taxa reviewed by Riddell (1985). Phylogenetic analysis needed.
Gonatidae	4(1)	19(1)	Genetic analysis by Lindgren et al. (2005) with some inconclusive results. Global review needed.
Histioteuthid families			
Histioteuthidae	2(2)	19(8)	Alpha taxonomy stabilised and 'species groups' recognised by Voss (1969) and Voss et al. (1998); NZ fauna reviewed morphologically by Horstkotte (2008). Phylogenetic analysis needed.
Psychroteuthidae	1(0)	1(0)	One known species; additional undescribed taxa may exist (Roper et al., 1969). Phylogenetic analysis needed.

Table 1—Continued.

Oegopsid (deep sea) squid taxa	Known genera (# known in NZ)	Known species (# known in NZ)	Systematic status
Lepidoteuthid families			
Lepidoteuthidae	1(1)	1(1)	One known species, but additional taxa exist (Kelly, pers. comm.). Global integrative taxonomic revision nearing completion (Kelly, in prep.).
Octopoteuthidae	2(2)	14(5)	Genus *Octopoteuthis* reviewed by Stephen (1985). Global integrative taxonomic revision of the family nearing completion (Kelly, in prep.).
Pholidoteuthidae	1(1)	2(2)	Two known species, but additional taxa exist (Kelly, pers. comm.). Global integrative taxonomic revision nearing completion (Kelly, in prep.).
Ommastrephidae	11(7)	20(9)	Phylogenetic analysis by Wakabayashi et al. (2012). Genus-level phylogeny in press (Santos et al.). Global integrative taxonomic review needed.
Onychoteuthidae	7(4)	~34(10)	Recent global morphology-based revision (Bolstad, 2010) followed by phylogenetic analysis (Bolstad et al., in press).
Thysanoteuthidae	1(0)	1(0)	One known species, but multiple BINs (pers. obs.) suggest phylogenetic revision is needed.

of New Zealand's marine habitats. Those most targeted by commercial fisheries have been well sampled, such as the Chatham Rise, although new cephalopod taxa are still being discovered in this region (e.g. Bolstad et al., 2018). Other regions, such as the far northern Kermadec Islands region, remain relatively unexplored and the biodiversity of this area is still not well known.

Accurate portraits of our teuthofauna (i.e., squid biodiversity)—and in particular, deep-sea taxa—have historically been precluded both by limited sample availability, and by lack of taxonomic clarity in the locally occurring groups. Sampling is difficult in the deep sea because of its sheer vastness—an area that comprises ~95% of the volume of the ocean and ~70% of the available living space on the planet. In addition, nets only sample part of the total fauna living in the area (e.g., Wenneck, Falkenhaug, & Bergstad, 2008), and highly mobile cephalopods are able to evade them (Wormuth & Roper, 1983). Predators are much more efficient at capturing cephalopods, making them more reliable sources of cephalopod specimens and ecological information (Cherel, Duhamel, & Gasco, 2004). Most human-obtained deep-sea cephalopod samples are damaged during capture because of their fragile nature, and it is therefore difficult to obtain high-quality specimens. Some deep-sea taxa are rarely encountered, are only known from single, juvenile, or semi-digested specimens (e.g., *Asperoteuthis lui*; Salcedo-Vargas, 1999) or in extreme cases only from images (e.g., adult *Magnapinna* Vecchione & Young, 1998). Biodiversity assessments of deep-sea cephalopod diversity are therefore difficult because they rely on identifying badly damaged specimens or fragmentary remains, which is further complicated by a lack of systematic resolution in many families.

Thus a Catch-22 situation arises: the infrequent capture and fragmentary nature of deep-sea squid specimens contribute to systematic confusion, and when material is collected, much of it can be difficult to identify, because the taxa remain poorly understood due to a lack of material. However, the field of integrative taxonomy, which uses molecular data in combination with traditional morphological characters, shows great promise as a tool for resolving cephalopod taxonomy at both the species and higher levels. The first integrative taxonomic analysis of cephalopods from New Zealand waters was only recently conducted (Braid, McBride, & Bolstad, 2014; Braid & Bolstad, 2015). Braid et al. (2014) used three mitochondrial genes (cytochrome *c* oxidase subunit I [COI], 16S rRNA, and 12S rRNA) to review the higher systematics in the family Mastigoteuthidae. This analysis was followed by a revision of the species-level systematics of this family in New Zealand waters (Braid & Bolstad, 2015). These

studies relied primarily on DNA barcoding—sequencing the standardised 648 bp region from the 5′ end of COI (Hebert, Cywinska, Ball, & deWaard, 2003)—and their results indicated that the combination of genetics and morphology for cephalopod taxonomy is far superior to using either technique alone. While traditional morphology-based taxonomy remains useful, and can provide substantial insight into taxon relationships, this approach may overlook subtle differences in characters. Conversely, studies based only on genetics can either oversplit or lump species and genera and cannot (alone) shed insight into shared morphological features. Together, molecular and morphological characters appear to form a strong evidence base for resolving problematic taxa; however, this is a young science and it is not yet clear which gene (or combinations of genes) is best for systematics and species identification.

This study aims to test the usefulness of integrative taxonomy for various systematic and ecological applications. Two of the cephalopod taxa that are most in need of review—the chiroteuthid families and the histioteuthids—will be analysed using integrative taxonomy. These analyses will be used to establish reference libraries and to determine which mitochondrial gene (or set of genes) can be used for species identification. Ultimately, integrative taxonomy will be tested as a tool for assessing biodiversity, using the Kermadec Islands as a case study.

The Kermadec Islands region

The Kermadec Islands region represents the northern-most part of New Zealand's Exclusive Economic Zone (EEZ) (Fig. 1). The Kermadec Arc comprises a chain of volcanic islands and many seamounts on the ridge bordering the Kermadec trench (the second-deepest marine trench in the world). This region contains the only subtropical intertidal and shallow subtidal marine ecosystem in New Zealand. Within the surrounding deep sea, the water from the northwestern side of the ridge is warmer and more saline than that of the southeastern side at the same depth (Sutton et al., 2012), with highly diverse biological communities inhabiting this convergence (Duffy & Ahyong, 2015). A small proportion of this area is currently protected by the Kermadec Islands Marine Reserve, which was established in 1990 and protects 12 nautical miles around each of the five main islands in the Kermadec Arc (Ministry for the Environment, 2016). In 2007, a Benthic Protected Area was also established for the Kermadec Islands region, which prohibits fishing within 100m of the seafloor (Satyanand, 2007).

The proposed Kermadec–Rangitāhua Ocean Sanctuary

New Zealand presently manages and monitors 44 marine reserves, which are classified as Type 1 Marine Protected Areas (MPAs) (Department of Conservation, n.d.a). This is the highest level of protection offered in New Zealand waters. A second type of MPA, Type 2, provides protection from commercial fisheries (Department of Conservation, n.d.a). In addition, a range of lower-level protection tools protect subsets of the locally occurring habitats and/or biodiversity (sometimes temporarily). There are currently 17 Benthic Protection Areas, which offer protection from dredging and bottom trawling in these areas (Department of Conservation, n.d.b). The purpose is mainly to protect the seafloor, particularly hydrothermal vents, so midwater trawling is still allowed, but closely monitored (Department of Conservation, n.d.b). This type of protected area would be helpful for benthic cephalopods, such as octopus, but is unlikely to protect pelagic squid species. Similarly, there are 17 Seamount Area Closures, which are designed to protect the benthic biodiversity of seamounts by banning all types of trawling in these areas (Department of Conservation, n.d.b). These closures may provide some protection for cephalopods species associated with seamounts. There are also six Marine Mammal Sanctuaries, where fishing may be restricted, aiming to protect marine mammals from anthropogenic activity (Department of Conservation, n.d.b).

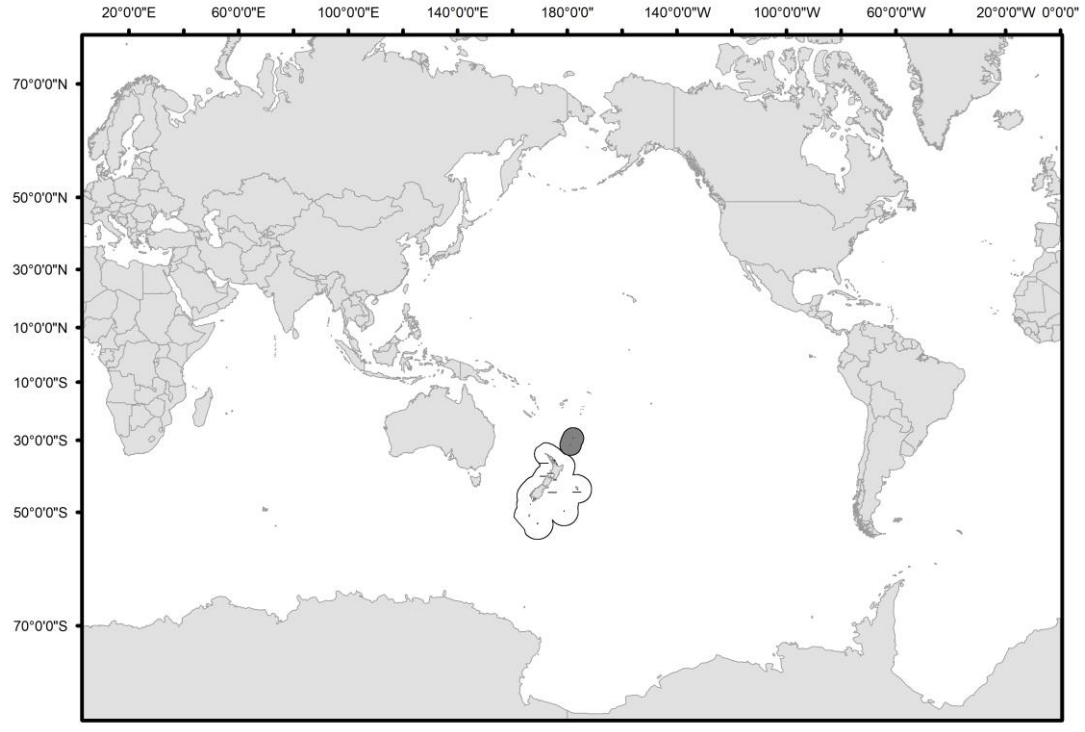

Fig. 1—Locatility of the New Zealand Exclusive Economic Zone (outlined in black), including the Kermadec Islands region (shaded in dark grey).

Recently, a new Kermadec–Rangitāhua Ocean Sanctuary was proposed, which would extend the area of protection offered by the existing Kermadec Marine Reserve to extend 200 nautical miles from the land, covering a total area of 620,000 square kilometres (Ministry for the Environment, 2016; Fig. 1). Many of the area's diverse habitats are nearly pristine (never subjected to intensive commercial fishing), and are oceanographically and biologically unique within the New Zealand EEZ (Clark et al., 2017). Although a lower level of protection would be offered in the wider Sanctuary when compared with the existing Marine Reserve, it would still prohibit fishing and mining in this area (Ministry for the Environment, 2016). This large, relatively unexplored and relatively unimpacted area therefore represents the rare chance to protect a wide range of unique habitats in New Zealand, before they may be disrupted by large-scale human activities. The few studies conducted in the region to date indicate that high diversities and unique assemblages of organisms are found in this part of New Zealand's EEZ, including sponges (Kelly, Amirapu, Mills, Page, & Reiswig, 2015), decapod crustaceans (Ahyong, 2015), fish (Francis & Duffy, 2015), and cephalopods (Bolstad, 2016); Bolstad (2016) found twice as many cephalopods as those currently included in checklists in an uncritical review of the cephalopod listings in local collections from the Kermadec region. This area represents a unique environment in New Zealand waters that needs protection; however, before the Sanctuary can be established, the biodiversity of the Kermadec region needs to be reviewed (Golder, 2016). The deep-sea fauna in this region is most poorly known, with only ~5% of the Kermadec ocean area explored to date (Golder, 2016).

Species richness

Recently, marine biodiversity of the Kermadec region has been the subject of considerable scientific and public interest (Golder & Connell, 2016). Following recent biodiversity surveys in the area, studies have been published on a wide range of taxa. These include two cephalopod checklists, which are considered to represent the most up-to-date understanding of cephalopod biodiversity in the Kermadec region. Duffy and Ahyong (2015) compiled a checklist of the known marine biodiversity of this region (including cephalopods), while Reid and Wilson (2015) created a similar checklist but only for cephalopods, and reported four octopus taxa based on specimens collected in shallow waters.

While useful, the limitations of these types of lists should also be recognised—for example, they necessarily rely on synthesising a wide body of published literature,

may or may not include information from unpublished theses, and must often uncritically accept published records that may prove dubious. Neither list reported any species in the chiroteuthid families or in the Histioteuthidae, although species from both groups had been identified in the region, by Mensch (2010) and Horstkotte (2008), respectively. Conversely, a number of taxa were included based on a global catalogue of cephalopods, the FAO 'Cephalopods of the world' published by Jereb and Roper (2010), and Jereb, Roper, Norman, and Finn (2014). Like the Kermadec checklists on a much larger scale, the FAO guide represents a massive and impressive synopsis of information about cephalopod taxa and distributions, but naturally requires some inferences to be drawn based on patchy data, and inevitably includes some inaccuracies. Even some region-specific, cephalopod-focused studies can contain inaccurate information, such as that of Imber (1978) who undertook a local revision of the notoriously problematic Cranchiidae based on paralarvae, juvenile specimens, and beaks from bird stomach contents. This study has been heavily criticised for synonymising valid species and misidentifying specimens (Voss, 1980). Nonetheless, Imber (1978) was used in both Kermadec checklists (Duffy & Ahyong, 2015; Reid & Wilson, 2015). While it is not necessarily expected for scientists outside the field of teuthology to be aware of the criticisms of this study, it appears safe to say some of our understanding of cephalopods in the Kermadec region is outdated and would benefit greatly from an updated assessment of the region's teuthofauna through direct observations on physical specimens. The last such studies remain those conducted over 100 years ago by Berry (1913, 1916).

Focus families

In order to gain an accurate picture of cephalopod diversity in the Kermadec Islands region, it is necessary to be able to adequately distinguish among species likely to be encountered. In an assessment of the locally occurring oegopsid families (Table 1; see also Hoving et al., 2014), two abundant and diverse clades, the chiroteuthid families and the family Histioteuthidae, stand out as requiring systematic attention. As previously stated, these have not been formally reported from the Kermadecs (Duffy & Ahyong, 2015; Reid & Wilson, 2015), but morphology-based reviews have encountered specimens from both groups in New Zealand, including the Kermadec region (Chiroteuthidae: Mensch, 2010; Histioteuthidae: Horstkotte, 2008).

The Histioteuthidae are a family of deep-sea squids whose distinctive asymmetrical, photophore-studded appearance has given rise to a wide range of common names, including ‘jewelled’, ‘strawberry’, ‘cockeyed’, and ‘violet’ squids. The family is presently thought to comprise 19 species (Young & Vecchione, 2013a), and is considered to be one of the deep-sea cephalopod families most in need of review (Hoving et al., 2014). Locally, the genus *Histioteuthis* was reviewed (based on morphology) by Horstkotte (2008), which included an analysis of the ecological role that each resident species plays in New Zealand waters. Seven species were identified from New Zealand waters, along with beaks of two additional unidentified ‘types’ that could not be attributed to known species. Therefore, despite several focused studies on the Histioteuthidae, its systematics is still not completely resolved either globally or locally.

Histioteuthids are highly abundant, and play a particularly important role in the local diets of many marine predators, particularly whales, including pygmy sperm whales (*Kogia breviceps* [de Blainville, 1838]; Beatson, 2007) and sperm whales (*Physeter macrocephalus* Linnaeus, 1758; Gómez-Villota, 2007). Horstkotte (2008) provided detailed descriptions and illustration of beak morphology in local species of histioteuthids along with regression equations for four species to estimate mantle length and weight. This information is essential for dietary analyses relying on beak identifications and in determining the relative importance of each species in the diet. Although cephalopod beaks can be used for species identification (Xavier & Cherel, 2009), this technique remains difficult (Xavier et al., 2007). Soft prey remains can be even more difficult to identify visually from stomach contents; however, a combination of visual identification and DNA barcoding can significantly increase the successful and accurate identification of prey remains (Bartley et al., 2015; Méheust, Alfonsi, Le Ménec, Hassani, & Jung, 2015). Recently, several studies (Waap et al., 2017; Alonso et al., 2014) have used molecular tools to identify histioteuthids from stomach contents, although their results were limited by a lack of reference material (see Chapter 4). Because of their ecological role, it is important to be able to accurately identify histioteuthids morphologically and genetically.

The ‘chiroteuthid families’—the other focus group in this thesis—are a clade of six families united by the absence of a primary tentacle club and the presence of a secondary tentacle club (Young & Vecchione, 2017). This clade contains: the Chiroteuthidae, the Mastigoteuthidae, the Joubiniteuthidae Naef, 1922, the Promachoteuthidae Naef, 1912, the Batoteuthidae Young and Roper, 1968, and the

Magnapinnidae Vecchione and Young, 1998, which together comprise 13 genera and ~43 species, but are one of the groups identified as being most in need of revision by Hoving et al. (2014). Locally, recent taxonomic revisions have been undertaken for *Chiroteuthis* (Mensch, 2010, based on morphology) and the Mastigoteuthidae (Braid et al., 2014; Braid and Bolstad, 2015; integrative taxonomic reviews). However, a genetic analysis of the wider chiroteuthid families group has not been completed. Similar to the Histioteuthidae, species 'groups' have been proposed for the species within *Chiroteuthis* (Roper, Young, & Vecchione, 2017).

A better understanding of the systematics of this clade is needed to fully understand their role in the ecosystem, particularly since the two most speciose of these families (the Mastigoteuthidae and the Chiroteuthidae) are among the most abundant deep-sea squid families worldwide (Young, Vecchione, & Donovan, 1998). These two families are commonly found in the diets of marine mammals (e.g., Gómez-Villota, 2007; González, Lopez, Guerra, & Barreiro, 1994; West, Walker, Baird, Mead, & Collins, 2017), fish (e.g., Goldsworthy et al., 2002; Potier et al., 2007) and seabirds (e.g., Xavier, Croxall, Trathan, & Rodhouse, 2003; West & Imber, 1986). The Mastigoteuthidae and the Chiroteuthidae likely also play a significant role as predators, but this aspect of their biology remains largely unknown. The unusual modified tentacle structures present in these families suggest a specialised feeding strategy (Young et al., 1998). For example, the chiroteuthid *Grimalditeuthis bonplandi* (Vérany, 1839) has unique tentacle clubs that lack suckers; Hoving et al. (2013) found that this species consumed crustaceans and cephalopods, and appeared to use its tentacles as a lure to attract prey. The photophore present at the end of the tentacles in other *Chiroteuthis* species has also been suggested to act as a lure for prey (Young et al., 1998), but generally, the feeding behaviour and dietary habits of this group remain largely unknown, apart from a handful of studies opportunistically reporting on the diets of specific taxa. A better understanding of the taxonomy of the chiroteuthid families, and a library of reference sequences, is necessary to fully understand the role they play in marine food webs.

Integrative taxonomy

Integrative taxonomic methods

Taxonomy underpins all biological research and accurate species identification is fundamental for understanding important ecological relationships and for species

conservation. Species conservation is impacted by taxonomy because a species must first be identified before it can be protected, and the higher-level systematics of a species could impact the management choices for the protection of biodiversity (May, 1990). Morrison et al. (2009) found that taxonomic changes can have three outcomes: (1) increased protection from splitting species; (2) no effect on conservation in the case of charismatic species, species in protected areas, and species that are economically valuable; (3) decreased protection from lumping species and identifying hybridisation between species. Integrative taxonomy uses the powerful combination of molecular and morphological characters to resolve both species and higher-level systematics, and can also be used to analyse species distribution patterns. For example, *Magnoteuthis* (‘*Mg*.’) *magna* (Joubin, 1913) was believed to range from the North Atlantic down to New Zealand waters until a genetic analysis revealed the presence of a previously unrecognised species (*Mg. osheai* Braid & Bolstad, 2015) and all known species in this genus now appear allopatric (Braid & Bolstad, 2015). However, studies that only rely on genetics may also be problematic. Song, Buhay, Whiting, and Crandall (2008) reported the presence of nuclear mitochondrial pseudogenes (numts), which amplify with DNA barcode primers and can lead to the over-splitting of species. Therefore, it is clear that, whenever possible, taxonomic work should ideally use both genetic and morphological characters in combination.

Integrative taxonomy for conservation

The Earth is on the brink of a sixth mass extinction (Barnosky et al., 2011), and cephalopods are faced with stressors such as climate change (Rodhouse, 2013), ocean acidification (Northern, 2016), fisheries pressures (Rodhouse et al., 2014), and pollution (Unger, Harvey, Vadas, & Vecchione, 2008). The conservation of biodiversity is a crisis discipline and therefore requires rapid and accurate species identification (DeSalle & Amato, 2004). DNA barcoding has been proposed as a rapid, high-throughput, low-cost method for accurate species identification (Hajibabaei et al., 2005). However, the use of a single mitochondrial gene for managing biodiversity has been criticised because of the inherent limitations in this approach (Rubinoff, 2006). For cephalopods, Strugnell and Lindgren (2007) suggested that because of the potential for the presence of multiple copies of COI and numts, multiple lines of evidence (such as additional genes, like 16S rRNA or 12S rRNA, and morphology) should be used in conjunction with COI to ensure that the species added to the Barcode of Life Data System (BOLD; Ratnasingham & Hebert, 2007) are accurately identified.

One beneficial outcome of integrative taxonomic studies is the creation of reference libraries of DNA sequences, using expertly identified material (ideally with vouchered, traceable parent specimens); these sequences and specimens are then available for use and comparison in a wide range of future studies. For example, these sequences can enable environmental DNA (eDNA) detection of a target species, to determine in a non-invasive way whether the species is present in a given location, such as the use of quantitative PCR (qPCR) in detecting the endangered fish redside dace (*Clinostomus elongatus* [Kirtland, 1840]) from stream water (Serrao, Reid, & Wilson, 2017). However, eDNA detection using qPCR can only be applied to known species. Protecting threatened species using this type of evidence therefore requires (1) recognition of the taxon, and (2) sequences being available for comparison with collected data. However, effective protection and management also require a further layer of information: an understanding of the organism's role in the ecosystem.

Thesis objectives and organisation

The overall aim of this thesis is to apply (and evaluate) integrative taxonomic techniques in order to improve systematic resolution in two of the most diverse and poorly understood deep-sea squid groups occurring in New Zealand, with the ultimate goal of providing an improved (and taxonomically sound) account of the biodiversity of the Kermadec Islands region to aid with the conservation of this area. These objectives will be accomplished through the following four studies, each comprising one chapter (Fig. 2):

1. Resolution of a problematic, wide-ranging, ecologically important species in the large-bodied chiroteuthid genus *Asperoteuthis* (*A. lui* Salcedo-Vargas, 1999).
2. An integrative taxonomic analysis of the poorly understood chiroteuthid families in New Zealand and the wider Pacific Ocean.
3. A global integrative taxonomic analysis of the family Histioteuthidae, resolving the family's higher-level systematics.
4. An analysis of the (currently underreported) cephalopod biodiversity of the Kermadec Islands.

Within these chapters, integrative taxonomic techniques will be evaluated and refined by answering the following questions:

1. Can mitochondrial genes (COI, 16S rRNA, and 12S rRNA) and morphological characters in combination provide a strong evidence base for species-level taxonomy in cephalopods?
2. Depending on their observed variability, is a subset of the mitochondrial genes 16S rRNA, 12S rRNA, and COI sufficient for systematic applications in cephalopods?
3. Can cephalopod species be identified using COI and the Barcode Index Number (BIN) system?
4. Does optimised integrative taxonomy reveal a unique enough assemblage of cephalopods in Kermadec region to support establishment of the proposed Kermadec–Rangitāhua Ocean Sanctuary?

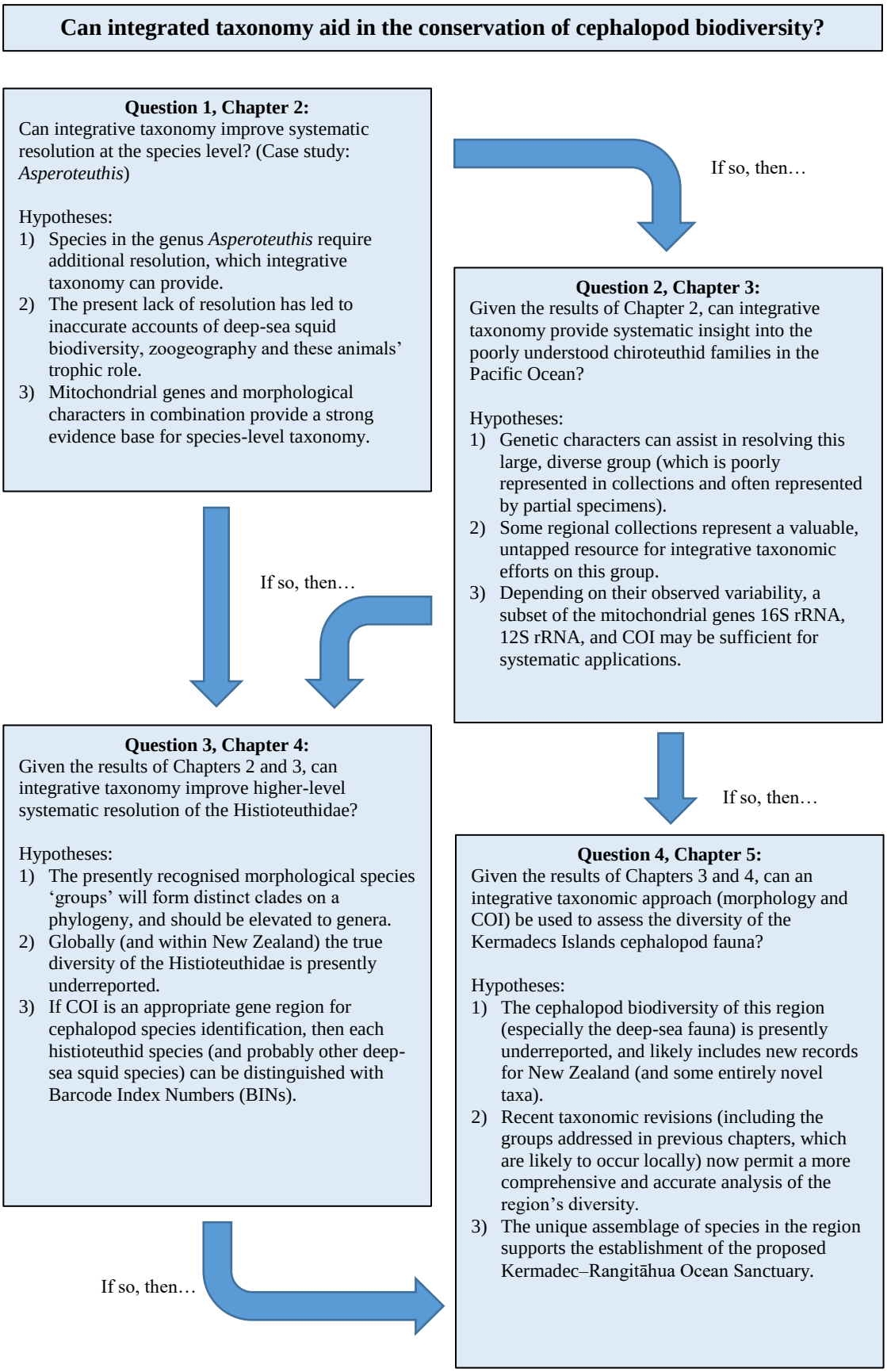

Fig. 2—A conceptual diagram of the flow of this thesis with the research question and approach for each chapter.

Chapter 2: Resolving the taxonomic status of *Asperoteuthis lui* Salcedo-Vargas, 1999 (Cephalopoda, Chiroteuthidae) using integrative taxonomy

Abstract:

The biology and systematics of the squid genus *Asperoteuthis* are poorly known. Although there have been four named and five described species in this genus, it now appears that there are only three valid species: *A. acanthoderma*, *A. mangoldae*, and *A. lui*. Using a combination of mitochondrial DNA sequences (cytochrome *c* oxidase subunit I [COI], 16S rRNA, and 12S rRNA) and morphology, *A. nesisi* Arkhipkin and Laptikhovsky, 2008, and Clarke's (1980) '?*Mastigoteuthis* A' both appear to be junior synonyms of *A. lui*. The most distinctive feature of this species is the aboral tentacle club photophore distribution, which is chiral, with more photophores dorsally (~11–16) than ventrally (~9–12). Genetically, there is low intraspecific variation within *A. lui* and higher interspecific variation between this species and other chiroteuthids. Previously only known from the type description, *A. lui* now appears to have a circumpolar distribution in the Southern Ocean and is the most commonly encountered *Asperoteuthis* species in the diet of marine predators.

Introduction:

Asperoteuthis Nesis, 1980, is an enigmatic genus of poorly-known meso-bathypelagic squids in the family Chiroteuthidae Gray, 1849. This genus is characterised by the presence of a tail structure, the absence of arm photophores, and unique tentacle clubs, each of which lacks suckers on the proximal half and is surrounded by a wide protective membrane (Young & Roper, 2015). The exact use of asperoteuthid tentacles remains unknown and there are currently no data available on the feeding ecology of *Asperoteuthis*.

The type species of *Asperoteuthis* was originally described as '*Chiroteuthis*' *acanthoderma* Lu, 1977. Nesis (1980) established a new genus for this species after examining additional specimens of *A. acanthoderma*. However, he incorrectly synonymised *A. acanthoderma* with *'Chiroteuthis' famelica* Berry, 1909 (now *Echinoteuthis famelica*, see Joubin, 1933; Braid et al., 2014), which was poorly known at the time, because of similarities with Berry's description (*e.g.*, skin tubercles and lanceolate fin) despite previous publications that supported the validity of this species (*e.g.*, Roper & Young, 1975; Young, 1978). Young (1991) supported the validity of *E. famelica* and its place in the family Mastigoteuthidae, and suggested that the type

species for the genus *Asperoteuthis* should be '*Chiroteuthis*' *acanthoderma*. Young, Vecchione, and Roper (2007a) officially fixed '*C.*' *acanthoderma* as the type species for *Asperoteuthis*.

There are currently four named species in this genus. *Asperoteuthis acanthoderma* was described from two immature individuals collected in the Celebes Sea in the Western Pacific Ocean (Lu, 1977). This species has also been reported from the Northwestern Pacific Ocean near Okinawa (Tsuchiya & Okutani, 1993), the Eastern Pacific near Hawaii (Young & Roper, 2010), the North Atlantic Ocean in the Gulf of Mexico (Judkins, Ingrao, & Roper, 2009), Straits of Florida (Judkins et al., 2009), the Caribbean (Judkins et al., 2009), and the Indian Ocean off western Australia (Judkins et al., 2009). *Asperoteuthis lui* Salcedo-Vargas, 1999, was described from a single specimen from Cook Strait in New Zealand waters. *Asperoteuthis mangoldae* Young et al., 2007a, was described from specimens captured in Hawaiian waters. Most recently, *Asperoteuthis nesisi* Arkhipkin and Laptikhovsky, 2008, was described from a single specimen that was captured around the Falkland Islands in the Southern Ocean. A fifth species was reported by Clarke (1980) as '?*Mastigoteuthis* A', but is now believed to belong in *Asperoteuthis* rather than in the family Mastigoteuthidae Verrill, 1881 (Arkhipkin & Laptikhovsky, 2008).

The purpose of this chapter is to review the systematics of *A. lui*, which was originally described from a single, partial specimen that was from the stomach contents of a ling (*Genypterus blacodes* [Forster, 1801]), and was damaged due to digestion (Salcedo-Vargas, 1999). A redescription was possible because of additional, recently collected, material. The status of the species described in the genus *Asperoteuthis* is assessed using a combination of mitochondrial genes (cytochrome *c* oxidase subunit I [COI], 16S rRNA, and 12S rRNA) and morphology. In addition, these genes are tested for their value in integrative taxonomy for resolving taxa at the species level.

Methods:

Genetic analysis

DNA was extracted from eight specimens. Frozen tissue was available for four specimens (one whole individual, three tentacles only), which were identified by tentacle-club morphology as *Asperoteuthis lui* (Table 2). Tissue fixed in 80% ethanol was available for the other four specimens (one whole individual without tentacles, three that were beaks and buccal mass only), which were identified as '?*Mastigoteuthis* A' based on beak morphology (by Darren Stevens, NIWA) (Table 2). Additional

Table 2—Specimen information for *Asperoteuthis* sequences used in this study. Under the original morphological ID (identification), the feature used for the identification is indicated in brackets. Identifications based on beak morphology were conducted by Darren Stevens from the National Institute of Water and Atmospheric Research Ltd. (NIWA). The Barcode of Life Data Systems (BOLD) ID is the unique identifier given to each specimen.

Original morphological ID	**Genetic ID**	**Museum ID**	**BOLD ID**	**GenBank ID**			**Reference**
				COI	**16S rRNA**	**12S rRNA**	
'?*Mastigoteuthis* A' (beak)	*A. lui*	NIWA 95040	ALUI008-16	KX675425	KX675433	KX675441	Present study
'?*Mastigoteuthis* A' (beak)	*A. lui*	NIWA 95039	ALUI007-16	KX675426	KX675434	KX675442	Present study
'?*Mastigoteuthis* A' (beak)	*A. lui*	NIWA 93270	ALUI006-16	KX675427	KX675435	KX675443	Present study
'?*Mastigoteuthis* A' (beak)	*A. lui*	NIWA 95041	ALUI005-16	KX675428	KX675436	KX675444	Present study
A. lui (tentacle club)	*A. lui*	NIWA 96166	ALUI004-16	KX675429	KX675437	KX675445	Present study
A. lui (tentacle club)	*A. lui*	NIWA 96168	ALUI003-16	KX675430	KX675438	KX675446	Present study
A. lui (tentacle club)	*A. lui*	NIWA 93268	ALUI002-16	KX675431	KX675439	KX675447	Present study
A. lui (tentacle club)	*A. lui*	NIWA 97258	ALUI001-16	KX675432	KX675440	KX675448	Present study
A. nesisi (type description)	*A. lui*	BMNH 20070615	GBCPH775-09	EU421718	EU421719	EU421720	Arkhipkin & Laptikhovsky (2008)
A. mangoldae	*A. mangoldae*	FMNH 278099	CHSQX003-16	KX783173	KX783231	KX783199	Chapter 3

sequences were obtained from GenBank for the holotype of *A. nesisi*, and the outgroup species *A. mangoldae*, which was chosen based on the results of Braid, Kubodera, and Bolstad (2017), which showed some support for a relationship between *A. lui* and *A. mangoldae*. This outgroup was included to show the relationship between *A. lui*, *A. nesisi*, and '?*Mastigoteuthis* A'. *Asperoteuthis acanthoderma* was not included in this phylogeny because it does not form a monophyletic clade with these species, but comparative sequences are available for this species on BOLD and GenBank (Braid et al., 2017).

DNA was extracted using EconoSpin (Epoch Life Science) columns with QIAGEN reagents following protocols for the DNeasy Blood & Tissue Kit (QIAGEN). Three mitochondrial gene regions (COI, 16S rRNA, and 12S rRNA) were amplified and sequenced following protocols and using primers outlined in Braid et al. (2014). Bidirectional sequencing reactions were performed by Macrogen (Korea) using the same primers used for the PCR. Sequences were assembled into contigs and edited using Sequencher v. 4.9 (Gene Codes) and then uploaded to the Barcode of Life Data System (BOLD; Ratnasingham & Hebert, 2007) public project titled 'Resolving the taxonomic status of *Asperoteuthis lui*' (project code: ALUI) and subsequently submitted to GenBank (Table 2). Sequences were checked for potential contamination using the Basic Local Alignment Search Tool (BLAST) through GenBank.

Sequences were aligned separately for each gene using the Multiple Alignment using Fast Fourier Transform (MAFFT) online server (Katoh & Standley, 2013), then concatenated in SequenceMatrix 1.8 (Vaidya, Lohman, & Meier, 2011). The concatenated alignment was analysed in PartitionFinder 1.1.1 (Lanfear, Calcott, Ho, & Guindon, 2012) to determine optimal partitioning and models using all substitution models included under Bayesian Information Criterion. Each gene, as well as all codon positions for COI, was searched separately for a maximum of five partitions. The model TrN was chosen for both 16S rRNA and 12S rRNA. Different models were chosen for each codon position of COI as TrNef, F81, and HKY respectively. A maximum-likelihood combined phylogeny with 1000 bootstrap replicates was created in GARLI 2.0.1 (Zwickl, 2006). A consensus tree was created in Geneious 7.1.7 (Biomatters, Auckland, New Zealand) and branches were collapsed when bootstrap support values were less than 50. The final phylogeny was visualised in FigTree 1.4.0 (Rambaut, 2012). The Barcode Index Number (BIN) system was also used to test the species boundaries of *A. lui*, *A. nesisi*, and '? *Mastigoteuthis* A' (Ratnasingham & Hebert, 2013). Intra- and interspecific distances for each gene region (COI, 16S rRNA, and 12S

rRNA) were calculated MEGA 6.06 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) using the Tamura-Nei (1993) model with gamma correction.

Morphological analysis

Original type descriptions for all *Asperoteuthis* species were reviewed. The holotype for *A. lui* from the Museum of New Zealand Te Papa Tongarewa (NMNZ) was examined, and photographs of the holotype for *A. nesisi* from the British Museum of Natural History (BMNH) were observed. All specimens that were sequenced in this study were examined. A specimen of *A. mangoldae* was examined from the Field Museum of Natural History (FMNH). The entire national collections of asperoteuthid specimens were loaned and examined from the National Museum of New Zealand Te Papa Tongarewa (NMNZ) and the National Institute of Water and Atmospheric Research, Ltd. (NIWA), in Wellington. Additional non-morphological abbreviations used are: MNHN—Muséum National d'Histoire Naturelle; RV—research vessel; Stn—station; USNM—Smithsonian Institution National Museum of Natural History, USA.

Distribution maps of *Asperoteuthis* species (Fig. 3) and *A. lui* specimens, found in New Zealand waters (Fig. 4), were created with ArcGIS 10.2 (Environmental Systems Research Institute [ESRI], Redlands, CA). The distribution for *A. acanthoderma* is based on specimen records from USNM (USNM 1111098, USNM

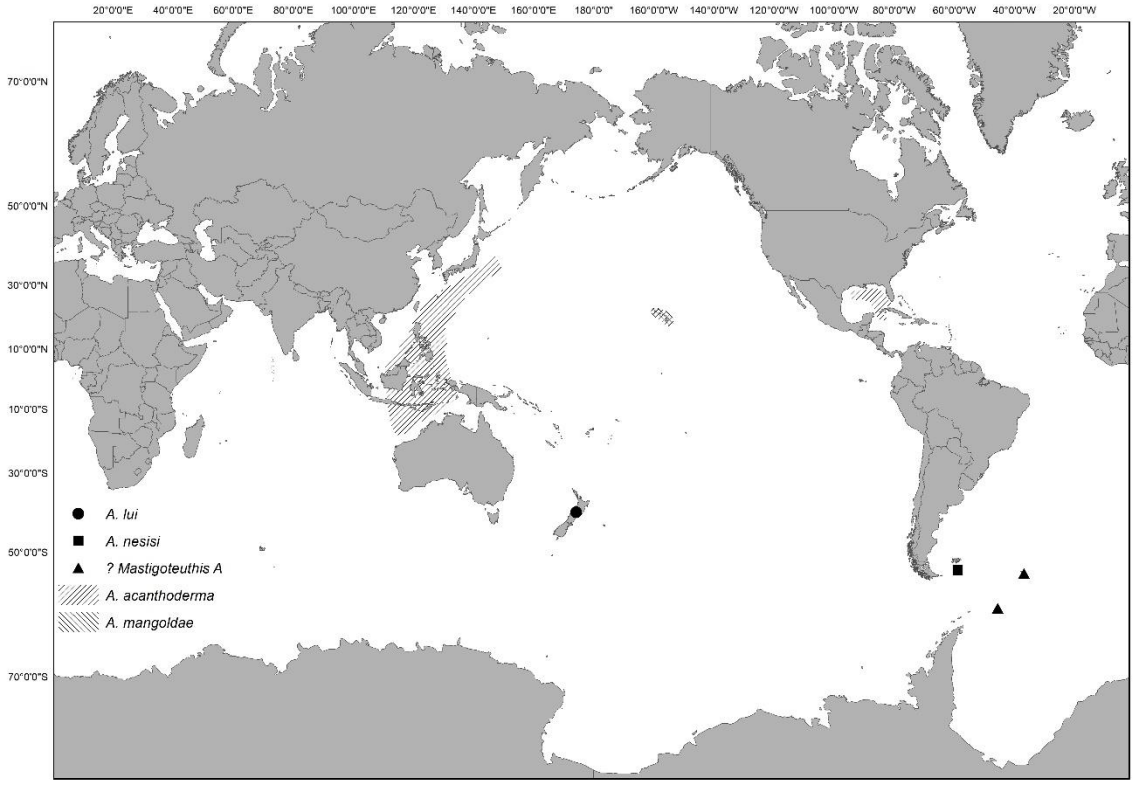

Fig. 3—Distribution of *Asperoteuthis* species.

1179399, USNM 1179402, USNM 1179422, USNM 1179632, and USNM 1179696) and Muséum National d'Histoire Naturelle (MNHN-IM-2002-2266), and localities from Lu (1977), Nesis (1980), Tsuchiya and Okutani (1993), Judkins et al. (2009), Young and Roper (2010), and Chapter 3. The distribution for *A. mangoldae* is based on Young and Roper (2011). The locality for ?*Mastigoteuthis* A (Clarke 1980) and the type localities for *A. nesisi* (BMNH 20070615) and *A. lui* (NMNZ M.143859) are indicated.

Collection data for some specimens were not available (ex-gut-content material). Collection dates are listed as dd/mm/yyyy. Specimens are listed by order of decreasing latitude, and secondarily by lower rostral length (LRL). Specimens were sexed when viscera were present, while badly damaged specimens or beak-only specimens where sex could not be determined were designated ‘sex indet.’. Measurements were taken from the most complete side of the specimen and ranges are given in the format of lowest value (X), mean (*Y*), and largest value (Z) in the format of X–*Y*–Z; when the range was less than 5% ML, only the mean is provided. Measurements of damaged features are indicated by an asterisk (*). Morphological examinations focused on both internal (beak, palps, and radula) and external anatomy following Braid and Bolstad (2015).

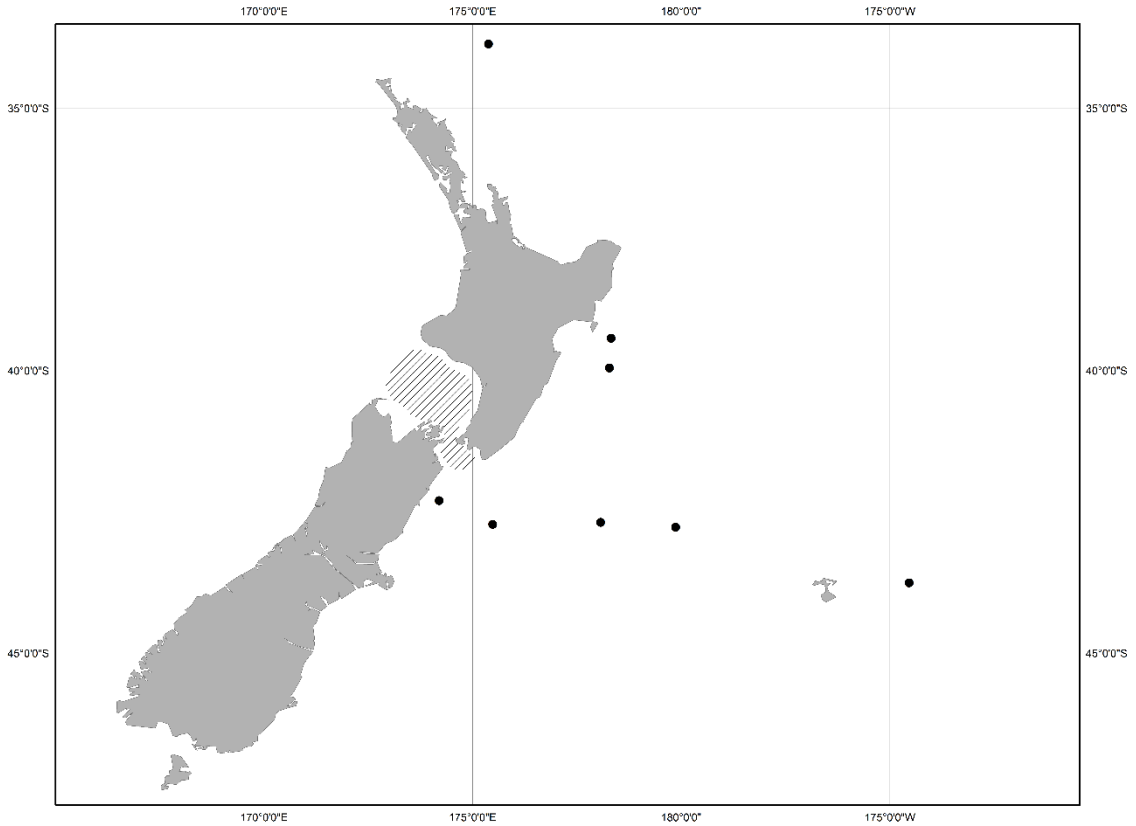

Fig. 4—Distribution of *Asperoteuthis lui* specimens examined in Chapter 2. The shading indicates Cook Strait, where two specimens were caught without specific locality data.

The species description was made in accordance with the guidelines provided by Roper and Voss (1983) with some modification (see Braid & Bolstad, 2015). Beaks were described following Clarke (1986), and drawn using a camera lucida. For scanning electron microscopy (SEM), specimens were critical-point dried at the University of Auckland, then platinum plated and imaged at the Auckland University of Technology. Sucker descriptions were based on Salcedo-Vargas (1995) with some modifications (see Braid & Bolstad, 2015). Palatine teeth on the lateral buccal palps were described following Bolstad (2010). Radular tooth descriptions followed Bolstad (2010) with some modifications (see Braid & Bolstad, 2015).

Specimen measurements used in text and tables include the following: ML—dorsal mantle length (measured to the end of the fin); MW—mantle width; FL—fin length; FW—fin width; HL—head length (measured from anterior tip of nuchal cartilage to separation of Arms I); HW—head width; ED—eye diameter; AL—arm length (arms measured from proximal-most sucker to arm tip); TnL—tentacle length; CL—tentacle club length; LRL—lower rostral length; URL—upper rostral length.

Results:

Genetic analysis

Bidirectional sequences were successfully recovered from all eight individuals for COI, 16S rRNA, and 12S rRNA. COI sequences were all 658 bp and did not contain stop codons or indels. The 16S rRNA sequences were 517 bp and the 12S rRNA sequences were 403 bp. All COI sequences for *A. nesisi*, *A. lui*, and '?*Mastigoteuthis* A' were assigned to the same Barcode Index Number (BOLD:AAJ9359) and these sequences of also formed a single clade on the combined maximum-likelihood phylogeny that was distinct from *A. mangoldae* (Fig. 5). Within the sequences used in this study for *A. nesisi*, *A. lui*, and '?*Mastigoteuthis* A?': COI showed a mean divergence of 0.5%, with a minimum of 0%, and a maximum of 1.4%; 16S rRNA showed no variation; and 12S rRNA showed a mean divergence of 0.1%, with a minimum of 0%, and a maximum of 0.3%. For the divergence between this clade and *A. mangoldae*: COI had a mean interspecific divergence of 19.4%, with a minimum of 19.0%, and a maximum of 20.9%; 16S rRNA showed a divergence of 5.4%; and 12SrRNA showed a mean divergence of 7.3%, with a maximum of 7.6%, and a minimum of 7.2%.

Systematics

Genus *Asperoteuthis* Nesis, 1980

Asperoteuthis Nesis, 1980: 613. Type species *Chiroteuthis acanthoderma* Lu, 1977, by subsequent designation of Young et al. (2007a:357).

Diagnosis: Mantle length at maturity 100 mm to >1030mm. Fins circular to oval in outline when considered together; fin length ~40–65% ML; tail structure present. Funnel-locking cartilage inverted Y-shaped groove, comma shaped, or ear shaped, with weak tragus, anti-tragus variably present; funnel pocket absent; buccal formula DDVV. Mantle-locking cartilage inverted Y-shaped, crescent, or approximately oval. Arm suckers arranged in two distinct series, with sharp or blunt teeth; arm length approximately subequal (~50–115% ML). Tentacular suckers present only on distal portion of club; trabeculate protective membrane present, expanded on proximal half of club; terminal-club photophore present, aboral club with small embedded photophores near lateral edges, photophores present on tentacular stalk. Photophore present on ventral surface of eye. Integumental photophores absent from mantle, fins, head, and arms.

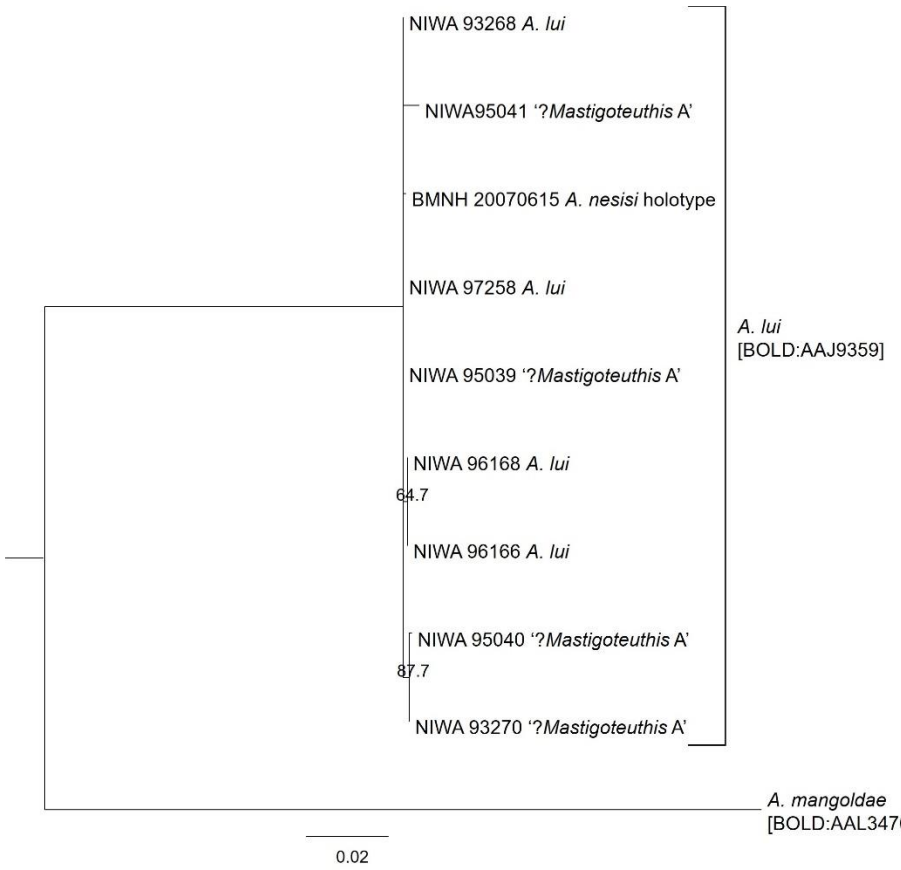

Fig. 5—Combined maximum-likelihood phylogeny based on COI, 16S rRNA, and 12S rRNA for specimens identified as *Asperoteuthis nesisi*, *A. lui*, and '?*Mastigoteuthis* A', with *A. mangoldae* as an outgroup, with 1000 bootstrap replicates.

Remarks: This diagnosis is based on descriptions of *A. acanthoderma* (Lu, 1977), *A. nesisi* (Arkhipkin and Laptikhovsky 2008), *A. mangoldae* (Young et al. 2007a), and *A. lui* (Salcedo-Vargas 1999). Additional information was taken from Young and Roper (2011), and the present findings.

***Asperoteuthis lui* Salcedo-Vargas, 1999** (Tables 3, 4, Figs 5–10)

'*?Mastigoteuthis* A' Clarke, 1980: 191–194, figs 155, 156; Clarke (1986): 160, 161, fig. 83.

Asperoteuthis lui Salcedo-Vargas, 1999: 48, 49, fig. 1.

Asperoteuthis nesisi Arkhipkin and Laptikhovsky, 2008: 203–205, figs 1, 2.

Type material examined: **NMNZ M.143859**, *A. lui* holotype, sex indet., head only, LRL 6.62 mm, no locality data, Cook Strait, *Genypterus blacodes* stomach content. Photographs of **BMNH 20070615**, *A. nesisi* holotype, ♀, ML 363 mm, 53.73°S, 58.77°W, 913 m, RV *Dorada*, pelagic trawl fishing near-bottom, 20/07/05, Stn 2132, cruise ZDLH1-07-2005, '*Asperoteuthis nesisi*'.

Additional local material examined (10 specimens): **NIWA 95041**, ♀, beak only, LRL 7.34 mm, 39.40°S, 178.32°E, 998 m, RV *Tangaroa*, 26/03/2010, Stn TAN1003/64; **NIWA 93270**, sex indet., beak only, LRL 6.70 mm, 39.95°S, 178.28°E, 1285 m, RV *Tangaroa*, 26/03/2010, Stn TAN1003/65; **NIWA 95040**, sex indet., ML 345 mm, LRL 7.70 mm, 42.35°S, 174.20°E, 1226 m, RV *Tangaroa*, 01/04/2010, Stn TAN1003/119; **NIWA 96168,** sex indet., tentacle only, CL 138 mm, 42.74–42.75°S, 178.07–178.05°E, 868–822 m, RV *Tangaroa*, 16/11/2011, TAN1116/115; **NIWA 96166**, sex indet., tentacle only, CL 144 mm, 42.74–42.75°S, 178.07–178.05°E, 868–822 m, RV *Tangaroa*, 16/11/2011, TAN1116/115; **NIWA 97258**, sex indet., tentacle only, CL 170 mm, 42.77°S, 175.48°E, 886–889m, RV *Tangaroa*, 20/01/2014, TAN1401/102; **NIWA 95039**, sex indet., beak only, LRL 5.16 mm, 42.82°S–42.83°S, 179.87–179.83°E, 960–962 m, RV *Tangaroa*, 16/06/2010, Stn TAN1008/04; **NIWA 93268**, ♀, fins missing, CL 79 mm, LRL 5.43 mm, 43.79°S, 174.54°W, 810–811m, RV *Tangaroa*, 11/01/2014, Stn TAN1401/56; **unaccessioned,** ♀, ML 315 mm, LRL 6.51 mm, no locality data, New Zealand; **NMNZ M.302215**, sex indet., head only, LRL 3.26 mm, no locality data, Cook Strait, *Genypterus blacodes* stomach content.

Comparative material examined: *Asperoteuthis mangoldae*, **FMNH 278099**, ♂, ML 144* mm, 21.33–21.58°N, 158.33–158.58°W, 975–1040 m RV *New Horizon*, 04/07/1996, Stn 962-sta#76, mother tucker trawl. *Asperoteuthis acanthoderma*, **unaccessioned**, sex indet., ML 1030* mm, Indian Ocean, no locality data.

Distribution: Circumglobal distribution in the Southern Ocean; New Zealand in Cook Strait and the Chatham Rise; South Atlantic around the Falkland Islands.

Diagnosis: Fins circular in outline when considered together, length ~64% ML, width 52–*60*–68% ML; circular skin depressions present. Single elongate photophore on ventral surface of eye. Funnel-locking cartilage ear shaped, with weak tragus, strong antitragus; mantle-locking cartilage approximately oval. Largest suckers of all located mid Arms II and III (~75% arm width); arm suckers with 7–11 blunt, rectangular or sharp, conical teeth. Tentacles with ~120–160 suckers with 5–7 sharp, conical teeth; aboral tentacle-club surface midline with five smaller proximal and two larger distal photophores; small embedded photophores near lateral edges bilaterally asymmetrical with more photophores dorsally (~11–16) than ventrally (~9–12); trabeculae present on protective membrane, trabeculae on distal portion of expanded proximal membrane fused to form a solid muscular area.

Description: Mantle conical anteriorly, with mantle cavity terminating approximately one third of FL from anterior of fins (thereafter gladius and surrounding musculature continue as narrow cylinder), widest (~28% ML) at anterior margin; dorsal anterior mantle margin triangular with point produced over nuchal-locking cartilage. Fins circular in outline when considered together, length ~64% ML, width 52–*60*–68% ML; anterior lobes absent; tail structure missing due to damage on all specimens examined. Integumental photophores absent from mantle, fins, head, and arms; skin tubercles not observed (skin always damaged); circular skin depressions present on dorsal and ventral surface of fins, and all external surfaces of mantle, head, and funnel (absent from collar).

Head narrowly conical, length 24–*32*–39% ML, widest posteriorly (width at midline ~11% ML). Olfactory papilla cylindrical. Eye diameter ~8% ML. Single elongate photophore on ventral surface of eye (Figs 5D–F). Funnel widely conical with recurved end, width ~13% ML, length ~13% ML; aperture posterior to eyeball; funnel pocket absent. Funnel-locking cartilage ear shaped (Fig. 6A), ~5% ML; anterior groove

Table 3—Measurements for *Asperoteuthis lui*, and mean indices for *A. lui*, *A. acanthoderma*, and *A. mangoldae*. Measurements based on previous studies for Whale 524 specimens 1 and 2 (Clarke, 1980), BMNH 20070615 (Arkhipkin & Laptikhovsky, 2008), NMNZ M.143859 (Salcedo-Vargas, 1999). Mean indices were sourced from previous studies for *A. acanthoderma* (Lu, 1977; Nesis, 1980; Tsuchiya & Okutani, 1993) and *A. mangoldae* (Young et al., 2007a, b). All specimens measured on the right side except for NIWA 93268, and Arm IV on NMNZ M.143859.

Specimen ID:	**Whale 524, specimen 1**	**Whale 524, specimen 2**	**BMNH 20070615**	**NMNZ M.143859**	**unaccessioned**	**NIWA 95040**	**NIWA 93268**	**Mean Indices**			
									A. lui	***A. acanthoderma***	***A. mangoldae***
Type status	none	none	holotype	holotype	none	none	none				
Sex	M	M	F	sex indet.	F	sex indet.	F				
ML	175	185	363	–	315	345	–				
MW			100	–	90	70	55	MWI	25	16	16
FL	115	118	220	–	218	210	–	FLI	64	51	44
FW	92	96	246	–	215	205	–	FWI	60	37	54
HL	62	56.5	88	–	103	135	72	HLI	32	27	38*
HW	20	18.3	36	40	38	35	26	HWI	11	10	11*
ED			27	30	30	35*	–	EDI	8	12	17
Arm I	150	150	312	255	258	310	153	ALI1	84	109	55
Arm II	166	195	335	304	310	375	195	ALI2	102	123	62
Arm III	215	210	303	327	297	392	197	ALI3	108	128	63
Arm IV	175	177	377	354 (L)	362	390*	224	ALI4	102	139	91
TnL	–	–	–	2065	–	–	986	TnLI	~570	605	–
CL	–	–	–	138	–	–	79	CLI	~40	24	19

* indicates a damaged feature
– indicates a missing feature

Table 4—Comparison of characters for *Asperoteuthis* species. Characteristics of *A. acanthoderma* based on the type description by Lu (1997). Characteristics of *A. mangoldae* based on the type description by Young et al. (2007a) and Young, Vecchione, and Roper (2011).

Character	***A. acanthoderma***	***A. mangoldae***	***A. lui***
Fin shape	Oval	Circular	Circular
Photophores			
Eye	Oval	Large oval patch	Elongate
Medial club	Large, at club tip	Small, at club tip	Series along midline, enlarged photophore at club tip
Aboral lateral club margins	12–13 on each side	8 distal, 5 proximal on each side	More on dorsal (9–12) than ventral (11–16)
Funnel-locking cartilage	Inverted Y	Comma-shaped	Ear-shaped
Tragus	Present	Present	Present, weak
Anti-tragus	Present	Absent	Present
Mantle-locking cartilage	Inverted Y	Crescent	Approximately oval
Arm suckers			
Dentition	3 or 4 rounded to truncated teeth	9 or 10 separate, truncated teeth	7–11 blunt, rectangular or sharp, conical teeth
Location of largest sucker	Arms II and III	Arms III	Arms II and III
Tentacles			
Sucker dentition	3 or 4 triangular teeth	Truncated teeth, 8 large distally, 17 small proximally	5–7 sharp, conical teeth
Number of suckers	~50	~50	~120–160
Skin morphology			
Circular depressions	Absent	Absent	Present
Tubercles	Present	Absent	Not observed in this study*

*skin tubercles were reported for *A. nesisi* in the type description (Arkhipkin & Laptikhovsky, 2008), but no tubercles were observed on specimens examined herein; however, the skin was badly damaged on all specimens examined.

concave due to strong antitragus; weak tragus along inner/medial margin; nearly straight along outer/lateral margin. Mantle-locking cartilage approximately oval (Fig. 6B), ~4% ML; posteriorly undercut.

Arm formula IV≥III≥II>I; arm length 83–*99*–108% ML (Table 3); arms of approximately subequal thickness with Arms IV thickest and Arms I thinnest; oral faces of arms bordered by membranes, trabeculae absent; aboral keels present on Arms I–III; expanded lateral membrane present on Arms IV. Each arm with ~122–206 suckers in two series; largest suckers of all located on Arms II (~75% arm width) at about pair 18–21 (~30–40% arm length), and Arms III (~75% arm width) at about pair 18–23 (~30–40% arm length).

Arm-sucker infundibular rings (Fig. 7) proximally adentate, distally with ~7–11 blunt, rectangular or sharp, conical teeth. Polygonal processes on oral surface of sucker papillated ring often damaged, in ~2–4 concentric rings; distally, central and

intermediate rings with ovate, porous pegs; proximally, central and intermediate rings nearly flat or slightly raised proximally, peripheral ring with flat rectangular or ovate processes.

Tentacle length ~570% ML, club length ~6% TnL (~40% ML), sucker covered surface ~75% club length; proximal protective membrane ~25% club length, widest portion of club surface ~30% maximum membrane width; stalk width at base of club ~70% club surface width, mid-stalk width ~40~% club surface width. Distal club with ~120–160 suckers in four series (Fig 7). Proximal rounded expanded portion of protective membrane trabeculate, distally fused to form a solid muscular area; distal non-expanded protective membrane trabeculate; medial aboral club with proximal series of ~6–8 smaller photophores, medium-sized photophore distally, large club-tip photophore; lateral aboral club with more ventral photophores (~11–16) than dorsal (~9–12); tentacle-stalk photophores alternating between larger (~75–100% stalk width) and smaller (~25% stalk width) along length of stalk, most distal ~5% stalk length with only smaller photophores, which decrease in size distally. Sucker infundibular rings (Figs 8D–M) proximally adentate, distally with ~5–7 sharp, conical teeth; proximal polygonal processes in papillated ring flat, often irregularly shaped varying from ovate to spindle shaped, in ~4–6 concentric rings; distal polygonal processes approximately circular to rectangular, slightly elevated proximally, in ~3 rings.

Lower beak, lateral profile (Figs 9A, D, G, J, M): lower rostral length ~43% wing length, rostral edge with strong curve, rostral tip without hook, rostral tip behind leading edge of wing by 20–*28*–35% baseline; wing angle slightly obtuse (nearly right angle), jaw angle obscured by prominent wing fold, shoulder groove present; height 79–

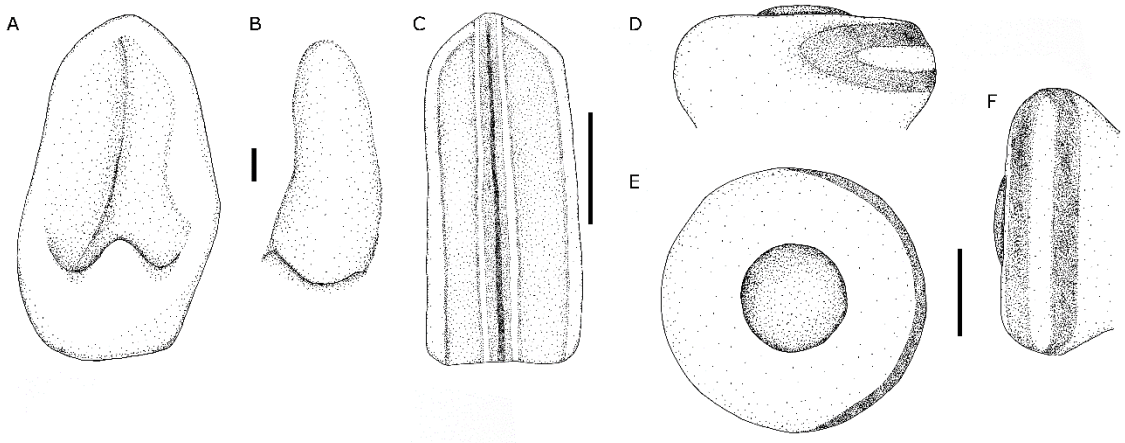

Fig. 6—*Asperoteuthis lui*. A–C) NIWA 93268, ♀, LRL 5.43 mm; D–F) NMNZ M.143859, sex indet., LRL 6.62 mm. A) Right funnel-locking cartilage; B) right mantle-locking cartilage; C) nuchal-locking cartilage; D) left eye photophore, anterior view; E) left eye photophore, lateral view; F) left eye photophore, ventral view. Scale bars = A, B) 1 mm; C) 5 mm; D–F) 10 mm.

87–92% baseline; hood close to crest, hood length ~64% crest length, crest length 54–*57*–63% of baseline, visible portion of crest nearly straight; broad lateral-wall fold extending to posterior edge of lateral wall; no or slight notch in lateral wall. Lateral oblique view (Figs 9B, E, H, K, N) with wing narrowest level with jaw angle, 48–*58*–68% of greatest width. Ventral view with broad notch in hood, free corners well separated. Wings remain entirely clear at LRL 3.26 mm, anterior edge of wing below shoulder clear at LRL 5.43 mm, anterior and posterior edge of wing remains clear through at least LRL 7.70 mm.

Upper beak, lateral profile (Figs 9C, F, I, L, O): upper rostral length ~32% hood length; hood length ~67% beak length; hood height ~39% beak width. Lateral-wall fold absent; shoulder produced into point or smooth curve, shoulder step 6–43–92% URL; jaw edge slightly curved; jaw angle nearly right angle.

Radula (Fig. 9A) with tricuspid rachidian, base width ~60% height, proximal margin of base rectangular, with broad, sharp triangular mesocone and small, sharp lateral cusps, slightly laterally directed, their height ~45% mesocone height. First lateral

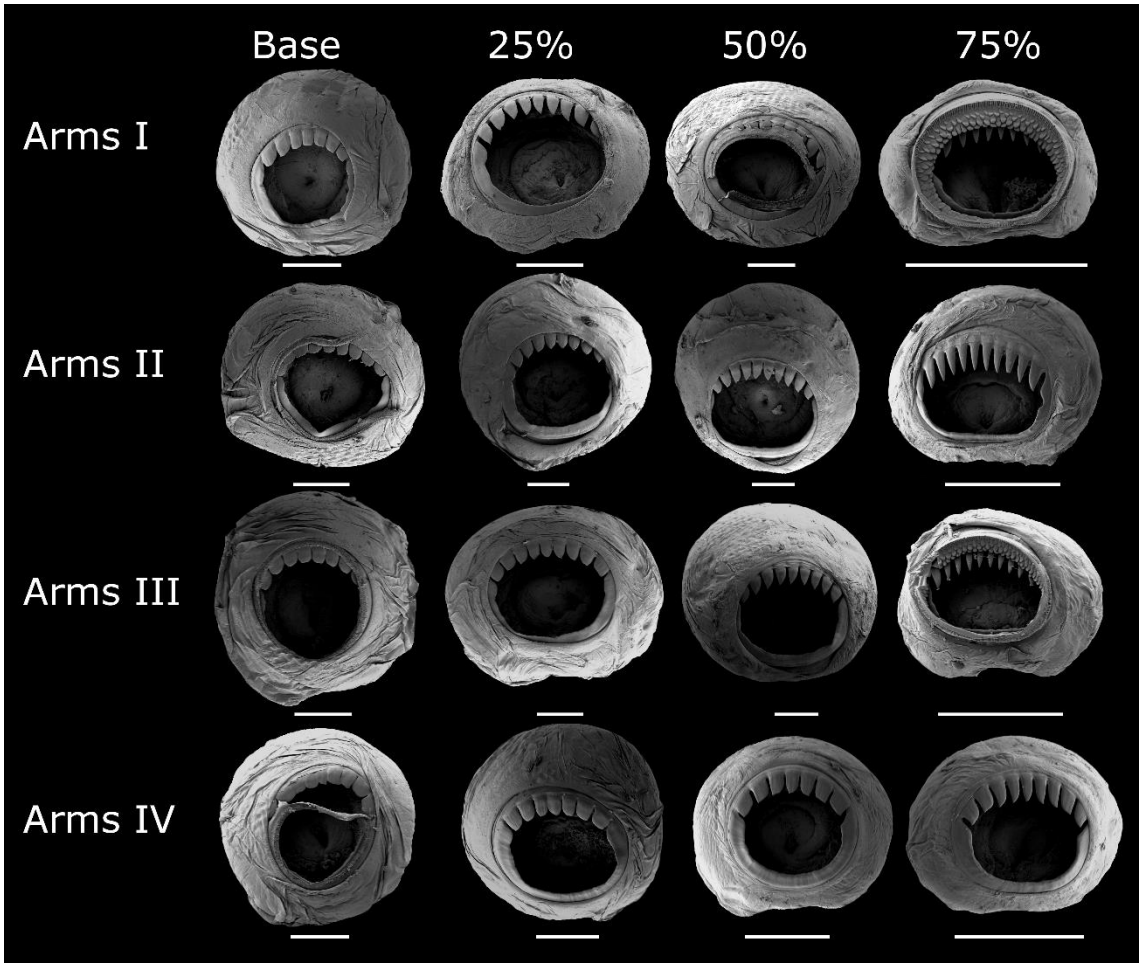

Fig. 7—*Asperoteuthis lui* arm suckers, NIWA 93268, ♀, LRL 5.43 mm. Placement along arm indicated as percent of arm length from the base. Scale bars = 500 μm.

tooth strongly bicuspid; inner cusp broad, triangular, slightly curved towards rachidian, its height ~100% that of overall rachidian; outer cusp sharply pointed, medially directed, its height ~60% that of inner cusp. Second lateral tooth simple, curved slightly towards rachidian, ~125% rachidian height. Marginal tooth simple, straight, ~145% height of rachidian. Marginal plate absent (Fig. 9B). Palatine palp (Fig. 9C) with ~70 narrow, flat teeth, each ~15–45% rachidian height, evenly distributed over palp.

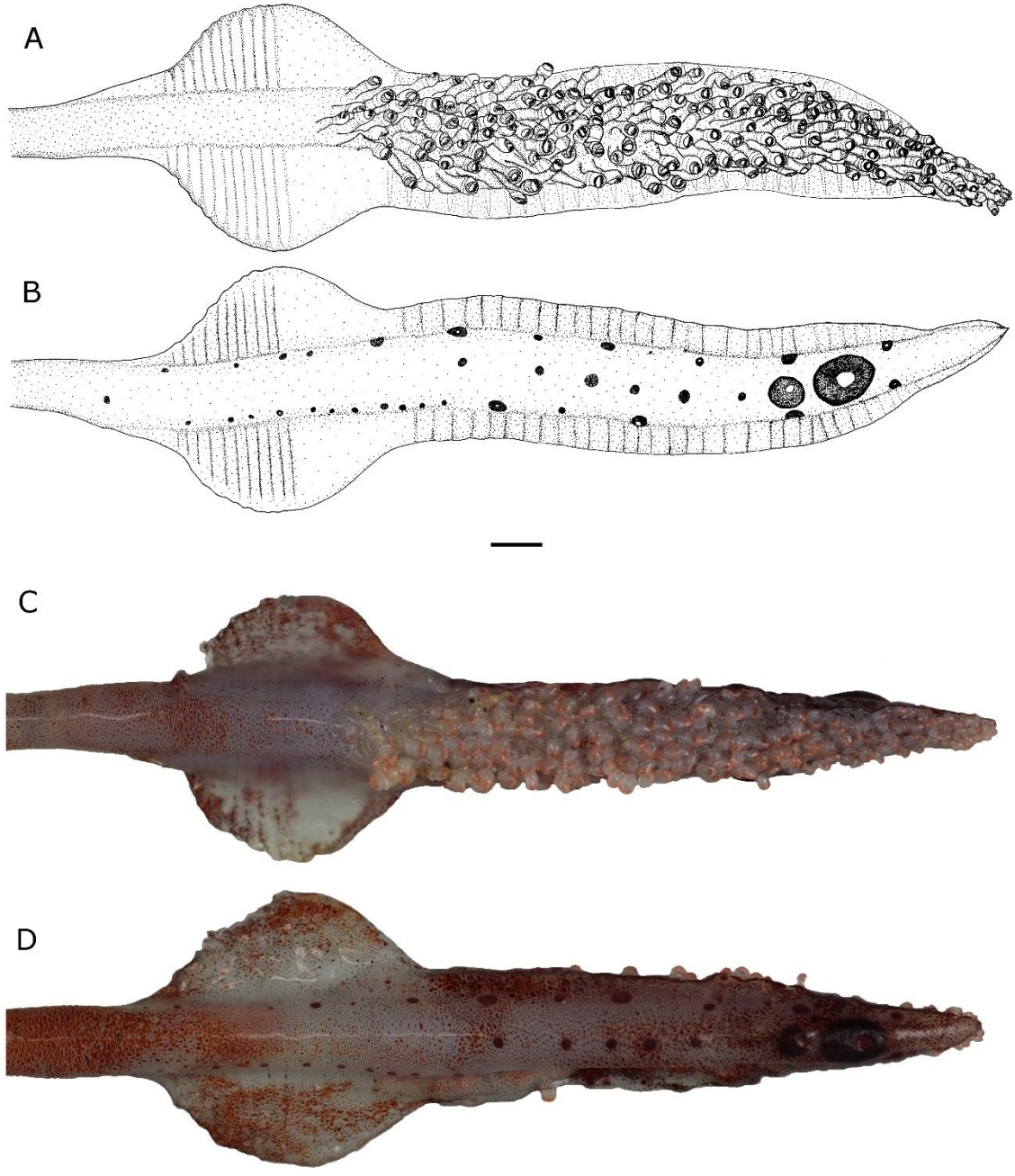

Fig. 8—*Asperoteuthis lui* tentacle club NIWA 93268, ♀, LRL 5.43 mm. A, C) Oral view; B) D) aboral view. C, D) Photographs by Darren Stevens. Scale bars = 5 mm.

Epidermis damaged on all examined material. Translucent white when fresh, yellow when preserved; purple and red chromatophores densely and evenly distributed on all exterior surfaces, absent from internal mantle.

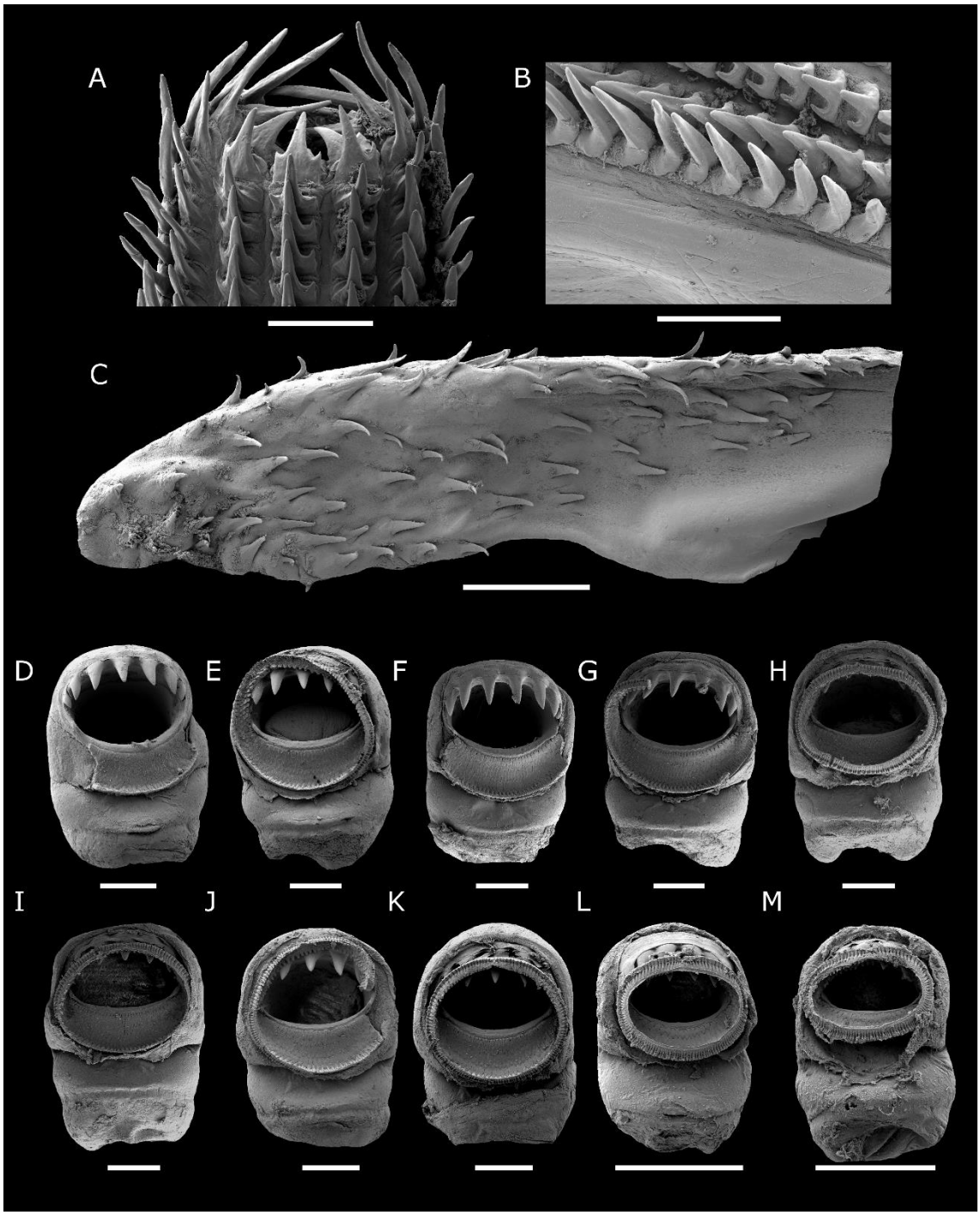

Fig. 9—*Asperoteuthis lui*. A–C) NIWA 93268, ♀, LRL 5.43 mm; D–M) NIWA 97258, sex indet., tentacle only, CL 170 mm. A) Radula; B) radula margin; C) palatine palp; D) proximal-most tentacle sucker; E) outer tentacle sucker close to base; F) inner sucker 25% from proximal-most sucker; G) outer sucker 25% from proximal-most sucker; H) inner sucker 50% from proximal-most sucker; I) outer sucker 50% from proximal-most sucker; J) inner sucker 75% from proximal-most sucker; K) outer sucker 75% from proximal-most sucker; L) tentacle-tip sucker from inner series; M) tentacle-tip sucker from outer series. Scale bars = 500 μm.

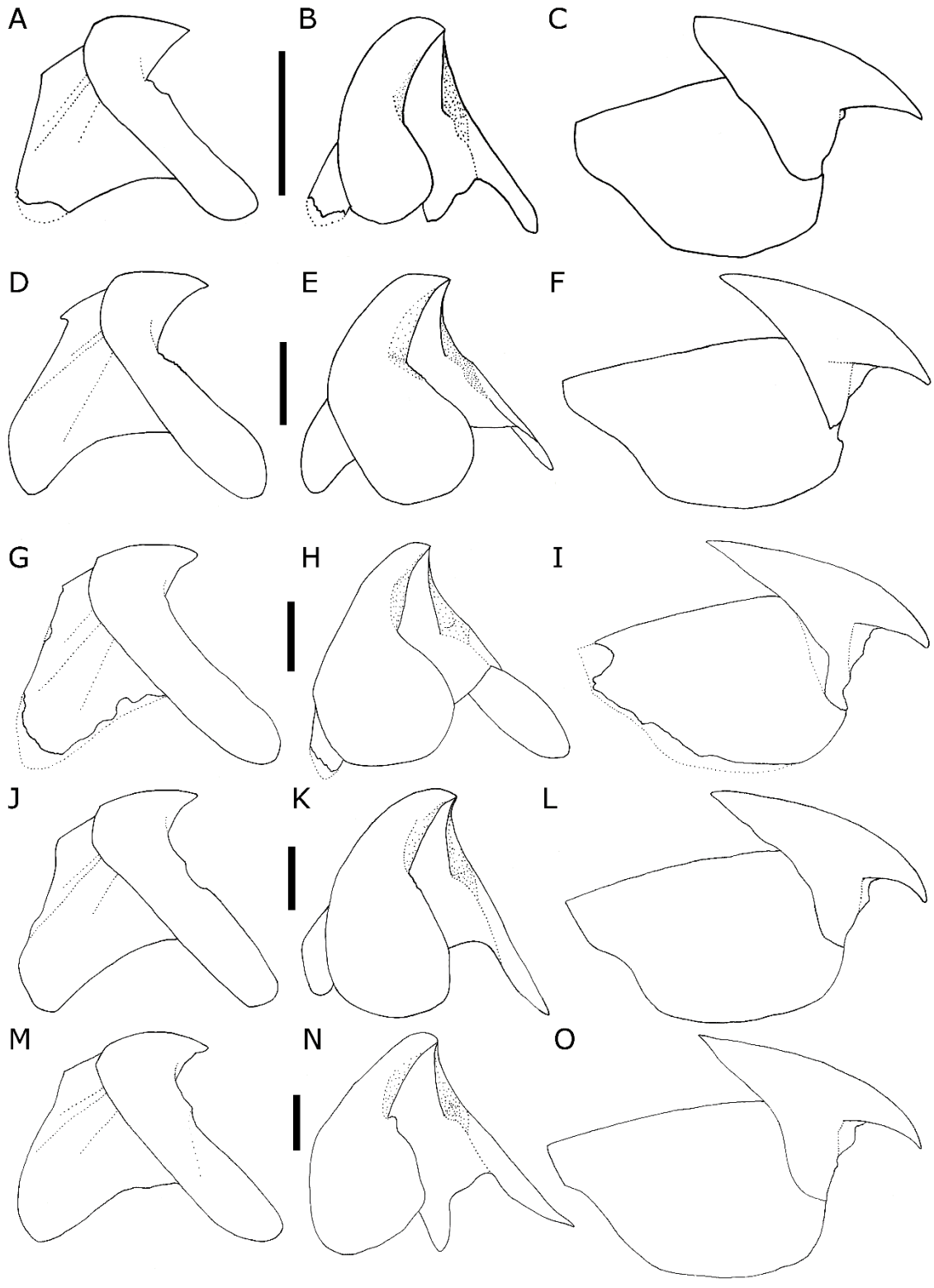

Fig. 10—*Asperoteuthis lui* beaks. A–C) NMNZ M.302215, sex indet., head only, LRL 3.26 mm; D–F) NIWA 93268, ♀, LRL 5.43 mm; G–I) NMNZ M.143859, sex indet., LRL 6.62 mm; J–L) NIWA 95041, ♀, beak only, LRL 7.34 mm; M–O) NIWA 95040, sex indet., beak only, LRL 7.70 mm. A, D, G, J, M) Lower beaks in lateral profile view; B, E, H, K, N) lower beaks in lateral oblique view; C, F, I, L, O) upper beaks. Scale bars = 5 mm.

Taxonomic Remarks:

Additional, recently collected specimens made this review possible. The most complete specimen available for genetic analysis was in good condition except that it lacked fins and eyes (NIWA 93268; Fig. 11). The tentacles (Fig. 8) and beaks (Figs 9D–F) of this specimen are morphologically consistent with those of the holotype for *A. lui* (Figs 9G–I). Some of the specimens examined herein consisted of only beaks (with buccal masses, which allowed for genetic analysis) or tentacles; these specimens were used to examine variation in beak morphology (Fig. 10) and tentacle-club photophore patterns, respectively. It appears that the tentacle-club photophores show some

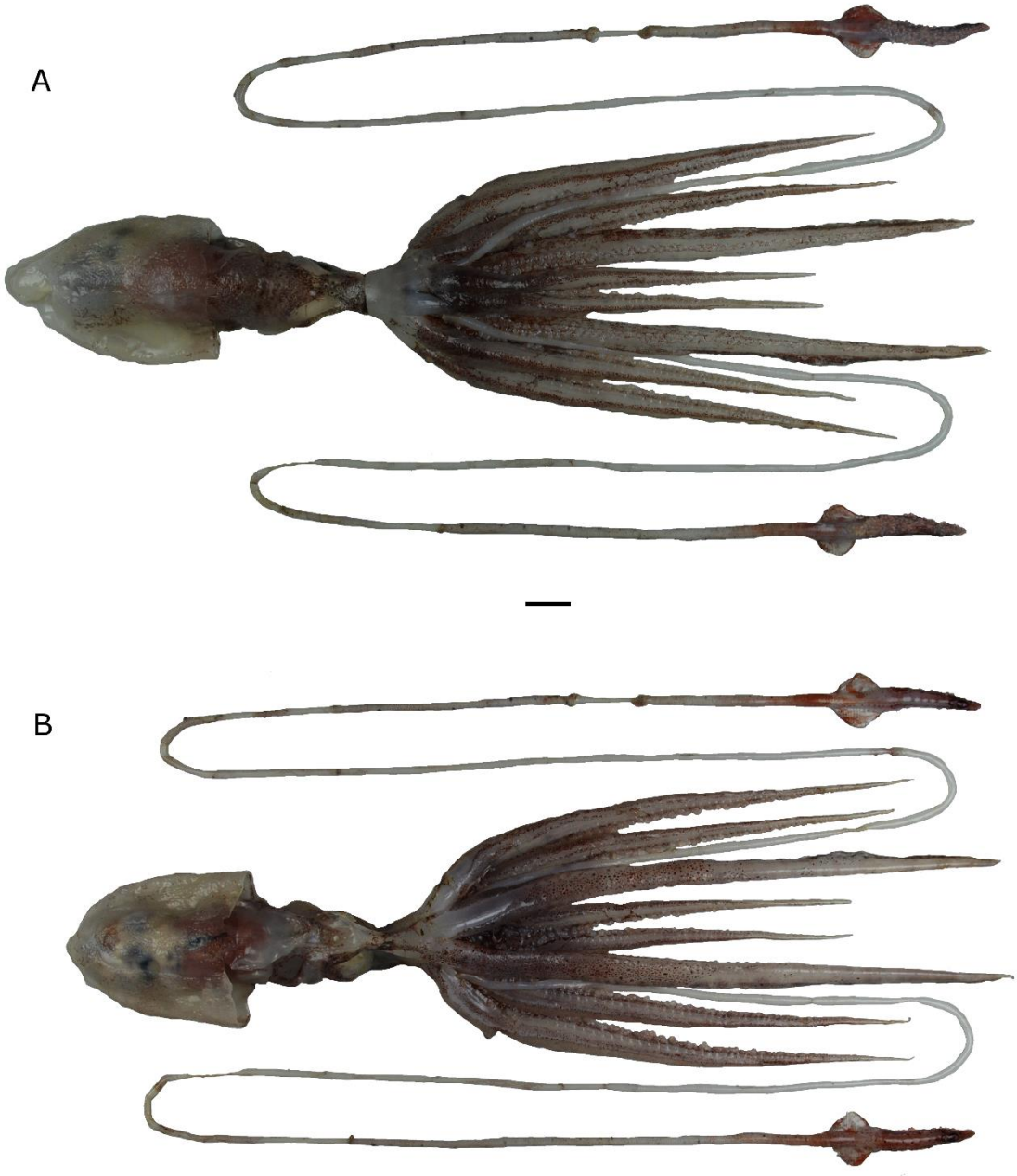

Fig. 11—*Asperoteuthis lui* NIWA 93268, ♀, LRL 5.43 mm. A) Dorsal; B) ventral. Photographs by Darren Stevens. Scale bar = 20 mm.

intraspecific variation, but a greater sample size will be needed to determine whether this is related to sex, growth, or locality.

The original description of *A. lui* was based on a single, incomplete specimen that was taken from the stomach of a ling (*Genypterus blacodes*) (Salcedo-Vargas, 1999). The specimen only consists of a head and arm crown, including two slightly damaged eyes and one tentacle (Salcedo-Vargas, 1999). Unfortunately, the sucker rings from the tentacle club and arms were degraded due to digestion. The only images in the type description were of the eye photophore and the tentacle club and stalk (Salcedo-Vargas, 1999). There are some inconsistencies with the type description for *A. lui* and the observations on that specimen made herein. The description of *A. lui* stated that the suckers on mid-Arms II and III were enlarged (Salcedo-Vargas, 1999); however, they appear to be simply the largest suckers on the animal, rather than truly enlarged suckers. The eyeball photophore was described as a patch (Salcedo-Vargas, 1999), but the examination herein found that it is elongate (Figs 5D–F). Salcedo-Vargas (1999) stated that there was no tentacle-club-tip photophore; however, there is a photophore located near the tentacle-club tip (Figs 7B, D), which is a characteristic of this genus. Two additional important features that appear to have been overlooked in the original description were the small embedded photophores on the lateral edges of the aboral surface of the club (Figs 7B, D) and the beak morphology (Fig. 10).

The characters that Arkhipkin and Laptikhovsky (2008) used to distinguish *A. nesisi* from other species of *Asperoteuthis* were based on features that were missing from the *A. lui* holotype (mantle musculature, fin shape, mantle skin texture, funnel-locking cartilage morphology, and arm-sucker dentition), or were not included in the original description of *A. lui* (arm sucker count and beak morphology). The specimens identified herein as *A. lui* have morphological characters that are consistent with those of *A. nesisi*, with some exceptions due to damage or size. The eye photophore was described as a single longitudinal photophore on the ventral surface of the eye (Arkhipkin & Laptikhovsky, 2008), which is consistent with the present findings (Figs 5D–F). The arm suckers described for *A. nesisi* had 12–14 sharp triangular teeth (Arkhipkin & Laptikhovsky, 2008), while the specimen examined herein had 7–11 blunt, rectangular or sharp, conical teeth (Fig. 7); this difference is likely related to size, because the specimen examined in the present study was smaller. Arkhipkin and Laptikhovsky (2008) reported small cartilaginous tubercles on the skin of the head and mantle, which were not observed herein, likely due to the damaged skin on all specimens examined in the present study. They also suggested that tentacles may be

absent in maturing specimens; however, this seems unlikely because the holotype for *A. lui*, which has similar arm lengths and head width, has a tentacle attached (CL 138mm) and even larger tentacles have been found (NIWA 97258, CL 170mm).

Arkhipkin and Laptikhovsky (2008) proposed that, due to a similar appearance, *A. nesisi* was probably synonymous with Clarke's (1980) '?*Mastigoteuthis* A', which is supported by the results of the present study; few differences were observed between Clarke's (1980) description and the specimens examined herein. Clarke's (1980) description is consistent with *A. lui* based on the characteristic gelatinous tissue that overlies the posterior portion of the mantle, the acutely pointed dorsal mantle margin, the long and narrow head, radula morphology, funnel- and mantle-locking-cartilage morphology, eye photophore shape, beak morphology, and general appearance. However, Clarke (1980) described a small muscular pad on each side of the posterior end of the mantle in both of the specimens he examined, which was not observed herein or reported by Arkhipkin and Laptikhovsky (2008). It is possible that this was either an artefact due to damaged caused by being eaten and partially digested, or possibly a character that is only associated with males (the specimens examined in this study were female or sex indet.). No swimming membranes were described on the arms, but these are easily damaged.

The taxonomic placement of '?*Mastigoteuthis* A' remained unclear for some time. Nesis (1987) suggested that his new species, '*Chiroteuthis*' n. sp. Nesis, 1974, from the South Atlantic, was synonymous with '?*Mastigoteuthis* A', and placed this species in a new unnamed genus, 'n. gen. B'. However, several morphological differences distinguish Neisis's (1974) '*Chiroteuthis*' n. sp. from *Asperoteuthis*: six series of suckers on the developing club; photophore present on the ink sac; Arms IV longer than other arms; and funnel-locking cartilage with rounded anti-tragus and without tragus (Nesis 1974). Because of these differences, the species reported by Nesis (1974) is now considered to belong in a new, as-yet undescribed genus in the family Chiroteuthidae (Nesis & Nikitina, 1999).

The only known specimen representing 'New Genus C' remains a badly damaged brachial crown from Antarctic waters (Young & Roper, 2000a), which is within the distribution for *A. lui*. There are many similarities between tentacle suckers of *A. lui* and those of the chiroteuthid genus 'New Genus C', which could not previously be compared to *A. lui* because the present study is the first report to describe the tentacle suckers in *A. lui* (Young & Roper, 2000a). The tentacle suckers for both have approximately the same number of conical teeth and are surrounded by a wide

papillated ring (Young & Roper, 2000b). It is possible that the 'New Genus C' is actually *A. lui*. In order to resolve this, a morphological comparison of additional suckers from along the length of the arms and the tentacles will be required.

A comparison of characters found in *A. lui*, *A. mangoldae*, and *A. acanthoderma* is summarised in Table 4 and mean indices are found in Table 3. *Asperoteuthis lui* is distinguished from both of these species by: 1) an elongate ventral eye photophore; 2) a medial row of photophores on the aboral tentacle club; 3) chiral photophores on the lateral margins of the aboral tentacle club, with more ventral photophores than dorsal; 4) ear-shaped funnel-locking cartilage with a weak tragus; 5) approximately oval mantle-locking cartilage; 6) tentacle suckers with 5–7 sharp, conical teeth; 7) more than twice as many tentacle-club suckers (~120–160); 8) circular skin depressions; 9) a relatively wide mantle; 10) a relatively longer club; and 11) a lack of trabeculae in the distal portion of the proximal, expanded region of the tentacle-club protective membrane. *Asperoteuthis lui* and *A. mangoldae* both have approximately circular fins, while *A. acanthoderma* has an oval fin, but the fin of *A. lui* is relatively larger. *Asperoteuthis acanthoderma* is additionally distinguished from the two other species by a larger club-tip photophore, inverted Y-shaped funnel-locking and mantle-locking cartilages, fewer teeth on arm and tentacle suckers, and a larger size at maturity (Lu, 1977). *Asperoteuthis acanthoderma* and *A. mangoldae* both have an oval ventral-eye photophore and lack circular skin depressions. *Asperoteuthis acanthoderma* has the longest arms relative to mantle length, *A. mangoldae* has the shortest arms, and *A. lui* arms are intermediate in length.

There are many commonalities in the aboral tentacle club photophores in the genus *Asperoteuthis*. The tentacle-club structure of all three species shares a lack of suckers in the proximal region of the tentacle, but the structure of the tentacles of *A. acanthoderma* is much more similar to *A. mangoldae* in terms of trabecula distribution, sucker count, club-length index, and photophores. Photophores in the lateral margins of the tentacle club have been reported for *A. mangoldae* (Young et al., 2007a), *A. acanthoderma* (Lu, 1977), and were found herein for *A. lui*. However, unlike the other species in this genus, *A. lui* photophores are chiral, which is a type of asymmetry in which a structure cannot be superimposed on its mirror image. In *A. lui*, the left and right tentacle clubs have an asymmetric distribution of photophores and the clubs are mirror images of each other—more photophores are present along the ventral margin (~11–16) than along the dorsal margin (~9–12). Although chirality is common in squid tentacles, the small embedded aboral photophores are symmetrical in *A. acanthoderma*

(Young & Roper, 2010) and *A. mangoldae* (Young et al., 2007a). In addition, bilaterally asymmetric photophore patterns are found in other squid; for example, the photophore patterns of histioteuthids (Horstkotte, 2008). *Asperoteuthis lui* has a series of medial club photophores with approximately five to eight smaller proximal photophores and two larger photophores distally, while *A. mangoldae* and *A. acanthoderma* only have a single medial club-tip photophore.

Discussion:

The taxonomic study of chiroteuthids is especially difficult because specimens are not frequently caught and are almost always damaged by capture. In addition, species in this family are especially delicate, and nearly all *Asperoteuthis* specimens lose their tail structure and tentacles during capture, which are important morphological features for their identification. Genetic analyses, such as DNA barcoding (Hebert et al., 2003), can be used for species identification of even badly damaged (St-Onge, LaRue, & Charpentier, 2008) and juvenile specimens (Victor et al., 2009). COI, along with 16S rRNA and 12S rRNA, has been helpful in species delimitation and the recognition of new species in the chiroteuthid families (Young et al., 2008; Braid et al., 2014; Chapter 3). Herein, COI, 16S rRNA, and 12S rRNA, in conjunction with a morphological analysis, have been used to determine that *A.* '*nesisi*' and '?*Mastigoteuthis* A' are junior synonyms of *A. lui*.

Asperoteuthis 'nesisi' and *A. lui* were both described from single, damaged specimens that were unfortunately each missing different morphological features: *A. lui* had a tentacle present, but the arm and tentacle suckers were damaged, and it lacked a mantle (Salcedo-Vargas, 1999); while *A. 'nesisi'* was intact other than the missing tentacles, tail, and damaged fin (Arkhipkin & Laptikhovsky, 2008). Because the description for *A. lui* primarily focused on the tentacle-club morphology, the relationship between *A. 'nesisi'* and *A. lui* was not clear when *A. 'nesisi'* was described. Fortunately, the type description for *A. 'nesisi'* included GenBank accession numbers for three mitochondrial genes from the holotype (Arkhipkin & Laptikhovsky, 2008). There were no DNA sequences associated with the *A. lui* holotype because this specimen was formalin fixed and described before DNA sequences were regularly included with species descriptions (a practice that is still not always undertaken or, when all available specimens are formalin fixed, possible).

Sequences for COI, 16S rRNA, and 12S rRNA from the holotype of *A.* '*nesisi*' were compared with specimens collected in New Zealand waters with tentacle club morphology consistent with *A. lui*, and with beaks with morphology consistent with '?*Mastigoteuthis* A', and they all formed a single cluster on the maximum-likelihood phylogeny, with very little variation within that clade, and a large gap between that clade and *A. mangoldae* (Fig. 5). These three taxa were also assigned the same Barcode Index Number (BIN) (Fig. 5), and there is a high concordance between BINs and species (Ratnasingham & Hebert, 2013). BINs have been successfully used to delimitate species in other genera in the Chiroteuthidae (Chapter 3), and in the closely related squid family Mastigoteuthidae (Braid et al., 2014).

The relationship among the three valid species in this genus remains unclear. Arkhipkin and Laptikhovsky (2008) suggested that, based on morphology, *A. acanthoderma* and *A. 'nesisi'* have a closer relationship to each other than to *A. mangoldae*. Herein, it appears that there are more similarities in the tentacle-club morphology of *A. acanthoderma* and *A. mangoldae*, than with *A. lui*. However, Braid et al. (2017) found the genus *Asperoteuthis* to be polyphyletic, and their Bayesian phylogeny showed some support for a sister relationship between *A. lui* and *A. mangoldae*. These results suggest the possibility that there may be additional species of *Asperoteuthis* that have not been sequenced yet, or that this genus needs to be reassessed.

There are very few records of specimens identified as *Asperoteuthis* in stomach contents of predators. However, they have been reported from the gut contents of sperm whales (*Physeter macrocephalus* Linnaeus, 1758; Gómez-Villota, 2007), ling (*Genypterus blacodes*; Salcedo-Vargas, 1999), and blue sharks (*Prionace glauca* [Linnaeus, 1758]; Kubodera, Watanabe, & Ichii, 2006). It was only recently recognised that Clarke's (1980) '?*Mastigoteuthis* A' is an *Asperoteuthis* species (Arkhipkin & Laptikhovsky, 2008; Young, 2015); therefore, this species has been previously incorrectly attributed to the family Mastigoteuthidae in dietary analyses. Even recent ecological studies continue to apply the original incorrect mastigoteuthid classification (e.g., Alvito et al., 2015; Bloom, 2012; Guerreiro et al., 2015). Beaks identified as '?*Mastigoteuthis* A' have been reported from the stomach contents of southern bottlenose whales (*Hyperoodon planifrons* Flower, 1882; Clarke & Goodall, 1994), sperm whales (Clarke, 1980; Pascoe et al., 1990), Grey-headed albatross (*Thalassarche chrysostoma* [Forster, 1785]) (Cherel, Weimerskirch, & Trouvé, 2002; Richoux, Jaquemet, Bonnevie, Cherel, & McQuaid, 2010; Alvito et al., 2015), wandering

albatross (*Diomedea exulans* [Linnaeus, 1758]; Guerreiro et al., 2015; Rodhouse, Clarke, & Murray, 1987; Xavier, Phillips, & Cherel, 2011), white-chinned petrels (*Procellaria aequinoctialis* Linnaeus, 1758; Bloom, 2012), Antarctic fur seals (*Arctocephalus gazella* Peters, 1875; Lea et al., 2002), Patagonian toothfish (*Dissostichus eleginoides* Smitt, 1898; Cherel et al., 2004), and porbeagles (*Lamna nasus* [Bonnaterre, 1788]; Cherel & Duhamel, 2004). Herein, '?*Mastigoteuthis* A' has been recognised as a junior synonym of *A. lui*. Therefore, the previous importance of '?*Mastigoteuthis* A' in the diets of marine predators must be transferred to *A. lui*, indicating that the role of *Asperoteuthis* in the feeding ecology of other species has been dramatically underestimated.

Using a combination of morphology and mitochondrial genes, the identity of *A. lui*, *A. nesisi*, and '?*Mastigoteuthis* A' has been resolved. This demonstrates the importance of including genetic sequences with species descriptions whenever possible. The ecological importance of *Asperoteuthis* in the diets of marine mammals, birds, and fish has been underestimated because the identity of '?*Mastigoteuthis* A' remained unknown until recently. It appears that the biodiversity of the genus *Asperoteuthis* has been overestimated, with a previous estimate of five species (Young & Roper, 2015). Currently, only three valid species recognised in this genus: *A. acanthoderma*, *A. mangoldae*, and *A. lui*. This study highlights the significance of critical taxonomic revisions as additional material for poorly known species becomes available. Accurate species identification is fundamental for all biological research, with ramifications for predator-prey relationships and conservation. The combination of the three mitochondrial genes (COI, 16S rRNA, and 12S rRNA) used in this study and morphology was successfully used for alpha taxonomy, and has the potential to also aid in beta taxonomy.

Chapter 3: One step closer to understanding the chiroteuthid families in the Pacific Ocean

Abstract:

The chiroteuthid families are a clade united morphologically by the absence of a primary tentacle club and the presence of a secondary tentacle club, comprising six families: the Chiroteuthidae, Mastigoteuthidae, Joubiniteuthidae, Promachoteuthidae, Batoteuthidae, and Magnapinnidae. This study provides new information on the group's biodiversity in the Pacific Ocean and the interrelationships among these taxa and those from other locations, using fresh and ethanol-fixed specimens collected from Japan, Hawaii, California, and New Zealand from three institutions. Sequences were obtained for cytochrome *c* oxidase subunit I (COI), 16S rRNA, and 12S rRNA, nearly doubling the available sequences for the chiroteuthid families. Although the genera *Chiroteuthis* and *Asperoteuthis* did not resolve into monophyletic clades, this analysis did find support for the '*C. veranyi*' group and the '*C. pictetii*' group—identifying additional unnamed species in both—and the mastigoteuthid genera. A close relationship was found between *Echinoteuthis atlantica* and *Mastigotragus pyrodes*, with the latter reported herein for the first time from Japanese waters. The genus *Idioteuthis* appears to contain at least two species, making *I.* '*cordiformis*' a species complex in need of resolution. A catalogue of all specimens in this clade (representing 12 species across four families) registered in the collections of the National Museum of Nature and Science, Tokyo (NSMT) is also provided.

Introduction:

The 'chiroteuthid families' comprise a group of related deep-sea oegopsid squids that share morphological similarities (Young, 1991): ventral attachment of the buccal connectives to Arms IV, a gladius with a secondary conus (except in the Promachoteuthidae Naef, 1912), and the absence of a primary tentacle club (Vecchione & Young, 1998). The clade is formed by six families: the Chiroteuthidae Gray, 1849, the Mastigoteuthidae Verrill, 1881, the Joubiniteuthidae Naef, 1922, the Promachoteuthidae, the Batoteuthidae Young and Roper, 1968, and the Magnapinnidae Vecchione and Young, 1998. Within this group, the families Chiroteuthidae and Mastigoteuthidae appear to be the most speciose, and are certainly the more often encountered and better represented in collections. Much work remains to be done, however—the generic status of mastigoteuthids has recently been stabilised (Braid et

al., 2014; Young, Vecchione, & Braid, 2014), but a worldwide review of all species in the family is still needed, and the Chiroteuthidae (whose taxonomy has been quite unstable; Young, 1991; Salcedo-Vargas, 1996; Braid & Bolstad, 2015) remains one of the deep-sea cephalopod families most in need of revision (Hoving et al., 2014).

Chiroteuthids are particularly challenging due to the drastic morphological changes that occur during their development and because specimens are frequently damaged during capture (Young, 1991). Some taxa remain so poorly known that Roper and Young (2013) have suggested no new species be named in the genus *Chiroteuthis* d'Orbigny [in Férussac & d'Orbigny], 1841, until our understanding of the morphological variation within each of the known species improves. One genus in this family, *Asperoteuthis*, was examined using integrative taxonomy—morphology and three mitochondrial genes (cytochrome *c* oxidase subunit I [COI], 16S rRNA, and 12S rRNA)—in Chapter 2. This approach was successful in increasing resolution at the species level (Chapter 2), and has also been used for both alpha- and beta-taxonomy in the closely-related family Mastigoteuthidae (Braid et al., 2014). This family could therefore benefit greatly from a thorough re-examination using integrative taxonomic methods.

Recent studies on the Chiroteuthidae have tended to focus on specific regions, primarily the United States (e.g., Young et al., 2007a), the Falklands (Arkhipkin & Laptikhovsky, 2008), and New Zealand (Mensch, 2010). Of these, only Arkhipkin and Laptikhovsky (2008) included genetic data; they included three sequences in their description of *Asperoteuthis nesisi* Arkhipkin & Laptikhovsky, 2008 (=*A. lui*, see Chapter 2). In total, sequences are publicly available for just eight of the 19 known chiroteuthid species, and these have been generated for inclusion in molecular phylogenetic analyses (Lindgren, 2010; Lindgren, Pankey, Hochberg, & Oakley, 2012; Braid et al., 2014), rather than for taxonomic insight into the Chiroteuthidae. Among the five other closely related families, sequences are publicly available for 15 of the ~43 known species, and the majority of these (11) are mastigoteuthids, most of which were compiled as part of a recent local revision of that family (Braid et al., 2014; Braid & Bolstad, 2015).

This study therefore aims to increase global knowledge of the chiroteuthid families by providing new information on the group's representatives in the Pacific Ocean, as a step toward a larger eventual revision of the group (Lindgren et al., in prep.). This study substantially increases the number of DNA sequences available for specimens in these families, presenting sequences for COI, 16S rRNA, and 12S rRNA

from primarily Pacific specimens sourced from the National Museum of Nature and Science, Tokyo (NSMT); the Field Museum of Natural History (FMNH), Chicago, USA; and the National Institute for Water and Atmospheric Research Ltd. (NIWA), in Wellington, New Zealand. In addition, a catalogue of all chiroteuthid families material available in the NSMT collections (more than 140 lots) has been provided, identified using morphology and current classifications. This institution—which houses over 7000 cephalopod specimens and over 400 cephalopod tissue samples fixed in ethanol for genetic analysis—is a particularly valuable source of material for integrative taxonomic efforts on this group. These sequences and the catalogue were generated with the hope that they will be helpful to future researchers aiming to resolve this challenging clade. Finally, the combination of three mitochondrial genes (COI, 16S rRNA, and 12S rRNA) and morphology was assessed for its systematic resolution above the species level.

Methods:

Genetic analysis

Specimens sequenced in this study came from three separate collections (Table 4). Tissue from 10 samples fixed in 100% ethanol were available from the NSMT. Eleven specimens representing five taxa were available from FMNH, fixed in 95% ethanol or RNAlater®. Tissue samples of seven specimens caught in New Zealand waters came from NIWA, and were fixed in 100% ethanol or frozen until DNA extraction. Additional sequences from species in the chiroteuthid families were included from the Barcode of Life Data System (BOLD) and GenBank (Table 5). The outgroup species, *Octopoteuthis nielseni* (Robson, 1948), was chosen from the lepidoteuthid families clade because this clade is a sister clade to the chiroteuthid families clade (Lindgren, 2010; Lindgren et al., 2012).

DNA was extracted using either EconoSpin (Epoch Life Science) columns with QIAGEN reagents following protocols for the DNeasy Blood & Tissue Kit (QIAGEN), or with alkaline lysis following Ivanova et al. (2009). Three mitochondrial gene regions (COI, 16S rRNA, and 12S rRNA) were amplified following protocols and primers in Braid et al. (2014). When amplification was unsuccessful, a secondary PCR was performed using internal primers with the cephalopod-specific primers from Braid et al. (2014) to amplify the DNA barcode region in two halves (LCO1490_CephF/mCephR [GCTCCTCTTTCTACAGCTGA]; mCephF

Table 5—Specimen information for sequences used in Present study.

Genetic ID	Specimen ID	BOLD ID	GenBank			Reference
			COI	16S rRNA	12S rRNA	
Batoteuthidae						
Batoteuthis						
Batoteuthis skolops		N/A	AY557527	EU735200	N/A	Lindgren et al. (2004) for COI; Lindgren (2010) for 16S rRNA
Chiroteuthidae						
Asperoteuthis						
A. acanthoderma	S004-40	CHSQX001-16	KX783172	KX783230	KX783198	Present study
A. acanthoderma	NSMT184	CHSQX002-16	KX783171	KX783229	KX783197	Present study
A. acanthoderma	REF_Carib		KT326921	N/A	N/A	Lindgren et al. (unpublished)
A. mangoldae	FMNH 278099	CHSQX003-16	KX783173	KX783231	KX783199	Present study
A. lui	NIWA 97258	ALUI001-16	KX675432	KX675440	KX675448	Chapter 2
A. lui	NIWA 93268	ALUI002-16	KX675431	KX675439	KX675447	Chapter 2
A. lui	NIWA 96168	ALUI003-16	KX675430	KX675438	KX675446	Chapter 2
A. lui	NIWA 96166	ALUI004-16	KX675429	KX675437	KX675445	Chapter 2
A. lui	NIWA 95041	ALUI005-16	KX675428	KX675436	KX675444	Chapter 2
A. lui	NIWA 93270	ALUI006-16	KX675427	KX675435	KX675443	Chapter 2
A. lui	NIWA 95039	ALUI007-16	KX675426	KX675434	KX675442	Chapter 2
A. lui	NIWA 95040	ALUI008-16	KX675425	KX675433	KX675441	Chapter 2
A. lui	BMNH 20070615	GBCPH775-09	EU421718	EU421719	EU421720	Arkhipkin & Laptikhovsky (2008)
Chiroteuthis						
C. calyx	FMNH 329592	CHSQX004-16	KX783179	KX783237	KX783205	Present study
C. calyx	FMNH 330004	CHSQX005-16	KX783180	KX783238	KX783206	Present study
C. calyx	FMNH 330063	CHSQX006-16	KX783181	KX783239	KX783207	Present study

Table 5—Continued.

Genetic ID	Specimen ID	BOLD ID	GenBank			Reference
			COI	16S rRNA	12S rRNA	
C. calyx	FMNH 330049	CHSQX007-16	KX783176	KX783234	KX783202	Present study
C. calyx	FMNH 330054	CHSQX008-16	KX783175	KX783233	KX783201	Present study
C. calyx	FMNH 330061	CHSQX009-16	KX783174	KX783232	KX783200	Present study
C. calyx	FMNH 330065	CHSQX010-16	KX783178	KX783236	KX783204	Present study
C. calyx	NSMT Mo.71605	CHSQX011-16	KX783177	KX783235	KX783203	Present study
C. calyx	BeringCalyx	GBCPH1066-10	EU735372	EU735237	N/A	Lindgren (2010)
C. mega	NIWA 76669	MPMTG022-12	KC860951	KC860982	KC861168	Braid et al. (2014)
C. mega	DE 0506 (sta. 19)	GBCPH1076-10	EU735362	EU735225	N/A	Lindgren (2010)
C. picteti	NSMT Mo.85541	CHSQX012-16	KX783182	KX783240	KX783208	Present study
C. veranyi	DE0304 (Sta. 4)		AY557529	N/A	N/A	Lindgren et al. (2004)
C. veranyi	MO78622	BIM387-14	N/A	N/A	N/A	Rinkevich (unpublished)
C. aff. *veranyi*	NIWA 105204	CHSQX014-16	KX783184	KX783242	KX783211	Present study
C. aff. *veranyi*	NIWA 105205	CHSQX015-16	KX783190	KX783248	KX783217	Present study
C. aff. *veranyi*	NIWA 92499	CHSQX016-16	KX783189	KX783247	KX783216	Present study
C. aff. *veranyi*	NIWA 92498	CHSQX017-16	KX783188	KX783246	KX783215	Present study
C. aff. *veranyi*	NIWA 105206	CHSQX018-16	KX783187	KX783245	KX783214	Present study
C. aff. *veranyi*	NIWA 96168	CHSQX019-16	KX783186	KX783244	KX783213	Present study
C. aff. *veranyi*	NIWA 96179	CHSQX020-16	KX783185	KX783243	KX783212	Present study
C. sp	NSMT260	CHSQX021-16	KX783183	KX783241	KX783210	Present study
C. sp	NSMT Mo.74587	CHSQX013-16	N/A	N/A	KX783209	Present study
Chiroteuthidae sp.	FMNH278015	GBCPH0129-06	AF075413	N/A	N/A	Anderson (2000)

Table 5—Continued.

Genetic ID	Specimen ID	BOLD ID	GenBank			Reference
			COI	16S rRNA	12S rRNA	
Grimalditeuthis						
G. bonplandi	DE0506 (sta. 11)	GBCPH1075-10	EU735363	EU735226	N/A	Lindgren (2010)
G. bonplandi	UCONN:Mo35.1	GBCPH1523-14	GU145075	N/A	N/A	Bucklin et al. (unpublished)
Planctoteuthis						
Pl. cf. *danae*	UCONN:Mo37.1.1	GBCPH1520-14	GU145077	N/A	N/A	Bucklin et al. (unpublished)
Pl. cf. *danae*	FMNH 278103	CHSQX022-16	KX783196	KX783254	KX783224	Present study
Pl. levimana	Mar-Eco #003372	GBCPH1054-10	EU735384	EU735247	N/A	Lindgren (2010)
Joubiniteuthidae						
Joubiniteuthis						
J. portieri	FMNH 278104	CHSQX028-16	KX783193	KX783251	KX783220	Present study
J. portieri	DE0304 (Stat. 14)	GBCPH784-09	EU201163	EU201153	EU201142	Young et al. (2008)
Magnapinnidae						
Magnapinna						
Mn. sp.	*Magnapinna* sp. ARL-2008	GBCPH1074-10	EU735364	EU735227	N/A	Lindgren (2010)
Mn. sp.	UCONN:Mo32.1.1	GBCPH1524-14	GU145072	N/A	N/A	Bucklin et al. (unpublished)
Mastigoteuthidae						
Echinoteuthis						
E. atlantica	USNM 1191216	MPMTG016-12	KC860970	KC861001	KC861187	Braid et al. (2014)
Idioteuthis						
I. cf. *latipinna*	NSMT Mo.75595	CHSQX023-16	KX783192	KX783250	KX783219	Present study
I. cf. *cordiformis*	NIWA 71437	CHSQX024-16	KX783191	KX783249	KX783218	Present study

Table 5—Continued.

Genetic ID	Specimen ID	BOLD ID	GenBank			Reference
			COI	16S rRNA	12S rRNA	
I. cf. *cordiformis*	NMNZ M.306356	MPMTG018-12	KC860952	KC860983	KC861169	Braid et al. (2014)
I. cf. *cordiformis*	NMNZ M.306355	MPMTG017-12	KC860955	KC860986	KC861172	Braid et al. (2014)
I. cf. *cordiformis*	NIWA 84390	MPMTG023-12	KC860953	KC860984	KC861170	Braid et al. (2014)
I. cf. *cordiformis*	NMNZ M.306358	MPMTG019-12	KC860954	KC860985	KC861171	Braid et al. (2014)
Magnoteuthis						
Mg. magna	USNM 1191198	MPMTG001-12	KC860963	KC860994	KC861180	Braid et al. (2014)
Mg. magna	USNM 1191199	MPMTG002-12	KC860962	KC860993	KC861179	Braid et al. (2014)
Mg. magna	USNM 1191200	MPMTG003-12	KC860961	KC860992	KC861178	Braid et al. (2014)
Mg. cf. *magna*	NSMT147	CHSQX025-16	N/A	N/A	KX783221	Present study
Mg. microlucens	FMNH 278100	CHSQX026-16	KX783194	KX783252	KX783222	Present study
Mg. microlucens	Keahole Pt., Hawaii		EU201161.1	EU201150.1	EU201139.1	Young et al. (2008)
Mg. osheai	NIWA 76653	MPMTG021-12	KC860964	KC860995	KC861181	Braid et al. (2014)
Mastigopsis						
Mp. hjorti	USNM 1191214	MPMTG014-12	KC860960	KC860991	KC861177	Braid et al. (2014)
Mp. hjorti	USNM 1191215	MPMTG015-12	KC860959	KC860990	KC861176	Braid et al. (2014)
Mp. hjorti	USNM 1191213	MPMTG013-12	KC860956	KC860987	KC861173	Braid et al. (2014)
Mp. hjorti	USNM 1191202	MPMTG005-12	KC860958	KC860989	KC861175	Braid et al. (2014)
Mp. hjorti	USNM 1191201	MPMTG004-12	KC860957	KC860988	KC861174	Braid et al. (2014)
Mastigoteuthis						
Mt. agassizii	USNM 1191203	MPMTG006-12	KC860965	KC860996	KC861182	Braid et al. (2014)
Mt. agassizii	USNM 1191207	MPMTG010-12	KC860969	KC861000	KC861186	Braid et al. (2014)
Mt. agassizii	USNM 1191208	MPMTG011-12	KC860967	KC860998	KC861184	Braid et al. (2014)

Table 5—Continued.

Genetic ID	Specimen ID	BOLD ID	GenBank			Reference
			COI	16S rRNA	12S rRNA	
Mt. agassizii	USNM 1191209	MPMTG012-12	KC860968	KC860999	KC861185	Braid et al. (2014)
Mt. agassizii	USNM 1191206	MPMTG009-12	KC860966	KC860997	KC861183	Braid et al. (2014)
Mt. agassizii	USNM 1191205	MPMTG008-12	KC860980	KC861007	KC861193	Braid et al. (2014)
Mt. agassizii	USNM 1191204	MPMTG007-12	KC860981	KC861008	KC861194	Braid et al. (2014)
Mt. cf. *dentata*	NIWA 95870	NZMTG011-14	KP725210	KP725215	KP725224	Braid & Bolstad (2015)
Mt. cf. *dentata*	NIWA 95868	NZMTG012-14	KP725211	KP725216	KP725225	Braid & Bolstad (2015)
Mt. cf. *dentata*	NIWA 95869	NZMTG013-14	KP725212	KP725217	KP725226	Braid & Bolstad (2015)
Mt. cf. *dentata*	NIWA 95871	NZMTG007-14	KP725213	KP725218	KP725227	Braid & Bolstad (2015)
Mt. cf. *dentata*	NIWA 95872	NZMTG008-14	KP725214	KP725219	KP725228	Braid & Bolstad (2015)
Mt. psychrophila	NIWA 44293	MPMTG024-12	KC860972	KC861002	KC861188	Braid et al. (2014)
Mt. psychrophila	NIWA 44303	MPMTG025-12	KC860973	KC861003	KC861189	Braid et al. (2014)
Mt. psychrophila	NIWA 44301.2	MPMTG026-12	KC860976	KC861004	KC861190	Braid et al. (2014)
Mt. psychrophila	NIWA 44301.3	MPMTG027-12	KC860977	KC861005	KC861191	Braid et al. (2014)
Mt. psychrophila	NIWA 44304	MPMTG028-12	KC860978	KC861006	KC861192	Braid et al. (2014)
Mastigotragus						
Mr. pyrodes	NSMT Mo.71606	CHSQX027-16	KX783195	KX783253	KX783223	Present study
Octopoteuthidae						
Octopoteuthis						
O. nielseni		GBCPH0080-06	AF000055	AY616983	AY616957	Carlini & Graves (1999) for COI; Strugnell et al. (unpublished) for 16S rRNA and 12S rRNA

[GAGCACCAGATATAGCATTCCCACG]/ HCO2198_CephR). The sequenced reaction was performed using the same primers that were used for the PCR (Macrogen, Korea). Bidirectional sequence contig assemblies were created and edited in Sequencher v. 4.9 (Gene Codes). Sequences were uploaded to the Barcode of Life Data System (BOLD; Ratnasingham & Hebert, 2007) public project titled 'Chiroteuthid Families' (project code: CHSQX) and subsequently submitted to GenBank (Table 5). Sequences were checked for potential contamination using a Basic Local Alignment Search Tool (BLAST) search through GenBank.

Sequences were aligned in Geneious 7.1.7 (Biomatters, Auckland, New Zealand) using the MAFFT algorithm (Katoh, Misawa, Kuma, & Miyata, 2002), and subsequently manually trimmed and concatenated. PartitionFinder 1.1.1 (Lanfear et al., 2012) was run on the concatenated alignment with all substitution models included under Bayesian Information Criterion. Each codon position for COI was searched separately, as well as each rRNA gene, which could lead to a maximum of five partitions. Different models were chosen for each codon position of COI as TrNef + I + G, F81, and TIM + G, respectively. A single model, GTR + I + G, was chosen for both 16S rRNA and 12S rRNA. A combined maximum-likelihood combined phylogeny was created in GARLI 2.0.1 (Zwickl, 2006) with 1000 bootstrap replicates.

A Bayesian combined phylogeny was created using the same data in BEAST 1.8.0 (Drummond, Suchard, Xie, & Rambaut, 2012) using the substitution model SRD06 (Shapiro, Rambaut, & Drummond, 2005) for COI, and HKY + G + I for both rRNA genes, which were determined through Tracer 1.6 (Rambaut et al., 2014). Three independent runs of 10 000 000 iterations were performed, with the first 1 000 000 removed as burn-in. Both partitions used uncorrelated lognormal relaxed clocks and assumed a Yule Process for tree prior. Tracer 1.6 (Rambaut et al., 2014) was used to determine the correct burn-in and to check for convergence. The tree files were concatenated using LogCombiner 1.8.0 and the maximum clade credibility tree was then selected from the combined output using TreeAnnotator 1.8.0. Nodes with low support (below posterior probability below 0.5) were collapsed TreeGraph 2 (Stöver & Müller, 2010), and the final phylogeny was visualised in FigTree 1.4.0 (Rambaut, 2012).

Species boundaries were tested using the Barcode Index Number (BIN) system, which uses a clustering algorithm to create operational taxonomic units based on COI, which have a high concordance with species (Ratnasingham & Hebert, 2013). BINs are automatically generated by BOLD for barcode sequences (Ratnasingham & Hebert,

2013). Because COI could not be recovered from two *Chiroteuthis* specimens (preventing these specimens' inclusion in the BIN analysis), their species boundaries were tested using 12S rRNA. For this analysis, a single-gene phylogeny was created using the parameters specified above for 12S rRNA using GARLI (Zwickl 2006) with 1000 bootstrap replicates. This phylogeny was used to determine species delimitations using the maximum-likelihood solution based on the Bayesian Poisson tree processes (bPTP) model (Zhang, Kapli, Pavlidis, & Stamatakis, 2013).

Catalogue of specimens

The entire collection of specimens in the chiroteuthid families from NSMT were examined (Appendix 1). Specimens were identified to species or to the lowest possible taxon following Nesis (1987), Mensch (2010), Roper and Young (2013), and Braid and Bolstad (2015). Some badly damaged specimens could not be confidently attributed to species; some individuals could only be attributed to species complexes because further work is needed to resolve these taxa. Specimens from the *Mastigoteuthis* ('*Mt.*') *agassizii* Verrill, 1881, species complex are here reported as *Mt.* cf. *dentata* Hoyle, 1904, because *Mt. dentata* is currently considered a valid Pacific species, but may eventually prove a junior synonym of the Atlantic *Mt. agassizii* (Braid & Bolstad, 2015). *Idioteuthis* Sasaki, 1916, specimens from Japan are reported as *I.* cf. *latipinna* Sasaki, 1916, because the present analysis revealed at least two species in this genus, with Japan being the type locality for *I. latipinna*. *Idioteuthis* specimens from New Zealand waters are conservatively attributed to *I.* cf. *cordiformis* (Chun, 1908) (type locality Indian Ocean) but future analyses may reveal these to represent a separate locally occurring species requiring description. Collection coordinates for the NSMT material (where available; see Appendix 1) were plotted on a world map (Fig. 12) and specimens from Japanese waters on an additional more detailed map (Fig. 13) using ArcGIS 10.2 (Environmental Systems Research Institute [ESRI], Redlands, CA).

To verify the identification of *Mastigotragus* ('*Mr.*') *pyrodes* Young, 1972, the type description (Young, 1972) was reviewed along with photographs from the Santa Barbara Museum of Natural History (SBMNH) of the holotype [SBMNH 34983, ♂, 110 mm ML, 33.53°N, 118.38°W, 02/02/1961, Velero Stn 7279] and paratypes [SBMNH 34986, ♀, 148 mm ML, 28.90°N, 117.82°W, 25/11/1965, Velero Stn 10844; SBMNH 34987, ♀, 180* mm ML, 29.67°N, 118.80°W, 02/08/1966, Velero Stn 11181]. A comparative specimen from the Zoological Museum Hamburg (ZMH) [ZMH 3827/1,

sex indet., 105*mm ML, 30.67°N, 117.83°W, 280 m,16/04/1975, RV *Weser*, Stn 425] was also examined.

Specimens are listed by order of taxon (alphabetically grouped by family, then genus, and species), secondarily by decreasing latitude, and subsequently by dorsal mantle length (ML). In cases where the mantle was too damaged to measure, or was missing, the lower rostral length (LRL) (if the beak was already removed) or the tentacle club length (CL) (if present and undamaged) is provided. The sex of each specimen was identified, except for juveniles and individuals with damaged viscera (indicated as 'sex indet.'). Notes on specimen condition include condition of tentacles, arms, skin, eyes, and photophores, including absent features (e.g., missing fins, tentacles), damaged features (e.g., arm tips), and overall condition.

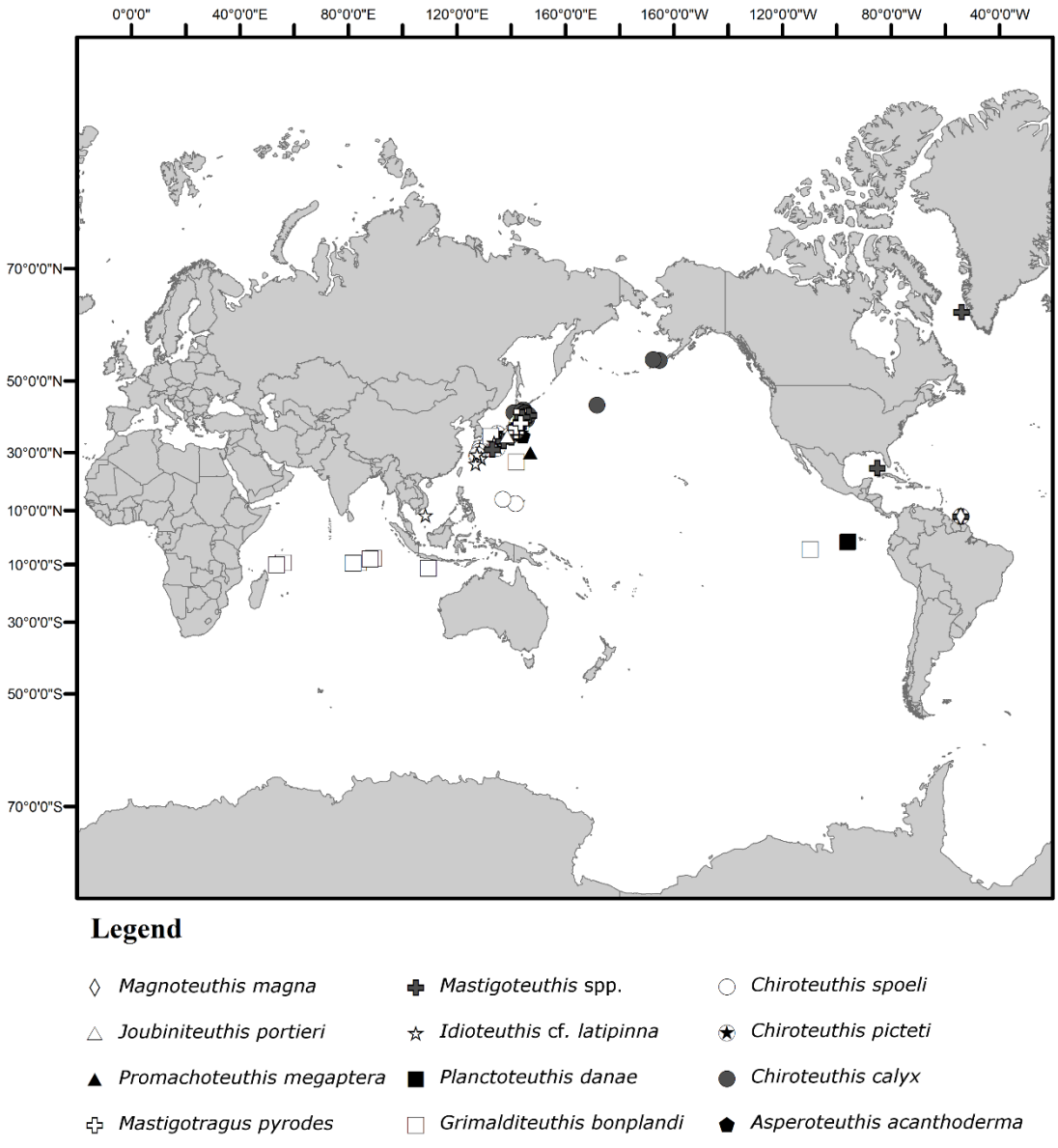

Fig. 12—Map of all specimens in the chiroteuthid families clade held in the National Museum of Nature and Science, Tokyo (NSMT) collections.

Results:

Genetics

COI and 16S rRNA sequences were successfully recovered for 26 out of the 28 specimens, and all 28 individuals were successfully sequenced for 12S rRNA. Two specimens (NSMT Mo.74587 and NSMT147) failed to amplify for COI and 16S rRNA (no visible band on the agarose gel) or were contaminated. COI sequences were 658 bp (except for NSMT152) and did not contain stop codons or indels. COI for both specimens of *Asperoteuthis acanthoderma* (Lu, 1977) could only be recovered with the use of internal primers and the sequence for NSMT152 was only 480 bp because the

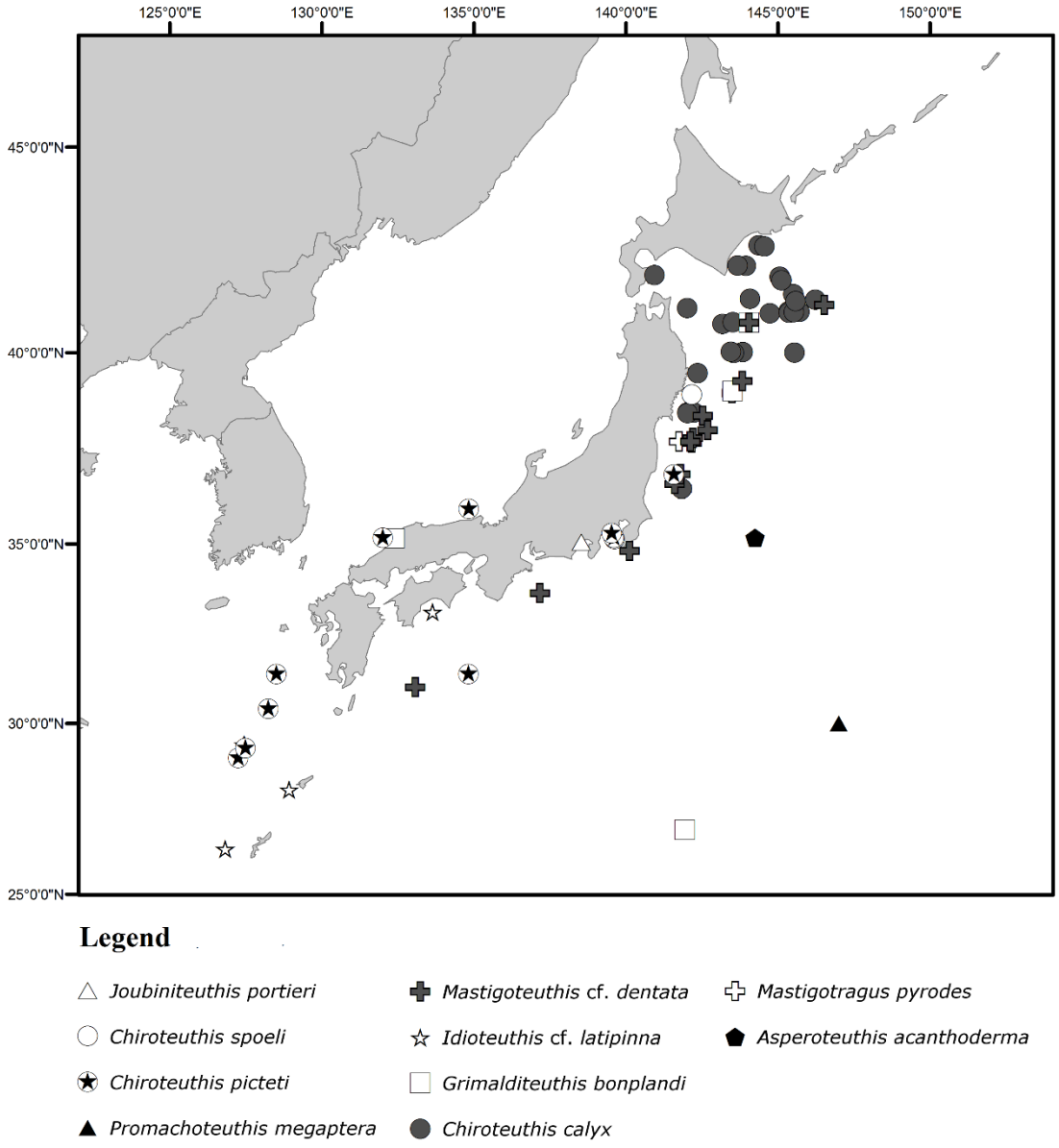

Fig. 13—Map of specimens in the chiroteuthid families clade collected from Japanese waters held in the National Museum of Nature and Science, Tokyo (NSMT) collections.

sequencing reaction for mCephR resulted in a failed sequence. 16S rRNA and 12S rRNA sequences included indels and ranged from 513 to 520, and 402 to 406 bp, respectively.

The Bayesian phylogeny showed support for the Chiroteuthidae and the Mastigoteuthidae (data not shown), whereas the maximum-likelihood phylogeny showed support only for the Chiroteuthidae (Fig. 14; Fig. 15). Within the Mastigoteuthidae, both phylogenies supported the five genera of Mastigoteuthidae established by Braid et al. (2014), with a close association found between the sixth, monotypic genus *Mastigotragus* Young, Vecchione, and Braid, 2014, and *Echinoteuthis atlantica* Joubin, 1933. Both species of *Magnapinna* (‘*Mn.*’) Vecchione and Young, 1998, formed a clade with *Joubiniteuthis portieri* (Joubin, 1916) on both phylogenies. The relationship of *Batoteuthis skolops* Young and Roper, 1968, to other clades in the chiroteuthid families was not resolved. The two species of *Planctoteuthis* (‘*Pl.*’) Pfeffer, 1912, did not form a clade on either phylogeny. *Asperoteuthis* Nesis, 1980, did not resolve into a single clade; there was a well-supported relationship between *A. mangoldae* Young, Vecchione, and Roper, 2007, and *A. lui* Salcedo-Vargas, 1999, on the Bayesian phylogeny, but this was not supported by the maximum-likelihood phylogeny. Although the genus *Chiroteuthis* did not resolve into a single clade, the ‘*C. veranyi*’ group and the ‘*C. picteti*’ group (Roper & Young, 2013) were well supported by both phylogenies.

In the chiroteuthid families clade, 28 Barcode Index Numbers (BINs) were recognised (Fig. 14). A single taxon was recognised for the monotypic family Batoteuthidae. Two species were recognised for the Magnapinnidae. The Mastigoteuthidae contained 11 species, two of which have been sequenced here for the first time: *I.* cf. *latipinna*, separate from the BIN for *I.* ‘*cordiformis*’ from New Zealand waters, and *Mr. pyrodes*, which had not been previously sequenced. The Chiroteuthidae had 13 taxa, with *A. acanthoderma* sequenced from its type locality for the first time. The specimens identified from New Zealand waters as *C.* aff. *veranyi* Férussac, 1835, were assigned a separate BIN from *C. veranyi* (with sequences from the Mediterranean Sea and the North Atlantic) and *C. calyx* Young, 1972. The specimen of *C. calyx* from Japan had the same BIN as individuals from California and the Eastern Bering Sea. *Chiroteuthis picteti* Joubin, 1894, was sequenced herein for the first time and was assigned a separate BIN from *C. mega* (Joubin, 1932), and another closely related species, whose identity is currently uncertain (the specimen could not be found for morphological examination). A specimen misidentified as ‘*Joubiniteuthis portieri*’ from

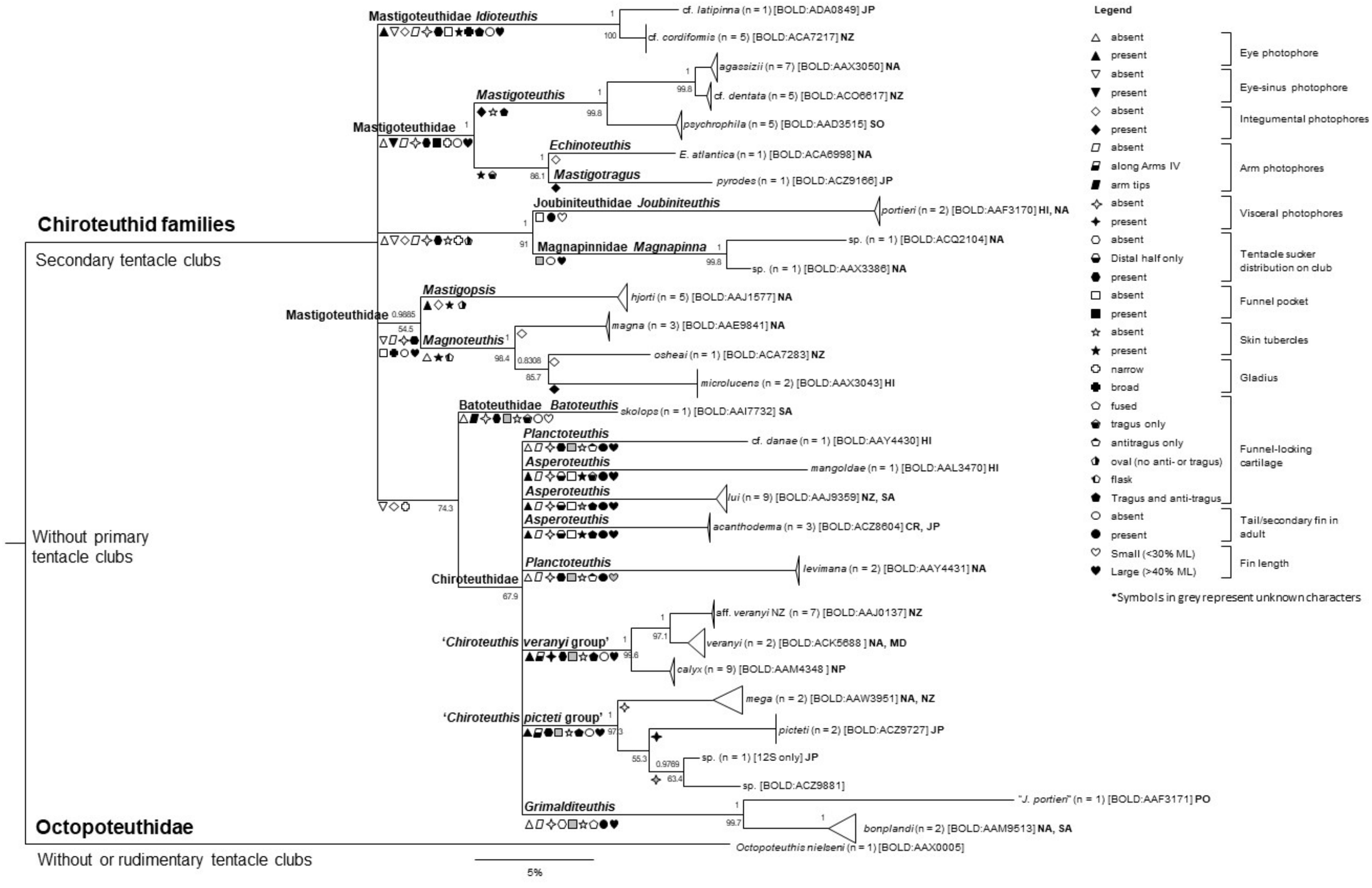

Fig. 14—Combined phylogeny of all the specimens of the chiroteuthid families clade sequenced for Chapter 3 and previously published sequences for COI, 12S rRNA, and 16S rRNA (see Table 4 and Fig. 15) made with 1000 bootstrap replicates. Upper node values indicate Bayesian posterior probabilities, and lower node values indicate maximum-likelihood bootstrap support, and branches with values lower than 50% have been collapsed. Barcode Index Numbers (BINs) are included beside species names (in square brackets). Clades are identified to the highest taxonomic level. The following abbreviations are used to indicate locality data: Caribbean (CR); Hawaii (HI); Japan (JP); Mediterranean (MD); North Atlantic (NA); North Pacific (NP); New Zealand (NZ); Pacific Ocean (PO); South Atlantic (SA); Southern Ocean (SO).

a previous study (Anderson, 2000), was assigned to a separate BIN from specimens of *J. portieri*, and the true identity of this specimen is uncertain. *Asperoteuthis mangoldae* was assigned the same BIN as a specimen identified as *Grimalditeuthis bonplandi* (Verany, 1839) (from Anderson, 2000), but these sequences came from the same specimen, which has recently been reidentified morphologically by Rebecca Mensch,

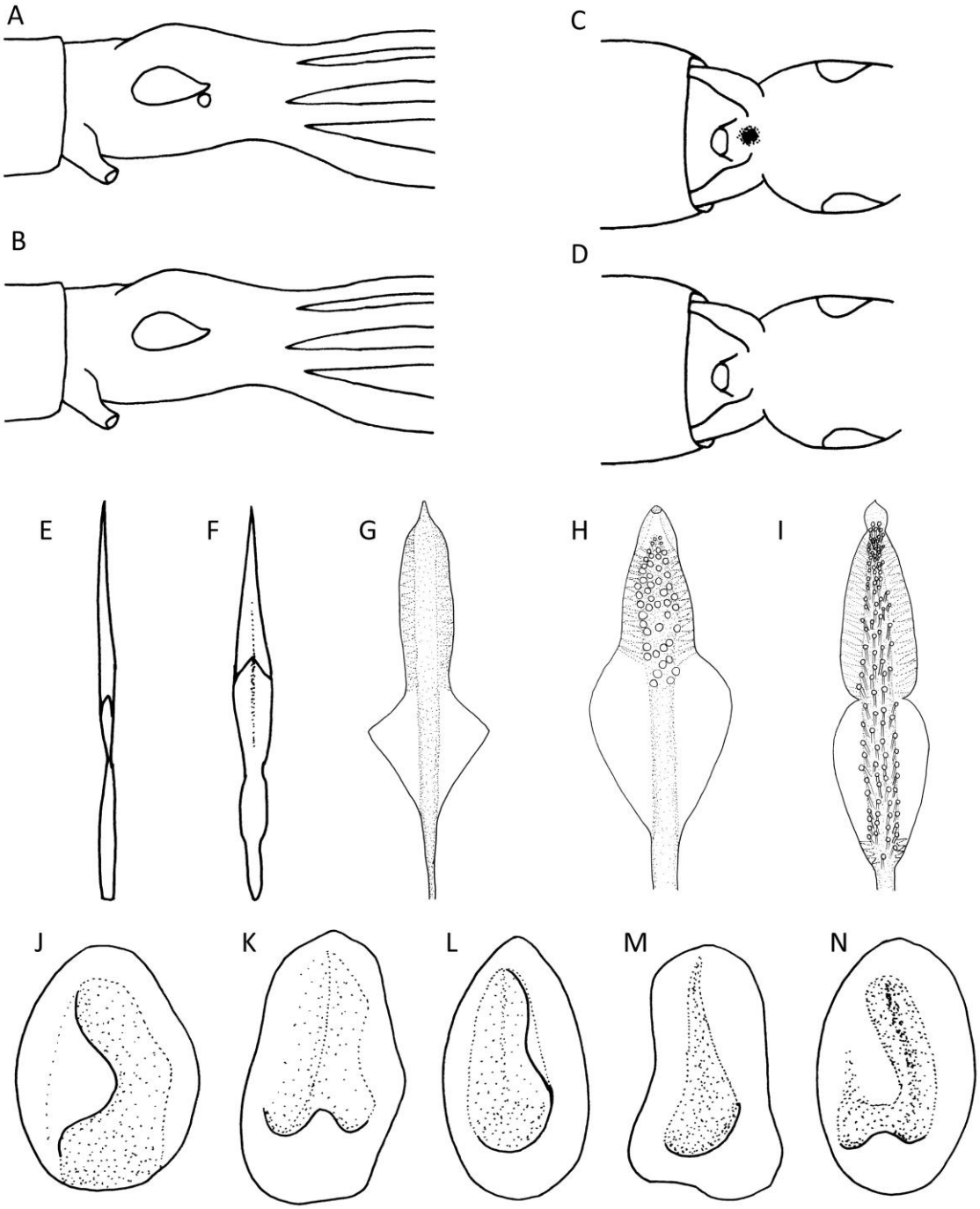

Fig. 15—Morphological features in the chiroteuthid families. A) Eye-sinus photophore present; B) Eye-sinus photophore absent; C) funnel pocket present; D) funnel pocket absent; E) narrow gladius; F) broad gladius; G) tentacle club without suckers (modified from Young et al., 1998); H) tentacle club with suckers present only in the distal half (modified from Young et al., 1998) I) tentacle club with suckers present (modified from Young et al., 1998); J) funnel-locking cartilage with tragus; K) funnel-locking cartilage with anti-tragus; L) oval shaped funnel-locking cartilage; M) flask shaped funnel-locking cartilage; N) funnel-locking cartilage with tragus and antitragus.

and confirmed in the current study as *A. mangoldae*. There are currently two BINs for *G. bonplandi* on BOLD due to this misidentification.

The Bayesian Poisson tree processes (bPTP) analysis of 12S rRNA (a more conserved gene) did not recognise as many separate taxa as the BIN analysis. Two *Idioteuthis* species identified as separate BINs based on COI grouped together as a single taxon according to 12S rRNA, as did *Mt. agassizii* and *Mt*. cf. *dentata*. This analysis also did not distinguish *C. calyx* from *C.* aff. *veranyi*, but did recognise four separate species in the '*C. picteti*' group, which included *C. mega*, *C. picteti*, and two additional *Chiroteuthis* specimens that were unavailable for morphological analysis.

Catalogue of chiroteuthid families material in NSMT collections

A catalogue of all of the specimens in the chiroteuthid families from the National Museum of Nature and Science (NSMT) was compiled (Appendix 1). In the family Chiroteuthidae, specimens were found representing *C. calyx*, *C. picteti*, *C. spoeli* Salcedo-Vargas, 1996, *G. bonplandi*, and *Pl. danae* (Joubin, 1931). Two specimens of *Chiroteuthis* and one mastigoteuthid could not be confidently identified to species due to damage. There was a single specimen of Joubiniteuthidae, representing the only known species in this family, *Joubiniteuthis portieri*. *Idioteuthis* specimens are attributed to *I.* cf. *latipinna*, because the type locality of this species is Japan, and because the genetic results show this species to be separate from the *Idioteuthis* species found in New Zealand waters. Two specimens from the southwest Atlantic were identified as *Magnoteuthis* ('*Mg*.') *magna* (Joubin, 1913), and no specimens of *Magnoteuthis* Salcedo-Vargas and Okutani, 1994, were available from Japan. Three specimens of *Mastigoteuthis* Verrill, 1881, from the Atlantic Ocean were identified as *Mt. agassizii*, and all of the *Mastigoteuthis* specimens from Japanese waters were identified as *Mt*. cf. *dentata* because the status of *Mt. dentata* is still unresolved (Braid & Bolstad, 2015).

Discussion:

Interfamilial relationships

This analysis included sequences for specimens from five of the six chiroteuthid families; no sequences are currently available for the Promachoteuthidae. Two monotypic families included in the genetic analysis were the Joubiniteuthidae and the Batoteuthidae, with the latter represented by the sole sequence presently available for

Batoteuthis skolops. The maximum-likelihood analysis found a sister relationship between the Batoteuthidae and the Chiroteuthidae with some support (bootstrap support of 74.3, Fig. 14), but this relationship was not recovered with the Bayesian phylogeny. The Joubiniteuthidae formed a sister relationship with two species of Magnapinnidae, supported by both the Bayesian (posterior probability of 1; Fig. 14) and maximum-likelihood (bootstrap value of 86.1; Fig. 14) phylogenies. Although the Mastigoteuthidae formed a clade with some support on the Bayesian phylogeny (posterior probability of 0.6038; Fig. 14), the maximum-likelihood phylogeny did not recover this family as a single clade (Fig. 14). The Chiroteuthidae formed a well-supported clade on both phylogenies (Fig. 14).

The Chiroteuthidae

In the Chiroteuthidae, subdivisions within the genus *Chiroteuthis* are currently debated. Roper and Young (2013) divided this genus into three groups—the '*C. joubini*' group, the '*C. picteti*' group, and the '*C. veranyi*' group—on the basis of eye photophore number and morphology, tentacle club morphology and pigmentation, and tentacle club sucker dentition. Salcedo-Vargas (1996) divided *Chiroteuthis* into two subgenera; however, due to internal inconsistencies, this classification has not been widely accepted (e.g., Mensch, 2010). The '*C. joubini*' group consists of *C. joubini* Voss, 1967, *C. spoeli*, and an unnamed species (Roper & Young, 2013). This group is characterised by eyeball photophores arranged in two series, tentacle club protective membranes in three distinct sections, most pigmentation on tentacle clubs being due to epithelial cells rather than chromatophores, and tentacle suckers without a central tooth (Roper & Young, 2013). In contrast, Salcedo-Vargas (1996) grouped *C. joubini* and *C. spoeli* together, in the same subgenus as *C. veranyi* and *C. calyx*. Unfortunately, although formalin-fixed specimens of *C. spoeli* are available in collections, no tissue was available for DNA sequencing, so the placement of species in the '*C. joubini*' group could not be tested in the present study (and should be the focus of future studies). In this analysis, the genus *Chiroteuthis* did not form a single clade on the combined phylogenies (Fig. 14), but the species that were included form two separate, well-supported clades, which are consistent with two previously recognised subdivisions in this genus (the '*C. picteti*' group, and the '*C. veranyi*' group).

The '*C. picteti*' group consists of *C. picteti* and *C. mega* (Roper & Young, 2013). This group is characterised by eyeball photophores in three series or stripes, tentacle club protective membranes in two distinct sections (with the proximal section

being shorter), clubs pigmented by chromatophores rather than epithelial cells, and tentacle suckers with a single, central tooth (Roper & Young, 2013). For these species, Salcedo-Vargas (1996), resurrected *Chirothauma* Chun, 1910, as a subgenus for *C. picteti picteti*; *C. picteti somaliensis* Salcedo-Vargas, 1996; *C. imperator* Chun, 1908; and *C. capensis* Voss, 1967 (= *C. mega* fide Salcedo-Vargas, 1997). The present results show support for four genetically distinct species in this group (Fig. 14). The Barcode Index Number (BIN) analysis identified three separate BINs, with an additional species (for which COI could not be recovered) supported by the Bayesian Poisson tree processes (bPTP) analysis of 12S rRNA. The BIN analysis suggested that *C. mega* material from the North Atlantic and from New Zealand represent the same species (BIN BOLD:AAW3951), which is distinct from *C. picteti* (Fig. 14). A third BIN was also identified in this clade; although the specimen could not be located for morphological analysis and the identity remains unknown, it now appears that two separate species in this group are present in Japanese waters. The fourth species was identified through bPTP analysis of 12S rRNA, which is a more conserved gene (evolves more slowly) and thus generally shows smaller differences among taxa than those shown by COI analyses (in fact, some valid species were lumped together according to the bPTP of 12S rRNA, e.g., *C. calyx* and *C.* aff. *veranyi*). Although this fourth species could not be included in the COI analysis, its identification using the more conserved 12S rRNA strongly suggests that it is distinct from other taxa. The identity of this taxon remains unknown, however, because the parent specimen could not be located for morphological analysis. This clade now appears to have four genetically distinct species, and future studies will be required to determine whether they represent previously named or new species.

The '*C. veranyi*' group presently consists of *C. veranyi* and *C. calyx* (Roper & Young, 2013). This group is characterised by eyeball photophores in two stripes with an intermediate series of round photophores, tentacle club protective membranes in two distinct, subequal sections, and tentacle suckers with a single, central tooth (Roper & Young, 2013). These two species, along with *C.* aff. *veranyi*—a morphologically similar but genetically distinct species found in New Zealand waters—form a distinct and well-supported clade (Fig. 14). *Chiroteuthis veranyi* was originally described from the Mediterranean Sea (Férussac, 1835) and has since been reported throughout the Atlantic, Indian, and Pacific Oceans (but not the North Pacific) (Nesis, 1987), with some morphological differences noted. Mensch (2010) observed that the New Zealand material she attributed to the 'cosmopolitan' species *C. veranyi* had more teeth on the

arm sucker rings (16–27) than had been previously reported for *C. veranyi sensu stricto* (12–16). In the present study, sequences from New Zealand were assigned a separate BIN from Mediterranean and North Atlantic *C. veranyi* (Fig. 14), indicating that this name is currently being applied to at least two separate species. The status of the other species in this clade, *C. calyx*, presently appears more straightforward. It was originally described from Santa Catalina Basin, off Southern California (Young, 1972), and is distributed throughout the North Pacific, from Southern California to the Gulf of Alaska, and across to Honshu, Japan (Nesis, 1987). Herein, *C. calyx* individuals from waters off California were assigned the same BIN as the single sequenced *C. calyx* specimen from the west coast of Japan (NSMT Mo.71605), supporting the previously recognised distribution of this species.

The genus *Asperoteuthis* currently contains three species that are united by the presence of a secondary fin, and a tentacle club that only bears suckers in the distal half (Chapter 2). Surprisingly, this genus did not form a single clade on either phylogeny (pers. obs.), indicating that it may be in need of revision. The Bayesian phylogeny found strong support for the relationship between *A. lui* and *A. mangoldae* (posterior probability of 0.9885), but this was not recovered with the maximum-likelihood phylogeny. In the Bayesian phylogeny, *A. acanthoderma* did not group with the other *Asperoteuthis* species and instead showed a weak sister relationship to *Pl. levimana* (Lönnberg, 1896) (posterior probability of 0.7); *A. acanthoderma* did not show a sister relationship to any other species in the maximum-likelihood phylogeny (Fig. 14). The reason these species did not resolve into a single clade is not clear—it is possible that they truly belong in separate genera, or that additional, unrecognised species exist and their inclusion in a more complete analysis would cause these species to group into a single clade. Further investigation is certainly warranted, as genetic information has only recently become available for this taxon: this study provides the first sequences of *A. acanthoderma* from Japanese waters, which is the type locality (Lu, 1977). Recently, Judkins et al. (2009) reported the first records of *A. acanthoderma* from the North Atlantic Ocean, which dramatically expanded its range (previously known only from the North Pacific). The present genetic analysis confirms this wide, multi-ocean distribution, because the specimens from Japan and the sequence available for an *A. acanthoderma* from the Caribbean Sea showed low variation (Fig. 14). Because the Caribbean Sea *A. acanthoderma* sequences are not on BOLD, it could not be included in the BIN analysis, but when this sequence is searched in the BOLD identification

engine, it has a maximum divergence of 0.32% (p-distance), and a minimum of 0.02% (p-distance) from the *A. acanthoderma* specimens sequenced in the current study.

The two chiroteuthid genera *Planctoteuthis* and *Grimalditeuthis* both have representatives in the NSMT collections, but tissue was not available for either genus from Japanese waters. One specimen of *Pl.* cf. *danae* from the FMNH (collected in Hawaii) was sequenced and included in the present analysis, along with another previously sequenced species, *Pl. levimana*, but these species did not form a clade on either phylogeny (Fig. 14). Presently, three other species have been recognised in this genus (Young et al., 2014), which have not been sequenced and their inclusion in future phylogenies may help resolve this clade. The genus *Grimalditeuthis* is presently monotypic, and the two sequences currently available for *G. bonplandi* have the same BIN, but they also appear to have a close relationship with a specimen that was sequenced and misidentified as '*J. portieri*' (in Anderson, 2000), but which does not match the BIN for what appears to be true *J. portieri* (which was assigned to one specimen from the present study and one from Young et al. [2008]). This suggests the presence of a second species of *Grimalditeuthis*. Both specimens identified as *G. bonplandi* were from the Atlantic Ocean (Young et al., 2008; present study), while Anderson's (2000) specimen was from the Pacific Ocean. This clade is well supported by both the Bayesian phylogeny (posterior probability of 1; Fig. 14) and the maximum-likelihood phylogeny (bootstrap value of 99.7; Fig. 14). The identity of Anderson's specimen is unknown, but it may represent a previously unrecognised species in this genus, or a closely related taxon.

The Joubiniteuthidae

Joubiniteuthis portieri, presently the sole member of this family, was originally described from the North Atlantic Ocean and appears to have a circumglobal distribution (Jereb & Roper, 2010). The present analyses included one specimen of *J. portieri* from Hawaii and one from the North Atlantic (Table 5), which appear to represent a single species based on the BIN analysis (Fig. 14). This lends support to the hypothesised monotypy of the family across multiple oceans (but additional material from across its known distribution should be included in future studies for comparison). It therefore appears that some members of the chiroteuthid families are widespread with little genetic variation (see also *A. acanthoderma* above), while others, such as taxa in the mastigoteuthid genus *Magnoteuthis*, are morphologically similar in different oceans but appear to represent genetically distinct, geographically restricted species (Braid &

Bolstad, 2015). As noted in the previous paragraph, the sequence attributed to '*J. portieri*' by Anderson (2000) from the Pacific Ocean likely represents an undescribed species of *Grimalditeuthis* (Fig. 14).

The Magnapinnidae

The family Magnapinnidae is a poorly known family with a single genus recognised at present (Vecchione & Young, 2006); three species have been named, and two additional species have been identified but remain unnamed due to a lack of material. Publicly available sequences for two specimens of *Magnapinna* were included in the present analysis and appear to represent two separate species, having been assigned to separate BINs (Fig. 14). However, these specimens have not been identified to species level (Lindgren, 2010; Bucklin et al., unpublished) so their exact identities remain unknown. Because specimens are so rarely encountered, and are frequently badly damaged (Vecchione & Young, 2006), integrative taxonomy will be crucial in resolving this family. As techniques for recovering DNA from formalin-fixed museum cephalopod specimens advance (see Hoving et al., 2014), it may be possible to obtain sequences from material currently stored in collections.

The Mastigoteuthidae

The family Mastigoteuthidae was recently reclassified into five genera (Braid et al., 2014), with a sixth genus, *Mastigotragus* subsequently established for *Mr. pyrodes* (Young et al., 2014). The present study provides the first known sequences for *Mr. pyrodes* and provides some new information on mastigoteuthid interrelationships. Morphologically, *Mr. pyrodes* shares some superficial characteristics with species in the genus *Mastigoteuthis*—integumental photophores, eye-sinus photophore, and a tragus in the funnel-locking cartilage—but these genera are separated by the structure of the integumental photophores, size of the eye-sinus photophore, and differences in the shape of the mantle-locking cartilage (Young et al., 2014). The phylogenetic analyses now show a close relationship between *E. atlantica* and *Mr. pyrodes* (posterior probability of 1; bootstrap support of 86.1, Fig. 14), which together form a sister clade to the genus *Mastigoteuthis*. Several morphological characteristics are shared between *Echinoteuthis* Joubin, 1933, and *Mastigotragus*: funnel pocket present; large eye-sinus photophores; the presence of skin tubercles in some life stages; skin colouration due to dense covering of chromatophores on skin rather than pigmentation; strong tragus and no anti-tragus; and C-shaped mantle-locking cartilage (pers. obs). The most striking

difference between these genera is the presence of integumental photophores in *Mastigotragus*. However, it would not be unusual for a single mastigoteuthid species in a genus to have integumental photophores while the others do not—for example, *Mg. microlucens* (Young, Lindgren, & Vecchione, 2008) is the only known species in the genus *Magnoteuthis* that has integumental photophores (Young et al., 2008). Therefore, the close relationship found in this analysis between *Mastigotragus* and *Echinoteuthis* suggests that they may form a single clade, but other species of *Echinoteuthis* should be included in future studies to determine the status of the genus *Mastigotragus*.

The NSMT collections include representatives of the genus *Mastigoteuthis* from both the Atlantic and the Pacific (Japanese waters). The specimens from the Pacific have been identified herein as *Mt.* cf. *dentata* because the status of this species is currently unresolved (Braid & Bolstad, 2015). Tissue of *Mt. dentata* from the type locality, the Gulf of Panama (Hoyle, 1904), will be essential in resolving the status of this species. Unfortunately, there were no tissue samples available for this genus from Japanese waters in the NSMT.

Three species in the genus *Magnoteuthis* have been previously sequenced (Braid et al., 2014). These species have disjunct distributions: *Mg. magna* is from the North Atlantic (Joubin, 1913); *Mg. microlucens* is from Hawaii (Young et al., 2008); and *Mg. osheai* Braid and Bolstad, 2015, is from New Zealand waters (Braid & Bolstad, 2015). Two additional taxa have been reported, but no tissue has been available for sequencing for *Mg. inermis* (Rancurel, 1972), from the Eastern North Atlantic (Rancurel, 1972) or *Mg*. 'type beta' from the South Atlantic (Young & Vecchione, 2014). Although *Magnoteuthis* is known to occur in Japanese waters (Salcedo-Vargas, 1993), the only representatives of this genus in NSMT collections are *Mg. magna* from the Atlantic. Salcedo-Vargas (1993) identified the species in Japan as *Mg. magna*; however, two species have been added to this genus since that review so the identity of the species in Japanese waters is not currently known but should be reviewed in future studies.

The genus *Idioteuthis* was originally established by Sasaki (1916) for *I. latipinna*, but the validity of this species has been debated (Salcedo-Vargas & Okutani, 1994; Salcedo-Vargas, 1997; Braid & Bolstad, 2015). *Idioteuthis cordiformis* was described from the Indian Ocean near Sumatra (Chun, 1908), while the type locality for *I. latipinna* is Japan (Sasaki, 1916). In New Zealand waters, males of *I.* '*cordiformis*' appear to mature around ML ~500–600 mm (Braid & Bolstad, 2015), while a mature male specimen of *I.* cf. *latipinna* was found in the NSMT collections at ML 234 mm, which is consistent with a previous report of a small, mature male *Idioteuthis* specimen

from Japanese waters at ML 318 mm (Salcedo-Vargas, 1993). Genetically, the species in Japanese waters is distinct from the species from New Zealand waters and belongs in the same genus, with each being assigned a separate BIN but showing a close sister relationship on both phylogenies (Fig. 14). It appears that there are at least two species in this genus, but it is not clear whether *I. latipinna* is distinct from *I. cordiformis sensu stricto*, potentially creating a species complex. Although a previous genetic analysis of the Mastigoteuthidae found little support for the inclusion of *Idioteuthis* in this family (Braid et al., 2014), this current analysis found some support for the Mastigoteuthidae (including *Idioteuthis*) as a clade in the Bayesian analysis (posterior probability 0.6038), but not in the maximum-likelihood phylogeny (Fig. 14). This indicates that the inclusion of additional taxa has aided (and will likely continue to aid) our evolving understanding of the relationship between *Idioteuthis* and the other mastigoteuthid genera. Therefore, ongoing sampling of chiroteuthid family specimens from additional taxa and locations is recommended, in the interest of eventually resolving interrelationships in this group.

NSMT catalogue

A review of specimens in the chiroteuthid families found in the collections of the National Museum of Nature and Science (NSMT) has revealed 12 species found across four families (Appendix 1). This included five species of Chiroteuthidae (representing *Chiroteuthis*, *Grimalditeuthis*, and *Planctoteuthis*), one specimen from the monotypic family Joubiniteuthidae, five species of Mastigoteuthidae (representing *Idioteuthis*, *Mastigoteuthis*, *Magnoteuthis*, and *Mastigotragus*), and one specimen of Promachoteuthidae (*Promachoteuthis* [‘*Pr*.’] *megaptera* Hoyle, 1885a). In addition to the 98 specimens collected from Japanese waters (Fig. 13), 44 specimens from other regions are also part of this collection (Fig. 12), representing a valuable resource for future studies and revisions of the chiroteuthid families.

Conclusion:

The chiroteuthid families form a diverse clade, whose relationships and taxonomy are not yet fully understood. Herein, this study contributes COI, 16S rRNA, and 12S rRNA sequences for material from the waters around Japan, Hawaii, California, and New Zealand. This nearly doubles the number of publicly available sequences for the chiroteuthid families, and in combination with existing sequences from previous studies on BOLD and GenBank, comprises the largest phylogenetic study

on the chiroteuthid families to date. This phylogenetic analyses reveal that the genera *Chiroteuthis* and *Asperoteuthis* may not be monophyletic, but support was found for the '*C. veranyi*' group and the '*C. picteti*' group, with additional species found in both groups that require attention. Sequences are needed from specimens in the '*C. joubini*' group in particular, because none are currently available. Additional specimens of the Joubiniteuthidae from across its entire range will be necessary to determine whether this is truly a monotypic family. Similar to *A. acanthoderma*, *J. portieri* appears to be present in the Pacific and Atlantic Oceans with little genetic variation, which contrasts with the geographically restricted species in the genus *Magnoteuthis*. Specimens of the Magnapinnidae are rarely encountered, and integrative taxonomy will be essential in resolving this family, as specimens are often badly damaged and very few exist in collections. The Mastigoteuthidae still requires a global revision because of several species complexes, and to resolve the status of the genus *Mastigotragus*. This study has additionally compiled a catalogue of all of the specimens available in this clade in the NSMT collections (Appendix 1), representing 12 species across the Chiroteuthidae, the Joubiniteuthidae, the Mastigoteuthidae, and the Promachoteuthidae. Three mitochondrial genes were used herein (COI, 16S rRNA, and 12S rRNA) to resolve genus-level placements of species in this clade. However, given the low variation found in 12S rRNA, it is likely that the other two genes used in combination could resolve beta taxonomy in other oegopsid taxa.

Chapter 4: Molecular phylogenetic analysis of the squid family Histioteuthidae (Mollusca, Cephalopoda)

Abstract:

Histioteuthid squids are an important part of marine food webs, being abundant in the diets of many apex predators. Although they represent a substantial biomass in the deep sea, their systematics are not fully understood; damaged (especially ex-gut-content) specimens are difficult to identify morphologically, since most morphological characters presently used to distinguish species involve external photophore patterns. The purpose of this study was to test a morphological hypothesis for the division of the family Histioteuthidae into species groups using two mitochondrial genes (cytochrome *c* oxidase subunit I [COI] and 16S rRNA). Both the Bayesian and maximum-likelihood analyses supported the division of this family into six genera (formalising previously hypothesised species groups): *Calliteuthis*, *Fragariateuthis* gen. nov. *Histioteuthis*, *Histiothauma*, *Navia* gen. nov., and *Stigmatoteuthis*. Barcode Index Numbers (BINs) based on COI and 16S rRNA were used to distinguish 17 currently accepted species, and revealed up to nine additional species, including potentially new, unnamed species. A DNA barcode reference library of sequences generated in this study is available on the Barcode of Life Data System (BOLD), which can be used to confirm identifications or identify damaged specimens, such as those from gut contents. This study is the largest, most complete phylogenetic analysis of this family to date.

Introduction:

The Histioteuthidae Verrill, 1881, commonly known as ‘jewelled’, ‘violet’, ‘cockeyed’, or occasionally ‘strawberry’ squid, is a mesopelagic family characterised by asymmetrically sized eyes and integument covered in photophores. Currently, two genera—*Histioteuthis* (*Hi*.) d'Orbigny [in Férussac & d'Orbigny], 1841, and *Stigmatoteuthis* Pfeffer, 1900—and 19 species (one not yet formally named; ‘sp. A’ *fide* Young & Vecchione, 2010a) are accepted in the family (Young & Vecchione, 2013a). In addition, the genus *Histioteuthis* has been divided into six species ‘groups’ (Voss, Nesis, & Rodhouse, 1998; Young & Vecchione, 2013a). Historically, histioteuthids remained poorly known and their systematics unstable, due to a lack of available specimens in collections, until a review by Voss (1969). A more recent review by Voss et al. (1998) further clarified the systematics of this family. Nevertheless, Hoving et al. (2014) identified the Histioteuthidae as one of the cephalopod families most in need of a

focused taxonomic revision, while Voss et al. (1998) suggested that future work on this family should include a phylogenetic analysis.

Historically, species eventually recognised as histioteuthids have been placed in a variety of different families including the Chiroteuthidae Gray, 1949, and the Mastigoteuthidae Verrill, 1881. The first named histioteuthid species, *Hi. bonnellii* (Férussac, 1834), was originally placed in the genus *Cranchia* Leach, 1817. Although eight genera have been named in this family, only two are currently accepted (Young & Vecchione, 2013a). At least five additional genera have been described but are not currently considered valid: *Lolidona* Risso, 1854; *Calliteuthis* Verrill, 1880; *Histiopsis* Hoyle, 1885a; *Meleagroteuthis* Pfeffer, 1900; and *Histiothauma* (*Ha.*) Robson, 1948. The holotype for an additional 'histioteuthid' genus—*Histiochromius* Pfeffer, 1912—is not a histioteuthid (originally identified by Chun [1910] as a brachioteuthid; the true systematic status of this species remains unclear), and has been excluded from subsequent studies on the Histioteuthidae (Voss, 1969). In the most recent studies, Voss (1969) and Voss et al. (1998) only considered *Histioteuthis* to be valid (but established species groups within this genus); Young and Vecchione (2015a) subsequently moved the three species in the '*hoylei*' group to the genus *Stigmatoteuthis*.

Histioteuthids play a substantial role in marine food webs, being an important food source for many apex predators. For example, histioteuthids are the most important cephalopod prey in the diet of sperm whales (Clarke, 1983; Gómez-Villota, 2007) and '*Hi.*' *atlantica* (Hoyle, 1885b) was found to be one of the most important cephalopod prey species in the diet of porbeagles (*Lamna nasus* [Bonnaterre, 1788]; Cherel & Duhamel, 2004). Although histioteuthid beaks are commonly used for species identification from gut contents, soft prey remains are much more difficult to identify. A combination of genetics and morphology together can be used to increase the accuracy and success of gut-content identification (Bartley et al., 2015; Méheust et al., 2015). Amassing a reference library of sequences, with reliably identified parent specimens, is a natural prerequisite of prey tissue identification using DNA. Fortunately, the Barcode of Life Data System (BOLD; Ratnasingham & Hebert, 2007) is a platform that has been used to create DNA barcode reference libraries that can be used for species identification (Ratnasingham & Hebert, 2007). Currently, there are only seven histioteuthid COI sequences from phylogenetic analyses (not including sequences from dietary analyses) publically available on GenBank, with one additional species available on BOLD, representing six of the currently accepted 19 species in the family.

A genetic analysis of the Histioteuthidae has not been previously undertaken; the few species sequenced to date were included in larger studies on cephalopod evolution (Lindgren, 2010; Lindgren et al., 2012). Thus, the aim of this chapter is to provide the first genetic study to focus specifically on the Histioteuthidae to date, with three specific objectives: (1) to test the morphological hypothesis of species groups as genera, using two mitochondrial genes; (2) to test species delimitation using COI and 16S rRNA; and (3) to establish a DNA barcode reference library for species in this family, which will enable subsequent comparison and more accurate identification of histioteuthid sequences from prey remains (especially soft tissue) in predator gut contents. In addition, previous genetic reviews of squid families have revealed additional, unnamed taxa (Braid et al., 2014; Chapter 3), which may well also exist in the Histioteuthidae.

Methods:

Specimens

Tissue from 194 specimens sequenced in this study was sourced from the Field Museum of National History (FMNH), the Monterey Bay Aquarium Research Institute (MBARI), the National Institute of Water and Atmospheric Research Ltd. (NIWA), the National Museum of Nature and Science, Tokyo (NSMT), and the Smithsonian Institution National Museum of Natural History (USNM) (Table 6). Specimens were either frozen or fixed (in 100% ethanol or RNALater) and stored at -20˚C or -80˚C until DNA extraction. All high-quality, publically available histioteuthid sequences from BOLD and GenBank were also included in the analysis for a total of 218 specimens (Table 6). Additional histioteuthid sequences that were available on GenBank from dietary analyses were not included in the present phylogenetic analysis because they were represented by a single gene and, due to the nature of being semi-digested, yielded shorter sequences. These sequences were from Waap et al. (2017) and Alonso et al. (2014), and were compared with the sequences from the present analysis in a neighbour-joining phylogeny (data not shown). The outgroup species is *Psychroteuthis glacialis* Thiele, 1920, the sole species in the family Psychroteuthidae Thiele, 1920, which has shown a sister relationship to the Histioteuthidae in previous phylogenetic studies (Lindgren, 2010; Lindgren et al., 2012).

Table 6—Specimen information for sequences used in Chapter 4. Specimen ID (identification) indicates the museum accession number, GenBank accession number, or field ID, when the specimen was unaccessioned; BIN indicates the Barcode Index Number (only available for COI), BOLD ID is available only for COI sequences on the Barcode of Life Data System (BOLD); and 16S rRNA indicates whether a sequence for this gene was available.

Identification	Specimen ID	BIN	BOLD ID (COI)	16S rRNA	Reference
Calliteuthis					
C. aff. *atlantica*	NIWA 121864 B	BOLD:AAX1287	KERCE052-17	No	Chapter 5
C. aff. *atlantica*	NIWA 121864 A	BOLD:AAX1287	KERCE053-17	No	Chapter 5
C. aff. *atlantica*	NIWA 118607 B	BOLD:AAX1287	KERCE064-17	No	Chapter 5
C. aff. *atlantica*	NIWA 118838 B	BOLD:AAX1287	KERCE073-17	No	Chapter 5
C. aff. *atlantica*	NIWA 105590	BOLD:AAX1287	MPHIS001-17	Yes	Present study
C. aff. *atlantica*	TAN0509/30	BOLD:AAX1287	MPHIS008-17	No	Present study
C. aff. *atlantica*	TAN0509/38	BOLD:AAX1287	MPHIS009-17	No	Present study
C. aff. *atlantica*	TAN0509/53	BOLD:AAX1287	MPHIS011-17	No	Present study
C. aff. *atlantica*	TAN0806/79/01	BOLD:AAX1287	MPHIS018-17	No	Present study
C. aff. *atlantica*	TAN0806/79/03/3 of 3	BOLD:AAX1287	MPHIS019-17	No	Present study
C. aff. *atlantica*	TAN1003/33	BOLD:AAX1287	MPHIS022-17	No	Present study
C. aff. *atlantica*	NIWA 106166 A	BOLD:AAX1287	MPHIS033-17	No	Present study
C. aff. *atlantica*	NIWA 106166 B	BOLD:AAX1287	MPHIS034-17	No	Present study
C. aff. *atlantica*	NIWA 92517	BOLD:AAX1287	MPHIS040-17	No	Present study
C. aff. *atlantica*	NIWA 106166 C	BOLD:AAX1287	MPHIS049-17	No	Present study
C. aff. *atlantica*	NIWA 106171	BOLD:AAX1287	MPHIS050-17	No	Present study
C. aff. *atlantica*	NIWA 106181 A	BOLD:AAX1287	MPHIS051-17	Yes	Present study
C. aff. *atlantica*	NIWA 85921 B	BOLD:AAX1287	MPHIS054-17	No	Present study
C. aff. *atlantica*	NIWA 96174 D	BOLD:AAX1287	MPHIS055-17	No	Present study
C. aff. *atlantica*	NIWA 96174 E	BOLD:AAX1287	MPHIS056-17	No	Present study
C. aff. *atlantica*	NIWA 96174 F	BOLD:AAX1287	MPHIS057-17	No	Present study
C. aff. *atlantica*	NIWA 96217 C	BOLD:AAX1287	MPHIS058-17	Yes	Present study
C. aff. *atlantica*	NIWA 96217 D	BOLD:AAX1287	MPHIS059-17	No	Present study

Table 6—Continued.

Identification	Specimen ID	BIN	BOLD ID (COI)	16S rRNA	Reference
C. aff. *atlantica*	NIWA 96174 B	BOLD:AAX1287	MPHIS075-17	No	Present study
C. aff. *atlantica*	NIWA 96187 C	BOLD:AAX1287	MPHIS082-17	No	Present study
C. aff. *atlantica*	NIWA 92520	BOLD:AAX1287	MPHIS087-17	No	Present study
C. aff. *atlantica*	TAN1401/47 A	BOLD:AAX1287	MPHIS088-17	No	Present study
C. aff. *atlantica*	TAN1401/47 B	BOLD:AAX1287	MPHIS091-17	No	Present study
C. aff. *atlantica*	TAN1003/12	BOLD:AAX1287	MPHIS103-17	No	Present study
C. aff. *atlantica*	NIWA 96159	BOLD:AAX1287	MPHIS114-17	No	Present study
C. aff. *atlantica*	NIWA 96176 A	BOLD:AAX1287	MPHIS115-17	Yes	Present study
C. aff. *atlantica*	NIWA 96176 B	BOLD:AAX1287	MPHIS116-17	No	Present study
C. aff. *atlantica*	NIWA 96182 B	BOLD:AAX1287	MPHIS117-17	No	Present study
C. aff. *atlantica*	NIWA 96182 C	BOLD:AAX1287	MPHIS118-17	No	Present study
C. aff. *atlantica*	NIWA/105575/B	BOLD:AAX1287	MPHIS123-17	No	Present study
C. aff. *atlantica*	VSQTAN1601/7M	BOLD:AAX1287	MPHIS127-17	No	Present study
C. aff. *atlantica*	NIWA 96164	BOLD:AAX1287	MPHIS171-17	No	Present study
C. aff. *atlantica*	NIWA 96171 B	BOLD:AAX1287	MPHIS173-17	No	Present study
C. aff. *atlantica*	NIWA 96171 D	BOLD:AAX1287	MPHIS175-17	No	Present study
C. aff. *atlantica*	NIWA 96175	BOLD:AAX1287	MPHIS178-17	No	Present study
C. aff. *atlantica*	NIWA 96182 A	BOLD:AAX1287	MPHIS182-17	No	Present study
C. aff. *atlantica*	NIWA 96182 D	BOLD:AAX1287	MPHIS183-17	No	Present study
C. aff. *atlantica*	NIWA 96273 A	BOLD:AAX1287	MPHIS194-17	No	Present study
C. aff. *eltaninae*	SG03-58	BOLD:AAX1301	BASMC092-09	No	Collins (unpublished)
C. atlantica	NIWA 105591	BOLD:ADH0880	MPHIS002-17	Yes	Present study
C. atlantica	NIWA 85921 A	BOLD:ADH0880	MPHIS003-17	No	Present study
C. atlantica	NIWA 85960/2 of 3	BOLD:ADH0880	MPHIS004-17	No	Present study
C. atlantica	NIWA 85960/3 of 3	BOLD:ADH0880	MPHIS005-17	No	Present study
C. atlantica	NIWA 85960	BOLD:ADH0880	MPHIS006-17	No	Present study

Table 6—Continued.

Identification	Specimen ID	BIN	BOLD ID (COI)	16S rRNA	Reference
C. atlantica	NIWA 85961	BOLD:ADH0880	MPHIS007-17	No	Present study
C. atlantica	TAN0509/44	BOLD:ADH0880	MPHIS010-17	No	Present study
C. atlantica	TAN0709/79	BOLD:ADH0880	MPHIS012-17	No	Present study
C. atlantica	TAN0709/91	BOLD:ADH0880	MPHIS013-17	No	Present study
C. atlantica	TAN0806/05	BOLD:ADH0880	MPHIS014-17	No	Present study
C. atlantica	TAN0806/140	BOLD:ADH0880	MPHIS015-17	No	Present study
C. atlantica	TAN0806/47	BOLD:ADH0880	MPHIS016-17	Yes	Present study
C. atlantica	TAN0806/56/01	BOLD:ADH0880	MPHIS017-17	No	Present study
C. atlantica	TAN1001/48	BOLD:ADH0880	MPHIS020-17	No	Present study
C. atlantica	TAN1001/50	BOLD:ADH0880	MPHIS021-17	No	Present study
C. atlantica	TAN1003/49	BOLD:ADH0880	MPHIS023-17	No	Present study
C. atlantica	TAN1008/12	BOLD:ADH0880	MPHIS024-17	No	Present study
C. atlantica	NIWA 92564	BOLD:ADH0880	MPHIS030-17	No	Present study
C. atlantica	NIWA 92524	BOLD:ADH0880	MPHIS031-17	No	Present study
C. atlantica	NIWA 92562	BOLD:ADH0880	MPHIS032-17	No	Present study
C. atlantica	NIWA 92573	BOLD:ADH0880	MPHIS035-17	No	Present study
C. atlantica	NIWA 92561	BOLD:ADH0880	MPHIS036-17	No	Present study
C. atlantica	NIWA 92574	BOLD:ADH0880	MPHIS037-17	No	Present study
C. atlantica	TAN1401/40	BOLD:ADH0880	MPHIS038-17	No	Present study
C. atlantica	NIWA 92525	BOLD:ADH0880	MPHIS039-17	No	Present study
C. atlantica	NIWA 92565	BOLD:ADH0880	MPHIS042-17	No	Present study
C. atlantica	NIWA 105552	BOLD:ADH0880	MPHIS044-17	No	Present study
C. atlantica	NIWA 105553	BOLD:ADH0880	MPHIS045-17	No	Present study
C. atlantica	NIWA 105569	BOLD:ADH0880	MPHIS047-17	No	Present study
C. atlantica	NIWA 105579	BOLD:ADH0880	MPHIS048-17	No	Present study
C. atlantica	NIWA 106181 B	BOLD:ADH0880	MPHIS052-17	Yes	Present study

Table 6—Continued.

Identification	Specimen ID	BIN	BOLD ID (COI)	16S rRNA	Reference
C. atlantica	NIWA 106197	BOLD:ADH0880	MPHIS053-17	No	Present study
C. atlantica	TAN0509/52	BOLD:ADH0880	MPHIS060-17	No	Present study
C. atlantica	NIWA 92521	BOLD:ADH0880	MPHIS062-17	No	Present study
C. atlantica	NIWA 92523	BOLD:ADH0880	MPHIS063-17	No	Present study
C. atlantica	NIWA 92522	BOLD:ADH0880	MPHIS064-17	No	Present study
C. atlantica	NIWA 92519	BOLD:ADH0880	MPHIS065-17	No	Present study
C. atlantica	NIWA 92526	BOLD:ADH0880	MPHIS066-17	No	Present study
C. atlantica	NIWA 92572	BOLD:ADH0880	MPHIS067-17	No	Present study
C. atlantica	NIWA 92571	BOLD:ADH0880	MPHIS068-17	No	Present study
C. atlantica	NIWA 92569	BOLD:ADH0880	MPHIS069-17	No	Present study
C. atlantica	NIWA 96173 C	BOLD:ADH0880	MPHIS070-17	No	Present study
C. atlantica	NIWA 96173 F	BOLD:ADH0880	MPHIS073-17	No	Present study
C. atlantica	NIWA 96174 A	BOLD:ADH0880	MPHIS074-17	No	Present study
C. atlantica	NIWA 96183 B	BOLD:ADH0880	MPHIS077-17	No	Present study
C. atlantica	NIWA 96183 C	BOLD:ADH0880	MPHIS078-17	No	Present study
C. atlantica	NIWA 96187 A	BOLD:ADH0880	MPHIS080-17	No	Present study
C. atlantica	NIWA 96188 B	BOLD:ADH0880	MPHIS083-17	Yes	Present study
C. atlantica	NIWA 96188 C	BOLD:ADH0880	MPHIS084-17	No	Present study
C. atlantica	NIWA 96188 D	BOLD:ADH0880	MPHIS085-17	No	Present study
C. atlantica	TAN1117/29	BOLD:ADH0880	MPHIS092-17	No	Present study
C. atlantica	TAN1301/26	BOLD:ADH0880	MPHIS093-17	No	Present study
C. atlantica	NIWA 89387	BOLD:ADH0880	MPHIS095-17	No	Present study
C. atlantica	NIWA 89383	BOLD:ADH0880	MPHIS096-17	No	Present study
C. atlantica	NIWA 103965	BOLD:ADH0880	MPHIS097-17	No	Present study
C. atlantica	NIWA 103966	BOLD:ADH0880	MPHIS098-17	Yes	Present study
C. atlantica	NIWA 103967	BOLD:ADH0880	MPHIS099-17	No	Present study

Table 6—Continued.

Identification	Specimen ID	BIN	BOLD ID (COI)	16S rRNA	Reference
C. atlantica	NIWA 96183 D	BOLD:ADH0880	MPHIS119-17	No	Present study
C. atlantica	NIWA 96188 A	BOLD:ADH0880	MPHIS121-17	No	Present study
C. atlantica	NIWA 105577	BOLD:ADH0880	MPHIS124-17	No	Present study
C. atlantica	NIWA 105580 A	BOLD:ADH0880	MPHIS125-17	No	Present study
C. atlantica	NIWA 105580 B	BOLD:ADH0880	MPHIS126-17	No	Present study
C. atlantica	NIWA 96171 A	BOLD:ADH0880	MPHIS172-17	No	Present study
C. atlantica	NIWA 96171 C	BOLD:ADH0880	MPHIS174-17	No	Present study
C. atlantica	NIWA 96173 A	BOLD:ADH0880	MPHIS177-17	No	Present study
C. atlantica	NIWA 96178	BOLD:ADH0880	MPHIS179-17	No	Present study
C. atlantica	NIWA 96180 A	BOLD:ADH0880	MPHIS180-17	No	Present study
C. atlantica	NIWA 96183 A	BOLD:ADH0880	MPHIS184-17	No	Present study
C. atlantica	NIWA 96186 C	BOLD:ADH0880	MPHIS191-17	No	Present study
C. atlantica	NIWA 96186 A	BOLD:ADH0880	MPHIS192-17	No	Present study
C. atlantica	NIWA 96297 B	BOLD:ADH0880	MPHIS196-17	No	Present study
C. cf. *reversa elongata*	EU735392 [COI]	BOLD:AAM9451	GBCPH1046-10	EU735256	Lindgren (2010)
C. cf. *reversa elongata*	EU735368 [COI]	BOLD:AAM9451	GBCPH1070-10	EU735231	Lindgren (2010)
C. cf. *reversa elongata*	DE0304 Sta 6	BOLD:AAM9451	MPHIS159-17	Yes	Present study
C. cf. *reversa elongata*	DE0506 elongata	BOLD:AAM9451	MPHIS161-17	Yes	Present study
C. cf. *reversa elongata*	DE0506 Sta 3	BOLD:AAM9451	MPHIS162-17	Yes	Present study
C. eltaninae	NIWA 92559	BOLD:ADH1219	MPHIS041-17	Yes	Present study
C. eltaninae	NIWA 105542	BOLD:ADH1219	MPHIS043-17	Yes	Present study
C. eltaninae	NIWA 105560	BOLD:ADH1219	MPHIS046-17	Yes	Present study
C. eltaninae	NIWA 96173 E	BOLD:ADH1219	MPHIS072-17	Yes	Present study
C. eltaninae	NIWA 96184 B	BOLD:ADH1219	MPHIS120-17	Yes	Present study
C. eltaninae	NIWA 96162 C	BOLD:ADH1219	MPHIS168-17	No	Present study
C. eltaninae	NIWA 96162 A	BOLD:ADH1219	MPHIS169-17	No	Present study

Table 6—Continued.

Identification	Specimen ID	BIN	BOLD ID (COI)	16S rRNA	Reference
C. eltaninae	NIWA 96162 B	BOLD:ADH1219	MPHIS170-17	No	Present study
C. eltaninae	NIWA 96173 B	BOLD:ADH1219	MPHIS176-17	No	Present study
C. eltaninae	NIWA 96180 B	BOLD:ADH1219	MPHIS181-17	No	Present study
C. eltaninae	NIWA 96184 C	BOLD:ADH1219	MPHIS185-17	No	Present study
C. eltaninae	NIWA 96184 A	BOLD:ADH1219	MPHIS186-17	No	Present study
C. eltaninae	NIWA 96186 B	BOLD:ADH1219	MPHIS193-17	No	Present study
C. eltaninae	NIWA 96301	BOLD:ADH1219	MPHIS197-17	No	Present study
C. cf. *reversa reversa*	PC1404 Sta 1 AB1 LN93	BOLD:ADI7337	MPHIS156-17	Yes	Present study
C. cf. *reversa reversa*	DE0506	BOLD:ADI7337	MPHIS157-17	Yes	Present study
C. cf. *reversa reversa*	DE0409 Sta 4	BOLD:ADI7337	MPHIS158-17	Yes	Present study
C. cf. *reversa reversa*	DE0304 Sta 7	BOLD:ADI7337	MPHIS160-17	Yes	Present study
Fragariateuthis					
F. aff. *inermis*	NSMT211	BOLD:ADH1220	MPHIS028-17	Yes	Present study
F. cf. *inermis*	EU735211	N/A	N/A	EU735211	Lindgren (2010)
F. cf. *inermis*	Mo.71595	BOLD:ADH1221	MPHIS027-17	Yes	Present study
F. pacifica	NSMT 261	BOLD:ADH1218	MPHIS110-17	Yes	Present study
F. pacifica	AM C.487271.002	BOLD:ADH1218	MPHIS130-17	Yes	Present study
Histiothauma					
Ha. aff. *meleagroteuthis* (NWAO)	AB1 LO34 PC1404 Sta 21	BOLD:ADJ5254	MPHIS142-17	Yes	Present study
Ha. aff. *meleagroteuthis* (NWAO)	AB1 LO14 PC1404 Sta 13	BOLD:ADJ5254	MPHIS143-17	Yes	Present study
Ha. aff. *meleagroteuthis* (NWAO)	MARECO 013221	BOLD:ADJ5254	MPHIS144-17	Yes	Present study
Ha. aff. *meleagroteuthis* (NWAO)	DE0611 Sta 6	BOLD:ADJ5254	MPHIS164-17	Yes	Present study
Ha. aff. *meleagroteuthis* (TWNP)	Mo.71596	BOLD:ADH0921	MPHIS106-17	Yes	Present study
Ha. aff. *miranda*	SajiHistio	BOLD:ADH1217	MPHIS122-17	Yes	Present study
Ha. cf. *meleagroteuthis*	NSMT 265	BOLD:ADH0922	MPHIS107-17	Yes	Present study

Table 6—Continued.

Identification	Specimen ID	BIN	BOLD ID (COI)	16S rRNA	Reference
Ha. heteropsis	MBARI2015heteropsis	BOLD:ADH2317	MPHIS111-17	Yes	Present study
Ha. heteropsis	MBARI2015Histio	BOLD:ADH2317	MPHIS112-17	Yes	Present study
Ha. heteropsis	MBARI MVH 001	BOLD:ADH2317	MPHIS141-17	Yes	Present study
Ha. miranda	EU735391 [COI]	BOLD:AAX1314	GBCPH1047-10	EU735255	Lindgren (2010)
Ha. miranda	NIWA 121877	BOLD:AAX1314	KERCE065-17	Yes	Chapter 5
Ha. miranda	NIWA 121878	BOLD:AAX1314	KERCE111-17	Yes	Chapter 5
Ha. miranda	TAN1208/40 miranda	BOLD:AAX1314	MPHIS108-17	Yes	Present study
Ha. miranda	TAN1210/078 miranda	BOLD:AAX1314	MPHIS109-17	No	Present study
Ha. miranda	AM C.500878.001	BOLD:AAX1314	MPHIS134-17	Yes	Present study
Ha. miranda	NIWA 121885	BOLD:AAX1314	MPHIS139-17	No	Present study
Ha. miranda	NIWA 121886	BOLD:AAX1314	MPHIS140-17	Yes	Present study
Ha. 'n. sp. large'	Mo.85130	BOLD:ADH1048	MPHIS029-17	Yes	Present study
Ha. 'n. sp. large'	NSMT 217	BOLD:ADH1048	MPHIS089-17	Yes	Present study
Ha. 'n. sp. large'	NSMT 309	BOLD:ADH1048	MPHIS090-17	Yes	Present study
Ha. oceania	AY616986	N/A	N/A	AY616986	Strugnell et al. (unpublished)
Histioteuthis					
Hi. aff. *bonnellii*	NIWA 118607 A	BOLD:ADH3734	KERCE063-17	Yes	Chapter 5
Hi. aff. *bonnellii*	NIWA 119197	BOLD:ADH3734	KERCE097-17	Yes	Chapter 5
Hi. aff. *bonnellii*	NIWA 119220 A	BOLD:ADH3734	KERCE112-17	Yes	Chapter 5
Hi. bonnellii	EU735385 [COI]	BOLD:AAX1286	GBCPH1053-10	EU735248	Lindgren (2010)
Hi. bonnellii	PC1401 Sta 4 AB1 LN93	BOLD:AAX1286	MPHIS145-17	Yes	Present study
Hi. bonnellii	DE0611 Sta 11	BOLD:AAX1286	MPHIS146-17	Yes	Present study
Hi. bonnellii	DE0506 Sta 10	BOLD:AAX1286	MPHIS147-17	Yes	Present study
Hi. bonnellii	DE0611 ta 6	BOLD:AAX1286	MPHIS148-17	Yes	Present study
Hi. macrohista	TAN1003/20	BOLD:ADG8263	MPHIS061-17	Yes	Present study

Table 6—Continued.

	Identification	Specimen ID	BIN	BOLD ID (COI)	16S rRNA	Reference
	Hi. macrohista	NIWA 96173 D	BOLD:ADG8263	MPHIS071-17	No	Present study
	Hi. macrohista	NIWA 96174 C	BOLD:ADG8263	MPHIS076-17	No	Present study
	Hi. macrohista	NIWA 96185 E	BOLD:ADG8263	MPHIS079-17	Yes	Present study
	Hi. macrohista	NIWA 96187 B	BOLD:ADG8263	MPHIS081-17	Yes	Present study
	Hi. macrohista	NIWA 96297 A	BOLD:ADG8263	MPHIS086-17	No	Present study
	Hi. macrohista	NIWA 89392	BOLD:ADG8263	MPHIS094-17	No	Present study
	Hi. macrohista	TAN0509 F macro	BOLD:ADG8263	MPHIS100-17	No	Present study
	Hi. macrohista	TAN0509/57 macro	BOLD:ADG8263	MPHIS101-17	No	Present study
	Hi. macrohista	TAN0509 macro	BOLD:ADG8263	MPHIS102-17	No	Present study
	Hi. macrohista	TAN1003/19 macro	BOLD:ADG8263	MPHIS104-17	No	Present study
	Hi. macrohista	TAN1003/29 macro	BOLD:ADG8263	MPHIS105-17	No	Present study
	Hi. macrohista	AM C.500877.001	BOLD:ADG8263	MPHIS131-17	No	Present study
	Hi. macrohista	AM C.500828.001	BOLD:ADG8263	MPHIS132-17	No	Present study
	Hi. macrohista	AM C.500838.001	BOLD:ADG8263	MPHIS133-17	No	Present study
	Hi. macrohista	AM C.483761.001	BOLD:ADG8263	MPHIS135-17	Yes	Present study
	Hi. macrohista	AM C.500845.001	BOLD:ADG8263	MPHIS136-17	Yes	Present study
	Hi. macrohista	NIWA 96185 A	BOLD:ADG8263	MPHIS187-17	No	Present study
	Hi. macrohista	NIWA 96185 B	BOLD:ADG8263	MPHIS188-17	No	Present study
	Hi. macrohista	NIWA 96185 C	BOLD:ADG8263	MPHIS189-17	No	Present study
	Hi. macrohista	NIWA 96185 D	BOLD:ADG8263	MPHIS190-17	No	Present study
Navia						
	N. corona cerasina	NIWA 118838 A	BOLD:ACG8287	KERCE072-17	Yes	Chapter 5
	N. corona corona	PC10-B0627-2789 MTB 064-DD	BOLD:ACG8287	MPHIS149-17	Yes	Present study
	N. corona corona	AB1 LO33 PC1404-21	BOLD:ACG8287	MPHIS150-17	Yes	Present study
	N. corona corona	PC10-B0627-2788 MTB 249-SD	BOLD:ACG8287	MPHIS151-17	Yes	Present study

Table 6—Continued.

Identification	Specimen ID	BIN	BOLD ID (COI)	16S rRNA	Reference
N. corona corona	PC10-01 Sta 088	BOLD:ACG8287	MPHIS153-17	Yes	Present study
N. corona corona	DE0611 Sta 3	BOLD:ACG8287	MPHIS154-17	Yes	Present study
N. corona corona	DE0304 Sta 13	BOLD:ACG8287	MPHIS155-17	Yes	Present study
Stigmatoteuthis					
S. aff. *hoylei*	X79581	N/A	N/A	X79581	Bonnaud et al. (1994)
S. aff. *hoylei*	AF000045 [COI]	BOLD:AAX1315	GBCPH0071-06	EU735212	Carlini & Graves (1999) for COI; Lindgren (2010) for 16S rRNA
S. aff. *hoylei*	FMNH286543	BOLD:AAX1315	MPHIS137-17	Yes	Present study
S. aff. *hoylei*	FMNH286564	BOLD:AAX1315	MPHIS138-17	Yes	Present study
S. arcturi	PC10-01 Sta 033	BOLD:ADJ2097	MPHIS152-17	Yes	Present study
S. arcturi	DE0511 Sta 3	BOLD:ADJ2097	MPHIS163-17	Yes	Present study
S. arcturi	AB1 LO11 PC1404 St 13	BOLD:ADJ2097	MPHIS165-17	Yes	Present study
S. arcturi	PC10-B0625-2888 MTB 082-DD	BOLD:ADJ2097	MPHIS166-17	Yes	Present study
S. arcturi	PC10-B0627-2788 MTB 249-SN	BOLD:ADJ2097	MPHIS167-17	Yes	Present study
S. cf. *hoylei*	NIWA 119219 A	BOLD:ADH3733	KERCE110-17	Yes	Chapter 5
S. cf. *hoylei*	NIWA 119220 B	BOLD:ADH3733	KERCE113-17	Yes	Chapter 5
S. dofleini	Mo.71594	BOLD:ADH3329	MPHIS025-17	Yes	Present study
S. dofleini	NSMT155	BOLD:ADH3329	MPHIS026-17	Yes	Present study
S. dofleini	MBARIStigmato	BOLD:ADH3329	MPHIS113-17	No	Present study
UnkNown ID					
?Histioteuthidae	GU220787	BOLD:ACH3185	GBCPH1087-13	No	Elliger et al. (unpublished)
?Histioteuthidae	GU220786	BOLD:ACH3185	GBCPH1088-13	No	Elliger et al. (unpublished)

DNA sequencing

DNA extraction was performed following protocols for the DNeasy Blood & Tissue Kit (QIAGEN) using EconoSpin (Epoch Life Science) columns. Amplification for COI and 16S rRNA followed protocols and primers in Braid et al. (2014). PCR products were sequenced using the same primer sets used for the PCR (Macrogen, Korea). Bidirectional sequences were assembled into contigs and manually edited in Sequencher v.4.9 (Gene Codes), and subsequently uploaded to BOLD (Ratnasingham & Hebert, 2007) in the public project titled 'Molecular Phylogenetic Analysis of the Histioteuthidae' (project code: MPHIS). Sequences were screened for contamination using the Basic Local Alignment Search Tool (BLAST) through GenBank. All histioteuthid sequences available on BOLD were added to the public dataset titled 'Dataset of All Histioteuthids' (dataset code: DS-HISTIO).

Phylogenetic analysis

Species boundaries were tested using the Barcode Index Number (BIN) system, which uses a clustering algorithm to generate operational taxonomic units based on COI, which have a high concordance with species (Ratnasingham & Hebert, 2013). BINs are automatically generated by BOLD for barcode sequences (Ratnasingham & Hebert, 2013). A single species ('*Hi*.' *oceani* [Robson, 1948]) was not included in the BIN analysis because it was represented by a single 16S rRNA sequence. Intra- and interspecific distances for COI were calculated using MEGA 7.0.26 (Tamura et al., 2013) using the Tamura–Nei (Tamura & Nei, 1993) model with gamma correction. The two individuals that represented a single, unidentified histioteuthid species that could not be assigned to a genus were omitted from the distance calculations.

For the phylogenetic analyses, a reduced dataset with 87 specimens was used; samples were only included when both genes were available or when a species was only represented by a single gene. Sequences were aligned separately for COI and 16S rRNA using the Multiple Alignment using Fast Fourier Transform (MAFFT) online server (Katoh & Standley, 2013), then concatenated in SequenceMatrix 1.8 (Vaidya et al., 2011). A maximum-likelihood phylogeny was created in RAxML 3.0 (Stamatakis, 2006) through Geneious 11.0 (http://www.geneious.com, Kearse et al., 2012), with 1000 rapid bootstrap pseudoreplicates, using the following options: -s <input file> -n <output file> -m GTRGAMMA -f a -x <random number seed for rapid bootstrapping> -N 1000 -p <random number seed for initial parsimony inferences>. Branches with low

bootstrap support (BS) (lower than 50%) were collapsed in TreeGraph 2 (Stöver & Müller, 2010) and the final phylogeny was visualised in FigTree 1.4.0 (Rambaut, 2012).

A Bayesian combined phylogeny was created in BEAST 1.8.0 (Drummond et al., 2012) using the substitution model SRD06 (Shapiro et al., 2005) for COI, and HKY+ G + I for 16S rRNA, which were determined through Tracer 1.6 (Rambaut et al., 2014). Four independent runs of 10,000,000 iterations were performed, with the first 1,000,000 removed as burn-in. Tracer 1.6 (Rambaut et al., 2014) was used to determine the correct burn-in and to check for convergence. The tree files were concatenated using LogCombiner 1.8.0 and the maximum clade credibility tree was then selected from the combined output using TreeAnnotator 1.8.0. Posterior probability (PP) values indicate the level of support for each branch.

Morphological analysis

The family and genus diagnoses are based on those of Voss (1969), Voss, Stephen, and Dong (1992), Voss et al. (1998), Young and Vecchione (2013a), and observations from the present study.

Results:

Genetics

From 218 individuals, 214 DNA barcodes were included (194 obtained from this thesis) and 69 16S rRNA sequences (66 obtained from this thesis). *Stigmatoteuthis* and four of the six previously identified species groups in *Histioteuthis* (‘*Hi*.’) were recovered as well-supported monophyletic clades on both the maximum likelihood and Bayesian phylogenies (Fig. 16; Fig. 17) and are therefore considered to represent generic divisions: *Calliteuthis* (the ‘*reversa*’ group; BS 83; PP 0.9985), *Fragariateuthis* gen. nov. (the ‘*celetaria*’ group; BS 99; PP 1), *Histioteuthis* (the ‘*bonnellii*’ group; BS 98; PP 0.9999), *Navia* gen. nov. (the ‘*corona*’ group; BS 100; PP 1), and *Stigmatoteuthis* (the ‘*hoylei*’ group; BS 100; PP 0.9908). The two other groups (the ‘*miranda*’ and ‘*meleagroteuthis*’ groups) formed a single, well-supported clade together (BS 86; PP 0.9953), forming the genus *Histiothauma* (‘*Ha*.’). The minimum divergence between genera ranged from 12.82 to 19.10% (Table 7). Within genera, the maximum divergence was 18.72% (in *Calliteuthis*), with the smallest maximum divergence between species found in *Fragariateuthis* (6.87%) (Table 8). *Navia* had a small maximum divergence of 0.31% (Table 8) because it contains two closely related

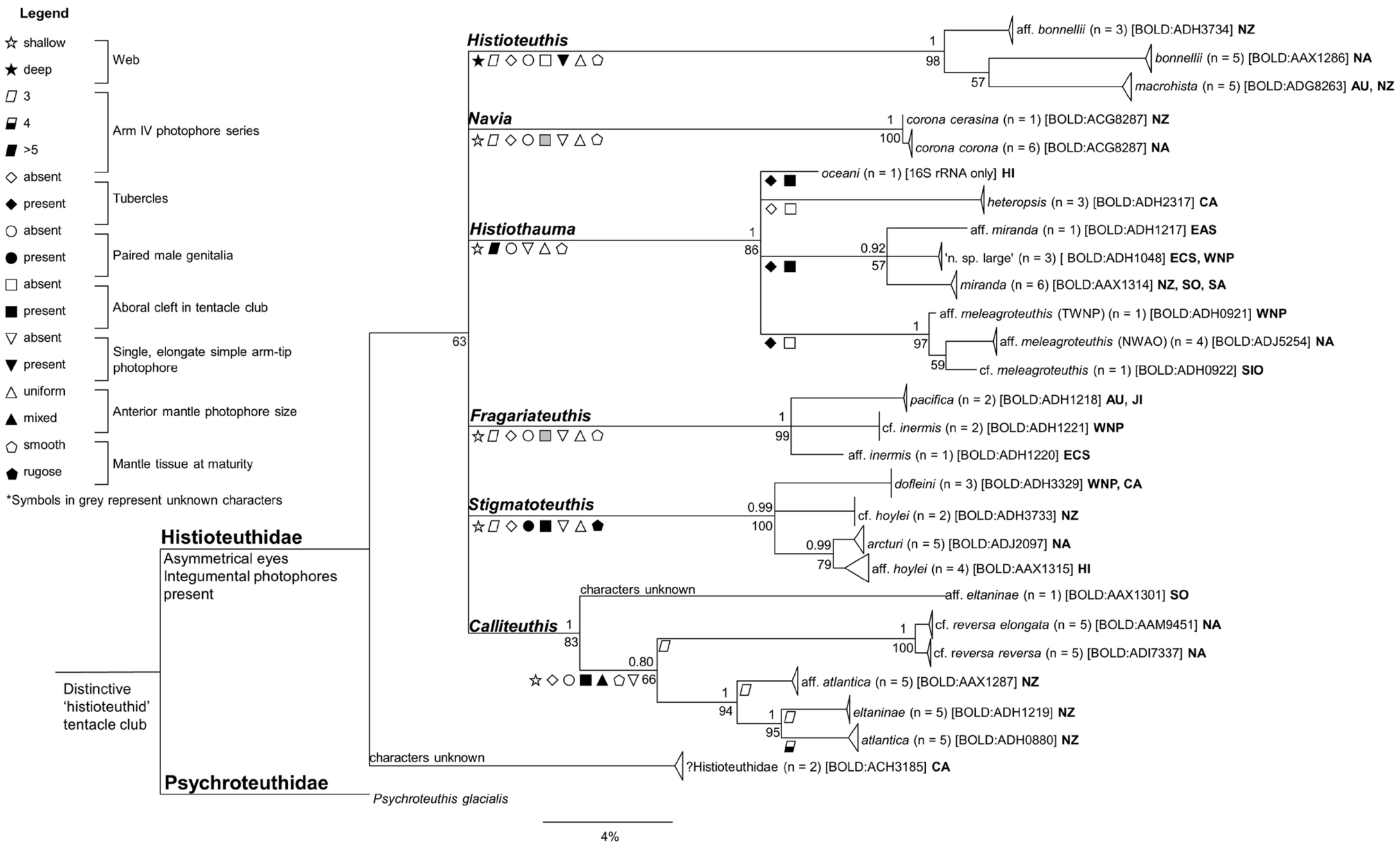

Fig. 16—Combined phylogeny of all histioteuthid specimens sequenced for this study and previously published sequences for cytochrome c oxidase subunit I (COI) and 16S rRNA (see Table 5 and Fig. 17). Upper node values indicate Bayesian posterior probabilities, and lower node values represent bootstrap support from the maximum-likelihood analysis, based on 1000 bootstrap replicates. Nodes with bootstrap support below 50% have been collapsed. Barcode Index Numbers (BINs) for COI are indicated. Morphological character states are represented by symbols. Abbreviations used in species names are defined as follows: NWAO – North West Atlantic Ocean; TWNP – Tohoku, western North Pacific. The following abbreviations are used to indicate locality data: Australia (AU); California (CA); East China Sea (ECS); Eastern Arabian Sea (EAS); Hawaii (HI); Java, off Indonesia (JI); New Zealand (NZ); North Atlantic (NA); Sumatra, Indian Ocean (SIO); South Africa (SA); Southern Ocean (SO); Western North Pacific (WNP).

subspecies, *N. corona corona* (Voss & Voss, 1962) and *N. corona cerasina* (Nesis, 1971). Intraspecific distances ranged from 0–*0.05*–0.51%, while interspecific divergences were larger, ranging from 1.42–*16.07*–24.43% (Table 9).

This analysis included 17 previously named species (including nine not previously available on BOLD, indicated with an asterisk): *C. atlantica**, *C. eltaninae** (Voss, 1969), *C.* cf. *reversa elongata** Voss and Voss, 1962, *C.* cf. *reversa reversa* Verrill, 1880; *F.* cf. *inermis* (Taki, 1964), *F. pacifica* (Voss, 1962); *Ha. heteropsis** (Berry, 1913), *Ha.* cf. *meleagroteuthis** (Chun, 1910), *Ha. miranda* Berry, 1918, *Ha. oceani*; *Hi. bonnellii*, *Hi. macrohista** Voss, 1969; *N. corona corona*, *N. corona cerasina**; *S. arcturi* Robson, 1948, *S. dofleini** Pfeffer, 1912, and *S.* cf. *hoylei** (Goodrich, 1896). Nine additional (possibly new) species were identified in the present

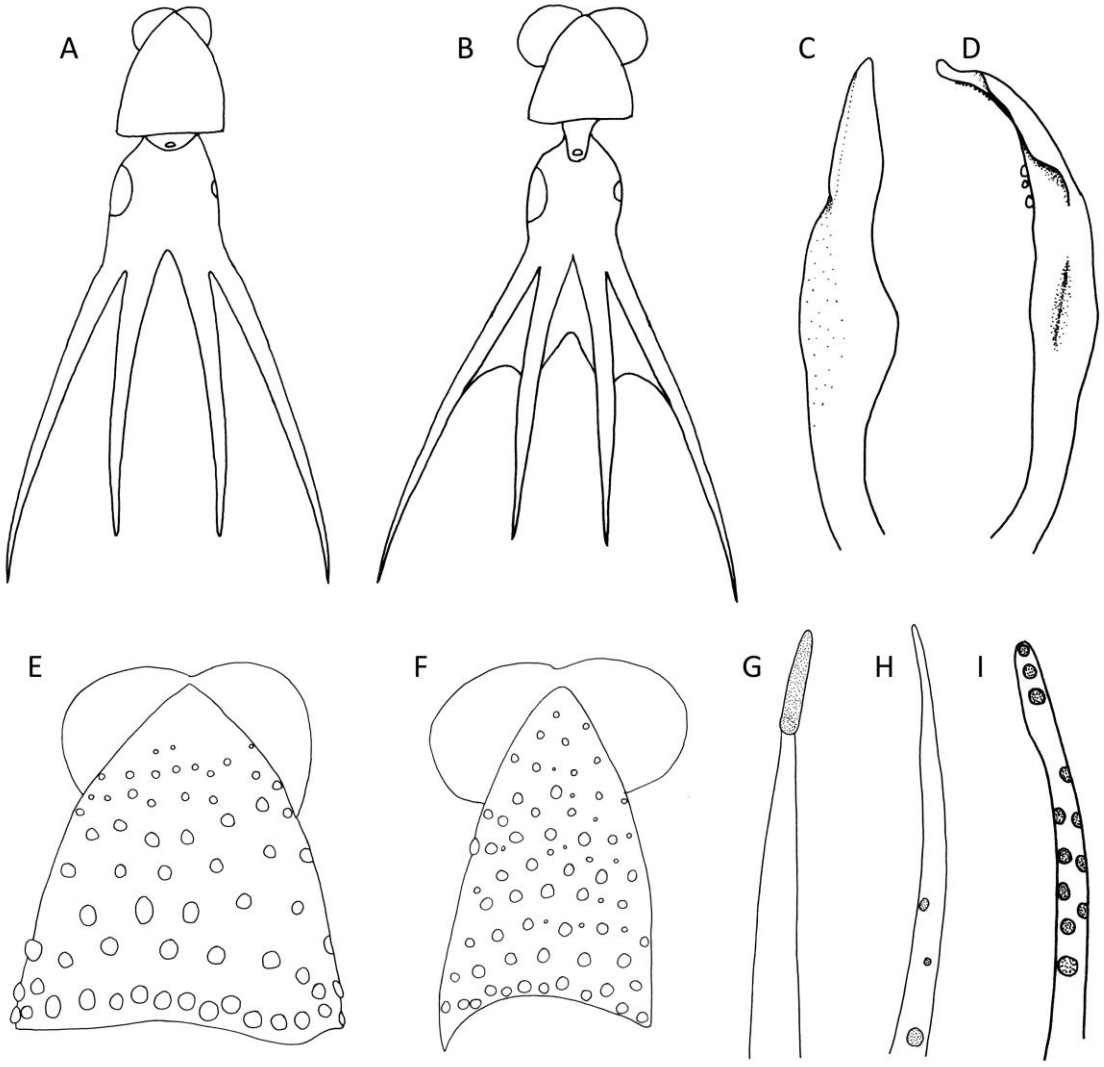

Fig. 17—Morphological features in the Histioteuthidae. A) Shallow web; B) deep web; C) club without aboral cleft; D) club with aboral cleft; E) anterior mantle photophore size uniform; F) anterior mantle photophores size mixed; G) single, elongate simple arm-tip photophore; H) arm tip without photophore; I) arm tip with multiple photophores.

Table 7—Minimum intergeneric distances (%) for cytochrome *c* oxidase subunit I (COI) among six genera of the Histioteuthidae.

	Stigmatoteuthis	***Histiothauma***	***Histioteuthis***	***Navia*** gen. nov.	***Fragariateuthis*** gen. nov.
Calliteuthis	14.47	16.77	19.10	18.57	14.23
Fragariateuthis gen. nov.	15.16	13.33	17.22	15.33	–
Navia gen. nov.	14.50	12.82	14.16	–	–
Histioteuthis	15.17	16.31	–	–	–
Histiothauma	13.73	–	–	–	–

Table 8—Intrageneric distances (%) for cytochrome *c* oxidase subunit I (COI) in six genera of the Histioteuthidae (*Navia* gen. nov. contains two subspecies both with the same Barcode Index Number, but are displayed here for comparison).

	Percent divergence		
	Min	Mean	Max
Calliteuthis	1.10	6.60	18.72
Fragariateuthis gen. nov.	5.03	6.18	6.87
Histioteuthis	10.06	10.49	11.19
Histiothauma	2.39	9.44	14.91
Navia gen. nov.	0.15	0.20	0.31
Stigmatoteuthis	1.42	4.80	7.46

Table 9—Pairwise intra- and inter-specific (%) evolutionary distances for cytochrome *c* oxidase subunit I (COI) across 18 and 25 species of the Histioteuthidae, respectively (minimum interspecific distance listed for a full species, *Navia corona cerasina* is 0.15% divergent from *Navia corona corona*, and the divergence between *Calliteuthis* cf. *reversa reversa* and *Calliteuthis* cf. *reversa elongata* is 1.10%).

	Percent divergence		
	Min	Mean	Max
Intraspecific	0	0.05	0.51
Interspecific	1.42	16.07	24.43

study: *C.* aff. *eltaninae, C.* aff. *atlantica*; *F.* aff. *inermis*; *Ha.* aff. *meleagroteuthis* (North West Atlantic Ocean [NWAO]), *Ha.* aff. *meleagroteuthis* (Tohoku, western North Pacific [TWNP]), *Ha.* 'n. sp. large', *Ha.* aff. *miranda*; *Hi.* aff. *bonnellii*, and *S.* aff. *hoylei*. Out of the 19 species currently recognised in this family, sequences were not available for three species: *F. celetaria* (Voss, 1960), *F.* 'sp. A', and *N. berryi* (Voss, 1969). An additional unidentified possible histioteuthid represented by two specimens from a previous study was also included (GU220786 and GU220787, from Elliger et al., unpublished), but did not group closely with any genus so their identification remains unclear. This species may not actually represent a histioteuthid, but groups closely with histioteuthids in a full database search in BOLD. A DNA barcode 'gap' (Meier, Zhang, & Ali, 2008) was observed (excluding the two subspecies found in *Navia* and *C.* cf.

reversa reversa and *C.* cf. *reversa elongata*), with a minimum interspecific distance of 1.42% across all sequenced histioteuthids and a maximum intraspecific distance of 0.51%.

The BIN analysis revealed 25 distinct BINs. One species (*Ha. oceani*) cannot yet be included in the BIN analysis because only 16S rRNA is available from previous studies and no additional tissue has been located to date, but will almost certainly represent a separate BIN when COI sequences become available. Thus, the family Histioteuthidae is likely to contain at least 29 species (26 identified herein, plus *F. celetaria*, *F*. 'sp. A', and *N. berryi*), including four subspecies (*C.* cf. *reversa reversa*, *C.* cf. *reversa elongata*, *N. corona corona*, and *N. corona cerasina*).

Following assembly of the reference library of sequences, species identifications from several previous studies could be compared. Three samples identified as '*Hi. reversa*' (KY793565, KY793564, and KY793556) from Waap et al. (2017) grouped within the clade of *C. reversa*; although *C.* cf. *reversa reversa* and *C.* cf. *reversa elongata* can be distinguished by COI and have been assigned separate BINs, these subspecies cannot presently be distinguished using 16S rRNA, which was the only available locus. A specimen initially identified as '*Hi. miranda*' (KY886223) by Sajikumar et al. (unpublished), appears to represent a new species, distinct from other sequenced individuals of *Ha. miranda* (from New Zealand waters, the Great Australian Bight, and off South Africa), and has been identified herein as *Ha*. aff. *miranda*. Two specimens previously identified as '*Hi. hoylei*' (X79581, AF000045) from Bonnaud, Boucher-Rodoni, and Monnerot (1994) and Carlini and Graves (1999) align closely with *S. arcturi* from the present study, but were assigned a separate BIN and are herein referred to as *S*. aff. *hoylei*. A single specimen identified as '*Hi. corona*' from Lindgren (2010) (EU735211) appears to represent *F*. cf. *inermis*. One specimen identified as '*Hi.* sp. RJ-2009' (GU145057) from Bucklin et al. (2010) is not a histioteuthid, but an octopoteuthid, and was not included in the present analysis.

Systematics

Family Histioteuthidae Verrill, 1881

Diagnosis: Small- to medium-bodied mesopelagic squids (mantle length [ML] ~40–330mm at maturity). Fins small, oval in outline when taken together, joined posteriorly. Head relatively large with asymmetrical eyes; left eye usually much larger than right

eye. Locking cartilages simple, funnel-locking cartilage slightly curved; buccal formula DDVD. Arms with biserial suckers; inner web present between oral surfaces. Hectocotylus usually present. Tentacles with suckers in ~5–8 series; club divided into manus and dactylus; median keel present on aboral surface of club; carpal-locking apparatus present. Numerous compound integumental photophores present on outer surface of mantle, head, and arms. Skin tubercles variably present. Gladius with posterior cupped coil.

Included species: *C. atlantica, C.* aff. *atlantica, C. eltaninae, C.* aff. *eltaninae, C. reversa elongata, C. reversa reversa*; *F. celetaria, F. inermis, F.* aff. *inermis, F. pacifica, F.* 'sp. A'; *Ha. heteropsis, Ha. meleagroteuthis, Ha.* aff. *meleagroteuthis* (NWAO], *Ha.* aff. *meleagroteuthis* (TWNP), *Ha. miranda, Ha.* aff. *miranda, Ha. oceani, Ha.* 'n. sp. large'; *Hi. bonnellii, Hi.* aff. *bonnellii, Hi. macrohista*; *N. berryi, N. corona corona, N. corona cerasina*; *S. arcturi, S. dofleini, S.* aff. *hoylei*, and *S.* cf. *hoylei*.

Remarks: This diagnosis is based on Voss (1969), Voss et al. (1992), Voss et al. (1998), Young and Vecchione (2013a), and the present findings.

***Calliteuthis* Verrill, 1880** (Table 10)

Calliteuthis Verrill, 1880, p. 393. Type species *Calliteuthis reversa* Verrill, 1880, by monotypy.

Histiopsis Hoyle, 1885b, p. 205. Type species *Histiopsis atlantica* Hoyle, 1885b, by monotypy.

Diagnosis: Mantle length at maturity ~50–260mm ML. Integumental photophores on ventral mantle intermixed large and small organs; 17 large and one small photophores around right eye; photophores on Arms IV in three or four series, row of multiple, simple arm-tip photophores on Arms I–III variably present. Tubercles absent. Smooth mantle tissue at maturity. Shallow inner web between arms. Male genitalia single.

Included species: *C. atlantica, C.* aff. *atlantica, C. eltaninae, C.* aff. *eltaninae, C. reversa elongata*, and *C. reversa reversa*.

Table 10—Morphological characteristics of the six proposed genera in the Histioteuthidae. Based on Voss et al. (1998), Young and Vecchione (2013), and the present findings.

	Calliteuthis	***Fragariateuthis*** gen. nov.	***Histioteuthis***	***Histiothauma***	***Navia*** gen. nov.	***Stigmatoteuthis***
ML at maturity (mm)	~50–260	~60–280	~50–330	~90–270	~110–188	~70–235
Anterior mantle photophores	intermixed*	uniform anterior 2/3 v mantle	uniform anterior 1/2 v mantle	uniform anterior 3/4 v mantle	uniform anterior 1/2 v mantle	uniform anterior 1/3 v mantle
Photophores around R eye	17 large + 1 small	16–18	15–18	17–22	16–18	17 (rarely 16)
Arm IV photophore series	3 or 4	3	3	5–10*	3 or 4	3
Elongate, simple arm-tip photophore	absent	absent	Arms I-III*	absent	absent	absent
Tubercles	absent	absent	absent	variably present*	absent	absent
Mantle tissue at maturity	smooth	smooth	smooth	smooth	smooth	rugose*
Inner arm web depth	shallow	shallow	deep between Arms I-III*	shallow	shallow	shallow
Paired male genitalia	absent	absent	absent	absent	absent	present*
Aboral cleft in tentacle club	present	unknown	absent	variable	unknown	present

*indicates a unique character within the family

Remarks: The diagnosis for *Calliteuthis* is based on characteristics of the '*reversa*' group as described by Voss et al. (1998), and Young and Vecchione (2013a). Unlike all other histioteuthids, which have uniformly sized photophores on the ventral anterior mantle, *Calliteuthis* species have intermixed large and small photophores in this region (Voss et al., 1998). The lateral wall of the lower beak in *Calliteuthis* species possesses a weakly developed median ridge (Young & Vecchione, 2010b).

***Fragariateuthis* gen. nov. Braid & Bolstad (in prep.)** (Table 10)

Fragariateuthis Braid & Bolstad (in prep.). Type species *Calliteuthis celetaria* Voss, 1960, by monotypy.

Diagnosis: Mantle length at maturity ~60–280mm ML. Integumental photophores on anterior 2/3 of ventral mantle large, uniform in size, and evenly spaced; 16–18 photophores around right eye; photophores on Arms IV in three series, simple arm-tip photophore absent. Tubercles absent. Smooth mantle tissue at maturity. Shallow inner web between arms. Male genitalia single.

Included species: *F. celetaria*, *F. inermis*, *F.* aff. *inermis*, *F. pacifica*, and *F.* 'sp. A'.

Remarks: The diagnosis for *Fragariateuthis* is based on characteristics of the '*celetaria*' group as described by Voss et al. (1998) and Young and Vecchione (2013a). Voss et al. (1998) were only able to examine nine specimens of *F. inermis*, and considered this taxon to represent a subspecies ('*Histioteuthis corona inermis*') in the '*corona*' group. However, Young and Vecchione (2000a) placed *F. inermis* in the '*celetaria*' group based on photophore size and pattern. Two specimens identified as *F. inermis* in the present study grouped with *F. pacifica* and not with *N. corona;* which supports the hypothesis of Young and Vecchione (2000a), *F. inermis* is accordingly placed in *Fragariateuthis* with the other '*celetaria*' group taxa in the present study.

Young and Vecchione (2013a) additionally characterised this group by the presence of the 'Type 1b' photophore pattern on the head, 9 or 10 photophores on the basal row of the head, and the absence of the right basal series of head photophores.

Etymology: This genus name reflects both the animals' photophore-studded exterior, and the aromatic nature of deep-sea squid specimens: *Fragaria* is the genus name for

strawberries (acknowledging one of the animals' common names of 'strawberry squid'), and means 'fragrant' in Latin.

***Histioteuthis* d'Orbigny [in Férussac & d'Orbigny], 1841** (Table 10)

Histioteuthis d'Orbigny, 1841 In Férussac and d'Orbigny, 1834–1848:xxxvii. Type species *Cranchia bonnellii* Férussac, 1834, by monotypy.

Lolidona Risso, 1854, p. 33. Type species *Lolidona euphrosina* Risso, 1854, by monotypy.

Diagnosis: Mantle length at maturity ~50–330mm ML. Integumental photophores present on ventral 1/2 of anterior mantle surface large and uniform in size; 15–18 photophores around right eye; Arms IV photophores in three series; single, simple, elongate arm-tip photophore present on Arms I–III. Tubercles absent. Smooth mantle tissue at maturity. Deep inner web between Arms I–III. Male genitalia single.

Included species: *Ha. heteropsis, Ha. meleagroteuthis, Ha.* aff. *meleagroteuthis* (NWAO], *Ha.* aff. *meleagroteuthis* (TWNP), *Ha. miranda*, *Ha.* aff. *miranda*, *Ha. oceani*, and *Ha.* 'n. sp. large'.

Remarks: The diagnosis for *Histioteuthis* is based on characteristics of the '*bonnellii*' group as described by Voss et al. (1998) and Young and Vecchione (2013a). This genus is distinguished from all other genera in the family by the deep inner web between the arms, and by having two or three large photophores on the left posteroventral margin of the head (Young & Vecchione, 2010c) and elongate simple photophores on arm tips. Young and Vecchione (2010c) found that *Hi. bonnellii* has the 'Type 1b' photophore pattern, but the pattern of other species in this genus are not confirmed. They also reported that this genus is characterised by the presence of 'large' compound photophores on the anterior half of the ventral mantle. Voss et al. (1998) found that these species have a single, elongate photophore on the ends of Arms I–III, two or three large photophores on the left side of the posteroventral head surface, and multiple attachments of the fourth supports to the buccal membrane.

***Histiothauma* Robson, 1948** (Table 10)

Histiothauma Robson, 1948, p. 123. Type species *Histiothauma oceani* Robson, 1948, by original designation.

Diagnosis: Mantle length at maturity ~90–270mm ML. Integumental photophores on anterior 3/4 of ventral mantle small, dense, uniform in size; 17–22 photophores around right eye; photophores on Arms IV in five to ten series; elongate simple arm-tip photophore absent. Tubercles variably present on mantle dorsal midline and aboral surface of Arms I–III. Smooth mantle tissue at maturity. Shallow inner web between arms. Male genitalia single.

Included species: *Hi. bonnellii*, *Hi*. aff. *bonnellii*, and *Hi. macrohista*.

Remarks: The diagnosis for *Histiothauma* is based on characteristics of the '*miranda*' and '*meleagroteuthis*' groups as described by Voss et al. (1998) and Young and Vecchione (2013a). Species from these two morphologically identified groups formed a single, well-supported clade on both phylogenies (Fig. 16). The '*meleagroteuthis*' group contains *Ha. meleagroteuthis* and *Ha. heteropsis*, and is distinguished by small, dense photophores on the ventral mantle, head, and arms IV (Voss et al., 1998). The '*miranda*' group contains *Ha. miranda* and *Ha. oceani* and is distinguished by the moderately dense photophores on the ventral side of the head, mantle, and arms IV. All members of this genus possess a greater number of Arm IV photophore series (five to ten) than the other histioteuthid genera (which all have three or four) (*fide* Voss et al., 1998).

***Navia* gen. nov. Braid & Bolstad (in prep.)** (Table 10)

Navia Braid & Bolstad (in prep.). Type species *Calliteuthis corona* Voss & Voss, 1962, by monotypy.

Diagnosis: Mantle length at maturity ~110–188mm ML. Integumental photophores on anterior 1/2 of ventral mantle surface uniform in size; 16–18 photophores around right eye; photophores on Arms IV in 3 or 4 series, simple arm-tip photophore absent. Tubercles absent. Smooth mantle tissue at maturity. Shallow inner web between arms. Male genitalia single.

Included species: *N. berryi*, *N. corona corona*, and *N. corona cerasina*.

Remarks: The diagnosis for *Navia* is based on characteristics of the '*corona*' group as described by Voss et al. (1998) and Young and Vecchione (2013a). There are three currently accepted species in this genus: *N. corona*, *N. berryi*, and *N. cerasina* (Young & Vecchione, 2000b). Voss et al. (1998) considered all species in this group as subspecies of *N. corona* (including *F. inermis*; see Remarks for *Fragariateuthis*), but Young and Vecchione (2000b) maintained the full species status of *N. berryi* and *N. cerasina*. In the present study, no specimens were available for *N. berryi*. A single specimen identified as *N. corona cerasina* from the Kermadec region in northern New Zealand waters was included. Because this specimen formed a single BIN with *N. corona corona* from the Atlantic Ocean and yet showed a sister relationship with *N. corona corona* on the phylogenies (Fig. 16), *N. corona cerasina* is considered a subspecies in the present study. However, no specimens of *N. corona cerasina* were available from its type locality (Eastern Pacific Ocean, off Ecuador). Specimens of *N. corona corona* and *N. corona cerasina* formed a clade distinct from other genera, suggesting that a genus-level grouping is appropriate for these species, as for the other 'species groups'.

Young and Vecchione (2000b) additionally characterised this group by 'Type 2' head photophores, 7 photophores in the basal head row, the presence of a right basal series of photophores, and the absence of a series of compound photophores at the tip of Arms IV. Voss et al. (1998) found that the suckers are smooth on Arms I–III, only becoming dentate distally, and with dentition on all Arm IV suckers.

Etymology: This genus is named for Nancy A. Voss (NAV), in honour of her significant contributions to histioteuthid systematics.

***Stigmatoteuthis* Pfeffer, 1900** (Table 10)

Stigmatoteuthis Pfeffer, 1900, p. 170. Type species *Histiopsis hoylei* Goodrich, 1896, by monotypy.

Meleagroteuthis Pfeffer, 1900, p. 170. Type species *Meleagroteuthis hoylei* Pfeffer, 1900, by monotypy.

Diagnosis: Mantle length at maturity ~70–235mm ML. Integumental photophores on anterior 1/3 of ventral mantle uniform in size; 17 (rarely 16) photophores around right eye; photophores on Arms IV in three series, simple arm-tip photophore absent.

Tubercles absent. Rugose mantle tissue at maturity. Shallow inner web between arms. Male genitalia paired.

Included species: *S. arcturi*, *S. dofleini*, *S.* aff. *hoylei*, and *S.* cf. *hoylei*.

Remarks: The diagnosis for *Stigmatoteuthis* is based on characteristics of the '*hoylei*' group as described by Voss et al. (1998), and on the genus *Stigmatoteuthis* as described by Young and Vecchione (2013a; 2016b). This clade was first recognised by Voss (1969); Young and Vecchione (2016b) resurrected Hoyle's *Stigmatoteuthis* as the correct name for the three known species characterised by the unique paired male genitalia and the photophore pattern ('Type 1a') on the head. The *Stigmatoteuthis* clade is strongly supported by both phylogenies (Fig. 16). Species in this genus are further distinguished by having enlarged (over four times as large as the marginal suckers) medial suckers on the tentacular clubs, and papillated skin (Voss et al., 1998).

Young and Vecchione (2016b) further characterised this genus by a basal row of head photophores with eight photophores and three 'sawteeth', the presence of the right basal series of photophores on the head, a lack of separate compound photophores on arms IV, and the presence of large uniformly sized photophores on the anterior half of the ventral mantle that are evenly spaced.

Discussion:

Six previously recognised 'species groups' in the Histioteuthidae now appear to represent a mix of previously named and new genera: *Calliteuthis* (the '*reversa*' group), *Fragariateuthis* gen. nov. (the '*celetaria*' group), *Histioteuthis* ('*Hi*,'; the '*bonnellii*' group), *Histiothauma* ('*Ha*.'; the '*miranda*' and '*meleagroteuthis*' groups), *Navia* gen. nov. (the '*corona*' group), and *Stigmatoteuthis* (the '*hoylei*' group). These groupings, which were previously identified on the basis of morphology by Voss et al. (1998), formed well-supported clades on the combined COI and 16S rRNA phylogenies (Fig. 16), supporting their status as natural subdivisions within the family. The intergeneric divergences for COI (12.82–19.10%) found for the Histioteuthidae in the present study are comparable to those previously found for the Octopoteuthidae Berry, 1912 (12.3–18.6%; J. Kelly unpubl. data), and the Onychoteuthidae Gray, 1847 (4.7–18.8%; Bolstad et al., 2018), but lower than those reported in the Mastigoteuthidae (18.9–34.1%, Braid et al. 2014). Members of each histioteuthid genus have a suite of morphological characters in common, which can be used to distinguish them from

members of other genera. As such, both morphological and molecular evidence support the elevation of the former 'species groups' to full genera. The relationships among genera in the Histioteuthidae are still not resolved.

In the present study, 25 BINs have been identified within the Histioteuthidae, with one additional species that was only represented by 16S rRNA, making a total of 26 genetically distinct taxa. Young and Vecchione (2013a) recognised 19 species in the Histioteuthidae, three of which were not available for inclusion in the present study. The species boundaries within the species complexes of *C. reversa*, *S.* aff. *hoylei*/*S. arcturi*, and *Hi. meleagroteuthis* are not fully understood. Therefore, it appears that at least 25–29 species are likely to exist within this family—a substantial increase in diversity from previous reports, as has been found recently in other oegopsid families where integrative taxonomic reviews have been undertaken (e.g., Chapter 3; Braid & Bolstad, 2015; Bolstad et al., 2018). The divergences found in COI in this family (Tables 9) are also comparable to recent reports for other oegopsid families. The intraspecific divergences found in the Histioteuthidae (0–*0.05*–0.51%) were similar to the divergences for the Mastigoteuthidae (0–*0.2*–1.0%; Braid et al., 2014), the Octopoteuthidae (0–*0.2*–0.4%; J Kelly, unpubl. data), and the Onychoteuthidae (0–*0.23*–2.6%; Bolstad et al., 2018). The interspecific divergences within the Histioteuthidae (1.42–*16.07*–24.43%) were also similar to the Octopoteuthidae (3.0–*16.9*–25.1%; J. Kelly, unpubl. data), the Onychoteuthidae (3.8–20.0–32.9%; Bolstad et al., 2018), and the Mastigoteuthidae (10.5–*25.6*–35.3%; Braid et al., 2014). The minimum interspecific distances were lowest in the Histioteuthidae, and highest in the Mastigoteuthidae, which could indicate that the species in the Histioteuthidae have diverged more recently.

The increase in the number of genera and species in this family has implications for conservation. This family appears to have a much higher biodiversity than previously believed, and species that previously appeared cosmopolitan seem to have much more restricted geographic boundaries. For example, *Hi. bonnellii* was previously believed to be widely distributed in the Atlantic, Indian, and western Pacific Oceans. However, it now appears that individuals from the North Atlantic represent a separate species from those in New Zealand waters (Fig. 16). Higher taxonomic rankings have been suggested as possible surrogates for species biodiversity in marine environments for establishing marine protected areas (e.g., Vanderklift, Ward, & Phillips, 1998). Therefore, it is important to increase the clarity of the higher taxonomy within families. The recognition of six genera in this family based on morphology and genetics where

only two were previously accepted (Young & Vecchione, 2013a) could help improve the protection of histioteuthid biodiversity. Currently, all histioteuthids listed on the IUCN Red List of Threatened Species (n.d.) have a status of least concern or are data deficient.

Calliteuthis

Calliteuthis (the '*reversa*' group) is presently understood to contain three species: *C. reversa*, *C. atlantica*, and *C. eltaninae*. These species formed a strongly supported clade on both phylogenies, along with *C.* cf. *reversa elongata* (a species recently synonymised with *C. reversa*, see Voss et al., 1998), and two previously unrecognised species, identified as *C.* aff. *atlantica* and *C.* aff. *eltaninae* (Fig. 16). The presence of two species morphologically resembling *C. atlantica* has been previously suspected based on the presence of small and large mature individuals in New Zealand waters and a bimodal distribution of lower rostral lengths in beaks collected from fish predators (D. Stevens, pers. comm.). *Calliteuthis* aff. *atlantica* appears widely distributed in New Zealand waters, with specimens collected to date from the Kermadec Islands to the Chatham Rise. Dell (1951) described '*Hi. cookiana*' from Cook Strait in New Zealand waters, which Voss (1969) synonymised with *C. atlantica*; however, it is possible that this species represents *C.* aff. *atlantica*. Although Voss et al. (1992) suggested that *Calliteuthis atlantica* may actually contain additional species or subspecies, Voss et al. (1998) found that the differences previously found in spermatophore structure were related to maturity, and thus concluded that *C. atlantica* represents a single species with a circumglobal distribution. Now, with two species resembling *C. atlantica* known from New Zealand waters alone, comparative material from other localities will be necessary to resolve species complex throughout its range.

Calliteuthis cf. *reversa elongata* was assigned a separate BIN from *C.* cf. *reversa reversa*. This suggests that two species are present. However, due to the low divergence between these species for COI (1.10%) and because they cannot be distinguished using 16S rRNA, these two species are presently considered subspecies rather than full species. They appear to occur a sympatrically, with specimens having both been collected in the same area (Bear Seamount, in the eastern North Atlantic). Although relatively low, the divergence found in COI for individuals found in the same area suggests that there are barriers to reproduction that exclude geography. *Calliteuthis elongata* was originally distinguished from *C. reversa* by the long, slender mantle, the presence of large black photophores on the ventral mantle, and a more symmetrical head

(Voss, 1969). Later, these species were synonymised because of similarities observed once specimens of compariable sizes were available (i.e., large *C. reversa* and small *C. elongata*). However, Toll (1982) found differences in the gladii of *C. reversa* specimens from different geographic locations; specimens from the Mediterranean had wider gladii than specimens from the tropical Atlantic. However, no specimens of *C. reversa* were available for sequencing in the present study from the Mediterranean for comparison. In addition, Toll (1982) noted that in the northwestern Atlantic, there was a greater width range in the gladii, which overlapped with the other two regions. Therefore, it is possible that this difference is due to the presence of both *C.*cf. *reversa reversa* and *C.* cf. *reversa elongata* in this area, and only a single species in the other two regions. More specimens from across the distribution of *C. reversa* are needed for both morphological examination and sequencing to determine the status of *C. reversa*.

The existence of a sixth *Calliteuthis* species is suggested by the COI sequence from a single specimen collected in the Scotia Sea by the British Antarctic Survey (BOLD ID BASMC092-09/BIN BOLD:AAX1301). This individual, which was initially identified as '*Histioteuthis eltaninae*' (termed *C.* aff. *eltaninae* herein) forms a strongly supported clade with the other *Calliteuthis* species. However, COI was the only gene available for this apparently unique taxon, and the parent specimen (if extant) could not be examined in this study. *Calliteuthis eltaninae* was originally described from the Pacific Ocean, just east of the New Zealand EEZ, and therefore it is more likely that the specimens from the Chatham Rise represent *C. eltaninae sensu stricto*, but a detailed morphological analysis and a comparison with type material will be required to resolve this species complex.

Fragariateuthis gen. nov.

There are four known species in *Fragariateuthis*, with one species presently unnamed: *F. celetaria*, *F. inermis*, *F. pacifica*, and *F.* 'sp. A' (Young & Vecchione, 2010a). In the present study, three species in this genus have been sequenced. A single specimen identified as *F. pacifica* from northern Australian waters formed a BIN (suggesting conspecifity) with a single specimen from Indonesia, off Java. The other two BINs are each morphologically consistent with *F. inermis*, suggesting that additional characters are needed for reliable differentiation of taxa within *Fragariateuthis*. The specimens that were captured closer to the type locality (off Japan) are called *F.* cf. *inermis*, while the single specimen from the East China Sea that formed a distinct BIN is called *F.* aff. *inermis* in the present study (Fig. 16). It is possible that

one of these species actually represents *F*. sp. A, which is known to be morphologically similar (differentiated only by the number of head photophores in the basal row and the size of the medial manal suckers) and currently only known from five specimens captured in Hawaiian waters (Young & Vecchione, 2010a). While some histioteuthid distributions span multiple oceans (e.g., *Ha*. *miranda*), is seems unlikely that one of the two '*F. inermis*' individuals might represent *F. celetaria,* which is only known from the Atlantic Ocean to date (Young & Vecchione, 2013b). *Fragariateuthis inermis* appears to have a close relationship with *F. pacifica*, rather than with *N. corona corona* (as suggested by the combined phylogenies where *F. pacifica* groups with *F. inermis*, rather than with *N. corona corona* and *N. corona cerasina*; Fig. 16).

Histioteuthis

Histioteuthis contains the species previously comprising the '*bonnellii*' group (Voss et al., 1998): *Hi. bonnellii* and *Hi. macrohista*. A third species in this genus, reported as *Hi.* aff. *bonnellii*, was identified from the Kermadec region, north of New Zealand. Voss et al. (1998) found geographic variation in the morphology of *Hi. bonnellii* individuals and suggested that separate populations of this species may exist. Specimens of *Hi*. aff. *bonnellii* formed a single BIN, distinct from *Hi. bonnellii* sequences from the North Atlantic (near the Mediterranean type locality), which suggests that they represent a separate species. However, morphological work is needed in order to confirm the status and specific characters of these animals.

Histiothauma

Histiothauma contains the species in the former '*meleagroteuthis*' group (*Ha. heteropsis* and *Ha. meleagroteuthis*) and the '*miranda*' group (*Ha. miranda* and *Ha. oceani*), which are united by a high number of photophores in the Arm IV series and by the presence of tubercles in most species. *Histiothauma heteropsis* is the only species in this genus without skin tubercles. The relationship between *Ha. oceani* and other species in this genus may be additionally clarified if COI sequences become available for this species. Although the relationships within this genus are still not fully resolved, there are two species that appear to represent species complexes: *Ha. meleagroteuthis* and *Ha. miranda*.

Although *Ha. meleagroteuthis* has been reported as having a circumglobal distribution, the great variation in COI and 16S rRNA sequences of specimens from different locations may signify different populations or possibly species. Four

specimens of *Ha.* aff. *meleagroteuthis* (NWAO) from the North Atlantic Ocean formed a unique BIN, distinct from the single specimen of *Ha.* cf. *meleagroteuthis* from the Indian Ocean, off Sumatra, and the BIN for the single specimen of *Ha.* aff. *meleagroteuthis* (TWNP) from the western Pacific Ocean off Tohoku, Japan. Unfortunately, the few specimens known from the type locality (New Zealand) are all formalin fixed, inhibiting sequencing to date. Voss et al. (1992) reported variation in the buccal membrane attachments of *Ha. meleagroteuthis* specimens from different geographic locations, but the significance of these differences was (and remains) unclear. Voss et al. (1998) found no consistent morphological characters that could be used to separate individuals of this species from different locations, but noted the lack of subadult and adult specimens available and suggested that the tentacle club suckers should be examined in future studies. The variations in the present *Ha. meleagroteuthis* complex COI sequences suggest that multiple species may be present, but a careful morphological review will be needed to determine if this variation represents species, subspecies, or population-level variation. However, given that the COI variation found among other histioteuthid species is often lower that that observed among different populations of *Ha. meleagroteuthis* (Tables 8, 9), it appears possible that additional species-level distinctions will be needed. However, there are currently species names available from two species that have been synonymised with *Ha. meleagroteuthis* for the Atlantic Ocean (*Ha. bruuni* Voss, 1969) and Japan (*Ha. separata* Sasaki, 1915), which could eventually prove valid.

Within the sub-clade representing the '*miranda*' group, three species appear to be present: *Ha. miranda*, *Ha.* aff. *miranda* (Indian Ocean), and *Ha*. 'n. sp. large' (*fide* T. Kubodera). Specimens identified as *Ha. miranda* from New Zealand waters (the type locality), off South Africa, and the Great Australian Bight appear to represent a species that is distinct from the single specimen of initially identified as '*Hi. miranda*' from the Indian Ocean (called '*Ha*. aff. *miranda*' herein). Voss et al. (1998) found no population-level variation in the morphology of *Ha. miranda* across its wide range; however, they noted that there were no unruptured spermatophores (one of the most reliable morphological characters) available from Australian or New Zealand specimens. It is clear that more work is needed to resolve the systematics of this genus.

Navia gen. nov.

Sequences of *N. corona corona* from the Atlantic Ocean were compared with those from a specimen identified morphologically as *N. corona cerasina* from the

Kermadec Islands region north of New Zealand (consistent with the morphology for this species reported by Horstkotte, 2008), and they formed a single BIN. *Navia corona corona* and *N. corona cerasina* share many morphological similarities, but they can be separated by geography and tentacle-club-sucker dentition (Young & Vecchione, 2000b) suggesting the possibility that these populations, which presently constitute a single species based on the BIN analysis, are in the process of diverging. Therefore, *N. corona cerasina* is maintained as a subspecies of *N. corona corona* in the present study, in recognition of its genetic similarity but also its observed morphological differences. No sequences are presently available for *N. berryi*, and its status as a species or subspecies is unclear. However, this species is easily distinguished from the others in this genus by the dentition on the manus suckers of the tentacle clubs (*fide* Voss et al., 1998), an additional photophore in the series on Arms IV (*fide* Voss et al., 1998), and the head photophore pattern (*fide* Young & Vecchione, 2006) and likely represents a separate species.

Stigmatoteuthis

Voss et al. (1998) only recognised *S. hoylei* and *S. arcturi* in the '*hoylei*' group (which was retained as part of *Histioteuthis*). However, Young and Vecchione (2016b) distinguished three species in this genus: *S. dofleini*, *S. hoylei*, and *S. arcturi*; sequences analysed in the present study support the existence of these three species, and a fourth distinct species, *S.* aff. *hoylei* from around Hawaii. Geographic location is routinely used to identify species in this genus, because the most reliable characters to distinguish them (hectocotylus and spermatophore morphology) are found only in mature males (Young & Vecchione, 2016b). Specimens of *S. arcturi* from the North Atlantic formed a sister relationship with specimens identified as '*S. hoylei*' from Hawaii (identified herein as *S.* aff. *hoylei*), and both populations formed distinct BINs. Because they can be readily distinguished using COI, and 16S rRNA shows some distinction (there is a single character in 16S rRNA that can be used to distinguish them), and because of their geographic isolation, they appear to represent separate species. Young and Vecchione (2016b) also noted that *Stigmatoteuthis* specimens from Hawaii had the same distinctive ejaculatory apparatus as *S. arcturi* from the Atlantic. This suggests that they are closely related and similar morphologically, and an integrative taxonomic review will be needed to distinguish these species.

At present, the *Stigmatoteuthis* species from New Zealand waters has been identified as *S.* cf. *hoylei*, and this species formed a BIN distinct from the other known

Stigmatoteuthis species (Fig. 16). The type locality for *S. hoylei* is near the Andaman Islands in the Indian Ocean, which is further north and west then New Zealand, but closer than Hawaii. It is not clear that what occurs in New Zealand waters truly represents *S. hoylei*; when possible, the morphology of mature male specimens should be compared for further insight.

Ecological studies

Species-level identification in the Histioteuthidae is important because of the role they play in marine food webs. Reference sequences are imperative for accurate species-level identifications in dietary studies that rely on sequences extracted from prey items. In a recent study, Waap et al. (2017) found histioteuthids to be one of the most important prey species found in the stomach contents of the Bulwer's petrel (*Bulweria bulwerii* [Jardine & Selby, 1828]) from the Northeast Atlantic Ocean, off Portugal. Specimens were identified using a combination of 16S rRNA sequences and morphology. They tentatively identified specimens as '*Hi.*' *reversa*, *S.* cf. *hoylei*, and three additional *Histioteuthis* operational taxonomic units. Through comparison with 16S rRNA sequences from the present study, a slight revision of these identifications is suggested, as follows: *C. reversa*, *S. arcturi*, *Ha.* aff. *meleagroteuthis* (NWAO), *N. corona*, and an additional taxon that is distinct from all the 16S rRNA sequences included in the present study. It is possible that this additional taxon belongs to the same species as the single unidentified histioteuthid species from Elliger, Lebaric, and Gilly (unpublished) (GU220786 and GU220787), but because only COI sequences are available for the latter, no direct comparison is possible.

Similarly, Alonso et al. (2014) analysed the stomach contents of Cory's shearwater (*Calonectris diomedea* [Scopoli, 1769]) in the Eastern North Atlantic Ocean, from off the coast of northwest Africa. Their '*Histioteuthis* sp.', identified using 16S rRNA, appears to represent *N. corona*. It should be noted (as the authors called their sequencing technique 'DNA barcoding'), that the 'DNA barcode' for animals is a term referring specifically to the 648 bp region from the 5′ end of COI (Hebert et al., 2003). Some studies use 16S rRNA for stomach content analysis, which is indeed useful because it amplifies more readily (and for some taxa more sequences are available on public databases), but for clarity this technique (and analysis of any gene regions other than COI) should not be termed 'barcoding'. Furthermore, 16S rRNA sequences were much more conserved and did not show the same level of species differentiation that was found with COI. In this family, COI appears to provide a higher resolution. This

analysis has included both genes, which will hopefully assist in the identification of histioteuthids in future ecological studies.

Conclusion:

This study, representing the largest phylogenetic analysis of the Histioteuthidae to date, reveals that this family appears to be far more speciose than previously believed. Until recently, this family was believed to contain about 19 species (Young & Vecchione, 2013a), but the present results suggest that there are at least 25–29 histioteuthid species. The morphological species groups identified by Voss et al. (1998) are supported in the present study and have been elevated to genera. Existing genus names have been resurrected where appropriate, and two additional genera have been established. Herein, 25 genetically distinct taxa have been found, including nine that likely represent previously unrecognised species, and 17 known 'species', eight of which now appear to comprise species complexes that will require an integrative taxonomic approach to resolve. A DNA barcode reference library (dataset DS-HISTIO) has been created on BOLD for comparative use in future studies. In addition, 16S rRNA sequences, which are often used in dietary studies, have also been sequenced and accessioned online for reference and use by other authors (Table 6). Results from this study have been used to compare histioteuthid identifications from several recent trophic studies, and have permitted lower-level identifications than were previously possible. Due to lower observed variability in 16S rRNA, COI is likely sufficient in combination with morphology for future species-level integrative taxonomy.

Chapter 5: Cephalopod biodiversity of the Kermadec Islands: implications for conservation

Abstract:

In order to establish the Kermadec–Rangitāhua Ocean Sanctuary, which will protect a large, unique, near-pristine northeastern section of New Zealand's marine environment, an improved understanding of the marine biodiversity of this area is required. The cephalopod biodiversity of the Kermadecs was previously directly assessed only once (over a century ago), although two updated species checklists (based on uncritical literature reviews) were published in the interim. A recent biodiversity survey of the Kermadec Islands collected over 150 cephalopod specimens, providing the first opportunity to assess locally occurring taxa using integrative taxonomy. Specimens were examined, morphologically identified, and DNA barcoded. DNA sequences were analysed using the Barcode Index Number (BIN) system in the Barcode of Life Data System (BOLD). The present results nearly double the previously known cephalopod biodiversity of the Kermadecs, adding 28 to the previously reported 42 species (bringing the total to 70); three cephalopod orders are also reported from this area for the first time. The BIN analysis highlighted several taxa that are badly in need of revision, including some monotypic genera that now appear to contain multiple species, and at least five species that appear to be new to science. The Kermadec region also hosts 34 cephalopod species that are not known to occur elsewhere in New Zealand waters. The results of this study strongly support the establishment of the Kermadec–Rangitāhua Ocean Sanctuary, which would offer protection to the pelagic, deep-sea taxa reported herein.

Introduction:

The Kermadec Islands region, located north-north-east of New Zealand (~26–34.5°S; ~177°E–174°W), represent a nearly pristine environment. The area is geologically diverse, containing (among other habitats) a chain of seamounts, and the second-deepest ocean trench in the world (Ministry for the Environment, 2016). Very little is known about the Kermadecs' biodiversity; only 5% of the Kermadec region is estimated to have been explored (Golder, 2016). This region is currently protected by the Kermadec Islands Marine Reserve, which was established in 1990 and extends 12 nautical miles around each island (including the smaller Macauley Island and outlying L'Havre and L'Esperance Rocks) (Ministry for the Environment, 2016). In this existing

7500 km^2 reserve, prohibited activities include fishing, mining, marine discharges and ballast exchange by ships and yachts, and laying of submarine cables (Ministry for the Environment, 2016). However, in 2015, the Kermadec–Rangitāhua Ocean Sanctuary was proposed, which would extend far beyond the existing reserve and cover an area from 12 to 200 nautical miles around each island. The sanctuary will increase the protection for this unique environment to a total area of 620,000 km^2 (more than 80 times larger) although at a lower level than within the reserve (which would remain unchanged) (Ministry for the Environment, 2016). Fishing and mining would be prohibited in the sanctuary, but other activities prohibited within the reserve would be permissible, subject to regulation (Ministry for the Environment, 2016). The total protected area, covering 15% of New Zealand's ocean environment, would be one of the world's largest protected areas (Ministry for the Environment, 2016). In order to make a scientific plan for the proposed sanctuary, scientists have recommended increased baseline sampling and collection efforts to better understand the biodiversity of this area (Golder, 2016).

Most of our current knowledge of the cephalopod biodiversity in the Kermadecs comes from two studies (Berry 1914, 1916) conducted over a century ago, in addition to several general invertebrate surveys conducted by divers on SCUBA, which added to our knowledge of the shallow-water octopod fauna (Reid and Wilson, 2015). While a critical review of all recent cephalopod specimens from the Kermadecs in collections is still needed (Bolstad, 2016), two recent checklists of Kermadec cephalopods have been published (Duffy & Ahyong, 2015; Reid & Wilson, 2015). These checklists, which were commendable undertakings representative of considerable effort, were (of necessity) uncritical summaries of records from other papers published in the interim, many of which inferred species' presence in the region, rather than directly observing material collected there. As a result, some dubious previously published records were maintained on the checklists; for example, both maintained the inclusion of two species of *Nautilus* Linneaus, 1758, which have been reported solely from shell fragments. Intact animals (i.e., with soft parts) have never been reported from the Kermadecs, so there is no evidence that live *Nautilus* occur there. As with other cephalopods that use shells for buoyancy, these are likely remains of shells that have drifted from elsewhere (see Reid, 2016). In addition, both checklists also use Imber (1978) as the basis for cranchiid records, when this study has been highly criticised (Voss, 1980). Bolstad (2016) suggested that a genetic analysis of the cephalopods in the Kermadecs would be particularly useful, because integrative taxonomic studies often reveal that

‘cosmopolitan’ cephalopod species (some of which have been reported from the Kermadecs) actually represent several geographically restricted species of similar morphology (e.g., Braid & Bolstad, 2015; Chapter 3).

The threat status of most cephalopod species in New Zealand waters remains unknown because of insufficient data. However, a recent review of the conservation status of marine invertebrates in New Zealand waters has expressed concern for five cephalopods (Freeman et al., 2014). Two octopus species—*Octopus kaharoa* O’Shea, 1999, and *Opisthoteuthis mero* O’Shea, 1999— are currently listed as ‘at risk’ due to predicted population declines, although their populations occupy a large area (Freeman et al., 2014). *Cirroctopus hochbergi* O’Shea, 1999, and *Opisthoteuthis chathamensis* O’Shea, 1999, are listed as ‘naturally uncommon’ and occur in small, widely scattered populations (Freeman et al., 2014); although, the listings for these two species are uncertain due to deficient data (Freeman et al., 2014). The giant mastigoteuthid squid, *Idioteuthis cordiformis* (Chun, 1908), is listed as both ‘at risk’ and ‘naturally uncommon’, with widely spaced, sparse populations (Freeman et al., 2014). Although this species is noted to be secure elsewhere, *Idioteuthis cordiformis* now appears to represent a species complex and suggested that the population found in New Zealand waters may prove to represent a distinct endemic species (Chapter 3). While *Nautilus* is listed in CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), there is no evidence that it actually occurs in the Kermadecs, with only shell fragments for evidence, which cannot be used to confirm the presence of this species (House 2010).

Systematics and accurate species identification are fundamental for conservation efforts, because both a lack of recognition of distinct taxa and over-splitting can have important consequences for the future of the species involved (e.g., May, 1990; Zachos, 2013). Integrative taxonomy, which uses a combination of genetics and morphology, has repeatedly proven helpful in species identification and classification, particularly in cephalopods (e.g., Allcock et al., 2011; Braid & Bolstad, 2015; Chapter 2). Among other loci, cytochrome *c* oxidase subunit 1 (COI) has recently been used successfully for resolving some systematic problems in deep-sea squids (*e.g*., Braid et al., 2014; Braid & Bolstad, 2015; Chapters 2, 3, and 4). An integrative taxonomic review of the Histioteuthidae Verrill, 1881, has revealed up to nine previously unrecognised species (Chapter 4). In contrast, a review of the genus *Asperoteuthis* Nesis, 1980, found that two previously named species were actually synonymous and, rather than being endemic, *A. lui* Salcedo-Vargas, 1999, now appears to occur circumglobally in the

Southern Hemisphere (Chapter 2). Therefore, cephalopod biodiversity studies should utilise integrative taxonomy whenever possible.

The aim of the present study is to improve our understanding of the cephalopod biodiversity of the Kermadec region, establishing a solid baseline that can be compared with historical data and with future surveys. These data will help support the information requirements of the proposed Kermadec–Rangitāhua Ocean Sanctuary. A recent costal, marine mammal, and deep-sea biodiversity survey of this region provided fresh material for examination and genetic analysis, storing the samples frozen on board until they could be examined by taxonomists (Clark et al., 2017). To maximise the rate and accuracy of specimen identification, cephalopod specimens were identified using both morphology and DNA barcoding. This study represents the first genetic analysis of the cephalopod biodiversity of the Kermadec Islands region.

Methods:

Specimens

A biodiversity study of the Kermadec Islands was undertaken on the National Institute of Water & Atmospheric Research (NIWA) Research Vessel *Tangaroa* in October and November 2016, on the 'Biodiversity of the Kermadec Islands and offshore waters of the Kermadec Ridge—a coastal, marine mammal and deep-sea survey (TAN1612)' expedition. Specimens were collected from waters surrounding Raoul Island, L'Esperance Rock, and Macauley Island using a variety of collection methods (Fig. 18, Table 11). Station locations were plotted on a map of the Kermadec Islands using layers (NZ Coastlines and Islands Polygons [Topo 1:50k]) from Land Information New Zealand (https://data.linz.govt.nz) and New Zealand's Exclusive Economic Zone (NZ Fisheries General Statistical Areas) from the Ministry of Primary Industries (https://www.mpi.govt.nz/) using ArcGIS 10.2 (Environmental Systems Research Institute [ESRI], Redlands, CA). Specimens were frozen on board and maintained at -20˚C until examination (Fig. 19). A total of 160 cephalopod specimens were collected in 69 lots. Specimens were identified morphologically by the systematists from the AUT Lab for Cephalopod Ecology & Systematics (ALCES). Where more than five individuals within a given lot appeared to represent the same taxon, a subset of five individuals were sampled for genetic analysis (lots NIWA 118605, NIWA 118136, and 119225 [unsequenced specimens now NIWA 121876]). Therefore, tissue was collected from a total of 131 specimens (Table 12).

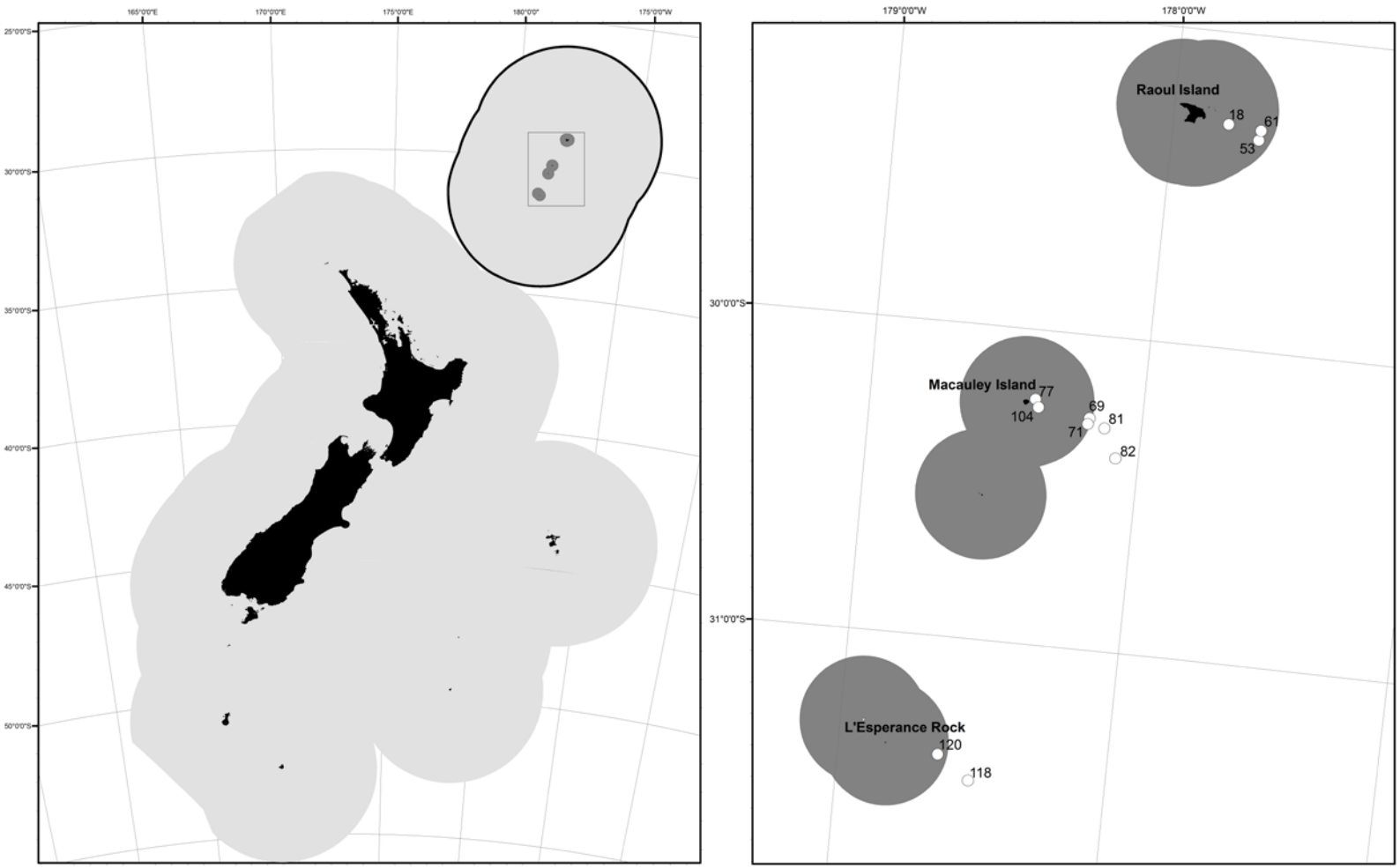

Fig. 18—Collection localities: A) New Zealand, with Exclusive Economic Zone (EEZ) in light grey, the existing Kermadec Islands Marine Reserve in dark grey, proposed Kermadec–Rangitāhua Ocean Sanctuary outlined in black, and a square showing sampling area; B) enlargement of sampling area, with station numbers where cephalopods were captured during the voyage TAN1612.

DNA barcoding

Tissue samples were fixed in 100% EtOH and maintained at -20˚C until analysis. Samples were extracted using EconoSpin (Epoch Life Science) spin columns with QIAGEN reagents following the protocols for the DNeasy Blood & Tissue Kit (QIAGEN). The DNA barcode region was amplified using Folmer, Black, Hoeh. Lutz, and Vrijenhoek (1994) primers LCO1490/HCO2198 following protocols in Braid et al. (2014). The sequencing reaction was performed by Macrogen (Korea) using the same primers used for PCR. Bidirectional sequences were assembled into contigs and edited in Sequencher v.4.9 (Gene Codes). Sequences were uploaded to the Barcode of Life Data System (BOLD; Ratnasingham & Hebert, 2007) in a public project titled 'DNA Barcoding Cephalopods of the Kermadecs' (project code: KERCE) (Table 12).

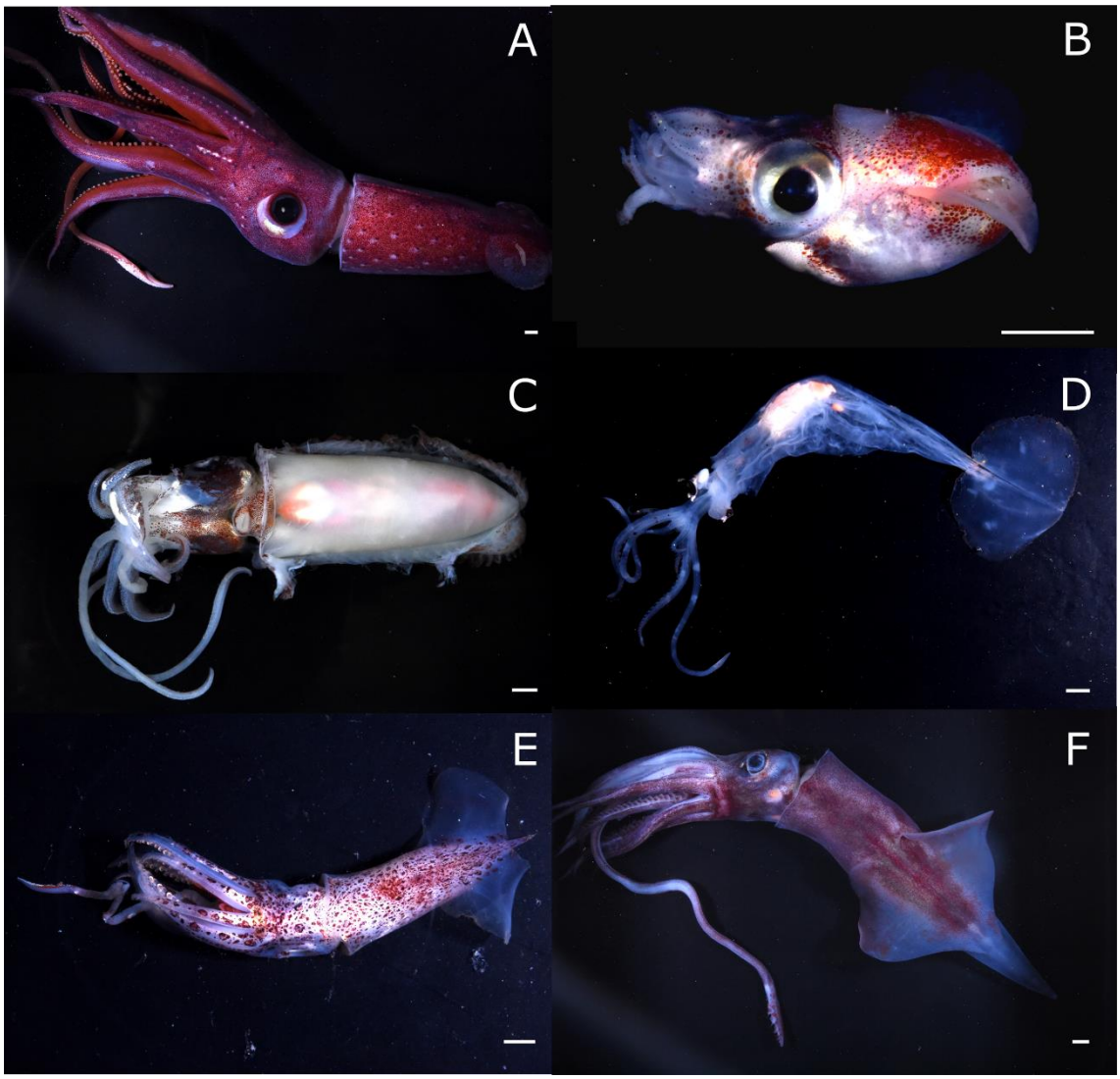

Fig. 19—Examples of the specimens examined in Chapter 5 captured during the recent expedition Biodiversity of the Kermadec Islands and offshore waters of the Kermadec Ridge—a coastal, marine mammal and deep-sea survey (TAN1612). A) *Histioteuthis miranda*, NIWA 121878; B) *Heteroteuthis* sp. KER2, NIWA 118128; C) *Chtenopteryx* sp. KER1, NIWA 118600; D) *Leachia separata*, NIWA 118129; E) *Pyroteuthis serrata*, NIWA 119224; F) *Enoploteuthis semilineata*, NIWA 119221. Scale bars = 5mm. A, E, F, photographs by Rob Stewart; B, C, D, photographs by Keren Spong.

Table 11—Collection data for specimens analysed in Chapter 5. ‘Island’ = closest island to the collection site; ‘depth’ = maximum depth (meters) recorded during collection (no closing nets were used, so specimens could have been captured at any depth between the maximum depth and the surface).

Station ID	Date	Latitude	Longitude	Depth (m)	Island	Gear
18	24/10/2016	-29.29	-177.80	1000	Raoul Island	Fine-mesh midwater trawl
53	26/10/2016	-29.33	-177.69	2000	Raoul Island	Fisheries midwater trawl
61	27/10/2016	-29.30	-177.68	0	Raoul Island	Hand line
69	28/10/2016	-30.26	-178.19	1000	Macauley Island	Fine-mesh midwater trawl
71	29/10/2016	-30.28	-178.20	1431	Macauley Island	Beam trawl
77	29/10/2016	-30.22	-178.40	101	Macauley Island	Beam trawl
81	29/10/2016	-30.29	-178.13	1826	Macauley Island	Beam trawl
82	29/10/2016	-30.38	-178.08	1992	Macauley Island	Fisheries midwater trawl
104	31/10/2016	-30.25	-178.38	135	Macauley Island	Fish bottom trawl
118	02/11/2016	-31.45	-178.51	2006	L'Esperance Rock	Fisheries midwater trawl
120	03/11/2016	-31.38	-178.63	1000	L'Esperance Rock	Fine-mesh midwater trawl

Sequences were screened for contamination using the Basic Local Alignment Search Tool (BLAST) through GenBank. Specimen IDs were primarily confirmed using the Barcode Index Number (BIN) analysis in BOLD, which uses a clustering algorithm to automatically generate operational taxonomic units based on COI, which have a high concordance with species (Ratnasingham & Hebert, 2013). In cases where sequences received unique BINs the sequences were searched through the Full Database and Tree Based Identification method in BOLD and through a BLAST search in GenBank to check their similarity with closely related taxa (in July, 2017). In order to examine relationships, neighbour-joining trees were created with the Tree Based Identification method in BOLD, or by using all available sequences for each group to create a neighbour-joining tree in BOLD. Specimens that could not be identified definitively to any species were given an interim name (i.e., 'sp. KER' when only one species was present in a genus, or numbered as 'sp. KER1' and 'sp. KER2' where multiple unidentified species appear to exist) so that future studies will have a clearer reference point.

Maximum-likelihood phylogenies were generated in MEGA 7.0 (Kumar et al. 2016), using all available specimens on BOLD for four selected groups that are not currently under review by the present authors, and for which the inclusion highlights important areas of future taxonomic priorities. These groups are the Bathyteuthoidea (with *Moroteuthopsis ingens* as the outgroup), the Bolitaeninae Chun, 1911 (with *Amphitretus pelagicus* as the outgroup), the Octopodidae (with *Argonauta nodosa* as the outgroup), and the enoploteuthid families (with *Dosidicus gigas* as the outgroup). Each dataset was run in jModelTest (under Bayesian Information Criterion) to determine appropriate models. For the Bathyteuthoidea and the enoploteuthid families General Time Reversible model (GTR) + G was chosen, for the Octopodidae GTR + I + G was chosen, and for the Bolitaeninae Hasegawa-Kishino-Yano (HKY) model + G was chosen.

Checklist

A checklist of cephalopod species that occur in the Kermadec Islands region was compiled using the results from the present and previous studies (Table 13). Although a recent global catalogue of cephalopods (Jereb & Roper, 2010; Jereb et al., 2014) included species found in the New Zealand region (FAO Area 81), these records were not included in the present checklist because its distributions are largely inferred from current (often patchy) understandings of wider taxon distributions, rather than based on

Table 12—Specimen data for individuals analysed in Chapter 5. Under 'Catalogue Number', NIWA = National Institute of Water and Atmospheric Research, Ltd, in Wellington. Individuals sequenced from multi-specimen lots are identified with unique letters (in parentheses). BIN is the Barcode Index Number assigned by the Barcode of Life Data Systems.

Taxon	BIN	Catalogue Number	BOLD Process ID	Station ID
Bathyteuthoidea				
Bathyteuthidae				
Bathyteuthis sp. KER	BOLD:ADB2642	NIWA 119196 (A)	KERCE095-17	118
Chtenopterygidae				
Chtenopteryx sp. KER1	BOLD:ADH6886	NIWA 118600	KERCE037-17	69
Chtenopteryx sp. KER2	BOLD:ADH6887	NIWA 118839	KERCE074-17	82
Chtenopteryx sp. KER2	BOLD:ADH6887	NIWA 119229	KERCE133-17	120
Chtenopteryx sp. KER2	BOLD:ADH6887	NIWA 121875	KERCE123-17	120
Myopsida				
Loliginidae				
Sepioteuthis australis	BOLD:AAF0818	NIWA 119062 (A)	KERCE089-17	104
Sepioteuthis australis	BOLD:AAF0818	NIWA 119062 (B)	KERCE090-17	104
Octopoda				
Amphitretidae				
Amphitretus pelagicus	BOLD:AAR3840	NIWA 118487 (A)	KERCE030-17	53
Bolitaena sp. KER1	BOLD:ABA4172	NIWA 118130	KERCE008-17	18
Bolitaena sp. KER1	BOLD:ABA4172	NIWA 118489	KERCE033-17	53
Bolitaena sp. KER1	BOLD:ABA4172	NIWA 118642	KERCE069-17	71
Bolitaena sp. KER1	BOLD:ABA4172	NIWA 119200 (B)	KERCE102-17	118
Bolitaena sp. KER2	BOLD:ADH3686	NIWA 118490	KERCE034-17	53

Table 12—Continued.

Taxon	BIN	Catalogue Number	BOLD Process ID	Station ID
Octopodidae				
Amphioctopus kagoshimensis	BOLD:ABA8783	NIWA 118711	KERCE070-17	77
Pinnoctopus sp. KER	BOLD:ADH6174	NIWA 119060 (A)	KERCE086-17	104
Pinnoctopus sp. KER	BOLD:ADH6174	NIWA 119060 (B)	KERCE087-17	104
Pinnoctopus sp. KER	BOLD:ADH6174	NIWA 119060 (C)	KERCE088-17	104
Tremoctopodidae				
Tremoctopus robsoni	BOLD:ADH3946	NIWA 118842	KERCE079-17	82
Oegopsida				
Brachioteuthidae				
Brachioteuthis sp. KER1	BOLD:ADH5612	NIWA 121863 (C)	KERCE054-17	69
Brachioteuthis sp. KER1	BOLD:ADH5612	NIWA 121863 (B)	KERCE055-17	69
Brachioteuthis sp. KER2	BOLD:ABU6933	NIWA 121863 (A)	KERCE056-17	69
Chiroteuthidae				
Chiroteuthis mega	BOLD:AAW3951	NIWA 118623 (A)	KERCE067-17	69
Cranchiidae				
Cranchia scabra	BOLD:AAJ6514	NIWA 118124	KERCE002-17	18
Galiteuthis sp. KER	BOLD:ADH4035	NIWA 118845 (A)	KERCE082-17	82
Helicocranchia sp. KER	BOLD:ADH6254	NIWA 118847	KERCE084-17	82
Helicocranchia sp. KER	BOLD:ADH6254	NIWA 118486 (C)	KERCE028-17	53
Leachia separata	BOLD:ADH5276	NIWA 118129	KERCE007-17	18
Leachia separata	BOLD:ADH5276	NIWA 118134	KERCE014-17	18
Leachia separata	BOLD:ADH5276	NIWA 118606 (A)	KERCE059-17	69

Table 12—Continued.

Taxon	BIN	Catalogue Number	BOLD Process ID	Station ID
Leachia separata	BOLD:ADH5276	NIWA 118606 (B)	KERCE060-17	69
Leachia separata	BOLD:ADH5276	NIWA 118606 (C)	KERCE061-17	69
Leachia separata	BOLD:ADH5276	NIWA 118817	KERCE071-17	81
Leachia separata	BOLD:ADH5276	NIWA 119200 (A)	KERCE101-17	118
Leachia separata	BOLD:ADH5276	NIWA 119228 (A)	KERCE130-17	120
Leachia separata	BOLD:ADH5276	NIWA 119228 (B)	KERCE131-17	120
Leachia separata	BOLD:ADH5276	NIWA 119228 (C)	KERCE132-17	120
Leachia separata	BOLD:ADH5276	NIWA 121871	KERCE083-17	82
Sandalops melancholicus	BOLD:ADH6536	NIWA 118602 (A)	KERCE039-17	69
Sandalops melancholicus	BOLD:ADH6536	NIWA 118602 (B)	KERCE040-17	69
Sandalops melancholicus	BOLD:ADH6536	NIWA 118843	KERCE080-17	82
Sandalops melancholicus	BOLD:ADH6536	NIWA 118848	KERCE085-17	82
Sandalops melancholicus	BOLD:ADH6536	NIWA 119226	KERCE124-17	120
Taonius expolitus	BOLD:ADH3662	NIWA 119227 (A)	KERCE125-17	120
Taonius expolitus	BOLD:ADH3662	NIWA 119227 (B)	KERCE126-17	120
Taonius expolitus	BOLD:ADH3662	NIWA 119227 (C)	KERCE127-17	120
Taonius expolitus	BOLD:ADH3662	NIWA 119227 (D)	KERCE128-17	120
Taonius expolitus	BOLD:ADH3662	NIWA 119227 (E)	KERCE129-17	120
Taonius tanuki	BOLD:ADH3663	NIWA 121868	KERCE062-17	69
Teuthowenia aff. *pellucida*	BOLD:ADH5304	NIWA 118601	KERCE038-17	69
Teuthowenia aff. *pellucida*		NIWA 118844	KERCE081-17	82

Table 12—Continued.

Taxon	BIN	Catalogue Number	BOLD Process ID	Station ID
Enoploteuthidae				
Abraliopsis tui	BOLD:ADH6894	NIWA 118125	KERCE003-17	18
Abraliopsis tui	BOLD:ADH6894	NIWA 118132	KERCE012-17	18
Abraliopsis tui	BOLD:ADH6894	NIWA 118136 (A)	KERCE017-17	18
Abraliopsis tui	BOLD:ADH6894	NIWA 118136 (B)	KERCE018-17	18
Abraliopsis tui	BOLD:ADH6894	NIWA 118136 (C)	KERCE019-17	18
Abraliopsis tui	BOLD:ADH6894	NIWA 118136 (D)	KERCE020-17	18
Abraliopsis tui	BOLD:ADH6894	NIWA 118136 (E)	KERCE021-17	18
Abraliopsis tui	BOLD:ADH6894	NIWA 118488	KERCE032-17	53
Abraliopsis tui	BOLD:ADH6894	NIWA 119201 (A)	KERCE103-17	118
Abraliopsis tui	BOLD:ADH6894	NIWA 119201 (B)	KERCE104-17	118
Abraliopsis tui	BOLD:ADH6894	NIWA 119225 (A)	KERCE118-17	120
Abraliopsis tui	BOLD:ADH6894	NIWA 119225 (B)	KERCE119-17	120
Abraliopsis tui	BOLD:ADH6894	NIWA 119225 (C)	KERCE120-17	120
Abraliopsis tui	BOLD:ADH6894	NIWA 119225 (D)	KERCE121-17	120
Abraliopsis tui	BOLD:ADH6894	NIWA 119225 (E)	KERCE122-17	120
Abraliopsis tui	BOLD:ADH6894	NIWA 121867	KERCE077-17	82
Enoploteuthis semilineata	BOLD:ADH5348	NIWA 119221	KERCE114-17	120
Enoploteuthis cf. *reticulata*	BOLD:ADH4238	NIWA 121865	KERCE022-17	18
Histioteuthidae				
Calliteuthis aff. *atlantica*	BOLD:AAX1287	NIWA 118607 (B)	KERCE064-17	69
Calliteuthis aff. *atlantica*	BOLD:AAX1287	NIWA 118838 (B)	KERCE073-17	82

Table 12—Continued.

Taxon	BIN	Catalogue Number	BOLD Process ID	Station ID
Calliteuthis aff. *atlantica*	BOLD:AAX1287	NIWA 121864 (B)	KERCE052-17	69
Calliteuthis aff. *atlantica*	BOLD:AAX1287	NIWA 121864 (A)	KERCE053-17	69
Histioteuthis aff. *bonnellii*	BOLD:ADH3734	NIWA 118607 (A)	KERCE063-17	69
Histioteuthis aff. *bonnellii*	BOLD:ADH3734	NIWA 119197	KERCE097-17	118
Histioteuthis aff. *bonnellii*	BOLD:ADH3734	NIWA 119220 (A)	KERCE112-17	120
Histiothauma miranda	BOLD:AAX1314	NIWA 121877	KERCE065-17	69
Histiothauma miranda	BOLD:AAX1314	NIWA 121878	KERCE111-17	120
Navia corona cerasina	BOLD:ACG8287	NIWA 118838 (A)	KERCE072-17	82
Stigmatoteuthis cf. *hoylei*	BOLD:ADH3733	NIWA 119219 (A)	KERCE110-17	120
Stigmatoteuthis cf. *hoylei*	BOLD:ADH3733	NIWA 119220 (B)	KERCE113-17	120
Mastigoteuthidae				
Mastigoteuthis cf. *dentata*	BOLD:ACO6617	NIWA 118486 (A)	KERCE026-17	53
Mastigoteuthis cf. *dentata*	BOLD:ACO6617	NIWA 118486 (B)	KERCE027-17	53
Mastigoteuthis cf. *dentata*	BOLD:ACO6617	NIWA 118486 (D)	KERCE029-17	53
Mastigoteuthis cf. *dentata*	BOLD:ACO6617	NIWA 118622	KERCE066-17	69
Mastigoteuthis cf. *dentata*	BOLD:ACO6617	NIWA 118840	KERCE075-17	82
Mastigoteuthis cf. *dentata*	BOLD:ACO6617	NIWA 119203 (B)	KERCE107-17	118
Mastigoteuthis cf. *dentata*	BOLD:ACO6617	NIWA 121870	KERCE015-17	18
Mastigoteuthis psychrophila	BOLD:AAD3515	NIWA 119203 (A)	KERCE106-17	118
Magnoteuthis osheai	BOLD:ACA7283	NIWA 118623 (B)	KERCE068-17	69
Magnoteuthis osheai	BOLD:ACA7283	NIWA 121862	KERCE057-17	69
Magnoteuthis osheai	BOLD:ACA7283	NIWA 121879	KERCE098-17	118

Table 12—Continued.

Taxon	BIN	Catalogue Number	BOLD Process ID	Station ID
Ommastrephidae				
Nototodarus gouldi	BOLD:AAI2536	NIWA 119063 (B)	KERCE092-17	104
Nototodarus gouldi	BOLD:AAI2536	NIWA 119063 (A)	KERCE091-17	104
Nototodarus gouldi	BOLD:AAI2536	NIWA 119063 (D)	KERCE094-17	104
Nototodarus gouldi	BOLD:AAI2536	NIWA 119063 (C)	KERCE093-17	104
Ommastrephes brevimanus	BOLD:ACH3929	NIWA 118149	KERCE023-17	18
Ommastrephes brevimanus	BOLD:ACH3929	NIWA 118150	KERCE024-17	18
Ommastrephes brevimanus	BOLD:ACH3929	NIWA 118151	KERCE025-17	18
Ommastrephes brevimanus	BOLD:ACH3929	NIWA 118512	KERCE036-17	61
Onychoteuthidae				
Onychoteuthis aff. *compacta*	BOLD:ADG9816	NIWA 121866	KERCE076-17	82
Onychoteuthis aff. *compacta*	BOLD:ADG9816	NIWA 119202	KERCE105-17	118
Onychoteuthis meridiopacifica	BOLD:ADH5825	NIWA 121869	KERCE109-17	118
Onychoteuthis meridiopacifica	BOLD:ADH5825	NIWA 121861	KERCE058-17	69
Pyroteuthidae				
Pterygioteuthis cf. *gemmata*	BOLD:ADH6415	NIWA 119204	KERCE108-17	118
Pterygioteuthis cf. *gemmata*	BOLD:ADH6415	NIWA 118126	KERCE004-17	18
Pterygioteuthis cf. *gemmata*	BOLD:ADH6415	NIWA 118605 (A)	KERCE047-17	69
Pterygioteuthis cf. *gemmata*	BOLD:ADH6415	NIWA 118605 (B)	KERCE048-17	69
Pterygioteuthis cf. *gemmata*	BOLD:ADH6415	NIWA 118605 (D)	KERCE050-17	69
Pyroteuthis aff. *margaritifera*	BOLD:AAX9745	NIWA 118605 (C)	KERCE049-17	69
Pyroteuthis aff. *margaritifera*	BOLD:AAX9745	NIWA 118605 (E)	KERCE051-17	69

Table 12—Continued.

Taxon	BIN	Catalogue Number	BOLD Process ID	Station ID
Pyroteuthis serrata	BOLD:ADH6416	NIWA 118127	KERCE005-17	18
Pyroteuthis serrata	BOLD:ADH6416	NIWA 118131 (A)	KERCE009-17	18
Pyroteuthis serrata	BOLD:ADH6416	NIWA 118131 (B)	KERCE010-17	18
Pyroteuthis serrata	BOLD:ADH6416	NIWA 118131 (C)	KERCE011-17	18
Pyroteuthis serrata	BOLD:ADH6416	NIWA 118135	KERCE016-17	18
Pyroteuthis serrata	BOLD:ADH6416	NIWA 119224	KERCE117-17	120
Sepiolida				
Sepiolidae				
Heteroteuthis dagamensis	BOLD:AAM7920	NIWA 118123	KERCE001-17	18
Heteroteuthis dagamensis	BOLD:AAM7920	NIWA 118133	KERCE013-17	18
Heteroteuthis dagamensis	BOLD:AAM7920	NIWA 118491	KERCE035-17	53
Heteroteuthis dagamensis	BOLD:AAM7920	NIWA 118604 (A)	KERCE043-17	69
Heteroteuthis dagamensis	BOLD:AAM7920	NIWA 118604 (B)	KERCE044-17	69
Heteroteuthis dagamensis	BOLD:AAM7920	NIWA 118604 (C)	KERCE045-17	69
Heteroteuthis dagamensis	BOLD:AAM7920	NIWA 118604 (D)	KERCE046-17	69
Heteroteuthis dagamensis	BOLD:AAM7920	NIWA 118841	KERCE078-17	82
Heteroteuthis dagamensis	BOLD:AAM7920	NIWA 119222	KERCE115-17	120
Heteroteuthis sp. KER	BOLD:ADH5539	NIWA 118128	KERCE006-17	18
Spirulida				
Spirulidae				
Spirula spirula	BOLD:AAI0193	NIWA 118603 (A)	KERCE041-17	69
Spirula spirula	BOLD:AAI0193	NIWA 118603 (B)	KERCE042-17	69

Table 12—Continued.

Taxon	BIN	Catalogue Number	BOLD Process ID	Station ID
Spirula spirula	BOLD:AAI0193	NIWA 119223	KERCE116-17	120
Vampyromorpha				
Vampyroteuthidae				
Vampyroteuthis infernalis	BOLD:ACQ2157	NIWA 119198 (A)	KERCE099-17	118
Vampyroteuthis infernalis	BOLD:ACQ2157	NIWA 119198 (B)	KERCE100-17	118

specific records of individuals collected and identified from a given region, and because presence in Area 81 does not necessarily correspond to presence in the Kermadec region. Records from Imber (1978) have been included with caution (see the Discussion and Voss, 1980), with independent verification sought when this publication was sole record for a given species. The current checklist does not include *Nautilus* because of the reasons outlined in the Introduction.

Results:

DNA barcodes were recovered from 130 out of the 131 sampled specimens (Table 12). A single specimen (NIWA 118844, morphologically identified as *Teuthowenia* aff. *pellucida*) showed low amplification, and failed to sequence. The BIN analysis revealed 43 separate BINs (Table 12), with the DNA barcodes of 28 species added to BOLD for the first time. All BIN details (e.g., inter- and intraspecific divergences) are available on BOLD (http://www.boldsystems.org/) in the BIN database. A total of 70 cephalopod species are now reported from the Kermadec region, with 28 species (and eight families) reported herein for the first time. Of these species, 13 were not previously reported from the New Zealand Exclusive Economic Zone (EEZ), and the 15 additional species had been previously found in New Zealand waters, but are reported for the first time from the Kermadec region (Fig. 20). The presence of 15 previously reported species was confirmed in the present study (Fig. 20, Table 13). Deep-sea oegopsid squids comprise the vast majority of the new records (18 species across seven families), but three orders not previously reported from the region were also encountered: Bathyteuthoidea Vecchione, Young, and Sweeney, 2004, Myopsida Naef, 1916, and Sepiolida Keferstein, 1866.

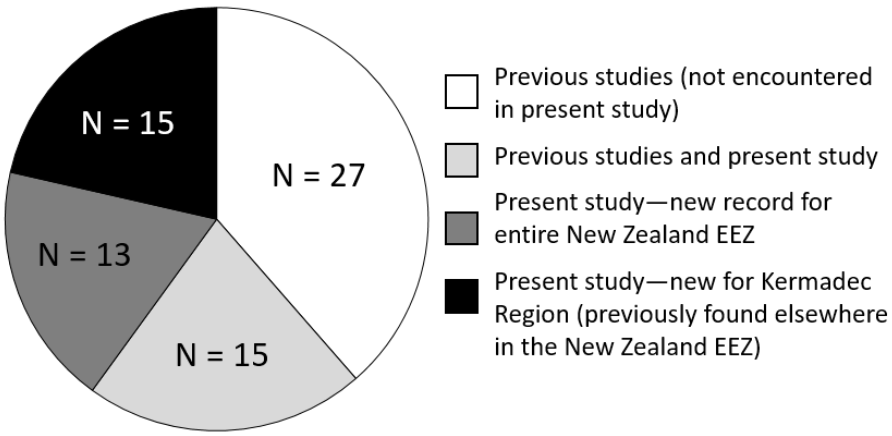

Fig. 20—Information sources for the 70 cephalopod species currently recorded from the Kermadec region in the New Zealand Exclusive Economic Zone (EEZ)—see Table 13.

Table 13—Checklist of the cephalopod fauna of the Kermadec Islands region. NRKR = new record for the Kermadec Islands region, where species have been previously reported from other areas in the New Zealand Exclusive Economic Zone (EEZ); NRNZ = new record for the Kermadec Islands region that have not been previously reported from the New Zealand EEZ; FBS = first Barcode of Life Data Systems (BOLD) sequence (present study provides the first sequence for this species on BOLD); FNZS = first NZ sequence (species previously sequenced from other localities [and available on BOLD], but present study provides the first sequences from New Zealand).

Taxon	NRKR	NRNZ	FBS	FNZS	TAN1612 Stn	Reference
Bathyteuthoidea						
Bathyteuthidae						
Bathyteuthis sp. KER		x	x		118	Present study
Chtenopterygidae						
Chtenopteryx sp. KER1		x	x		69	Present study
Chtenopteryx sp. KER2		x	x		82, 120	Present study
Myopsida						
Loliginidae						
Sepioteuthis australis	x			x	104	Present study
Octopoda						
Amphitretidae						
Amphitretus pelagicus				x	53	Present study; Murray (1895); Berry (1916); O'Shea (1999); Duffy & Ahyong (2015); Reid & Wilson (2015)
Bolitaena sp. KER1	x			x	18, 53, 71, 118	Present study
Bolitaena sp. KER2	x			x	53	Present study
Bolitaena pygmaea						O'Shea (1999) as *Eledonella pygmaea*
Japetella diaphana						O'Shea (1999); Duffy & Ahyong (2015)
Vitreledonella richardi						O'Shea (1999)
Argonautidae						
Argonauta argo						Berry (1916); O'Shea (1999); Duffy & Ahyong (2015); Reid & Wilson (2015)
Argonauta nodosa						Berry (1916); O'Shea (1999); Duffy & Ahyong (2015); Reid & Wilson (2015)

Table 13—Continued.

Taxon	NRKR	NRNZ	FBS	FNZS	TAN1612 Stn	Reference
Cirroteuthidae						
Cirroteuthis muelleri						Reid & Wilson (2015)
Megaleledonidae						
Graneledone challengeri						Murray (1895) as *Eledone verrucosa*; Berry (1916) as *Moschites challenger*; Voss (1976); O'Shea (1999); Duffy & Ahyong (2015); Reid & Wilson (2015)
Opisthoteuthidae						
Grimpoteuthis meangensis						Berry (1916); Sweeney & Roper (1998); O'Shea (1999); Collins & Villanueva (2006); Duffy & Ahyong (2015); Reid & Wilson (2015)—all same species under various genus names
Octopodidae						
Amphioctopus kagoshimensis		x		x	77	Present study
Callistoctopus kermadecensis					104	Berry (1914, 1916); O'Shea 1999; Duffy & Ahyong (2015); Reid & Wilson (2015)—all same species under various genus names (see Discussion)
Pinnoctopus sp. KER		x	x			Present study
Octopus huttoni						O'Shea (1999)
Octopus oliveri						Berry (1914) as *Polypus oliveri*; Berry (1916); O'Shea (1999); Duffy & Ahyong (2015); Reid & Wilson (2015)
Octopus sinensis						Reid & Wilson (2015) as *Octopus jollyorum* (Reid pers. comm.; see also Gleadall (2016)
Octopus 'sp. A'						Reid & Wilson (2015)
Octopus 'sp. 2' (NMNZ M.90311)						O'Shea (1999); Duffy & Ahyong (2015)
Ocythoidae						
Ocythoe tuberculata						O'Shea (1999); Duffy & Ahyong (2015); Reid & Wilson (2015)
Tremoctopodidae						
Tremoctopus robsoni			x		82	Present study; O'Shea 1999; Duffy & Ahyong (2015) as *Tremoctopus robsonianus*; Reid & Wilson (2015)

Table 13—Continued.

Taxon	NRKR	NRNZ	FBS	FNZS	TAN1612 Stn	Reference
Oegopsida						
Brachioteuthidae						
Brachioteuthis sp. KER1		x	x		69	Present study
Brachioteuthis sp. KER2		x	x		69	Present study
Chiroteuthidae						
Chiroteuthis mega	x				69	Present study
Cranchiidae						
Bathothauma cf. *lyromma*						Evans (pers. comm.); Imber (1978); Duffy & Ahyong (2015); Reid & Wilson (2015)
Cranchia scabra				x	18	Present study; Imber (1978); Duffy & Ahyong (2015); Reid & Wilson (2015)
Galiteuthis sp. KER			x		82	Present study; as *Galiteuthis armata* in Imber (1978), Duffy & Ahyong (2015), Reid & Wilson (2015)
Helicocranchia sp. KER	x		x		53, 82	Present study
Leachia separata			x		18, 69, 81, 82 118, 120	Present study; Imber (1978) as *Leachia eschscholtzi*; Duffy & Ahyong (2015) as *Leachia dislocata*
Liguriella pardus						Berry (1916) as *Megalocranchia pardus*; Reid & Wilson (2015)
Megalocranchia sp. indet.						Evans (pers. comm.); as *Megalocranchia maxima* in Imber (1978), Duffy & Ahyong (2015), and Reid & Wilson (2015)
Sandalops melancholicus			x		69, 82, 120	Present study; Imber (1978); Duffy & Ahyong (2015); Reid & Wilson (2015)
Taonius expolitus		x	x		120	Present study
Taonius tanuki			x		69	Present study; as *Taonius belone* in Imber (1978), Duffy & Ahyong (2015), and Reid & Wilson (2015)
Teuthowenia aff. *pellucida*		x	x		69, 82	Present study; Imber (1978) as *Fusocranchia pellucida* and *Teuthowenia megalops impennis*; Powell (1979) as *Megalocranchia pardus*; as *Teuthowenia pellucida* in Duffy & Ahyong (2015) and Reid & Wilson (2015)

Table 13—Continued.

Taxon	NRKR	NRNZ	FBS	FNZS	TAN1612 Stn	Reference
Enoploteuthidae						
Abralia astrolineata						Berry (1914, 1916); Duffy & Ahyong (2015); Reid & Wilson (2015)
Abralia 'sp. B'						Riddell (1985)
Abraliopsis tui			x		18, 53, 82, 118, 120	Present study; Riddell (1985)*; Reid & Wilson (2015); probably as *A. hoylei* in Berry (1914, 1916), Duffy & Ahyong (2015), and Reid & Wilson (2015)
Enoploteuthis '?*jonesi*'						Riddell (1985)
Enoploteuthis semilineata		x	x		120	Present study
Enoploteuthis cf. *reticulata*			x		18	Present study; Riddell (1985)
Histioteuthidae						
Calliteuthis aff. *atlantica*		x	x		69, 82	Present study
Histioteuthis aff. *bonnellii*	x		x		69, 118, 120	Present study; Horstkotte (2008) as *Histioteuthis bonnellii*
Histiothauma miranda	x		x		69, 120	Present study; Horstkotte (2008)
Navia corona cerasina	x		x		82	Present study; Horstkotte (2008) as *Histioteuthis corona cerasina*
Stigmatoteuthis cf. *hoylei*	x		x		120	Present study
Lycoteuthidae						
Lampadioteuthis megaleia						Berry (1916)*; Duffy & Ahyong (2015); Reid & Wilson (2015)
Nematolampas regalis						Berry (1913*; 1914; 1916); Duffy & Ahyong (2015); Reid & Wilson (2015)
Mastigoteuthidae						
Mastigoteuthis cf. *dentata*	x				18, 53, 69, 82, 118	Present study
Mastigoteuthis psychrophila	x				118	Present study
Magnoteuthis osheai	x				69, 118	Present study

* indicates type description

Table 13—Continued.

Taxon	NRKR	NRNZ	FBS	FNZS	TAN1612 Stn	Reference
Ommastrephidae						
Eucleoteuthis luminosa						Berry (1914) [and as a result probably Reid & Wilson (2015)] as *Sthenoteuthis oualaniensis*; Berry (1916); Duffy & Ahyong (2015)
Nototodarus gouldi	x				104	Present study; widely known to occur in Kermadecs but not previously formally reported
Ommastrephes brevimanus				x	18, 61	Present study; Berry (1914; 1916 as *Sthenoteuthis bartramii*); as *Ommastrephes bartramii* in Duffy & Ahyong (2015) and Reid & Wilson (2015)
Onychoteuthidae						
Onychoteuthis aequimanus						Berry (1914; 1916) as *Onychoteuthis banksii*; Bolstad (2010); Duffy & Ahyong (2015); Reid & Wilson (2015) as *Onychoteuthis banksii*
Onychoteuthis aff. *compacta*	x				82, 118	Present study
Onychoteuthis meridiopacifica		x	x		69, 118	Present study
Pyroteuthidae						
Pterygioteuthis cf. *gemmata*			x			Present study; Riddell (1985)
Pterygioteuthis giardi						Riddell (1985); Reid & Wilson (2015)
Pterygioteuthis microlampas					69	Riddell (1985)
Pyroteuthis aff. *margaritifera*			x		18, 120	Present study; Riddell (1985)
Pyroteuthis serrata			x		18, 69, 118	Present study; Riddell (1985); Reid & Wilson (2015)
Sepiolida						
Sepiolidae						
Heteroteuthis dagamensis		x	x		18, 53, 69, 82, 120	Present study
Heteroteuthis sp. KER		x	x		18	Present study

Table 13—Continued.

Taxon	NRKR	NRNZ	FBS	FNZS	TAN1612 Stn	Reference
Spirulida						
Spirulidae						
Spirula spirula				x	69, 120	Present study; Berry (1916); Powell (1979); Duffy & Ahyong (2015); Reid & Wilson (2015)
Vampyromorpha						
Vampyroteuthidae						
Vampyroteuthis infernalis					118	Present study; Powell (1979); Duffy & Ahyong (2015); Reid & Wilson (2015)

Maximum-likelihood phylogenies were generated for the Bathyteuthoidea (Appendix 2), the Bolitaeninae Chun, 1911 (Appendix 3), the Octopodidae (Appendix 4), and the enoploteuthid families (Appendix 5) based on all available sequences on BOLD.

Seven named species previously sequenced from other locations were sequenced from New Zealand for the first time in this study (*Sepioteuthis australis*, *Amphitretus pelagicus*, *Bolitaena* sp. KER1, *Amphioctopus kagoshimensis*, *Cranchia scabra*, *Ommastrephes brevimanus*, and *Spirula spirula*). A further five taxa previously believed attributable to known species now appear to represent closely related but separate (likely new) species (*Histioteuthis* aff. *atlantica*, *H.* aff. *bonnellii*, *Onychoteuthis* aff. *compacta*, *Pyroteuthis* aff. *margaritifera*, and *Teuthowenia* aff. *pellucida*). The known biodiversity of the cephalopods in the Kermadecs now includes 70 species in 24 families (Table 3).

Discussion:

Many of the 43 cephalopod species identified in the present study from the Kermadec region represent poorly understood taxa, including some likely novel species. Therefore, the conservation status of each of these taxa remains largely unclear; the following discussion highlights potentially new species and taxa that are in need of systematic revision. A conservative management approach for species identified as potentially new would be ideal because they may represent endemic species. Out of the 24 known cephalopod families in the Kermadecs (Table 13), 21 contain deep-sea species (Hoving et al., 2014). Because the current Kermadec Marine Reserve only extends 12 nautical miles around the islands with a maximum depth of ~2250 m (while the maximum depth of the proposed sanctuary is ~10000 m), the majority of the deep-sea environment in the region remains unprotected. The establishment of the Kermadec–Rangitāhua Ocean Sanctuary would provide protection between the islands and across many depth zones and habitats in this geologically diverse area, which is particularly important for deep-sea cephalopods.

One interesting recurring phenomenon is the presence of multiple confamilial (sometimes congeneric) species collected by single trawl events (e.g., amphitretids at station 53; mastigoteuthids and onychoteuthids, station 118; brachioteuthids and histioteuthids, station 69, and the latter also at station 120). This could be a simple result of the broad depth range—greater than 1000m at each of these stations—sampled by non-closing nets. In some regions, congeneric species have been reported to co-occur

geographically, but inhabiting different depth strata (e.g., *Hi. bonnellii* (Férussac, 1834) and *C. reversa* [Verrill, 1880] in the western Mediterranean; Quetglas, de Mesa, Ordines, & Grau, 2010). Another possibility is that closely related species could have asynchronous life cycles, co-occurring but with little competition due to the presence of different age/size cohorts; in several instances where congeners were collected in the same haul, different species were represented by individuals of markedly different size and maturity, such as the specimens of *Histioteuthis* d'Orbigny 1841, taken at Stations 69 and 120 (three species at each station), and the specimens of *Chtenopteryx* Appellöf, 1890, taken at station 120 (two species). Conversely, nearly identically sized individuals of two brachioteuthid and two mastigoteuthid species were also collected within single hauls (stations 69 and 118, respectively). Trawls could also have been conducted in specific areas of interest (*e.g*, near undersea features such as seamounts) conducive to high productivity and diversity compared to surrounding waters, in order to maximise sampling yield.

Groups are treated below in the same order as they appear in the checklist (Table 13) in sections internally organised by order, family group, or family.

Bathyteuthoidea

The Bathyteuthoidea is an order that contains two monogeneric families: Bathyteuthidae Pfeffer, 1900, and Chtenopterygidae Grimpe, 1922 (Young & Vecchione, 2016c). This order is united by the presence of suckers on the buccal membrane, branchial canals in the gills, and the lack of carpal-locking apparatus on the tentacles (Young & Vecchione, 2016c). Strong molecular support has been found for the relationship between these two families (Lindgren, 2010). Taxa in this clade had not been previously reported from the Kermadec region (nor formally from the New Zealand EEZ), while one species of *Bathyteuthis* Hoyle, 1885a, (preliminarily called '*B.* sp. KER') and two species of *Chtenopteryx* are reported herein (Tables 12, 13).

Seven species have been named in the Chtenopterygidae, with type localities for four in the Mediterranean Sea, two in the Atlantic Ocean, and one in the South Pacific (Sweeney & Young, 2009). Only three named species are currently accepted, but the presence of undescribed species in this genus is known (Young & Vecchione, 2010d) and supported by the seven BINs formed from the currently available *Chtenopteryx* sequences on BOLD (Appendix 2). The four specimens examined in the present study represent two of these BINs, which are distinct from all other *Chtenopteryx* sequences on BOLD. Similarly, three out of the four named *Bathyteuthis* species are presently

considered valid (Sweeney & Young, 2003), while the sequences on BOLD form five BINs (Appendix 2). The newest BIN is represented by one specimen from the Kermadec region, together with a single specimen from the Chatham Rise (NIWA 85956) (pers. obs.). This indicates that both families within Bathyteuthoidea are in need of revision, using integrative taxonomy.

Myopsida

The order Myopsida has not been formally reported from the Kermadec region until now. Four specimens identified as *Sepioteuthis* cf. *australis* Quoy & Gaimard, 1832, formed a BIN (BOLD:AAF0818) with other *S. australis* individuals collected from South Australia and New Zealand. The maximum divergence found within this BIN is 1.68% (p-distance). *Sepioteuthis australis* has been previously reported from around New Zealand's North Island as *Sepioteuthis bilineata* Quoy & Gaimard, 1832 (Dell, 1952; Powell, 1979).

Octopoda Leach, 1818

Previously, 14 octopod species have been reported from the Kermadecs (see Table 13). Of the six species encountered in the present study, four represent new records for the region (Table 13).

Four species in the mesopelagic, gelatinous family Amphitretidae Hoyle, 1886, are now known to occur in the Kermadec region (Table 13). In the subfamily Amphitretinae Hoyle, 1886, *Amphitretus pelagicus* Hoyle, 1885c, was found both in the present study, and by previous authors (Murray, 1895; Berry, 1916; O'Shea, 1999). The sequence for the Kermadec specimen was assigned the same BIN as the only other *A. pelagicus* specimen on BOLD (BOLD:AAR3840), collected from Hawaii. In the subfamily Bolitaeninae Chun, 1911, only *Japetella diaphana* Hoyle, 1885c, was previously reported from the Kermadecs, while two species of *Bolitaena* Steenstrup, 1859—*B. pygmaea* (Verrill, 1884) and *B. microtyla* Steenstrup in Hoyle, 1886—are known from west of the Kermadecs region, and east of Cook Strait (O'Shea, 1999). The *Bolitaena* specimens sequenced in the present study formed two separate BINs (BOLD:ABA4172 and BOLD:ADH3686), which were both distinct from the BIN (BOLD:AAW7444) of a specimen attributed to *B. pygmaea* from a previous study (Appendix 3; Carlini & Graves, 1999; unknown collection locality), so at least one additional unnamed taxon appears to exist in this genus. (Another online sequence from a specimen identified as '*B. pygmaea*' [GenBank ID GU145071] from the eastern North

Atlantic, off Liberia, forms a BIN (BOLD:AAW9122) with a specimen identified as *J. diaphana*. The nearest neighbour for this BIN is *J. heathi* (Berry, 1911), suggesting that this sequenced specimen was misidentified as '*B. pygmaea*'. All other known *Bolitaena* sequences do, however, group together and form a sister relationship with the *Japetella* sequences available on BOLD (Appendix 3). It is unclear what species of *Bolitaena* are present in the Kermadec region, and future studies using integrative taxonomy, which includes comparative genetic material from different regions, will be necessary to clarify their identifications.

In the family Octopodidae d'Orbigny, 1840, *Amphioctopus kagoshimensis* (Ortmann, 1888) is reported from New Zealand waters for the first time. The single specimen from the present study was assigned the same BIN (BOLD:ABA8783) as other BOLD sequences identified as *A. kagoshimensis* from China, Australia, and Japan. Three specimens identified as *Pinnoctopus* sp. KER in the present study formed a single, unique BIN (BOLD:ADH6174), for which *Pinnoctopus cordiformis* (Quoy and Gaimard, 1832) was the nearest neighbour (p-distance divergence of 4.01% in BOLD, BIN BOLD:ACH7358; Appendix 3). The validity of *Pinnoctopus* d'Orbigny, 1845 (type species *P. cordiformis*, by original designation) has been debated, with O'Shea (1999) resurrecting it for *P. cordiformis* and *P. kermdecensis* (Berry, 1914), while Norman and Hochberg (2005) subsequently considered *P. cordiformis* a junior synonym of '*Macroctopus maorum*' (Hutton, 1880), and placed *P. kermadecensis* in *Callistoctopus* Taki, 1964 (type species *Callistoctopus arakawai* Taki, 1964, accepted as *Callistoctopus ornatus* (Gould, 1852), by original designation). Although Norman and Hochberg (2005) considered *P. cordiformis* unresolved, O'Shea (1999) designated a neotype for *P. cordiformis*. Therefore, '*M. maorum*' is a junior synonym of *P. cordiformis*, and because *P. cordiformis* is the type species of *Pinnoctopus*, this genus has priority over *Macroctopus* Robson, 1928, and *Callistoctopus*. The close genetic relationship between our *Pinnoctopus* sp. KER specimens and *P. cordiformis* supports their status as congeners. Presently, the currently available sequences for all *Calliteuthis* species form a clade with very low support (Appendix 3). Sequencing additional specimens from other locations around New Zealand may provide further insight into relationships among these taxa. It is clear that the higher taxonomy within the Octopodidae is still badly in need of resolution.

Of the four species described in the genus *Tremoctopus* delle Chiaje, 1830, only one, *T. robsoni* Kirk, 1884, has been reported from New Zealand waters (O'Shea, 1999). A single male specimen identified as *T. robsoni* was sequenced in the present

study, and formed a unique BIN (BOLD:ADH3946), which showed a nearest-neighbour relationship with other *Tremoctopus* sequences (p-distance divergence of 13.48% in BOLD, BIN BOLD:AAJ8602). Comparative sequences are currently available on BOLD for specimens identified as both *T. gracilis* (Souleyet, 1852) (BOLD IDs CEPHW173-11 and CEPHW176-11, from Japan) and *T. violaceus* delle Chiaje, 1830 (GenBank ID AF377978, unknown locality), but the present analysis grouped these three sequences into a single BIN (BOLD:AAJ8602), suggesting that either systematic attention is needed within this group, or that misidentification has occurred. The fourth known species in this genus, *T. gelatus* Thomas, 1977, has not been sequenced, but is easily recognised morphologically by its gelatinous consistency, while other species in this genus are muscular (Mangold, Vecchione, & Young, 2016).

Brachioteuthidae Pfeffer, 1908a

Brachioteuthids are some of the most poorly understood and taxonomically confused oegopsids (Hoving et al., 2014), and they have not previously been formally reported from New Zealand waters (see Methods regarding Jereb & Roper, 2010, and Jereb et al., 2014). Among other problems, most species have been inadequately or incompletely described (often from single immature life stages), many specimens lack characters relied upon for identification (e.g., tentacle clubs), and some species exhibit sexual dimorphism (K. Bolstad, pers. comm.). Currently, two genera and seven species are tentatively accepted in this family (Hoving et al., 2014), but a complete review of the family has never been undertaken and is badly needed.

The Brachioteuthidae sequences on BOLD currently form six BINs, (BOLD:AAE8893, BOLD:AAW7811, BOLD:ABU6933, BOLD:ADH5612, BOLD:AAF9932, BOLD:ADH5813), with four from New Zealand waters (BOLD:ADH5612, BOLD:ADH5813, BOLD:ABU6933, and BOLD:AAW7811; Bolstad et al., in prep.). At least some species appear to be widely distributed; the specimen identified herein as *Brachioteuthis* sp. KER 2 formed a BIN (BOLD:ABU6933) together with paralarval specimens identified as *B. riisei* (Steenstrup, 1882) collected near Morocco and a specimen from the South Atlantic Ocean (NMNZ 39501). This broad-ranging species could truly be *B. riisei* (type locality: North Atlantic), but should not be attributed to this species name until the family's internal taxonomy can be stabilised and the distribution patterns clarified.

The other two Kermadec specimens, representing *B.* KER1, were assigned a unique BIN (BOLD:ADH5612), which did not show a close relationship with any other

brachioteuthid, and instead showed a distant nearest-neighbour relationship with *Notonykia africanae* (p-distance divergence of 13.48% in BOLD, BIN BOLD:AAE2122). However, these specimens have morphological characteristics that are consistent with other brachioteuthids. The New Zealand brachioteuthid fauna (presently under investigation by K. Bolstad; Table 1) now appears to comprise at least four species. A thorough local and global review of this family is desperately needed, and integrative taxonomy will be vital in this endeavour.

Chiroteuthid families

The chiroteuthid families consist of the Chiroteuthidae Gray, 1849, the Mastigoteuthidae Verrill, 1881, the Joubiniteuthidae Naef, 1922, the Promachoteuthidae, the Batoteuthidae Young and Roper, 1968, and the Magnapinnidae Vecchione and Young, 1998 (Young, 1991), none of which has been previously reported from the Kermadec region. Four species in the chiroteuthid families are reported here, all of which were placed in BINs with species already known to occur elsewhere in New Zealand waters (see Chapter 3). The family Chiroteuthidae was represented by a single specimen of *Chiroteuthis mega* (Joubin, 1932), which is rarely encountered in New Zealand waters (only five other specimens are present in local collections [pers. obs.]).

Three mastigoteuthid species in two genera were also found in the present study. Two *Mastigoteuthis* (‘*Mt.*’) Verrill, 1881, species were encountered: *Mt.* cf. *dentata* Hoyle, 1904, and *Mt. psychrophila* Nesis, 1977. The specimens of *Mt.* cf. *dentata* were placed in the same BIN (BOLD:ACO6617) as specimens of this species sequenced in a previous study (Braid & Bolstad, 2015), collected from the Chatham Rise and the east coast of the North Island. The relationship between *Mt. agassizii* Verrill, 1881, and *Mt.* cf. *dentata* remains unclear; specimens of *Mt. dentata* (*sensu stricto*) from the type locality are still required for comparison with integrative taxonomy. The presence of *Mt. psychrophila* was quite unexpected (BIN BOLD:AAD3515), because this species is otherwise known only from sub-Antarctic waters (Braid & Bolstad, 2015; Nesis, 1977). This is by far the most northern distribution record confirmed for this species; it was caught at the same station (118) as *Mt. cf. dentata* and *Magnoteuthis* (‘*Mg.*’) *osheai*, some 12° further north than its previously recognised northern limit in New Zealand waters (based on Braid & Bolstad, 2015). Likewise, the three specimens of *Mg. osheai* encountered represent the northernmost record for this species (BIN BOLD:ACA7283). The distributions of both the known species of *Magnoteuthis* Salcedo-Vargas and

Okutani, 1994, remain incompletely understood; the type locality for *Mg. osheai* is New Zealand (Braid & Bolstad, 2015), while the closely related *Mg. microlucens* (Young et al., 2008) occurs in Hawaiian waters (Young et al., 2008).

Cranchiidae Prosch, 1847

Cranchiids presently appear to be the most diverse cephalopod family represented in the Kermadecs, with 11 species reported, eight of which were found in the present study (Table 13). Most cranchiid records from previous checklists (Duffy & Ahyong, 2015; Reid & Wilson, 2015) were based on a revision by Imber (1978). These records are questionable (and have been strongly criticised by Voss, 1980), being based primarily on paralarvae, juvenile individuals, and beaks from bird stomach contents; furthermore, many valid taxa were incorrectly synonymised and many specimens were misidentified (Voss, 1980). Herein, species records from Imber (1978) have only been included when independent corroboration was available. Two species were reported by Imber but not found in the present study (*Bathothauma lyromma* Chun, 1906, and *Megalocranchia maxima* Pfeffer, 1884), but the presence of these genera (exact species unconfirmed) in the Kermadec region has been confirmed by A. Evans (pers. comm.) as part of an ongoing morphological systematic revision of the Pacific cranchiid fauna. A third previously reported species, *Liguriella pardus* (Berry, 1916) (Berry, 1916, as *Megalocranchia pardus*), was first described from the Kermadecs, but was not encountered in the present study.

Two species in the subfamily Cranchiinae Prosch, 1847, were found in the present study. One, *Cranchia scabra* Leach, 1817, is the sole recognised species in the genus, and appears circumglobally distributed in tropical to temperate waters. The sequence obtained herein formed a single BIN (BOLD:AAJ6514) with the six other *C. scabra* barcodes on BOLD (from the South Atlantic Ocean, Hawaii, and Japan), supporting the cosmopolitan distribution of this taxon. The more frequently encountered cranchiid, *Leachia separata* (Evans, in prep.) (11 collected and sequenced), appeared to represent a single, unique BIN (BOLD:ADH5276), closely related to the two other species of *Leachia* Lesueur, 1821, available on BOLD, which were identified as *L. lemur* (Berry, 1920; BIN BOLD:AAW9980), *L. pacifica* (Issel, 1908; BIN BOLD:ACH8015), and an unidentified *Leachia* species, ‘sp. RJ2009’ (BIN BOLD:ABX8833). Imber (1978) previously reported *L. eschscholtzi* (Rathke, 1833) from the Kermadec region but a recent review of the Pacific cranchiids has shown the

Leachia material collected in this region to represent a new species (*L. separata*), and not *L. eschscholtzii* (Evans in prep.).

In the subfamily Taoniinae Pfeffer, 1912, six species were encountered. *Sandalops melancholicus* Chun, 1906, is presently accepted as the only species in its genus, and has been sequenced herein for the first time (BIN BOLD:ADH6536). A single specimen of *Galiteuthis* Joubin, 1898, was sequenced, which was assigned a unique BIN (BOLD:ADH4035); the other sequences available on BOLD form six additional BINs (BOLD:AAB8549, BOLD:ADH5671, BOLD:ACQ8318, BOLD:ADH4034, BOLD:ABA4569, and BOLD:ACQ6814). Five *Galiteuthis* species are currently accepted (Young & Mangold, 2011a), but one or two additional species are believed to exist based on morphology (Voss et al., 1992), which is supported by the present results. Imber (1978) identified the Kermadec species as *Galiteuthis armata*, but that species is presently believed to be restricted to the Atlantic Ocean (Voss et al., 1992). Therefore, this taxon is presently called '*Galiteuthis* sp. KER', pending the forthcoming Pacific cranchiid revision by A. Evans.

The two specimens of *Helicocranchia* Massy, 1907, found in the present study formed a single, unique BIN (BOLD:ADH6254), which is distinct from the two other BINs formed by available *Helicocranchia* sequences on BOLD (BOLD:ACQ6631 from the Atlantic Ocean, BOLD:AAY2019 from the Pacific Ocean, off Hawaii). *Helicocranchia* had not been previously reported from the Kermadecs, although it was included in the New Zealand waters checklist by Spencer, Willan, Marshall, and Murray (2017), based on Jereb and Roper (2010). Many unnamed species are known to exist in this genus; Voss et al. (1992) hypothesised that the total number was approximately 14. As such, the Kermadec species has been assigned the temporary designation of '*Helicocranchia* sp. KER' until more information on the genus becomes available.

The BIN analysis identified two genetically distinct species among the six specimens of *Taonius* Steenstrup, 1861, sequenced in this study. One species, *Taonius expolitus* (Evans, in prep.), was placed in the same BIN (BOLD:ADH3662) as a specimen from the Chatham Rise, while the single individual representing *Taonius tanuki* received a unique BIN (BOLD:ADH3663). The *Taonius* sequences on BOLD form seven BINs (BOLD:ACD9245, BOLD:AAM9951, BOLD:AAK0251, BOLD:ADH3663, BOLD:ADH3660, BOLD:ADH3661, and BOLD:ADH3662), while only three species are currently recognised (Young & Mangold, 2011b); Voss et al. (1992) reported the existence of at least five species. Imber (1978) reported *Taonius belone* (Chun, 1906) from the Kermadecs, but specimens conforming to *T. belone*

morphology (as it is currently understood) have not been encountered in New Zealand waters to date (A. Evans, pers. comm.).

The single sequenced specimen of *Teuthowenia* Chun, 1910, from the Kermadecs shows a close genetic relationship with *T. pellucida* (Chun, 1910) (p-distance divergence of 2.33%, BIN BOLD:AAW6797), but it was placed into a distinct BIN (BOLD:ADH5304) along with a single specimen from the Great Australian Bight. There are currently 44 *T. pellucida* sequences present on BOLD, which show an intraspecific divergence of 0.31%. Sequences are presently available on BOLD for two out of the three known species in this genus: *T. pellucida* (from New Zealand and the Atlantic Ocean) and *T. megalops* (Prosch, 1847) (North Atlantic). Of the remaining two known species in the genus, sequences are presently available for *T. megalops* (Prosch, 1847) from near the United Kingdom (BIN BOLD:AAW6796), but not *T. maculata* (Leach, 1817), which is presently only known from the eastern tropical Atlantic Ocean (Voss *et al*. 1992). This information, combined with the low intraspecific diversity seen in *T. pellucida*, suggests that *T.* aff. *pellucida* likely represents a new, previously unrecognised species.

Enoploteuthid families

The 'enoploteuthid families' clade contains the Ancistrocheiridae Pfeffer, 1912, the Enoploteuthidae Pfeffer, 1900, the Lycoteuthidae Pfeffer, 1908b, and the Pyroteuthidae Pfeffer, 1912, which are united morphologically by the presence of numerous photophores and eight (or remnants of eight) buccal supports (Young & Vecchione, 2015b). Species in two of the enoploteuthid families were found in the present study. Three species in the Enoploteuthidae were encountered: single individuals of *Enoploteuthis* cf. *reticulata* Rancurel, 1970, and *E. semilineata* Alexeyev, 1994, and 16 specimens attributed to *Abraliopsis tui* Riddell, 1985. Three species in the Pyroteuthidae were also encountered: *Pterygioteuthis* (*Pt.*) cf. *gemmata*, *Pyroteuthis* (*Py.*) aff. *margaritifera*, and *Py. serrata* Riddell, 1985. Two species of Lycoteuthidae were previously reported from the Kermadecs, but were not encountered in the present study (Table 13).

Enoploteuthis d'Orbigny [in Rüppell], 1844, is known to occur in New Zealand waters (*E. galaxias* Berry, 1918, *E.*?*jonesi* Burgess, 1982, and *E. reticulata*; Riddell, 1985), but has not previously been reported from the Kermadecs. The specimen herein identified as *Enoploteuthis* cf. *reticulata* was given a unique BIN (BOLD:ADH4238), distinct from the BOLD sequence identified as *E. reticulata* from the northwest Pacific

Ocean by Carlini and Graves (1999) (GenBank ID AF000039; BIN BOLD:AAX6906). It is possible that *E. 'reticulata*' represents a species complex, or that the previously sequenced specimen was misidentified, particularly if the individual was small. Since *E. reticulata* was originally described from waters off New Guinea, and appears widely distributed in the tropical Indo-Pacific (Tsuchiya & Young, 2012), if one of these sequences represents *E. reticulata* (*sensu stricto*) it is more likely the specimen collected from the Kermadecs (rather than the individual from the NW Pacific).

Enoploteuthis semilineata, which has not been previously reported from New Zealand waters, formed a BIN (BOLD:ADH5348) together with a specimen from the East Coast of New Zealand's North Island (NIWA 76660). Three closely related *Enoploteuthis* species—*E. semilineata*, *E. chuni*, and *E. galaxias*—have similar photophore patterns, and without a clear understanding of the possible variability of the patterns through ontogeny, the question has been raised of whether these species are truly distinct, or whether they represent a single, variable species (Tsuchiya, 2014). These results suggest that at least two genetically distinct species do exist within this group (in New Zealand alone)—the specimens identified as *E. semilineata* in the present study grouped separately from two sequenced specimens morphologically identified as *E. galaxias* (BIN BOLD:ADI1174) from due east of Cook Strait (NIWA 95191) and the mid-west coast of the South Island (NIWA 89614). Thus, genetic data support the local presence of at least three species. A fourth *Enoploteuthis* species, '*E.* ?*jonesi*', has also been reported from New Zealand waters based on a single, damaged specimen (Riddell, 1985), so more species in this genus may be present in New Zealand waters, but a review is needed.

All specimens of *Abraliopsis* Joubin, 1896, were morphologically identified as *A. tui* Riddell, 1985, a species that was originally described from the Kermadecs (Riddell, 1985). These specimens formed a single, unique BIN (BOLD:ADH6894), with *A. morisii* (Vérany, 1839) from Morocco as the nearest neighbour (p-distance divergence of 8.35% in BOLD, BIN BOLD:ABW7719). This study provides the first sequences available for *A. tui*. Riddell (1985), in his original description, noted that *A. tui* is widely distributed and probably the most commonly encountered enoploteuthid in the New Zealand region, and is particularly common north of 32˚S. The only other known *Abraliopsis* species reported from New Zealand waters is *A. gilchristi* Robson, 1924, which is presently only known locally south of 36°S.

In the Pyroteuthidae, three species in two genera were found. Five specimens of *Pt.* cf. *gemmata* from the present study formed a single, unique BIN

(BOLD:ADH6415), whose nearest known (sequenced) neighbour at present is *Pt. giardi* Fischer, 1896, (GenBank ID GU145065) from the Atlantic Ocean (p-distance divergence of 8.33% in BOLD, BIN BOLD:ACQ3446). Both *Pt. gemmata* and *Pt. giardi* have previously been reported from the Kermadecs (Riddell, 1985). *Pterygioteuthis gemmata* is morphologically similar to *Pt. microlampas* Berry, 1913, which has also been previously reported from the Kermadecs (Riddell, 1985). However, sequences from two specimens attributed to *Pt. microlampas* from Hawaiian waters (GenBank IDs EU735387 and AY616887) formed a different BIN (BOLD:AAL2286) from our *Pt*. cf. *gemmata* specimens. The Kermadec specimens were morphologically attributed to *Pt.* cf. *gemmata* based primarily on the large size of mature female specimens (ML 31 mm) and the configuration of the arm suckers and hooks.

The specimens identified as *Py.* aff. *margaritifera* formed a BIN (BOLD:AAX9745) with another specimen identified as '*Py*. *margaritifera*' (Rüppell, 1844) from the Chatham Rise (BOLD ID CANTA256-08). These sequences cluster closely with *Py. margaritifera* sequences from Morocco (which is close to the Mediterranean type locality), although they were assigned a separate BIN (p-distance divergence of 1.34% on BOLD, BIN BOLD:ADH3719). It is possible that the differences among these specimens represent population-level differences; a thorough morphological analysis is needed for clarification. Six specimens of *Py. serrata* were also sequenced in the present study. These specimens formed a single, unique BIN (BOLD:ADH6416), with *Py. margaritifera* from Morocco as its nearest neighbour (p-distance divergence of 6.89% on BOLD). This species is morphologically distinctive and was originally described from the Kermadecs, but no other *Py. serrata* sequences are currently available for comparison. Although Riddell (1985) reported five pyroteuthid species to occur in the Kermadecs (see Table 13), recent checklists have either omitted members of this family altogether (Duffy & Ahyong, 2015), or have only reported *Pt. giardi* and *Py. serrata* (Reid & Wilson, 2015).

Although only seven species are currently accepted in the Pyroteuthidae, the specimens sequenced to date (present study and available online) form 12 BINs (Appendix 5), which do not form a single monophyletic group when a neighbour-joining tree is created on BOLD of the sequences in the enoploteuthid families (Appendix 5). The low support for many nodes on this phylogeny could suggest that many additional taxa are presently missing, and the inclusion of additional species will likely resolve this phylogeny further. The Pyroteuthidae is split into two clades, both of which contain a combination of *Pterygioteuthis* Fischer, 1896, and *Pyroteuthis* Hoyle,

1904, specimens. Thus, the two genera also do not appear to be monophyletic. Based on the molecular data, there are also many undescribed species present in this family and the higher systematics are badly in need of review.

Histioteuthidae Verrill, 1881

No histioteuthid species have been previously included in checklists of the Kermadecs (Duffy & Ahyong, 2015; Reid & Wilson, 2015), although three species were reported by Horstkotte (2008). Five histioteuthid species in five different genera were encountered in the Kermadecs region. Four of these represent the first BOLD sequences for those taxa, and one of these four appears to be new to science. Sequences were compared to previously named species gathered for Chapter 4. A comparison with specimens from across a wide geographic distribution was particularly helpful for species generally believed to have cosmopolitan distributions such as *Calliteuthis atlantica* (Hoyle, 1885b), *Histioteuthis* (‘*Hi.*’) *bonnellii*, and *Histiothauma* (*Ha*.) *miranda* (Berry, 1918).

Three Kermadec specimens were assigned to the same BIN as other previously sequenced specimens identified as *C.* aff. *atlantica* (BIN BOLD:AAX1287; a separate BIN from the local material which is currently considered most likely to represent *C. atlantica* [*sensu stricto*] [BIN BOLD:ADH0880], according to morphology). Specimens of *C.* aff. *atlantica* are commonly encountered on New Zealand’s mid-east coast and the Chatham Rise, and this taxon represents an additional species in *Calliteuthis* (Chapter 4).

Three specimens morphologically identified as ‘*Hi. bonnellii*’, which has been previously reported from New Zealand waters including the Kermadecs (Horstkotte, 2008), were assigned a separate BIN (BOLD:ADH3734) from *Hi. bonnellii* from the North Atlantic Ocean (BIN BOLD:AAX1286). Voss et al. (1998) found variation in the characters of *Hi. bonnellii* specimens from different regions and suggested that they may represent different populations. However, the New Zealand *Hi*. aff. *bonnellii* specimens formed a separate BIN from the North Atlantic *Hi. bonnellii*, which suggests that these are separate species, rather than separate conspecific populations. The type locality for *Hi. bonnellii* (*sensu stricto*) was the Mediterranean Sea, suggesting that the New Zealand taxon likely represents a new species.

The *Ha. miranda* specimens from the Kermadecs formed a single BIN (BOLD:AAX1314), which also included *Ha. miranda* specimens from off South Africa (GenBank ID EU735391), the Great Australian Bight (AM C.500878), the west coast of

New Zealand (beak in the NIWA Invertebrate Collection, trawl number TAN1210/078), and the Chatham Rise (beak in the NIWA Invertebrate Collection, trawl number TAN1208/40). This species was previously reported from the Kermadecs by Horstkotte (2008). Presently, *H. miranda* appears to be a notalian species.

One specimen identified as *Navia corona cerasina* Nesis, 1971, formed a BIN with specimens identified as *N. corona corona* (Voss & Voss, 1962) from the Gulf of Mexico and the eastern North Atlantic Ocean (Bear Seamount) (BIN BOLD:ACG8287). The status of the species in *Navia* (gen. nov.) has been debated, with some authors considering three separate taxa to be valid at the species level (*N. corona*, *N. berryi* Voss, 1969, and *N. cerasina* Nesis, 1971; see Young & Vecchione, 2000b), while others consider these to be subspecies of *N. corona* (see Voss et al. 1998, who also included *Fragariateuthis inermis* [Taki, 1964]). Horstkotte (2008) reported '*Hi. corona cerasina*' from the Kermadecs, stating that the morphology of New Zealand specimens was fully consistent with previous descriptions of this species. In all salient morphological characters, *N. corona cerasina* and *N. corona corona* appear identical, but have previously been considered separate taxa based on geographic separation, with *N. corona cerasina* known from the eastern Pacific Ocean, and *N. corona corona* believed to be restricted to the Atlantic Ocean (Voss et al., 1998). Genetic and morphological analyses of material from the type localities will be critical in resolving the status of *Navia* species.

Stigmatoteuthis hoylei (Goodrich, 1896) is rarely encountered in New Zealand waters (Voss et al., 1998), and a recent review of specimens in local collections did not report this species (Horstkotte, 2008). Two of the Kermadec specimens identified as *S.* cf. *hoylei* formed a single, unique BIN (BOLD:ADH3733) distinct from previously sequenced specimens attributed to *S. arcturi* Robson, 1948 (BIN BOLD:ADJ2097), *S. dolfleini* Pfeffer, 1912 (BIN BOLD:ADH3329), and *S.* aff. *hoylei* from Hawaii (BIN BOLD:AAX1315) (Chapter 4).

Ommastrephidae Steenstrup, 1857

The Ommastrephidae contains many species that are commercially fished. One of these, *Nototodarus gouldi* (McCoy, 1888), has been occasionally commercially fished in the Kermadec region for a number of years (Ministry of Primary Industries, 2017), but surprisingly has not been officially reported in previous checklists of this region (Duffy & Ahyong, 2015; Reid & Wilson, 2015). Four specimens of *N. gouldi* were found in the present study, which were placed in the same BIN (BOLD:AAI2536)

as specimens of *N. gouldi* from Taranaki Bight, New Zealand (GenBank ID AB270939; BOLD IDs CANTA266-08 and CANTA267-08). This species is formally reported on a checklist of Kermadec cephalopods here for the first time (Table 13).

A recent global analysis of the morphology and genetics of the *Ommastrephes bartramii* (Lesueur, 1821) species complex has revealed the presence of four distinct species (Fernandez-Alvarez, 2018). The specimens from the Kermadecs represent *O. brevimanus* Gould, 1852, and formed a BIN with specimens from the Cook Islands (BOLD:ACH3929); this BIN is distinct from the BIN formed by *O. bartramii* (*sensu stricto*) sequences from northern Hawaiian waters (BOLD:AAI1480), and from the BIN that contains a single *O. caroli* Furtado, 1887, specimen from Croatia (BOLD:ACW0102). This species complex is currently under review (Fernández-Álvarez in prep.).

Berry (1914) reported *Sthenoteuthis oualaniensis* (Lesson [in 1830–1831], 1830) from the Kermadecs. However, after re-examining the specimens, he determined that, although the Kermadecs is within the probable range of this species, his specimens actually represented *Eucleoteuthis luminosa* (Sasaki, 1915) (Berry, 1916). Although *E. luminosa* was not included in the checklist by Duffy and Ahyong (2015), it was present in the checklist by Reid and Wilson (2015). A single preserved specimen at the Museum of New Zealand Te Papa Tongarewa, collected in the Kermadecs, has previously been identified as *S. oualaniensis* (NMNZ M.287323). It appears, therefore, that the family Ommastrephidae is represented by at least four species in the Kermadec region: both *Eucleoteuthis luminosa* and *Sthenoteuthis oualaniensis*, and the two species encountered herein (*Nototodarus gouldi* and *Ommastrephes brevimanus*).

Onychoteuthidae Gray, 1847

The family Onychoteuthidae, which was the subject of a recent, global, morphology-based review (Bolstad, 2010), has recently also been assessed from a genetic standpoint (Bolstad et al., 2018). This is fortuitous, since comparing the onychoteuthid material from the Kermadecs with sequences from most of the named species in the family reveals that this group requires further systematic attention in the region.

Previously, two onychoteuthid species, both from the genus *Onychoteuthis* Lichtenstein, 1818, have been reported in Kermadec checklists: *O. aequimanus* Gabb, 1868, (Duffy & Ahyong, 2015) and *O. banksii* (Leach, 1817) (Reid & Wilson, 2015). In the current study, two different *Onychoteuthis* species were found. Two specimens of *O.*

meridiopacifica Rancurel & Okutani, 1990, represent a new species record for New Zealand, and have been sequenced herein for the first time (where both individuals formed a single, unique BIN BOLD:ADH5825). Two other specimens, which are designated as '*O*. aff. *compacta*', align morphologically with *O. compacta* (Berry, 1913) from Hawaii (see Bolstad, 2010), but were assigned a separate BIN (BOLD:ADG9816) from *O. compacta* (*sensu stricto*) and showed a close relationship with *O. aequimanus* (from New Zealand) and *O.* cf. *bergii* Lichtenstein, 1818 (from the Indian Ocean) (Bolstad et al., 2018). Two other specimens collected from the mid-east coast of New Zealand and northeast of the North Island (Bolstad et al., 2018) were assigned the same BIN as the two *O*. aff. *compacta* specimens from the Kermadecs, suggesting that this species may be distributed throughout at least the northern half of the New Zealand EEZ. Although *O. banksii* was previously included in a checklist of the Kermadecs (Reid & Wilson, 2015), this species is not known to occur in New Zealand waters (Bolstad, 2010); following a review of the genus that resolved the long-problematic *O.* '*banksii*' species complex, *O. banksii* (*sensu stricto*) is now believed to occur only within tropical to temperate Atlantic waters (Bolstad, 2008).

Sepiolidae Leach, 1817

Although sepiolids have not previously been included in checklists of the Kermadecs (Duffy & Ahyong, 2015; Reid & Wilson, 2015), two separate species of *Heteroteuthis* Gray, 1849, were encountered, a genus not yet formally reported from New Zealand waters (Spencer et al., 2017). Specimens of *Heteroteuthis dagamensis* Robson, 1924, were assigned to a single BIN (BOLD:AAM7920), along with a sequence for *H. dagamensis* from the Gulf of Mexico (GenBank ID KR606071) and an unidentified species of *Heteroteuthis* from Australian waters off Lizard Island, near northern Queensland (BOLD ID LIMX146-10). Although these specimens formed a single BIN, there is a large maximum intraspecific distance (p-distance divergence of 1.45%), suggesting that population-level differences exist within this species. The nearest neighbour for the *H. dagamensis* BIN is *Heteroteuthis dispar* (Rüppell, 1844) (p-distance divergence of 4.75% in BOLD, BIN BOLD:ABW9322). The single specimen of *H.* sp. KER was assigned a unique BIN (BOLD:ADH5539), which had *H. hawaiiensis* (Berry, 1909) as its nearest neighbour (p-distance divergence of 2.25% in BOLD, BIN BOLD:AAI8461). Sequences are available for two additional (out of the five named) *Heteroteuthis* species: *H. dispar* (BIN BOLD:ABW9322 from Morocco [BOLD IDs CEPAR206-11 and CEPAR205-11] and Israel [BIM456-15]) and *H.*

ryukyuensis Kubodera, Okutani and Kosuge, 2009 (BIN BOLD:ACH3445 from Japan; GenBank ID AB591074), which each formed distinct BINs. Of the two remaining species, *H. dagamensis* was described from off South Africa, and has been assumed to be the only species present in South Atlantic and South Pacific waters (Vecchione *et al*. 2013), and *H. nordopacifica* Kubodera and Okutani, 2011, is known only from a single specimen from the northwest Pacific Ocean (Kubodera and Okutani 2011). Another species, *H. serventyi* Allan, 1945, was described from Jervis Bay (just south of Sydney), Australia, but the status of this species is currently unknown. It is clear that this genus is in need of revision, and until that time, the correct name for *H.* KER from the Kermadecs remains unclear.

Spirulida Stolley, 1919

Spirula spirula (Linnaeus, 1758) is the only known living species in the order Spirulida Haeckel, 1896. Although several species have been named in this genus, a single species is currently accepted. Three specimens of *Spirula* Lamarck, 1799, were identified in the present study, supporting the occurrence of this species in the Kermadecs, as reported in previous checklists (Duffy & Ahyong, 2015; Reid & Wilson, 2015). The present material was assigned the same BIN (BOLD:AAI0193) as all other *Spirula* sequences available on BOLD from Australia and the Atlantic Ocean. This species has been reported from the tropical Atlantic and the Indo-West Pacific (Nesis, 1987), and the present results support its wide-spread distribution.

Vampyromorpha Robson, 1929

Vampyroteuthis infernalis Chun, 1903, is the only species currently accepted in the Vampyroteuthidae Chun, 1903, although seven genera and ten species have been named in this family (Sweeney & Young, 1998). A number of morphological differences have been found among specimens from the Gulf of Guinea, Africa, and California (Young, 1972). On BOLD, the DNA barcodes for *V. infernalis* (including two Kermadec specimens) form five BINs (BOLD:AAF0279, BOLD:AAF0280, BOLD:AAF0281, BOLD:ACH6701, and BOLD:ACQ2157); these morphological and genetic differences suggest that there may, in fact, be multiple species present in this family. The type locality for this species is the south Atlantic Ocean, and, rather surprisingly, the Kermadec material forms a BIN (BOLD:ACQ2157) with specimens from the northwest Atlantic Ocean and the central south Atlantic Ocean, distinct from BINs formed by specimens from Japan and the Sargasso Sea (BOLD:AAF0280),

Hawaii (BOLD:AAF0279), Vietnam (BOLD:AAF0281), and an unknown locality (BOLD:ACH6701; GenBank ID KC020187). It is clear that this order is in need of revision.

Conclusion:

The present study brings the total number of cephalopod species known to occur in the Kermadec region to 70, nearly double the number reported in previous checklists (Duffy & Ahyong, 2015; Reid & Wilson, 2015), and representing over half the known cephalopod diversity of the entire New Zealand EEZ. This study reports 14 new cephalopod records for the New Zealand EEZ (representing a >10% increase in our known cephalopod fauna), sequenced comparative material from New Zealand waters in order to clarify global distribution patterns, and flagged at least five species potentially new to science. Several historically monotypic genera may contain multiple species. DNA barcoding has assisted in confirming morphological species identifications, and the BIN system has allowed potentially new species to be identified. The name *Pinnoctopus cordiformis* has been resurrected, and a closely related, but presently unidentified species from the Kermadec region, has been identified. This study was enabled by a recent survey of the biodiversity of the Kermadec region; one of the main survey objectives—to gain a better understanding of the biodiversity of the central Kermadec Islands and ridge (Clark et al., 2017)—has certainly been met for the cephalopods. The results of this study support the establishment of the Kermadec–Rangitāhua Ocean Sanctuary, which will help protect the high cephalopod diversity of this region, especially pelagic, deep-sea species not fully protected by the current Reserve (including potentially new and poorly-known species). These results are based on fresh specimens from a single cruise, in order to enable integrative taxonomy. However, a thorough morphological review of all fixed cephalopod material from the Kermadec region is still needed. In addition to the Kermadec-specific insights gained, this study has highlighted several species- and higher-level taxa that need systematic attention, whose resolution will require integrated taxonomic methods.

Chapter 6: Overall discussion

In the introduction of this thesis, it was proposed that an increased taxonomic resolution could aid in the conservation of cephalopod biodiversity, which has now been demonstrated (Fig. 21). Conservation requires the proper identification of organisms, which depends on stable, resolved taxonomy. Consequently, this study focused on improving taxonomic resolution of two clades of deep-sea cephalopods most in need of revision: the chiroteuthid families (Chapters 2 and 3) and the Histioteuthidae Verrill, 1881 (Chapter 4). This resolution was attained using a combination of morphology and mitochondrial genes, and COI was found to be the gene with the highest variability and resolution. Therefore, a combination of morphology and COI was used to evaluate the cephalopod biodiversity of the Kermadec Islands region in New Zealand waters (Chapter 5), an area in which a better understanding of the biodiversity has been called for in order to establish a marine protected area (the Kermadec–Rangitāhua Ocean Sanctuary) (Clark et al., 2017). The cephalopod biodiversity of this area, and of the New Zealand Exclusive Economic Zone, was previously underestimated. This thesis has nearly doubled the known cephalopod biodiversity of the Kermadec Islands area, and added 12 new records to the New Zealand EEZ (Chapter 5).

Integrative taxonomy

Traditionally, cephalopod taxonomy and species identification have been based on morphology; however, genetics is becoming increasingly incorporated into these processes. Using morphological and genetic traits in combination is known as ‘integrative’ taxonomy. The overall aim of this thesis was to determine whether integrative taxonomy can aid in the conservation of cephalopod biodiversity. The first step was to determine whether integrative taxonomy could improve systematic resolution at the species level, using *Asperoteuthis* Nesis, 1980, as a case study. A combination of mitochondrial genes (COI, 16S rRNA, and 12S rRNA) and morphology were successfully used to resolve species in this genus (Chapter 2). These three genes were then used to examine the poorly understood chiroteuthid families in the Pacific Ocean (Chapter 3). The two most variable genes (COI and 16S rRNA) were used in

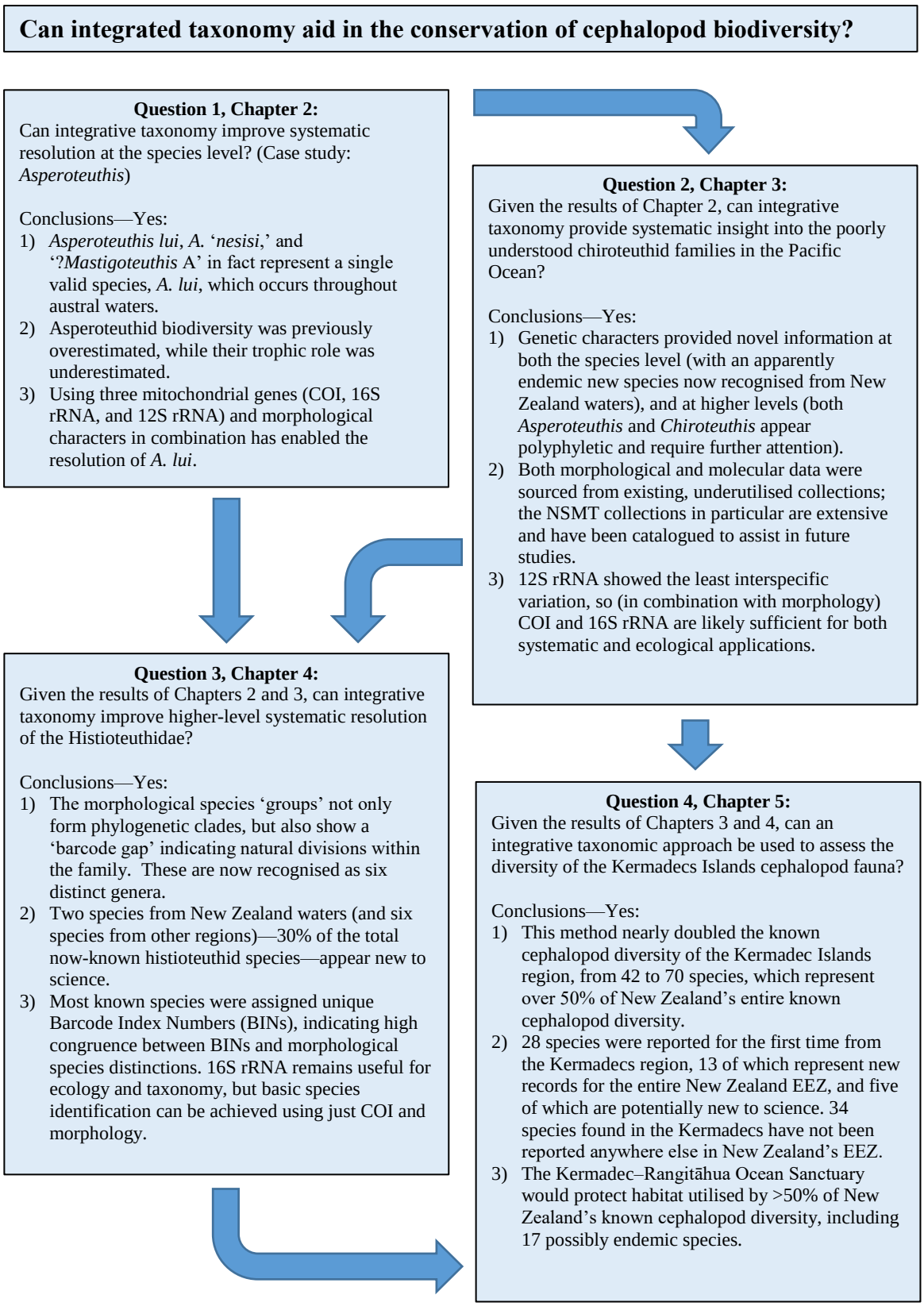

Fig. 21—A conceptual diagram of the flow of this thesis with the main conclusions from each chapter.

combination with morphological characters to resolve the higher systematics of the family Histioteuthidae (Chapter 4), one of the deep-sea cephalopod families most in need of revision (Hoving et al., 2014). This analysis found COI to show higher variability and resolution (Chapter 4). With the number of comparative sequences for these two clades nearly doubled, and following the increased taxonomic resolution, the cephalopod biodiversity of the Kermadec Islands region was reviewed using morphology and COI (Chapter 5). This analysis nearly doubled the previous estimates for the cephalopod biodiversity of this region and found 33 cephalopod species not presently known from other parts of the New Zealand EEZ, along with at least five species new to science (Chapter 5). The high cephalopod biodiversity found in the Kermadec Islands region supports the establishment of the Kermadec–Rangitāhua Ocean Sanctuary and demonstrates that integrative taxonomy can aid in the conservation of cephalopod biodiversity.

Integrative taxonomy is much more powerful than either morphology or genetics alone, which has been demonstrated throughout this thesis and in previous cephalopod studies (e.g., Braid & Bolstad, 2015; Bolstad et al., 2018; Allcock & Piertney, 2002). Both techniques are capable of over-splitting (e.g., *Asperoteuthis*, see Chapter 2; *Octopus tetricus* Gould, 1852, Amor, Norman, Cameron, & Strugnell, 2014) or over-lumping species (e.g., the Histioteuthidae, see Chapter 4). Within the genus *Asperoteuthis*, incomplete specimens and a lack of comparative sequences caused previous authors to overestimate the number of species (Chapter 2). Similarly, the diversity of the genus *Architeuthis* Steenstrup, 1857, was previously overestimated with 21 named species, until an analysis of mitochondrial DNA revealed a single, cosmopolitan species (Winkelmann et al., 2013). In contrast, genetics can also reveal the presence of previously unrecognised species. In the Chiroteuthidae, the analysis of the chiroteuthid families in Chapter 3 used mitochondrial DNA and revealed at least one species that is new to science (*C.* aff *veranyi*) and showed evidence for the recognition of a species that was previously synonymised (*I. latipinna* Sasaki, 1916). Similarly, nine potentially new species were found during the present analysis of the Histioteuthidae, a family whose diversity was previously underestimated due to conservative morphological features and a lack of adequate specimens from different geographic locations (Chapter 4). Other recent revisions of oegopsid families that used DNA have also revealed additional, previously unrecognised species (Braid & Bolstad, 2015; Bolstad et al., 2018). For example, the Onychoteuthidae Gray, 1847, which was recently revised using morphology (Bolstad, 2010), was also recently analysed using two

mitochondrial genes (COI and 16S rRNA) revealing several additional, morphologically cryptic species (Bolstad et al., 2018). Morphological studies are limited by the quality of material that can be studied, while genetic analyses are limited by available specimens and comparative sequences. Therefore, the combination of both techniques is ideal.

Beyond the species level, integrative taxonomy is also a key tool in establishing divisions within cephalopod families. Three mitochondrial genes were analysed for the chiroteuthid families, but a phylogeny of this clade based on only COI and 16S rRNA revealed a similar topology to that of a phylogeny generated using these two genes plus 12S rRNA, with the same resolution at the genus level (pers. obs.). In addition, these two mitochondrial genes (COI and 16S rRNA) were recently used to reclassify the higher taxonomy within the family Onychoteuthidae (Bolstad et al., 2018). Therefore, a combination of COI and 16S rRNA appears adequate for genus-level systematics within the oegopsid squids. These two genes were used for clarifying the genus-level systematics of the Histioteuthidae and revealed the presence of six genera (Chapter 4), which align with previously recognised morphological species 'groups' (Voss et al., 1998). Although the use of genetic characters has only recently become possible, and most existing classifications within cephalopod families were based solely on morphology, an integrative taxonomic approach is fast becoming the gold standard for cephalopod systematics.

Based on the results of this thesis (Chapters 2, 3, 4, and 5), integrative taxonomy appears to be the best practice for squid taxonomy and species identification. COI appears to be the most variable gene region when compared to 16S rRNA and 12S rRNA, and offers a higher resolution for closely related species or subspecies (Chapters 2, 3, and 4). However, although COI appears adequate for species identification, it has been recommended that additional genes—such as12S rRNA and 16S rRNA—be sequenced as well due to some of the potential problems with this gene region (see Strugnell & Lindgren, 2007). Furthermore, for some cephalopods, such as species of *Pareledone* Robson, 1932, COI has been found to show low interspecific variation (Allcock et al., 2011). Due to this potential problem, the chapters in this thesis that have focused on taxonomic revision have used COI along with at least one other mitochondrial gene (Chapters 2, 3, and 4). COI has shown higher interspecific distances than 16S rRNA (and 12S rRNA, when included) across a range of oegopsid clades: the chiroteuthid families (Chapter 3; Braid et al., 2014), the Histioteuthidae (Chapter 4), the lepidoteuthid families (J. Kelly, pers. comm.), and the Onychoteuthidae (Bolstad et al.,

2018). Therefore, it seemed appropriate to sequence COI to help identify species from the Kermadec Islands region (Chapter 5).

One of the additional advantages of using COI is that sequences can be added to the Barcode of Life Data System (BOLD), the most comprehensive global database of COI sequences, and incorporated into the search engine to facilitate easy species identification (Ratnasingham & Hebert, 2007), as well as identification using the Barcode Index Number (BIN) system (Ratnasingham & Hebert, 2013). In addition, BINs have shown high concordance with oegopsid species in this thesis (Chapters 2, 3, and 4) and previous studies (Bolstad et al., 2018; Braid et al., 2014). To assist in future work on the ecologically important chiroteuthid families and Histioteuthidae, DNA barcode reference libraries were established on BOLD for these groups (Chapters 3 and 4).

Often, 16S rRNA has been used in cephalopod genetic studies as a primary or secondary gene (e.g., Bonnaud et al. 1994; Allcock & Piertney, 2002; Lindgren, Katugin, Amezquita, & Nishiguchi, 2005). Previous studies have relied on 16S rRNA because it amplifies more readily than COI in animals (e.g., Vences, Thomas, van der Meijden, Chiari, & Vieites, 2005; Ivanova, Zemlak, Hanner, & Hebert, 2007; Chapter 3). Dietary analyses may choose a marker based on the availability of comparative sequences, such as the study by Alonso et al. (2014), where 16S rRNA was selected for cephalopods due to the higher number of comparative sequences available on GenBank compared to COI. (It should be noted that, at present, ~4300 cephalopod COI sequences are available on GenBank, while BOLD has ~6700 COI sequences [as of February 1, 2018]). However, 16S rRNA can be used to identify species but it is not always able to achieve the same level of resolution as COI (Lindgren et al., 2005; Chapter 4). The results of the present study on the Histioteuthidae were useful in refining the identification of histioteuthids from stomach contents from two previous ecological studies, but was not able to achieve the highest level of resolution (i.e., to sub-species level) because those studies used 16S rRNA (Chapter 4). Based on the variability found in the chiroteuthid families (Chapter 3) and the Histioteuthidae (Chapter 4), 16S rRNA makes an adequate secondary gene for genetic analyses.

While some studies on cephalopod genetics have used a combination of nuclear and mitochondrial genes (e.g., Strugnell, Norman, Jackson, Drummond, & Cooper, 2005; Lindgren, 2010; Lindgren et al., 2012), nuclear genes were not included in the present analysis. Although they would provide an additional line of evidence, they are often difficult to amplify due to low copy numbers and the presence of two copies of

each gene. There are presently only five histioteuthid specimens with nuclear gene sequences available on GenBank, and tissue or DNA is not available for three species included in this analysis (which were only sequenced for a single gene, either COI or 16S rRNA). However, the inclusion of nuclear genes should be considered in future analyses.

The Kermadec Islands region

The results of the cephalopod biodiversity assessment of the Kermadec Islands region (Chapter 5) strongly support the establishment of the proposed Kermadec–Rangitāhua Ocean Sanctuary. The proposed Sanctuary would extend the marine protection in the Kermadec region from 12 nautical miles to 200 nautical miles around each island in this area, with a deeper maximum depth (~10000 m) than is currently provided by the current Kermadec Marine Reserve (~2250 m) (Ministry of Primary Industries, 2016). In addition, the current Reserve only protects ~1% of the area that the proposed Sanctuary would cover (Ministry of Primary Industries, 2016), and this extended area is important for protecting pelagic animals with ranges that extend beyond the Reserve. At present, 34 species of cephalopod have been found in the Kermadec region, but have not been reported from other parts of the New Zealand Exclusive Economic Zone (Chapter 5); in total, the Sanctuary would offer protection to a range of taxa representing over half of New Zealand's known cephalopod biodiversity (or at least to portions of their populations). In addition, at least five cephalopod species reported in this area appear new to science (Chapter 5). The high and very diverse cephalopod biodiversity of the region is illustrated by the collection of 43 species at only 11 sampling stations, and the rarefaction curves do not plateau, which suggests that more species are likely to occur in this area (Fig. 22; Chapter 5); it is expected that future studies will reveal additional taxa beyond the present tally of 70 (as an uncritical review of Kermadec specimens held in national collections has already suggested; Bolstad, 2016). This pattern of high diversity in the region (including taxa not known from elsewhere within the EEZ) is not restricted to cephalopods. From the same voyage ('Biodiversity of the Kermadec Islands and offshore waters of the Kermadec Ridge—a coastal, marine mammal and deep-sea survey [TAN1612]'), 236 fish species were reported, including three new species and 20 new records for the New Zealand EEZ (Clark et al., 2017).

Protecting the Kermadec Islands region is also important for population connectivity. A recent genetic analysis of population connectivity of three species of coral from different regions in the New Zealand EEZ suggested that additional protected areas are required in order to maintain genetic diversity, and suggested that the establishment of the Sanctuary would offer some additional protection (Zeng, Rowden, Clark, & Gardner, 2017). The biodiversity of this area is still not fully understood, but initial investigations reveal a unique and high biodiversity in a relatively pristine area, which requires the protection of the Kermadec–Rangitāhua Ocean Sanctuary.

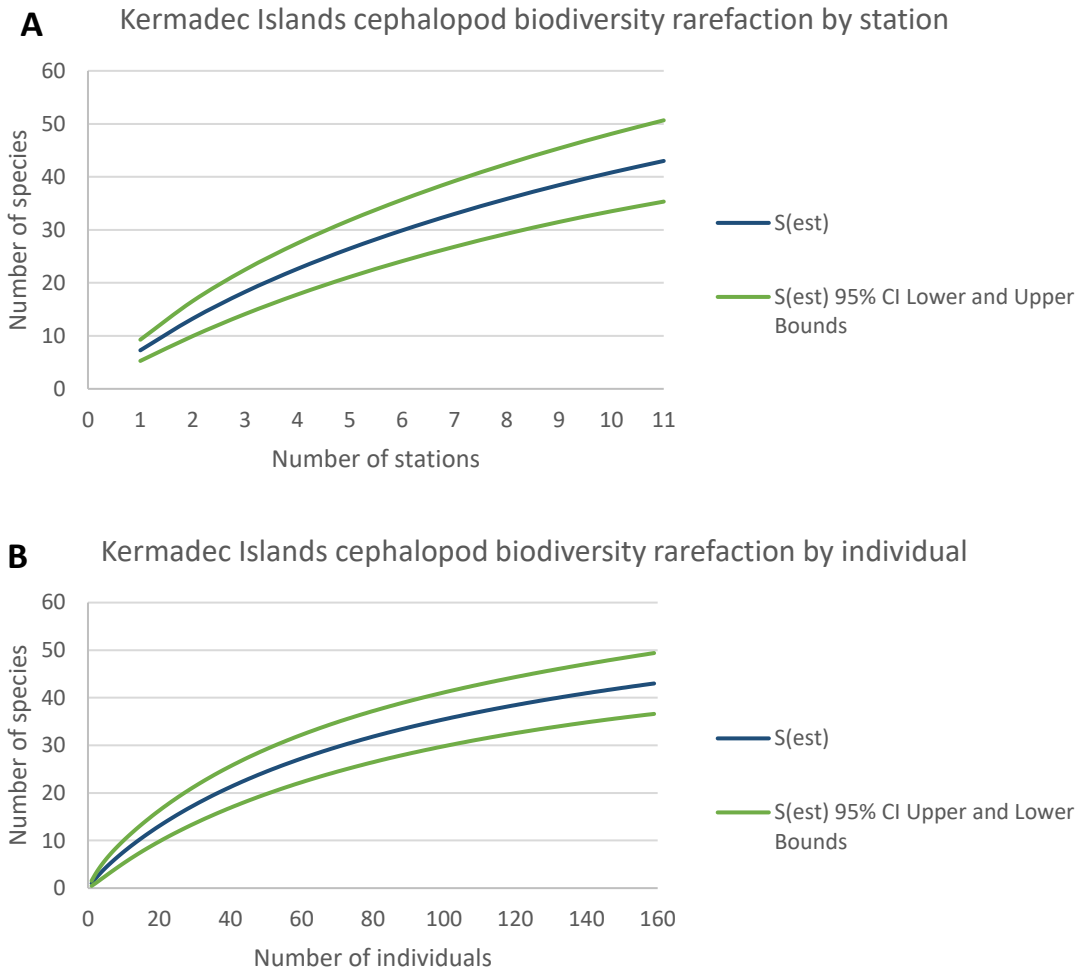

Fig. 22—Rarefaction curves for the cephalopod biodiversity of the Kermadec Islands region by: A) the number of stations; B) the number of individuals sampled. Rarefactions were calculated using EstimateS (Gotelli & Colwell, 2001; Colwell, 2013). S(est) is the estimated sample, and CI is the confidence interval.

Future work

The BIN analysis for the chiroteuthid families (Chapter 3), the Histioteuthidae (Chapter 4), and the cephalopod diversity of the Kermadecs (Chapter 5), revealed many taxa that still require systematic attention. The present work on the Histioteuthidae and previous work on the Mastigoteuthidae Verrill, 1881, (Braid et al., 2014) has shown that morphologically hypothesised species 'groups' often represent genera. Within the Pyroteuthidae Pfeffer, 1912, *Pterygioteuthis* Fischer, 1896, has been divided into two species 'groups' (Lindgren, Young, & Mangold, 2011), and *Pyroteuthis* Hoyle, 1904, also appeared to be paraphyletic and in need of systematic attention (Chapter 5). In the Chiroteuthidae Gray, 1849, both *Asperoteuthis* and *Chiroteuthis* d'Orbigny [in Férussac & d'Orbigny], 1841, appear to be paraphyletic, and are in need of revision (Chapter 3). The genus *Chiroteuthis* has been divided into three species 'groups' (Roper & Young, 2013), two of which appear to be supported by genetics. The third group, the '*C. joubini*/*C. spoeli* group', has yet to be sequenced and should be included in a phylogenetic analysis of this family when specimens become available especially from the South Atlantic Ocean, where all three species are known to occur (Roper et al., 2017). Based on the BIN analyses in Chapter 5 and comparison with public sequences on BOLD, the groups that are globally most in need of work are the Bathyteuthoidea Vecchione, Young, and Sweeney, 2004 (*Bathyteuthis* Hoyle, 1885a, + *Chtenopteryx* Appellöf, 1890), *Bolitaena* Steenstrup, 1859, the Pyroteuthidae Pfeffer, 1912, and the Sepiolidae Leach, 1817. Revisions for several additional groups are already underway by members of the AUT Lab for Cephalopod Ecology & Systematics (ALCES): the Brachioteuthidae Pfeffer, 1908a, the Chiroteuthidae, the Cranchiidae Prosch, 1847, the Histioteuthidae, and the lepidoteuthid families (Table 1).

One particularly important application of deep-sea squid taxonomy is prey identification from the gut contents of a variety of apex predators. To this end, DNA barcode reference libraries have been established for the chiroteuthid families and the Histioteuthidae; however, gut contents of predators often contain beaks without tissue, which can only be identified using morphology. Ideally, beaks from gut contents would be compared with beaks sourced from reference specimens that were identified using both morphology and genetics. New Zealand's National Institute for Water & Atmospheric (NIWA) houses one such reference beak collection, which ALCES lab members are currently in the process of sequencing, to further assist in future prey identification work. In this thesis, some progress has already been made on this front; in addition to five chiroteuthid and 33 histioteuthid beaks sequenced from this collection,

the beaks of several specimens of *A. lui* have been illustrated and described in detail for improved identification from predator gut contents (Chapter 2).

In addition to improved identification of ex-gut-content beaks and specimens, taxonomic resolution can permit the development of increasingly accurate biomass estimation equations, permitting calculation of the volume, weight, and dietary proportions of individual squid taxa. For example, histioteuthid beaks from species found in New Zealand waters were previously described—often with regression equations to estimate size and biomass—by Horstkotte (2008). Although this is a very valuable resource, results from this thesis indicate that his '*Histioteuthis atlantica*' equation combines two taxa, *C. atlantica* (Hoyle, 1885b) and *C.* aff. *atlantica*. These species need to be examined morphologically to determine characters that can be used to distinguish them and until the systematics is resolved, the known limitations of the '*Histioteuthis atlantica*' equations proposed by Horstkotte (2008) should be taken into consideration.

Cephalopod biogeographic patterns are still not well understood, partly because of unresolved taxonomy, and partly because many areas remain poorly sampled. Although there are some areas in various oceans that currently show especially high cephalopod diversity, it is unclear whether this is simply an artifact of considerable sampling efforts and/or focused biodiversity studies. In the Pacific, several regions show particularly high cephalopod biodiversity: Japan (Tittensor et al., 2010), Hawaii (Young et al., 1998; pers. obs.), and New Zealand (Spencer et al., 2017; pers. obs.). Across all cephalopod groups, biodiversity in the Pacific Ocean appears higher overall than the Atlantic Ocean (Tittensor et al., 2010), which could suggest a possible Pacific origin for cephalopods. A Pacific origin has been recently suggested specifically for an ommastrephid genus, *Ommastrephes* d'Orbigny [in 1834-1847], 1834 (Fernández-Álvarez, 2018).

Our current understanding of cephalopod distribution patterns could be clarified by the inclusion of genetic data. For example, the species *Histiothauma meleagroteuthis* (Chun, 1910) individuals from different geographic locations show sequence variation that indicates that additional subspecies may exist (Chapter 4). In contrast, some species, such as *Histiothauma miranda* (Berry, 1918) (Chapter 4) and *A. lui* Salcedo-Vargas, 1999 (Chapter 2), appear to truly have widespread distributions. As sampling efforts continue, collecting tissue samples for genetic analysis should be a top priority.

Conclusions:

This thesis has increased our knowledge of both local and global cephalopod biodiversity. There are now at least 33 cephalopods known from the Kermadec Islands region that are not found in other parts of the New Zealand EEZ. In addition, it appears that at least five cephalopod species found in this region are new to science. Cephalopods are negatively impacted by fishing and mining activity and the establishment of the Kermadec–Rangitāhua Ocean Sanctuary would offer protection to over half the cephalopod species found in the New Zealand EEZ, within at least a portion of their known ranges. This area is relatively unexplored, and our current biodiversity estimates of this area are likely conservative.

A genetic analysis of the chiroteuthid families was undertaken, and revealed the paraphyletic nature of *Chiroteuthis* and *Asperoteuthis*, a species complex in *Idioteuthis* Sasaki, 1916, and at least one new, unnamed species (*C.* aff. *veranyi*). It is clear that more work is needed on this clade, especially the Chiroteuthidae. Six histioteuthid genera have been established, which align closely with previously proposed morphological species 'groups'. This family was previously believed to consist of 19 species, but the genetic results of this thesis distinguished 17 named species, and revealed a further nine potentially new, unnamed species.

DNA barcode reference libraries for the chiroteuthid families and the Histioteuthidae were established to help future studies with species identification. Continued collection efforts for new specimens will be required in order to increase the systematic resolution of the taxa that have been highlighted in this thesis. Globally, cephalopods face many natural and anthropogenic pressures, and the resolution of their taxonomy is the first step in understanding and protecting their biodiversity.

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Appendix 1—Catalogue of the specimens in the chiroteuthid families clade from the National Museum of Nature and Science, Tokyo (NSMT) collections. Specimen ID is the museum registration, and for specimens that were sequenced herein the BOLD (Barcode of Life Data System) ID is also included underneath in bold. Sex is attributed as male (M), female (F), or undetermined (indet.). The dorsal mantle length (ML) is given, except when this was not possible and therefore the lower rostral length (LRL) or the club length (CL) is given. Notes are provided on specimen condition, focusing on important systematic characters (*e.g.*, tentacle clubs, photophores) and damage that would affect measurements/indices.

Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
Chiroteuthidae					
Chiroteuthis					
C. calyx	NSMT Mo.66639	54.83°N, 167.65°W, 990 m, 15/06/1979, BTT, collected by Yabe	F	97	Tentacle stalks without clubs; beaks removed and not with specimen; arm suckers in good condition; eyes damaged but photophores visible.
C. calyx	NSMT Mo.85671	54.63°N, 165.38°W, 117 m, 26/05/1976, FV *Mineshima-Maru*, trawl, collected by Y. Okada	F	168	No tentacles; skin in good condition; some arm tips damaged.
C. calyx	NSMT Mo.66651	43.97°N, 171.52°E, 10/05/1974, RV *Hakuho-Maru*, Stn 7 KH74-2, collected by M. Fukuchi	F	168*	Excellent overall condition; eye photophore well preserved; arms complete; locking cartilages in good condition; both tentacles attached with clubs; tail damaged.
C. calyx	NSMT Mo.71702	42.66°N, 144.37°E, 778 m, 20/07/1999, RV *Wakataka-Maru*, BTT, Stn D4, collected by T. Hattori	M	190	Head nearly detached from body; eyes damaged but photophores present; tentacles missing.
C. calyx	NSMT Mo.71703	42.63°N, 144.56°E, 744 m, 21/07/1999, RV *Wakataka-Maru*, BTT, collected by T. Hattori	F, Indet.	230, head only	Large female with one tentacle. Other specimen only head and arm crown; tentacles missing.
C. calyx	NSMT Mo.76357	42.17°N, 143.67°E, 300 m, 21/10/2003, SS *Eisyo-Maru*, OT	F	267	Good condition overall; tentacle stalks without clubs; skin in good condition; arm tips slightly damaged; eyes slightly damaged; locking cartilages in good condition.

Appendix 1—Continued.

Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
C. calyx	NSMT Mo.67864	42.16°N, 143.95°E, 990 m, 31/08/1992, TS *Oshoro-Maru*, BTT, collected by T. Kubodera	F	136	Eyes damaged; tentacle stalks without clubs; arm tips intact.
C. calyx	NSMT Mo.76276	41.93°N, 140.94°E, 1991, collected by Y. Sakurai	F	135*	Fair condition overall; tail damaged; both tentacles attached; eyes damaged.
C. calyx	NSMT Mo.71881	41.89°N, 145.06°E, 650 m, 23/07/1996, RV *Marusada-Maru*, MWT, tag 872	F	160*	both tentacles attached but damaged, eyes damaged, tail damaged
C. calyx	NSMT Mo.71882	41.89°N, 145.06°E, 650 m, 23/07/1996, RV *Marusada-Maru*, MWT, tag 874	Indet.	151*	Tentacles missing; eyes missing; arm tips damaged; tail damaged.
C. calyx	NSMT Mo.75068	41.89°N, 145.06°E, 650 m, 23/07/1996, RV *Marusada-Maru*, MWT, tag 996	F	175*	Tentacles missing; eyes damaged; tail damaged.
C. calyx	NSMT Mo.71883	41.89°N, 145.06°E, 650 m, 23/07/1996, RV *Marusada-Maru*, MWT, tag 873	Indet.	80*	Tentacles missing; eyes damaged; arm tips damaged; tail damaged; fins missing.
C. calyx	NSMT Mo.75069	41.81°N, 145.11°E, 650 m, 23/07/1996, RV *Marusada-Maru*, MWT, tag 1010	F	208	Tentacles missing; eyes damaged.
C. calyx	NSMT Mo.75070	41.81°N, 145.11°E, 650 m, 23/07/1996, RV *Marusada-Maru*, MWT, tag 1011	F	170*	Both tentacles attached; eyes damaged.
C. calyx	NSMT Mo.71857	41.46°N, 145.51°E, 500 m, 22/07/1996, RV *Marusada-Maru*, MWT, tag 871	F	135	Both tentacles attached; eyes damaged.
C. calyx	NSMT Mo.71856	41.46°N, 145.51°E, 500 m, 22/07/1996, RV *Marusada-Maru*, MWT, tag 870	M	205	Tentacles missing; eyes damaged.
C. calyx	NSMT Mo.71933	41.35°N, 144.08°E, 550 m, 25/07/1996, RV *Marusada-Maru*, MWT, tag 890	Indet.	130*	Tentacles missing; one eye missing, other eye damaged; tail damaged.

Appendix 1—Continued.

Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
C. calyx	NSMT Mo.71934	41.35°N, 144.08°E, 550 m, 25/07/1996, RV *Marusada-Maru*, MWT, tag 926	Indet.	137	One tentacle attached; eyes damaged.
C. calyx	NSMT Mo.75058	41.33°N, 146.23°E, 550 m, 09/07/1996, RV *Marusada-Maru*, MWT, tag 1023	M	139	Tentacles missing; eyes damaged.
C. calyx	NSMT Mo.75066	41.30°N, 145.58°E, 650 m, 22/07/1996, RV *Marusada-Maru*, MWT, tag 1036	F	192*	Tentacles missing; eyes damaged; tail damaged.
C. calyx	NSMT Mo.75067	41.30°N, 145.58°E, 650 m, 22/07/1996, RV *Marusada-Maru*, MWT, tag 1037	Indet.	90*	Tentacles missing; eyes damaged; tail damaged; viscera damaged; mantle nearly detached from head.
C. calyx	NSMT Mo.66911	41.12°N, 142.01°E, 1016-993 m, 11/06/1989, RV *Tansei-Maru*, collected by M. Terasaki	F	175	Excellent overall condition; eye photophore well preserved; arms complete; locking cartilages in good condition; both tentacles attached with clubs.
C. calyx	NSMT Mo.71803	41.04°N, 145.35°E, 650 m, 21/07/1996, RV *Marusada-Maru*, MWT	F	109	Tentacle stalks without clubs; eyes damaged; arm tips damaged.
C. calyx	NSMT Mo.71841	41.03°N, 145.70°E, 550 m, 22/07/1996, RV *Marusada-Maru*, MWT, tag 868	M	170*	Tentacles missing, arms damaged; eyes damaged; tail damaged.
C. calyx	NSMT Mo.71842	41.03°N, 145.70°E, 550 m, 22/07/1996, RV *Marusada-Maru*, MWT, tag 869	M	173*	Tentacles missing; arm tips damaged; tail damaged.
C. calyx	NSMT Mo.75065	41.02°N, 145.52°E, 650 m, 21/07/1996, RV *Marusada-Maru*, MWT, tag 995	F	240	One tentacle attached; eyes damaged.
C. calyx	NSMT Mo.72018	41.01°N, 145.36°E, 630 m, 07/07/1996, RV *Marusada-Maru*, MWT, tag 904	M	108*	One tentacle attached; eyes damaged; tail damaged.

Appendix 1—Continued.

Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
C. calyx	NSMT Mo.71756	40.99°N, 144.73°E, 550 m, 20/07/1996, RV *Marusada-Maru*, MWT, tag 884	Indet.	110*	Tentacles missing; eyes damaged; tail damaged; viscera damaged.
C. calyx	NSMT Mo.71959	40.77°N, 143.52°E, 550 m, 27/07/1996, RV *Marusada-Maru*, MWT, tag 875	F	250	Tentacles missing; eyes damaged.
C. calyx	NSMT Mo.71960	40.77°N, 143.52°E, 550 m, 27/07/1996, RV *Marusada-Maru*, MWT, tag 876	M	95*	Tentacles missing; eyes damaged; tail damaged
C. calyx	NSMT Mo.72044	40.72°N, 143.18°E, 530 m, 10/07/1996, RV *Marusada-Maru*, MWT	F	60*	Tail damaged; tentacles missing; one eye in good condition.
C. calyx	NSMT Mo.75059	40.03°N, 143.46°E, 570 m, 13/07/1996, RV *Marusada-Maru*, MWT, tag 1004	M	193	Tentacles missing; eyes damaged.
C. calyx	NSMT Mo.72057	40.02°N, 143.84°E, 530 m, 13/07/1996, RV *Marusada-Maru*, MWT, tag 947	F	198*	Tentacles missing; eyes damaged; tail damaged.
C. calyx	NSMT Mo.71742	40.00°N, 145.54°E, 600 m, 14/07/1996, RV *Marusada-Maru*, MWT, tag 938	F	223*	Tentacles missing; eyes damaged; tail damaged; locking cartilages in good condition.
C. calyx	NSMT Mo.71743	40.00°N, 145.54°E, 600 m, 14/07/1996, RV *Marusada-Maru*, MWT, tag 939	M	210	Tentacles missing; eyes damaged.
C. calyx	NSMT Mo.75063	39.99°N, 143.54°E, 530 m, 14/07/1996, RV *Marusada-Maru*, MWT, tag 971	F	160*	Tentacles missing; eyes damaged; tail damaged.
C. calyx	NSMT Mo.75064	39.99°N, 143.54°E, 530 m, 14/07/1996, RV *Marusada-Maru*, MWT, tag 972	Indet.	100*	One tentacle attached; eyes damaged; tail damaged.
C. calyx	NSMT Mo.75062	39.99°N, 143.54°E, 530 m, 14/07/1996, RV *Marusada-Maru*, MWT, tag 970	M	124*	One tentacle attached; eyes damaged; tail damaged.

Appendix 1—Continued.

Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
C. calyx	NSMT Mo.71604	39.48°N, 142.36°E, 751 m, 18/10/1997, RV *Wakataka-Maru*, BTT, collected by D. Kitagawa	Indet.	245	Eyes damaged, eye photophores present; head separate from mantle; tentacle stalks without clubs; locking cartilages in good condition; viscera missing.
C. calyx	NSMT Mo.71602	38.48°N, 142.15°E, 600 m, 23/04/1997, RV *Tanshu-Maru*, BTT, collected by G. Shinohara	M	156	Mantle in good condition; eyes damaged; tentacle stalks without clubs.
C. calyx	NSMT Mo.71601	38.47°N, 142.02°E, 400 m, 23/04/1997, RV *Tanshu-Maru*, BTT, collected by G. Shinohara	F	123	Tentacles missing; eyes damaged but photophores present; skin in good condition.
C. calyx	NSMT Mo.71603	38.47°N, 142.23°E, 700 m, 23/04/1997, RV *Tanshu-Maru*, BTT, collected by G. Shinohara	F	180	Tentacles missing; eyes damaged; locking cartilages in good condition.
C. calyx	NSMT Mo.72208	38.46°N, 142.03°E, 400 m, 12/04/1996, RV *Tanshu-Maru*, BTT, collected by T. Hattori	M	120	Excellent condition overall; eyes slightly damaged; both tentacles attached; locking cartilages in good condition.
C. calyx	NSMT Mo.71605 **CHSQX024-16**	37.77°N, 142.22°E, 759 m, 24/10/1998, RV *Wakataka-Maru*, BTT	Indet.	LRL 5.98	Head, arm crown, and mantle lumen present; fins missing; tentacle stalks without clubs; beaks removed and with specimen.
C. calyx	NSMT Mo.60685	36.48°N, 141.84°E, 36.52°N, 141.84°E, 600 m, 05/10/1982, RV *Kaiyo-Maru*	M	265	Head nearly detached from mantle; mantle in good condition; skin in good condition; tentacle stalks without clubs.
C. calyx	NSMT Mo.60686	36.48°N, 141.84°E, 36.52°N, 141.84°E, 600 m, 05/10/1982, RV *Kaiyo-Maru*	2 F	150, 230	Larger specimen with mantle separated from head; one tentacle attached. Smaller specimen whole; tentacles missing; eyes damaged.

Appendix 1—Continued.

Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
C. calyx	NSMT Mo.85670	14/02/1977, WRI, collected by Satake	2 F	105, 95	Larger specimen with one complete tentacle attached, other tentacle missing club; eyes damaged; skin damaged. Smaller specimen with two tentacle stalks attached, clubs missing; eyes damaged; skin damaged.
C. calyx	NSMT Mo.85672	13/02/1977, WRI, collected by Satake	F	93	Good condition overall; tentacles missing.
C. picteti	NSMT Mo.85541 **CHSQX012-16**	37.13°N, 137.05°E, BTT, FV *Ise-Maru No.15*, collected by H. Nishizaki	M	260	Excellent overall condition; tentacles missing; both eyes damaged.
C. picteti	NSMT Mo.75898	36.85°N, 141.58°E, 628 m, 19/10/2001, RV *Wakataka-Maru*, BTT	Indet.	230	Tentacles missing; eyes damaged.
C. picteti	NSMT Mo.85064	35.94°N, 134.83°E, 250 m, 04/06/2009, RV *Tanshu-Maru*, BTT, collected by Kubodera & Umezawa	M	300*	Good overall condition; one tentacle present in excellent condition but not attached; locking cartilages degraded; eyes damaged; tail damaged.
C. picteti	NSMT Mo.85461	35.29°N, 139.53°E, 29/08/2008, FN, collected by Nagai Suisan Co.	M	160*	One tentacle attached; arms in good condition; one eye with photophores in good condition; tail damaged.
C. picteti	NSMT Mo.74659	35.17°N, 139.60°E, 22/04/2001, SN, collected by K. Yamada	M	115*	Tentacle stalks without clubs; eyes damaged; tail damaged.
C. picteti	NSMT Mo.74660	35.17°N, 139.60°E, 26/11/2000, SN, collected by K. Yamada	M	165*	Both tentacles attached; arms in good condition; eyes damaged; locking cartilages in good condition; tail damaged.

Appendix 1—Continued.

Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
C. picteti	NSMT Mo.76057	35.17°N, 132.01°E, 139 m, 07/06/2001, Shimane Prefectural Fisheries Experimental Station	Indet.	72*, 90*	Both specimens with tentacles attached; eyes damaged; tails damaged.
C. picteti	NSMT Mo.74658	35.13°N, 139.62°E, 11/03/2001, FV *Marutomo-Maru*, SN, collected by K. Yamada	F	150*	One tentacle attached; arms in good condition; eyes damaged; tail damaged.
C. picteti	NSMT Mo.63978	31.40°N, 134.82°E, 11/08/1977, RV *Hakuho-Maru*, 10 foot IKMT oblique 400 m	Indet.	79*	Good overall condition; both tentacles attached; eye photophores damaged; tail damaged.
C. picteti	NSMT Mo.75209	31.40°N, 128.50°E, 500 m, 08/11/2002, RV *Yoko-Maru*, BTT	M, Indet.	190*, 195*	Both specimens with arms without skin; eyes damaged; tails damaged. Male specimen in one piece; locking cartilages in good conditon. Other specimen with head and mantle separate; viscera missing.
C. picteti	NSMT Mo.75210	30.41°N, 128.23°E, 500 m, 10/11/2002, RV *Yoko-Maru*, BTT	M	185*	Tentacles missing; mantle in good condition; viscera intact; arms in good condition; eyes damaged; tail damaged.
C. picteti	NSMT Mo.68874	29.29°N, 127.48°E, 650 m, 11/09/1993, RV *Yoko-Maru*, BTT, collected by H. Horikawa	lot of 28	172–237	Four specimens head and arm crown only; nine specimens mantle only; 15 whole individuals. All specimens with tentacle stalks only without clubs; some arm tips damaged; some skin missing; eyes damaged; most tails damaged.
C. picteti	NSMT Mo.75375	29.01°N, 127.24°E, 500 m, 08/11/2003, RV *Yoko-Maru*, BTT, collected by T. Kubodera	Indet.	140*	Tentacles missing; head separate from mantle; one eye damaged, other eye missing; viscera missing; tail damaged.

Appendix 1—Continued.

Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
C. picteti	NSMT Mo.67352	15°N, 85°E, 17/02/1990, RV *Hakuho-Maru*, IKMT, Stn 20 KH89-2	Indet.	65*	Both tentacles attached and in good condition; mantle nearly detached from head; eyes damaged; fins damaged.
C. picteti	NSMT Mo.85669	Yron Island, Okinawa, 3/10/1983, BTT, collected by N. Tsunoda	M	285	Both tentacles present but not attached; head separate from mantle; beaks removed and not with specimen.
C. picteti	NSMT Mo.85702	off Misaki, Miura-shi, Kanagawa Prefecture, Sagami Bay	F	165*	Good overall condition; both tentacles attached; left eye in good condition; tail slightly damaged.
C. picteti	NSMT Mo.66668	Suruga Bay, Shizuoka Prefecture, 11/04/1975, Stn A-2	M	150*	Specimen gelatinous; tentacles missing; photophores on left eye in good condition, right eye damaged; fins detached from mantle.
C. picteti	NSMT Mo.67814	Hiratsuka, Sagami Bay, 17/04/1968, set-net	lot of 16	75–150	Some specimens with tentacles present; skin missing; some with eyes in good condition.
C. picteti	NSMT Mo.67815	Hiratsuka, Sagami Bay, 17/04/1968, set-net	lot of 16	85-145	Some specimens with tentacles present; skin missing; one specimen has a mantle only; some with eyes in good condition.
C. picteti	NSMT Mo.62574	Suruga Bay (stranded), 03/1982, collected by T. Ueno	F	129*	Good overall condition; tentacles missing; one fin damaged; locking cartilages in good condition; tail damaged.
C. picteti	NSMT Mo.74588	off Mito, Miura, Kanagawa Prefecture, 23/11/1998, FV *Kyoei-Maru*, FN	Indet.	120*	Good overall condition; tentacle stalks without clubs; fins in good condition; tail damaged.

Appendix 1—Continued.

Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
C. picteti	NSMT Mo.66669	off Shizuoka Prefecture, Suruga Bay, 17/03/1979	2 F	75*, 102	Both specimens missing tentacles; eyes damaged. Larger specimen with tail intact. Smaller specimen with damaged tail.
C. picteti	NSMT Mo.85665	off Shizuoka Prefecture, Suruga Bay, 3/6/1983, FV *Daikoku-maru*	F	78	Tentacles missing; arm tips damaged; eyes and eye photophores in good condition.
C. picteti	NSMT Mo.66672	Suruga Bay, Shizuoka Prefecture, 16/06/1982	Indet.	65*	Specimen appears stretched; tentacles missing; eyes damaged; tail damaged.
C. picteti	NSMT Mo.66899	Tagonoura, Suruga Bay, 29/05/1979, FV *Kyousei-Maru*	Indet.	65*	Specimen appears stretched; both tentacles attached; eyes damaged; tail damaged.
C. picteti	NSMT Mo.85673	no data	2 F 2 Indet.	175, 188; 63, 158,	Four specimens with eye photophores in good condition; tails in good condition. Two specimens with both tentacles attached. One specimen with one tentacle. Smallest specimen without tentacles.
C. picteti	NSMT Mo.85701	no data	M	160*	Excellent overall condition; both tentacles attached; eyes in good condition; tail slightly damaged.
C. spoeli	NSMT Mo.75900	39.93°N, 142.16°E, 13/10/2000, RV *Wakataka-Maru*, BTT	F	156*	Specimen slightly desiccated; tentacle stalks without clubs; eye photophores in good condition; tail damaged.
C. spoeli	NSMT Mo.85136	14.02°N, 136.91°E, 150 m, 23/07/1995, RV *Hakuho-Maru*, IKPT-2	Indet.	86*	Excellent overall condition; both tentacles attached; eyes in good condition; tail slightly damaged.
C. spoeli	NSMT Mo.85612	12.52°N, 141.59°E, 12.52°N, 141.30°E, 07/06/2013, RV *Kaiyo-Maru*, MWT	M	85*	Specimen appears to have three eyes; tentacles missing; tail damaged.

Appendix 1—Continued.

Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
C. spoeli	NSMT Mo.85668	7.8°N, 54.13°W, 775 m, 28/09/1981, JAMARC	F	*89	End of damaged gladius exposed; tail damaged; eyes damaged with some photophores present.
C. spoeli	NSMT Mo.85667	7.78°N, 54.13°W, 632 m, 30/09/1979, JAMARC	F	97*	Tentacles missing, eyes damaged.
C. spoeli	NSMT Mo.60797	off Surnum, 7° IV No.88, T4-32, JAMARC	M	106	Tentacle stalks without clubs; eyes damaged.
***C.* sp.**	NSMT Mo.85630	39.38°N, 144.22°E, 7400 m, 30/09/2001, RV *Hakuho-Maru*, BTT	Indet.	LRL 2.36	Head and arm crown only; single tentacle stalk without club, not attached; beaks removed and with specimen.
***C.* sp.**	NSMT Mo.85666	7.85°N, 54.3°W, 815 m, 27/04/1980, JAMARC	F	50	Tentacles missing; arms damaged; eyes damaged.
Grimalditeuthis					
G. bonplandi	NSMT Mo.66706	40.76°N, 144.05°E, 04/10/1988, 10 foot IKMT, 3000 m wire out, Stn SR75	Indet.	30*	Tentacles missing, arms in good condition; eyes damaged; both sets of fins missing; jelly-like substance present in mantle.
G. bonplandi	NSMT Mo.75258	39.03°N, 143.51°E, 550 m, 30/07/1996, MWT, RV *Marusada-Maru*	Indet.	105	Tentacles missing; beak removed; secondary fins missing.
G. bonplandi	NSMT Mo.85455	35.15°N, 132.39°E, 23/04/2008, FN, collected by Y. Yuki	F	97*	Tentacles missing; arms in good condition; eyes damaged; tail damaged; secondary fins missing.
G. bonplandi	NSMT Mo.85137	26.91°N, 141.94°E, 10/10/2007, BTT, collected by K. Yamaguchi	Indet.	40*	Tentacles missing, head in good condition, eyes damaged; tail damaged; secondary fins missing.
G. bonplandi	NSMT Mo.62607	4.43°S, 109.82°W, 100 m, 07/03/1980, sample No. EPA	Indet.	152*	Tentacles missing; arm tips in good condition; parts of viscera missing; eyes damaged; tail damaged; secondary fins missing.

Appendix 1—Continued.

Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
G. bonplandi	NSMT Mo.85692	7.75°S, 89.24°E, 02/08/1975, FV *Shonan-maru*, collected by K. Fujita, sample No. CI4	Indet.	50*	Tentacles missing; arms slightly damaged; viscera missing; eyes damaged; tail damaged; secondary fins missing.
G. bonplandi	NSMT Mo.85694	7.86°S, 88.04°E, 03/08/1975, FV *Shonan-maru*, collected by K. Fujita, sample No.92-1	Indet.	65*	Mantle and primary fin separate from head; secondary fins missing; tentacles missing; tail damaged; secondary fins missing.
G. bonplandi	NSMT Mo.85696	9.25°S, 83.75°E, 28/07/1975, FV *Shonan-maru*, collected by K. Fujita, sample No. CI 165	Indet.	90*	Tentacles missing; eyes slightly damaged; tail damaged; primary fins damaged; secondary fins missing.
G. bonplandi	NSMT Mo.85693	9.25°S, 83.75°E, 28/07/1975, FV *Shonan-maru*, collected by K. Fujita, sample No. CI 53	Indet.	70*	Tentacles missing; eyes slightly damaged; tail damaged; secondary fins missing.
G. bonplandi	NSMT Mo.85698	9.30°S, 55.88°E, 23/10/1975, FV *Shonan-maru*, collected by K. Fujita, sample No. WI.164	Indet.	96*	Tentacles missing; tail damaged; secondary fins missing.
G. bonplandi	NSMT Mo.85695	9.35°S, 81.7°E, 06/08/1975, FV *Shonan-maru*, collected by K. Fujita, sample No. CI 111	Indet.	75*	Tentacles missing; eyes slightly damaged; tail damaged; secondary fins missing.
G. bonplandi	NSMT Mo.85697	10.03°S, 53.43°E, 17/10/1975, FV *Shonan-maru*, collected by K. Fujita, sample No. WI 154	Indet.	78*	Specimen slightly desiccated but rehydrated; tentacles missing; eyes damaged; tail damaged; secondary fins missing.
G. bonplandi	NSMT Mo.85699	11.27°S, 109.49°E, 25/12/1975, FV *Shonan-maru*, collected by K. Fujita, sample No. EI 75	Indet.	140*	Tentacles missing; most arm tips intact; eyes damaged; tail damaged; secondary fins missing.

Appendix 1—Continued.

Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
G. bonplandi	NSMT Mo.67350	27/07/1978, RV *Soyo-Maru*, Stn A17	M	91*	Tentacles missing; arms and head in good condition; gladius broken; tail damaged; secondary fins missing.
G. bonplandi	NSMT Mo.62608	Off Peru, Tuna stomach content, 10/1979–03/1980, sample No. EPZ-3	M	90*	Tentacles missing; arm tips in good condition; eyes slightly damaged; tail damaged; secondary fins missing.
Planctoteuthis					
Pl. danae	NSMT Mo.61912	1.45°S, 95.68°W, 100 m, 26/01/1981, JAMARC, collected by Shirasawa	F	82*	Both tentacles attached; arm tips damaged; most skin missing; eyes damaged; tail damaged.
Pl. danae	NSMT Mo.61911	1.70°S, 95.99°W, 100 m, 23/01/1981, JAMARC, collected by Shirasawa	2 Indet.	65*, 65*	One specimen has mantle and fins only; tail damaged. Other specimen whole; one tentacle attached; arms damaged; locking cartilages in good condition; eyes damaged; tail damaged.
Joubiniteuthidae					
Joubiniteuthis					
J. portieri	NSMT Mo.62576	Suruga Bay, stranded on Okitsu beach, lancet fish stomach content, 02/05/1977, sample No. 194	F	81 +135mm tail	Excellent overall condition; both tentacles present but detached; arms in good condition; eyes slightly damaged; tail intact.
Mastigoteuthidae					
Idioteuthis					
I.* cf. *latipinna	NSMT Mo.75595 **CHSQX023-16**	28.09°N, 128.92°E, 750 m, 14/05/2005, RV *Hakuho-Maru*, 4 m BTT, collected by T. Kubodera	F	193	Excellent overall condition; tentacles present; arm and tentacle suckers intact; most skin missing.
I.* cf. *latipinna	NSMT Mo.71698	33.14°N, 133.64°E, 600 m, 16/06/1999, RV *Kotaka-Maru*, BTT, collected by H. Saito	M	234	Right tentacle attached, left tentacle missing; most skin missing, some skin with tubercles present; eyes slightly damaged; mature male (terminal organ with spermatophores).

Appendix 1—Continued.

Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
I.* cf. *latipinna	NSMT Mo.68849	29.39°N, 127.44°E, 400 m, 03/10/1997, RV *Yoko-Maru*, BTT, collected by T. Kubodera	Indet.	102	Tentacles missing; most skin missing; eyes damaged; locking cartilages in good condition; immature.
I.* cf. *latipinna	NSMT Mo.68848	29.35°N, 127.47°E, 500 m, 04/10/1997, RV *Yoko-Maru*, BTT collected by T. Kubodera	F	115	Tentacles missing; most skin missing; eyes slightly damaged; locking cartilages in good condition; immature.
I.* cf. *latipinna	NSMT Mo.85565	26.35°N, 126.82°E, 612 m, 22/09/2013, deep-sea water intake pit, collected by Okinawa Prefectural Deep Sea Water Research Centre	Indet.	CL 410	Tentacle only from a 295* mm ML specimen, 2.714kg.
I.* cf. *latipinna	NSMT Mo.68899	off Tokunoshima Island, Kagoshima Prefecture, East China Sea, 08/08/1996, RV *Yoko-Maru*, collected by Y. Horikawa	M	102	Tentacles missing; skin missing; beaks removed and not with specimen; eyes damaged.
I.* cf. *latipinna	NSMT Mo.75482	8.10°N, 108.42°E, 546 m, 04/05/2005, BTT	F	277	Tentacles missing; most skin missing; eyes slightly damaged, immature.
Magnoteuthis					
Mg. magna	NSMT Mo.85675	7.85°N, 54.28°W, 810 m, 18/6/1980, JAMARC, BTT	F, Indet.	105*, 84	Both specimens missing tentacles; most skin missing; arm tips damaged. Larger specimen with damaged tail.
Mg. magna	NSMT Mo.85674	7.85°N, 54.25°W, 810 m, 21/06/1980, JAMARC, BTT	F, M	100*, 90*	Both specimens missing tentacles; most skin missing; arm tips damaged; tails damaged.
Mastigoteuthis					
Mt. agassizii	NSMT Mo.85359	63.75°N, 54.02°W, 1310 m, 07/05/1989, FV *Shinkai-Maru*, Trawl, collected by H. Saito	F	82*	Tentacles missing; some skin present; arm tips missing; tail damaged.

Appendix 1—Continued.

Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
Mt. agassizii	NSMT Mo.76281	24.98°N, 84.98°W, 3384 m, 08/12/1989, RV *Hakuho-Maru*, 10 foot IKMT, collected by T. Kubodera	2 Indet.	45, 63	Both specimens with tentacles missing; some skin present; tails intact.
Mt. agassizii	NSMT Mo.85679	7.87°N, 54.28°W, 825 m, 1/8/1979, JAMARC, BTT	F	95*	Specimen in three pieces (fins, mantle, and arm crown); tentacles missing; most skin missing; tail damaged.
Mt.* cf. *dentata	NSMT Mo.72043	41.20°N, 146.53°E, 700 m, 09/07/1996, RV *Marusada-Maru*, MWT	F	40*	Mantle only.
Mt.* cf. *dentata	NSMT Mo.66704	40.76°N, 144.05°E, 04/10/1988, 10 foot IKMT 3000 m wire out, Stn SR75	Indet.	58	One tentacle present, not attached; other tentacle missing; nearly all skin missing; ends of Arms IV damaged; eyes intact; tail damaged.
Mt.* cf. *dentata	NSMT Mo.75118	39.28°N, 143.83°E, 4800 m, 27/09/2001, RV *Hakuho-Maru*, 4 m BTT, collected by T. Kubodera	F	40*	Tentacles missing; arms damaged; most skin missing; fins missing.
Mt.* cf. *dentata	NSMT Mo.75349	39.00°N, 143.50°E, 650 m, 30/07/1996, RV *Marusada-Maru*, MWT	Indet.	77*	Mixed lot with three *Mr. pyrodes*. Tentacles missing; mantle lumen only with head and arm crown; fins missing; most skin missing; locking cartilages in good condition.
Mt.* cf. *dentata	NSMT Mo.85502	38.39°N, 142.53°E, 1200 m, 26/10/2007, RV *Wakataka-Maru*	F	62*	Tentacles missing; arm tips damaged; most skin missing; tail damaged.
Mt.* cf. *dentata	NSMT Mo.75901	38.02°N, 142.69°E, 2000 m, 13/06/2000, RV *Wakataka-Maru*, trawl, Stn 25	F	115*	Tentacles missing; arm tips damaged; most skin missing; beak removed and not with specimen; eyes damaged; fins separate from mantle; tail damaged.

Appendix 1—Continued.

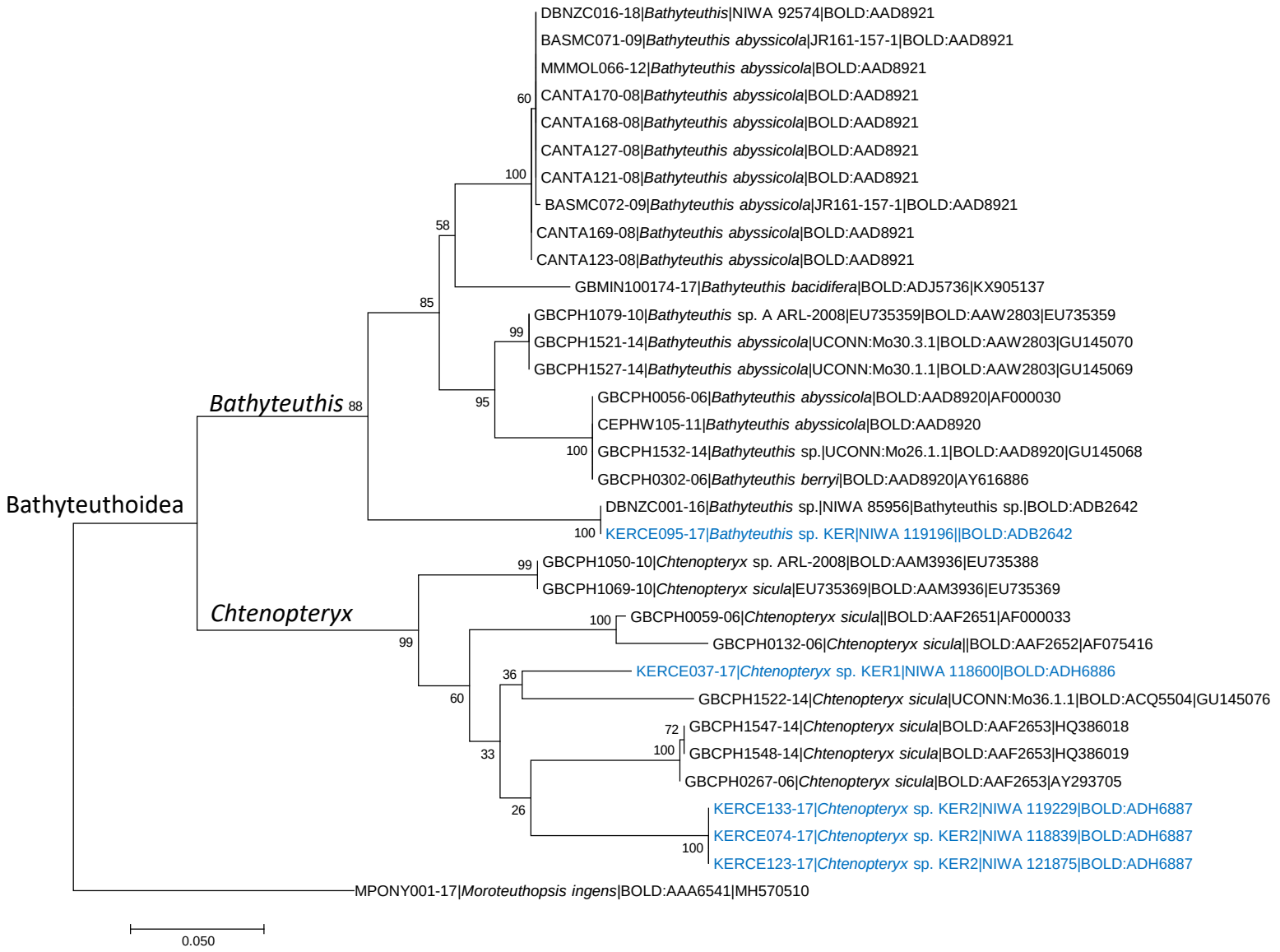
Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
Mt.* cf. *dentata	NSMT Mo.71734	37.81°N, 142.21°E, 763 m, 24/10/1999, RV *Wakataka-Maru*, BTT	Indet.	63	Tentacles missing; arms damaged; most skin missing; eyes damaged; tail intact.
Mt.* cf. *dentata	NSMT Mo.75903	37.72°N, 142.12°E, 604 m, 08/06/2000, RV *Wakataka-Maru*, trawl, Stn 6	Indet.	60*	Specimen partially desiccated; tentacles missing; most skin missing; eyes damaged; tail damaged.
Mt.* cf. *dentata	NSMT Mo.85458	36.86°N, 141.80°E, 1200 m, 31/10/2007, RV *Wakataka-Maru*, BTT	M	103*	Tentacles missing; most arms damaged; most skin missing; left eye in good condition, right eye missing; tail damaged.
Mt.* cf. *dentata	NSMT Mo.76170	36.60°N, 141.60°E, 1478 m, 15/11/2006, RV *Wakataka-Maru*, BTT	F	90*	Tentacles missing; arm tips damaged; some skin present; tail damaged.
Mt.* cf. *dentata	NSMT Mo.66702	34.82°N, 140.12°E, 06/07/1977, RV *Hakuho-Maru*, 10 foot IKMT 3000 m wire out	F	94*	One tentacle present but not attached, other tentacle missing; nearly all skin missing; arm tips damaged; tail damaged.
Mt.* cf. *dentata	NSMT Mo.66707	33.66°N, 137.17°E, 29/10/1988, Stn 27, 10 foot IKMT 3000 m wire out	F	86*	Both tentacles attached; arm tips slightly damaged; most skin missing; left eye in good condition, right eye slightly damaged.
Mt.* cf. *dentata	NSMT Mo.76279	31.03°N, 133.07°E, 800 m, 28/10/1988, RV *Hakuho-Maru*, IKMT with EMPS, collected by T. Kubodera	Indet.	118	Both tentacles missing; skin on mantle and arms in good condition; skin missing from fins.
Mt.* cf. *dentata	NSMT Mo.85681	1235 m, 5/7/1967, RV *Soyo-Maru*, BMT, Stn B2, collected by T. Kubodera	M	102*	Both tentacles present but not attached; skin missing; tail damaged; mature.
Mt.* cf. *dentata	NSMT Mo.85682	16/06/1974, RV *Kaiyo-Maru*, IKMT, Stn E, collected by T. Kubodera	F	98*	Tentacles missing; most skin missing; locking cartilages in good condition; tail damaged.

Appendix 1—Continued.

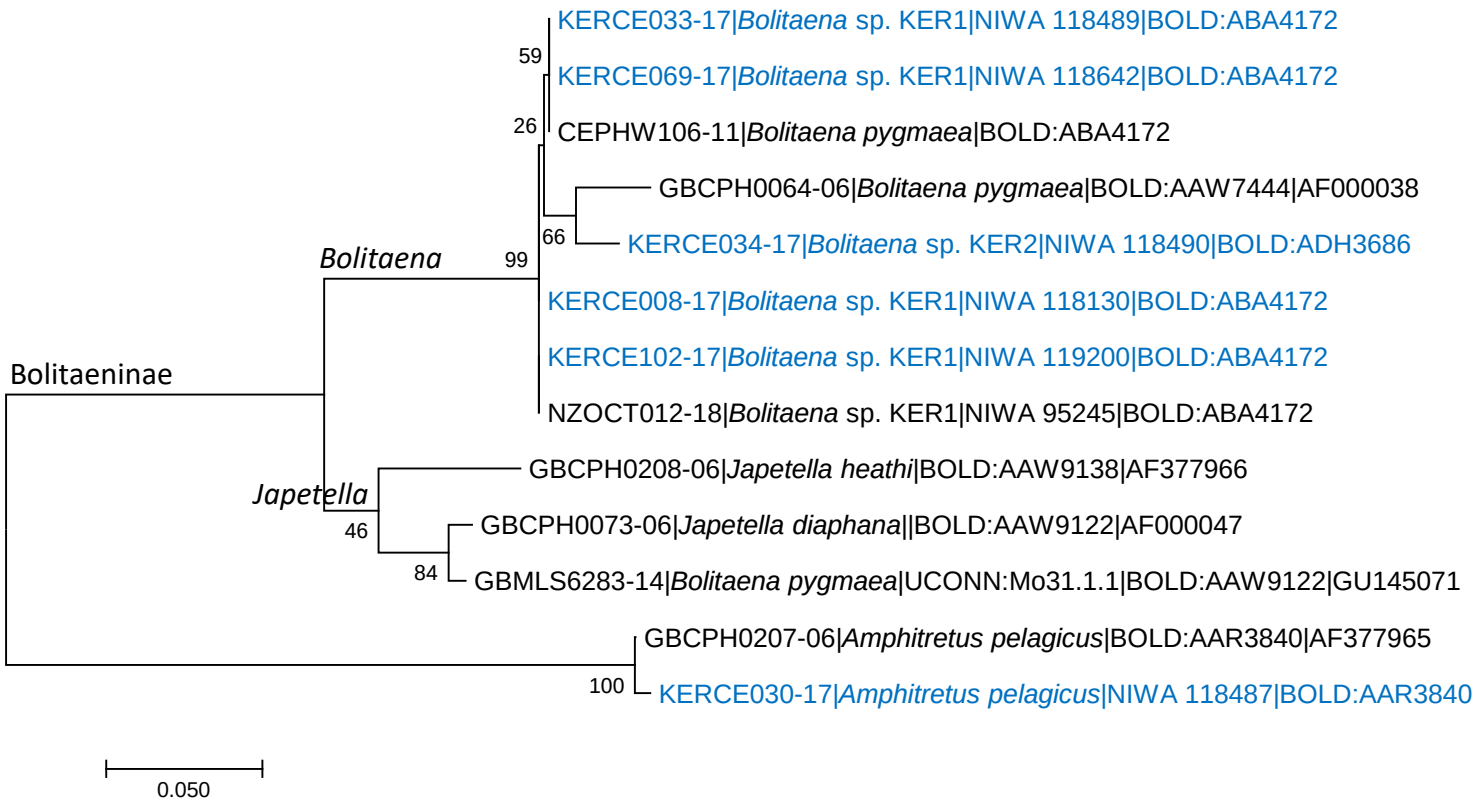
Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
Mt.* cf. *dentata	NSMT Mo.76260	1000–2000 m, 16/06/1979, RV *Kaiyo-Maru*, MWT, Stn K80F025, 79-K25	M	97*	Tentacles missing; skin missing; arm tips damaged; tail damaged.
Mt.* cf. *dentata	NSMT Mo.85680	Southeast Asian seas, 2000 m, 05–08/1972, RV *Hakuho-Maru*, KH72-1, Stn H207-2	F	97*	One tentacle attached, other tentacle present but not attached; some skin present; fins separate from mantle; tail damaged.
Mt.* cf. *dentata	NSMT Mo.85676	1000 m, 21/05/1973, RV *Kaiyo-Maru*, IKMT, Stn A	M, Indet.	76, 30	Mixed lot with *Vampyroteuthis infernalis* and *Pyroteuthis* sp. Both specimens with most skin missing. Smaller specimen without tentacles. Larger specimen with both tentacles present but not attached.
Mt.* cf. *dentata	NSMT Mo.62631	27/05/1973, RV *Kaiyo-Maru*, IKMT, Stn 45	F, M, 2 Indet.	95*, 46*, 55, 47	Female with two intact tentacles attached; most skin missing; eyes damaged; tail damaged. Male specimen mantle lumen, head, and arm crown only; fins missing. Two other specimens with nearly all skin missing, arm tips damaged; tails intact. One small, detached tentacle present.
Mt.* cf. *dentata	NSMT Mo.85451	22/11/1996, RV *Tansei-Maru*, Stn 3 day-1, IKPT-Deep	Indet.	39	Tentacles missing; tips of Arms IV damaged; most skin missing.
Mt.* cf. *dentata	NSMT Mo.76290	no data	M	86*	One tentacle present but not attached; skin missing; tail damaged.

Appendix 1—Continued.

Identification	Specimen ID	Collection data	Sex	ML (mm)	Notes on condition
Mastigotragus					
Mr. pyrodes	NSMT Mo.75349	39.00°N, 143.50°E, 650 m, 30/07/1996, RV *Marusada-Maru*, MWT	1M, 2 Indet.	M 102*, 83*, 88*	Mixed lot with one *Mt. agassizii* complex present. All specimens missing tentacles; some skin missing; arm tips damaged; eyes damaged; tails damaged.
Mr. pyrodes	NSMT Mo.75348	38.97°N, 143.49°E, 600 m, 30/07/1996, RV *Marusada-Maru*, MTW	F	84*	Tentacles missing; some skin damaged; eyes damaged; tail damaged.
Mr. pyrodes	NSMT Mo.71606 **CHSQX027-16**	37.72°N, 141.75°E, 607 m, 16/04/1998, RV *Wakataka-Maru*, BTT, collected by D. Kitagawa	M	172	Tentacles missing; skin damaged; locking cartilages in good condition; tail intact; mature.
Mr. pyrodes	NSMT Mo.71732	37.70°N, 142.17°E, 652 m, 24/10/1999, RV *Wakataka-Maru*, BTT, collected by D. Kitagawa	M	113	Tentacle stalks only, missing clubs; skin in good condition; eyes damaged; tail intact.
Mr. pyrodes	NSMT Mo.60684	36.48°N, 141.84°E, 36.52°N, 141.84°E, 600 m, 05/10/1982, RV *Kaiyo-Maru*	F	124	Tentacles missing; some skin present; tail intact.
Mastigoteuthidae sp.	NSMT Mo.75902	38.49°N, 142.36°E, 900 m, 13/10/2001, RV *Wakataka-Maru*, BTT	F	83*	Tentacles missing; skin missing; arm tips damaged; fins separate from mantle; eyes damaged; beaks removed and not with specimen; tail damaged.
Promachoteuthidae					
Promachoteuthis					
Pr. megaptera	NSMT Mo.61214	30.00°N, 147.00°E, 2750 m, 31/05/1980–01/06/1980, RV *Kaiyo-Maru*	F	34	Good overall condition; both tentacles attached.

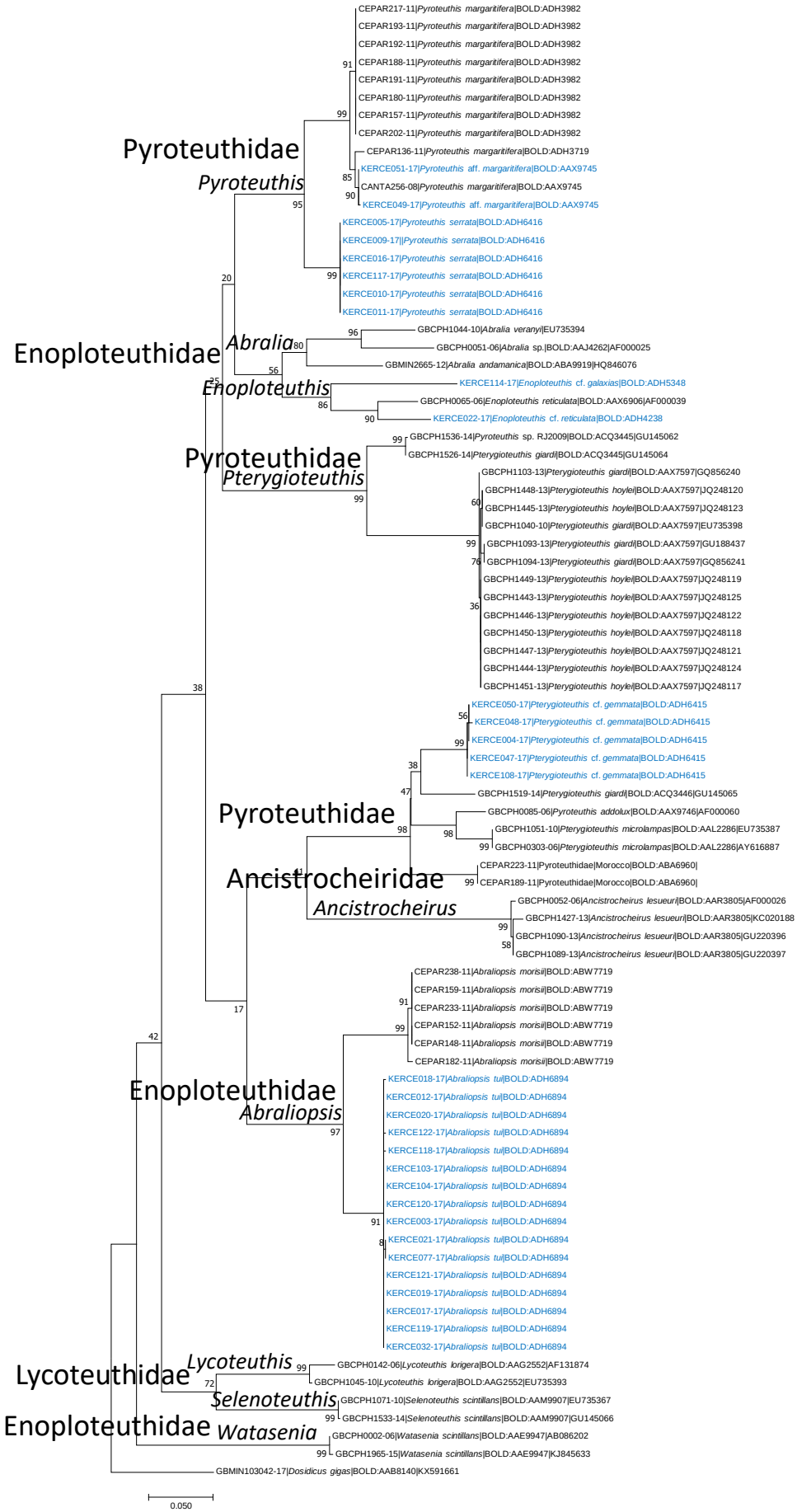


Appendix 2—Maximum-likelihood phylogeny for the order Bathyteuthoidea, with *Moroteuthopsis ingens* as the outgroup, with all presently available sequences of 658 bp region of cytochrome *c* oxidase subunit I (COI), based on 1000 bootstrap replicates. Each specimen is labelled with the following information, separated by pipe characters: BOLD ID, the original specimen identification, the museum ID (if present), the Barcode Index Number (BIN) beginning with 'BOLD:', and the GenBank ID (if present). Specimens from the Kermadec region are in blue.



Appendix 3—Maximum-likelihood phylogeny for the subfamily Bolitaeninae, with *Amphitretus pelagicus* as the outgroup, with all presently available sequences of 658 bp region of cytochrome *c* oxidase subunit I (COI), based on 1000 bootstrap replicates. Each specimen is labelled with the following information, separated by pipe characters: BOLD ID, the original specimen identification, the museum ID (if present), the Barcode Index Number (BIN) beginning with 'BOLD:', and the GenBank ID (if present). Specimens from the Kermadec region are in blue.

Appendix 4—Maximum-likelihood phylogeny for the family Octopodidae, with *Argonauta nodosa* as the outgroup, with all presently available sequences of 658 bp region of cytochrome *c* oxidase subunit I (COI), based on 1000 bootstrap replicates. Each specimen is labelled with the following information, separated by pipe characters: BOLD ID, the original specimen identification, the museum ID (if present), the Barcode Index Number (BIN) beginning with 'BOLD:', and the GenBank ID (if present). Specimens sequenced from the Kermadec region are in blue.



Appendix 5—Maximum-likelihood phylogeny for the enoploteuthid families, with *Dosidicus gigas* as the outgroup, with all presently available sequences of 658 bp region of cytochrome *c* oxidase subunit I (COI), based on 1000 bootstrap replicates. Each specimen is labelled with the following information, separated by pipe characters: BOLD ID, the original specimen identification, the museum ID (if present), the Barcode Index Number (BIN) beginning with 'BOLD:', and the GenBank ID (if present). Specimens from the Kermadec region are in blue.