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Biology and Environment: Proceedings of the Royal Irish Academy, Volume 123B, Number 1, 2023, pp. 1-12 (Article)

Published by Royal Irish Academy
DOI: *https://doi.org/10.1353/bae.2023.0000*

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Cite as follows: Taite, M., Dillon, L., Strugnell, J.M., Drewery, J. and Allcock, A.L. 2023 DNA barcoding reveals unexpected diversity of deep-sea octopuses in the North-east Atlantic. *Biology and Environment: Proceedings of the Royal Irish Academy* 123B (1).

Received 7 February 2022. Accepted 31 January 2023. Published 24 April 2023.

ABSTRACT

The taxonomy of *Bathypolypus* and *Muusoctopus* has long been confounded by poor original descriptions and difficulty in distinguishing among species morphologically. We aimed to use DNA barcoding in conjunction with species delimitation techniques and morphological identification of mature males to identify the species of *Bathypolypus* and *Muusoctopus* present in the North-east Atlantic and provide additional information on species distributions. From 298 specimens collected during biannual Deepwater Timeseries cruises and other aligned surveys undertaken by Marine Scotland onboard MRV Scotia between 2005–19, we identified *Bathypolypus arcticus, B. ergasticus, B. bairdii, B. sponsalis, B. pugniger, Muusoctopus normani* and *M. johnsonianus* as well as an unidentified *Muusoctopus* species that we conclude is likely to be a new species. We show the utility of DNA barcoding in identifying difficult to distinguish species such as deep-sea octopuses. Studies like ours are essential to provide clarity on the taxonomy of such groups and to determine the true diversity and distribution of species within them.

INTRODUCTION

Deep-sea benthic cephalopods are dominated by octopuses (Allcock *et al.* 2006). Difficulties in the sampling of deep-sea octopods has been one of the main factors in our lack of knowledge about their diversity, distribution, ecology and biology. In recent years, with the extension of commercial fishing into the deep sea and the advent of new technology such as Remotely Operated Vehicles (ROVs) there has been more opportunity to sample and observe deep-sea cephalopods (Drazen *et al.* 2003; Bush *et al.* 2012; Vecchione 2019). The extension of commercial fishing has also led governmental bodies to survey new and current areas to ensure that fishing is sustainable, and that fragile habitats are preserved. This has provided the opportunity for further scientific sampling of these areas (Eerkes-Medrano *et al.* 2020) and has resulted in new biological material, which has highlighted our lack of knowledge of these faunal communities.

North-east Atlantic incirrate (common) octopods include species within the genera *Bathypolypus* Grimpe, 1921, *Muusoctopus* Gleadall, 2004 and *Graneledone* Joubin, 1918, each of which represents a separate family (Bathypolypodidae, Enteroctopodidae and Megaleledonidae respectively) following Strugnell *et al.* (2014), which revised the long-standing classification of Voss (1988a). *Graneledone* is well defined, notably by its characteristic tubercles or warts (Allcock *et al.* 2003) and a single species, *Graneledone verrucosa*, occurs in the North Atlantic. It is not considered further herein. *Bathypolypus* and *Muusoctopus* (formerly *Benthoctopus*) have a more complex taxonomic history (Voss and Pearcy 1990; Muus 2002; Gleadall 2004; Strugnell *et al.* 2009, 2011; Gleadall *et al.* 2010). In 1921, Grimpe erected the genera *Bathypolypus* and *Benthoctopus* to accommodate species of octopus without an ink sac. Since then many studies have placed species into different genera (Voss and Pearcy 1990; Muus 2002), and have incorrectly created new species (Verrill 1879; Russel 1909; Robson 1927; Muus 1962), while the subfamilial (Robson 1927; Robson 1932; Thiele 1935; Voss 1988a,b) and familial (Strugnell *et al.* 2014) placements of these genera have also changed.

It is now well established that *Bathypolypus* is differentiated from *Muusoctopus* by having a laminated ligula on the male hectocotylised arm, shorter arms and distinguishable skin morphology, while *Muusoctopus* species have a smooth ligula, longer arms and smooth skin (Muus 2002; Voss 1988a,b; Allcock *et al.* 2006; Gleadall *et al.* 2010; Jereb *et al.* 2016).

Voss and Pearcy (1990) suspected the type specimen of *Benthoctopus*—*Octopus piscatorum* Verrill, 1879—belonged to the genus *Bathypolypus*. That claim was confirmed by Muus (2002) in a thorough review of deep-water incirrate octopods in the North-east Atlantic; Muus identified *Octopus piscatorum* as a junior synonym of *Bathypolypus bairdii*, thus rendering the genus *Benthoctopus* invalid. Voss and Pearcy (1990) and Muus (2002) argued for designation of a new type species and consequent

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retention of the name *Benthoctopus* in support of taxonomic and nomenclatural stability. Muus (2002) suggested that *Octopus januarii* Hoyle, 1885, known from the western Atlantic slope, would make a suitable type species of *Benthoctopus* as it had been revised and thoroughly redescribed by Toll (1981) and confirmed the description of *Benthoctopus* put forward by Voss and Pearcy (1990). Allcock *et al.* (2006) suggested *Polypus normani* Massy, 1907 from the North-east Atlantic slope would make a suitable type species as Massy had later synonymised *normani* with *Octopus piscatorum* (Massy, 1909) and also because the widely cited *Cephalopods of the world* (Nesis 1987) used Massy's *normani* drawing to illustrate the *Benthoctopus* ligula.

The new monotypic genus, *Muusoctopus,* was erected (Gleadall 2004), with *Octopus januarii* Hoyle, 1885 (Muus's preferred replacement type for *Benthoctopus*) as the type species. Gleadall did not include other species, reflecting the view of other authors (Norman *et al.* 1997; Voight 2002; Vecchione *et al.* 2009) that members of *Benthoctopus* were likely to be polyphyletic since they were united by mostly plesiomorphic characters. Nonetheless, this view is not reflected in molecular studies, for example the work of Strugnell *et al.* (2009) where eleven species of *Muusoctopus* form a well-supported clade, and subsequent papers by Ibáñez *et al.* (2016, 2020), so that *Muusoctopus* is now used for most species formerly included in *Benthoctopus* (e.g. WoRMS Editorial Board 2020, but cf. Jereb *et al.* 2016).

Muus (2002) produced a critical survey of all known North Atlantic *Bathypolypus,* and *Benthoctopus* species, identifying synonyms, misidentifications and taxonomic errors. He provided an updated generic diagnosis for *Bathypolypus* and identified five North-east Atlantic species: *Bathypolypus bairdii* (Verrill, 1873), *Bathypolypus arcticus* (Prosch, 1847), *Bathypolypus ergasticus* (Fischer and Fischer, 1892), *Bathypolypus sponsalis* (Fischer and Fischer, 1892), and *Bathypolypus pugniger* Muus, 2002. *Bathypolyus ergasticus* had previously been placed in *Benthoctopus*, but it has a distinctly laminated ligula. Muus's (2002) revisions, which included placing *Octopus piscatorum* in synonymy with *B. bairdii* and transferring *Benthoctopus ergasticus* to *Bathypolypus*, left no confirmed species of *Benthoctopus* in the North-east Atlantic.

Allcock *et al.* (2006) reassessed the collections of Collins *et al.* (2001), who had trawled extensively in British waters, considering Muus's findings and, while agreeing with all aspects of Muus's (2002) results, confirmed that specimens identified as *B. piscatorum* by Collins *et al.* (2001) matched the type specimens of *Polypus normani* and fitted the diagnosis of *Benthoctopus* (Muus, 2002: page 204). They further described specimens identified as *Benthoctopus* species A by Collins *et al.* (2001) as a new species *Benthoctopus johnsonianus*. Both are now recognised as *Muusoctopus* species. Therefore, according to Allcock *et al.* (2006), there are two *Muusoctopus* species in the North Atlantic, in addition to the five *Bathypolypus* species recognised by Muus (2002).

Throughout, many authors have recognised the difficulties of species identification in *Muusoctopus* and *Bathypolypus* (Voss and Pearcy 1990; Muus 2002; Allcock *et al.* 2006; Strugnell *et al.* 2009; Gleadall *et al.* 2010). Much emphasis has been placed on the morphology of the ligula and calamus of the hectocotylised arms of male specimens as many other counts and indices used in octopus taxonomy are plastic (Allcock *et al.* 2008). Muus (2002) identified characters of the hectocotylus that could clearly distinguish mature males of the five North-east Atlantic species of *Bathypolypus*, while Allcock *et al.* (2006) indicated how two sympatric North-east Atlantic *Muusoctopus* species could be discerned. Nonetheless, females and juveniles of these genera are very difficult to identify to species level, and here DNA barcoding can help.

In this paper we examine new collections of *Muusoctopus* and *Bathypolypus* from the North-east Atlantic, mainly collected during deep-water fisheries surveys over a twelve-year period. To avoid the problems of identification of juvenile and female specimens, we use DNA barcoding to identify species, which has previously been shown to be useful in separating octopod species (Allcock *et al.* 2011). Given the plasticity in morphology in octopuses and how few specimens have been examined, our aims were to determine whether the accepted number of species present is indeed correct, and to describe more fully the distribution of the species present.

METHODS

SAMPLE COLLECTION

All specimens were collected over the period 2008–19 (apart from one specimen collected in 2005) during the biannual Deepwater Timeseries cruise and other aligned surveys undertaken by Marine Scotland onboard the MRV Scotia. Deepwater Timeseries cruises are undertaken on eight downslope transects on the Malin-Hebrides slope between 55° and 59.5° N as well as on selected areas on the Rosemary Seamount. Complementary data were provided by irregular or one-off surveys to the Rockall Plateau, Wyville-Thompson Ridge, Ymir Ridge and the Faroe-Shetland Channel. On each of the Hebridean slope transects survey stations were undertaken at core depths of 500m, 1,000m, 1,500m, 1,800m and in later years to 2,000m depth. These were supplemented on an opportunistic basis with extra stations at intermediate depths. The surveys are fixed station in design and while generally the trawl is located very close to the same positions during each cruise, the practical difficulties of trawling on a slope and the influence

of slope currents as the trawl descends means that the actual position, and thus the depth of trawl settlement, varies slightly each time.

Trawls were conducted using a bottom trawl (model BT184, Jackson Trawls Ltd, Peterhead, UK) that incorporated a blinder of 20mm mesh inside the cod end. The net configuration included rock-hopper discs with a diameter of 53cm (2008 only) or 41cm (all other years), 1,700kg trawl doors, 100m sweeps and headline floats rated to 2,500m.

During trawls conducted on smooth ground, a small subsidiary net specialised for benthic capture and incorporating a 20mm blinder was attached below the central section of the main net just behind the rock-hoppers. Of the 316 specimens captured, 215 were caught in the main net and 101 were caught in the subsidiary net. The duration of each trawl varied with location. Trawls conducted on the well-characterised grounds of the Malin-Hebrides slope averaged 60 minutes, while those conducted in all other areas averaged 30 minutes due the uncertainty of seabed data and thus the higher risk of damaging the net. The associated specimen depth information presented here is that derived from the depth at the vessel position, however it is important to note that there may be a disparity between vessel position verses actual trawl position.

SPECIMEN IDENTIFICATION

Where possible, octopuses were identified to genus level at sea. Mature males tend to be easily identified to genus level on the basis of the morphology of the hectocotylus, the modification to the third right arm in males. Female and juveniles are harder to identify.

Tissue samples were either collected fresh or subsequently from frozen specimens. DNA was extracted from a sample of muscle tissue using a Purelink genomic DNA mini kit (Invitrogen) following the manufacturer's instructions. The Folmer region of the cytochrome oxidase c subunit I (COI) gene was amplified using primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer *et al.* 1994). Each PCR contained 12.5 µl of either GoTaq G2 Green Mastermix (Promega) or DreamTaq Green PCR Master Mix (Thermo Scientific), 0.5 µl of each primer (10 µM), 9 µl nuclease-free water (Promega or Thermo Scientific) and 2.5 µl DNA template. PCR conditions included a denaturation step of 94° for 2 mins, followed by 35 cycles of 94° for 40s, 50° for 40s and 72° for 90s. A final extension step of 72° for 10 mins completed each PCR. The size and quality of the PCR products were assessed by electrophoresis in 1.5% agarose gels stained with SYBR Safe DNA Gel Stain (Invitrogen). PCR products were purified using the PureLink PCR Purification Kit (Invitrogen) and sequenced by GATC Biotech (Constance, Germany) on a Sanger ABI 3730xl.

Sequences were aligned with Clustal W (Thompson *et al.* 1994) implemented in Ugene (Okonechnikov *et al.* 2012). There were no gaps or indels and no hard to align regions. This alignment containing COI with sequences from all 298 specimens was used to generate a haplotype network in TCS 1.21 (Clement *et al.* 2000) with the threshold of mutational changes set to 95%. Due to the marked differences between within- and among-species genetic variation in COI (the 'barcode gap'), statistical parsimony analysis applied to COI sequences tends to split the network into separate species networks. We included comparator sequences available on GenBank (Table 1). Specimens associated with GenBank sequences used for comparison were identified by either ALA, Mike Vecchione (Smithsonian Institute), or Dick Young (University of Hawaii). We further sequenced a specimen of *Bathypolypus pugniger* from the NW Atlantic identified by Mike Vecchione as an additional comparison (Table 1). Each network was assumed to represent a separate species, and, where possible, a species name was assigned to each network based on the inclusion in that network of a GenBank sequence, or based on the identification of mature males from within the samples by ALA, who has also examined relevant type material. In the genus *Bathypolypus,* the characteristics of mature males can easily distinguish between species

Table 1—Comparator specimens used in the final haplotype network analysis.

ID from this study	Identification given in GenBank for conspecific sequence	Accession number	Reference
Bathypolypus sponsalis	*Bathypolypus sponsalis*	EF016329	Allcock *et al.* 2006
Bathypolypus pugniger	*Bathypolypus pugniger*	OK489787	This study
Muusoctopus johnsonianus	*Benthoctopus johnsonianus*	EF016333	Allcock *et al.* 2006
	Benthoctopus johnsonianus	HM572172	Strugnell *et al.* 2011
Muusoctopus normani	*Benthoctopus normani*	EF016334	Allcock *et al.* 2006
	Benthoctopus normani	EF016335	Allcock *et al.* 2006

(see Muus 2002). Specifically, hectocotylised arm sucker counts separate *B. ergasticus* (70–85 suckers), *B. sponsalis* (50–65 suckers), from *B. arcticus, B. bairdii* and *B. pugniger* (all < 50 suckers). The number of laminae on the ligula of the hectocotylus then separates *B. pugniger* (4–6 laminae), *B. bairdii* (7–12 laminae), and *B. arcticus* (11–16 laminae). While an animal with fewer than 50 suckers on the hectocotylised arm and 11 or 12 laminae on the ligula could be either *B. arcticus* or *B. bairdii*, most mature males of the genus *Bathypolypus* can be easily identified to species level using this method. While, theoretically, combinations of the ratio of mantle length to total length, the number of gill lamellae, the presence of cirri over the eye, the presence of a crop diverticulum, and the shape of the funnel organ, can be used to distinguish females of these five species of *Bathypolypus* (see Muus 2002), identification using these characters is very difficult and potentially unreliable, and thus network identification was based on mature males only. The two recognised *Muusoctopus* species in the study area are more challenging to distinguish, although *M. johnsonianus* has a longer hectocotylised arm (>65% of length opposite arm versus <65% in *M. johnsonianus*) and more closely set suckers (Allcock *et al*. 2006), the latter being a character that can also be used to identify females if necessary, although we based network identification on mature males.

In the network figure, we coloured species using a colour-blind friendly colour ramp generated by the package *viridis* (Garnier *et al*. 2021) in R version 4.1.2 (R Core Team 2022). Phylogenetic trees of *Muusoctopus* and *Bathypolypus* were constructed using the unique haplotypes identified by TCS, the newly generated sequence of *Bathypolypus pugniger* (GenBank Accession Number OK489787), and a wider selection of comparator sequences of *Bathypolypus* and *Muusoctopus* from GenBank. *Bathypolypus* and *Muusoctopus* are not closely related (in different families) and, because the deep phylogeny of Octopoda is not well understood (e.g. Strugnell *et al*. 2014; Taite *et al*. 2023), it becomes necessary to root a tree including both genera with a cirrate octopod, which is a particularly distant, and therefore unsatisfactory, root. Thus, after an initial exploratory tree rooted on Cirrata to check genus assignments were correct (data not shown), we constructed a separate tree for each genus, rooting each on representatives of the other genus. The *Bathypolypus* tree was rooted with three *Muusoctopus* sequences from GenBank (EF01633 *M. johnsonianus*, FJ428012 *M. levis*, EF016334 *M. normani*); the *Muusoctopus* tree was rooted on two sequences of *Bathypolypus* from GenBank (AF000029 *B. arcticus,* EF016329 *B. sponsalis*). Maximum likelihood trees were generated in IQTree (Nguyen *et al*. 2015) using the -m MFP command, which calls ModelFinder (Kalyaanamoorthy *et al*. 2017) and then applies the best fit model, and 1,000 non-parametric bootstraps. Bayesian Inference Trees were generated in MrBayes v3.2.7 (Ronquist *et al*. 2012), with a default general time reversible model (since MrBayes has a limited selection of models and recent analyses show model selection has little influence on the outcomes when inferring evolutionary relationships; Abadi *et al*. 2019) with a proportion of invariant sites, two runs of four Markov Chain Monte Carlo (MCMC) for 1,000,000 generations, and tree sampling every 100 generations. Output was checked for stationarity in Tracer v.1.7.1 (Rambaut *et al*. 2018) and a burn-in of 25% applied.

In the figures, maximum likelihood trees are presented with nodes with less than 70% bootstrap support collapsed using TreeCollapserCL 4 (http://emmahodcroft.com/TreeCollapseCL.html). We do not present Bayesian trees separately but indicate posterior probability (PP) support for nodes in Bayesian analysis alongside the bootstrap support (BS) on the ML trees. Posterior probabilities are given to two decimal places, but rounded down, to avoid submaximal support appearing as maximal. To save space, outgroups are not included on figures.

We further conducted species delimitation analyses in ASAP **(**Assemble Species by Automatic Partitioning; Puillandre *et al*. 2021) using the same alignment files (one for *Bathypolypus*, one for *Muusoctopus*) as were used to build the phylogenetic trees. We applied the Kimura (K80) model and default settings. ASAP implements a hierarchical clustering algorithm using pairwise genetic distances and proposes several potential species partitions based on a ranked scoring system that accounts for the probability of proposed solutions actually having less diversity and the width of the barcode gap. We illustrate the 'best' solution (that with the lowest ASAP score) on the Maximum Likelihood trees, indicating species by name, but also by letter and colour as designated in the haplotype network analysis.

Finally, when all specimens were identified to the lowest possible taxon, we plotted the distributions of all *Muusoctopus* and *Bathypolypus* specimens collected over the twelve-year sampling campaign in QGIS 3.22.0 (QGIS Development Team 2020) to improve our knowledge on their distributions.

RESULTS

Our 298 specimens (GenBank Accession Numbers OK489470 - OK489786) formed eight haplotype networks (Fig. 1, A-H). Of the eight networks (Fig. 1), four were identified based on comparator sequences (Table 1) as representing *Muusoctopus normani* (G)*, Muusoctopus johnsonianus* (F)*, Bathypolypus sponsalis* (C) and *Bathypolypus pugniger* (D). The sequence of *Bathypolypus pugniger* from the

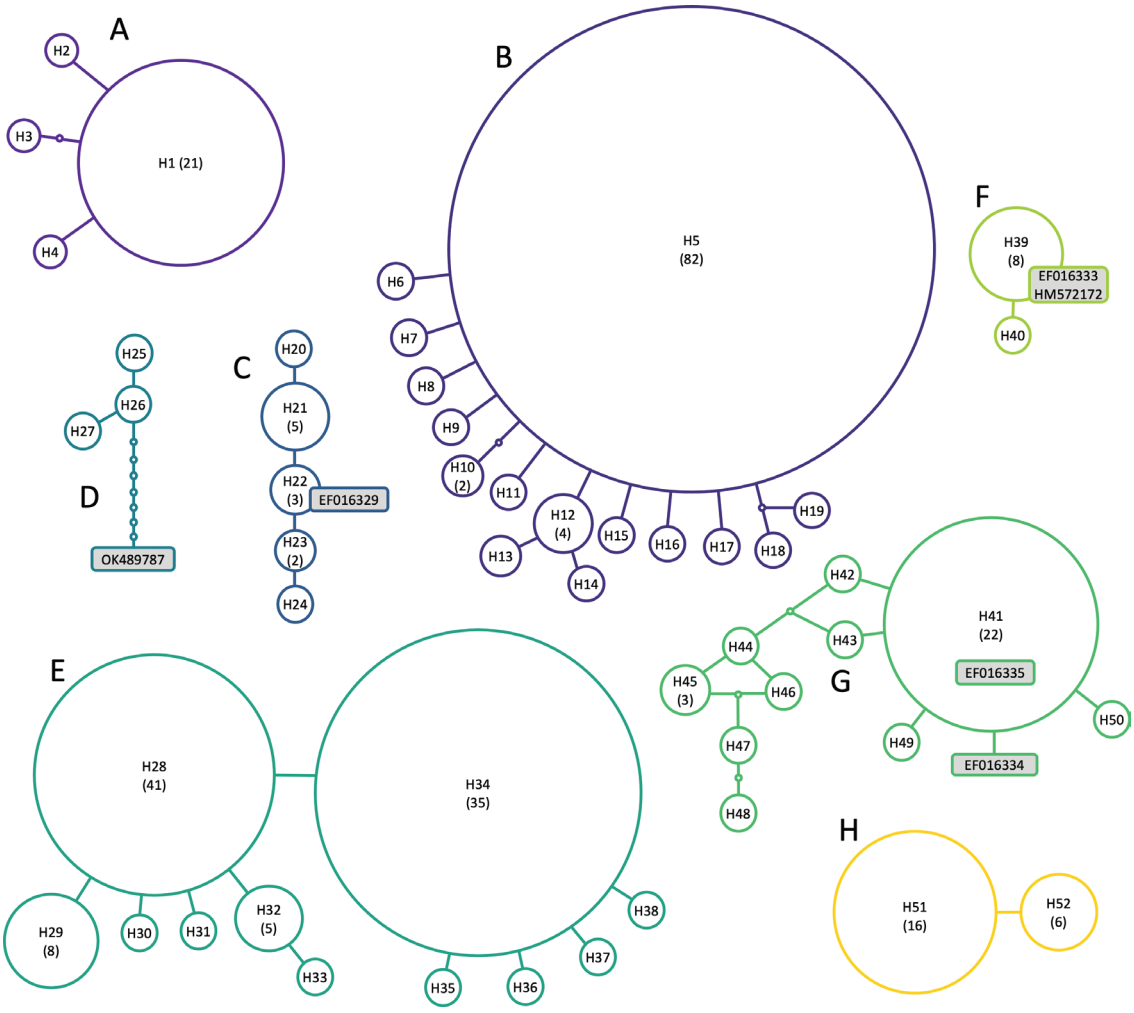

Fig. 1—Haplotype networks built using TCS 2.1 (Clement *et al.* 2000). Haplotype networks were labelled A-H and, within that, haplotypes were labelled H1 – H 52 for ease and direct comparison with phylogenetic trees. Networks: A, *Bathypolypus ergasticus*; B, *Bathypolypus arcticus*; C, *Bathypolypus sponsalis*; D, *Bathypolypus pugniger*; E, *Bathypolypus bairdii*; F, *Muusoctopus johnsonianus*; G, *Muusoctopus normani*; H, *Muusoctopus* sp. The numbers in parentheses represent the number of sequences i.e. specimens, in each haplotype. GenBank sequences shown in grey boxes.

North-west Atlantic was separated from our specimens by several steps in the network; unfortunately, no mature males were available for examination. Mature males were available in the samples for the first three species, and morphological identifications based on characters previously described supported the molecular conclusions. Three networks did not include comparator sequences from GenBank but the specimens on which they were based did include mature males in good condition and thus we were able to identify networks A, B and E as representing *Bathypolypus ergasticus, B. arcticus* and *B. bairdii* respectively based on hectocotylised arm sucker counts and ligula morphology. One haplotype network (H) in this analysis remained unidentified, and phylogenetic and species delimitation analysis (see below) confirmed that it represents a distinct species of *Muusoctopus.* Morphological examination (by ALA) could not associate these specimens to a known species of *Muusoctopus*, although fully mature males were not present in the available collection.

PHYLOGENETIC AND SPECIES DELIMITATION ANALYSES

The maximum likelihood tree of *Bathypolypus* (Fig. 2A) shows our specimens (as numbered haplotypes) in five distinct clades, as expected form the haplotype networks. Our four *Bathypolypus ergasticus* haplotypes (H01-H04) form a fully supported clade (BS = 100, PP = 1) and are distinguished as a separate species by ASAP. Specimens we identified

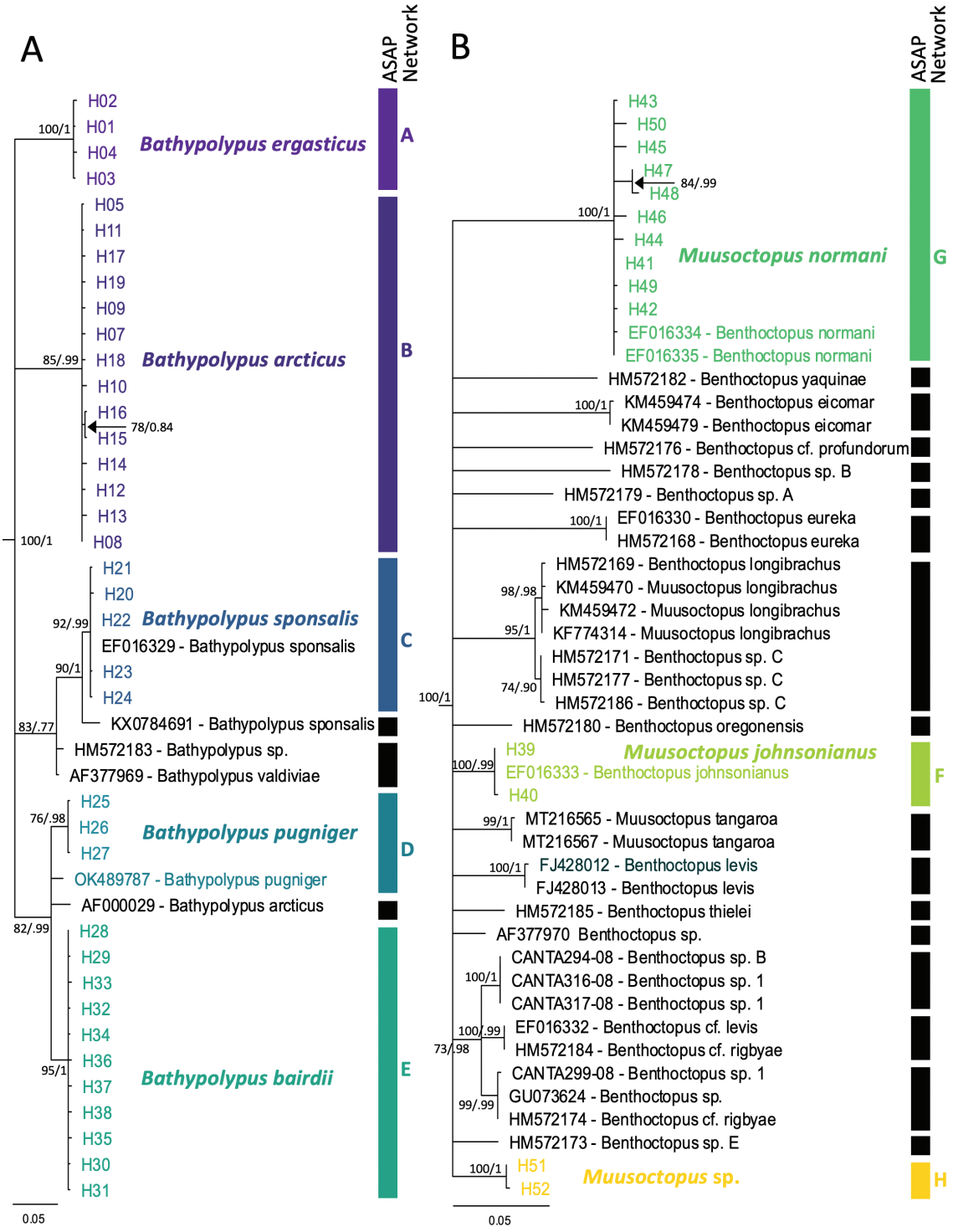

Fig. 2—Maximum likelihood trees depicting the phylogenetic relationships of (A) *Bathypolypus* specimens, rooted on *M. johnsonianus*, *M. normani* and *M. levis* (root not shown) and (B) *Muusoctopus* specimens rooted on *B. bairdii* and *B. sponsalis* (root not shown). Trees generated in IQTree. Bootstrap support indicated on nodes together with posterior probabilities from a separate Bayesian Inference analysis. Nodes with less than 70% bootstrap support collapsed. Names of GenBank specimens given as in GenBank. It is widely recognised that the correct genus name for all these *Benthoctopus* species is now *Muusoctopus*. Haplotypes labelled as in Figure 1. Species delimitation as determined by ASAP indicated as vertical bars, with the eight species from our samples also indicated with the network letter from Figure 1.

as *B. arcticus* (haplotypes H05-H19) form a highly supported clade (BS = 85, PP = 0.99) and are distinguished as a separate species by ASAP. *Bathypolypus sponsalis* forms a well-supported clade (BS = 90, PP = 1) including our specimens (haplotypes H20-H24) and the two available *B. sponsalis* sequences from GenBank. ASAP identifies GenBank sequence KX078469, which is from the Mediterranean (Fernando Fernández-Álvarez, pers. comm.), as a separate species. EF016329 is from the North-east Atlantic. The specimens we identified as *B. pugniger* (haplotypes H25-H27) in the haplotype network analysis do not form a monophyletic clade with the *B. pugniger* specimen from the NW Atlantic, but ASAP identifies these four termini as a single species. Our 11 *Bathypolypus bairdii* haplotypes (H28-H38), form a highly supported clade (BS = 95, PP = 1) and are supported as a single species by ASAP. ASAP identifies sequence AF000029 from GenBank, identified as *Bathypolypus arcticus*, as a separate species.

In the maximum likelihood tree of *Muusoctopus* (Fig. 2B), *M. normani* (represented by our haplotypes H41-H50 and two sequences from GenBank) forms a fully supported clade (BS =100, PP = 1) identified as a single species by ASAP. *Muusoctopus johnsonianus* (represented by our haplotypes H39-H40 and one sequence from GenBank) forms a highly supported clade (BS =100, PP = 0.99), also identified as a single species by ASAP. Our unidentified haplotypes (H51-H52; network H Fig. 1) form a separate lineage and are distinguished as a unique species by ASAP.

As expected in trees based on a single gene, deep relationships are not supported (Carlini and Graves 1999). An additional analysis (not shown) rooting the *Muusoctopus* tree on *Enteroctopus megalocyathus* (as a more closely related outgroup) did not resolve relationships more usefully.

Our statistical parsimony analysis (Fig. 1) and species delimitation analysis (Fig. 2) are completely congruent, indicating five species of *Bathypolypus* in our samples, which we identify as *B. ergasticus, B. arcticus, B. sponsalis, B. pugniger,* and *B. bairdii*, and three species of *Muusoctopus,* two of which we identify as *M. normani* and *M. johnsonianus.* We are unable to identify the third species of *Muusoctopus*, which may represent an as yet undescribed species.

DISCUSSION

The historically confused systematics of the genera *Muusoctopus* and *Bathypolypus* has led to issues with identification (Grimpe 1921; Massy 1909; Robson 1929; Robson 1932; Voss and Pearcy 1990), and a lack of knowledge of geographical distribution (Collins *et al.* 2001; Muus 2002; Ibáñez *et al.* 2016). Studies that focus on these aspects, as our study does, are required to further our knowledge of these understudied deep-sea octopods. Our study provides sequences for nine deep-sea benthic octopod species, four of which had not been sequenced for the DNA barcode gene COI prior to this study, and provides new information on the distribution of *Muusoctopus* and *Bathypolypus* in the North-east Atlantic.

BATHYPOLYPUS

Five *Bathypolypus* species are known to occur in the North-east Atlantic; *B. ergasticus*, *B. sponsalis, B. arcticus*, *B. bairdii* and *B. pugniger* (Table 2). All five were sampled in this study. Muus (2002) concluded that *B. arcticus* is confined to Norwegian Sea Deep Water (NSDW), which fills the Faroe-Shetland Channel and is then blocked by the Wyville Thomson Ridge, and described the new species *B. pugniger* based on specimens from further south. Collins *et al.* (2001) had previously noted two forms of *B. arcticus,* one restricted to the Faroe Shetland Channel, and one with a more southerly distribution. The latter probably represented what was subsequently described as *B. pugniger.* These distribution patterns agree with the distribution of our samples (Table 2, Fig. 3).

The sequence identified as *B. arcticus* on GenBank (Accession number AF000029 (Carlini and Graves 1999)) collected from the Gulf of Maine (Carlini and Graves 1999; Vecchione 2001) did not form a clade with specimens we identified as *B. arcticus*. *Bathypolypus arcticus*, as mentioned above, is confined to NSDW and was determined by Muus (2002) not to occur in the NW Atlantic. *Bathypolypus bairdii* has commonly been misidentified as *B. arcticus* (Kumpf 1958; Macalaster 1976; O'Dor and Macalaster 1983) particularly from specimens sampled in the western Atlantic (Muus 2002). Muus (2002) recognised these misidentifications as stemming from Kumpf's (1958) revision of *Bathypolypus* from the western Atlantic. Kumpf (1958) correctly concluded that all of the specimens he examined were conspecific, but there were no *arcticus* specimens in his American samples (as it does not occur there) and he applied the wrong name. Using the measurements of *B. arcticus* from Kumpf (1958) and Macalaster (1976), Muus (2002) concluded that all North-west Atlantic specimens are *B. bairdii,* and that *B. arcticus* does not occur there. GenBank sequence AF000029, submitted before Muus's (2002) revision, is thus possibly *B. bairdii*. This sequence forms a polytomy with our North-east Atlantic *B. bairdii* specimens, and *B. pugniger* (Fig. 2A), and was recovered as a unique species by the species delimitation analysis. This may suggest that the western Atlantic specimen (AF000029) and our eastern *B. bairdii* specimens have speciated or are in the process of speciating. *Bathypolypus bairdii* was described from a species collected in the Bay of Fundy,

Table 2—Study distribution versus known distribution of species sampled.

Species	Type locality	Distribution in current study	Known distribution
Bathypolypus bairdii (Verrill, 1873)	Bay of Fundy, Nova Scotia, Canada, North-west Atlantic Ocean	Malin-Hebrides slope into the upper Faroe Bank Channel, Ymir Ridge, Rosemary Bank and around the northern slope of Rockall Bank; 55°–60° N; 515–2,047m.	North Atlantic, cold water, off the coast of Newfoundland and USA, off the coast of Norway, Rockall Trough and Faroe-Shetland Channel in North-east Atlantic (Macalaster 1976, Muus 2002), 20m–2,700m (Collins *et al.* 2001; Muus 2002).
Bathypolypus ergasticus (P. Fischer and H. Fischer, 1892)	Sahara Banks, West Africa, North-east Atlantic Ocean	Malin slope, Rosemary Bank and western slope of the Rockall Bank; 55°–59° N; 840–1,259m.	North-east Atlantic, from the south-west of Ireland to Wyville Thomson ridge 510m–1,370m (Massy 1907; Collins *et al.* 2001; Muus 2002).
Bathypolypus sponsalis (P. Fischer and H. Fischer, 1892)	Sahara coast, West Africa, North-east Atlantic Ocean	Malin-Hebrides slope; 54°–58° N; 530–1,510m.	North-east Atlantic, 170m–1,250m, more commonly a Mediterranean species, type locality off the Cape Verde islands (P. Fischer and H. Fischer 1892; Collins *et al.* 2001; Muus 2002).
Bathypolypus arcticus (Prosch, 1849)	South-west Greenland, North Atlantic Ocean	North of the Wyville Thomson Ridge and eastern Faroe-Shetland Channel slope; 60°–61° N; 789–1,298m.	Arctic species – Greenland and northern Iceland, Norwegian deep-water in the Faroe-Shetland channel, 37–1,210m, shallower depths farther north (Muus 2002; Allcock *et al.* 2006).
Bathypolypus pugniger Muus, 2002	West Iceland, North Atlantic Ocean	Wyville Thomson Ridge and Faroe-Shetland Channel slope; 60° N; 524–740m.	Cold-water from Faroe-Shetland Channel and South-west Iceland, 200m–1,000m (Muus 2002).
Muusoctopus johnsonianus (Allcock, Strugnell, Ruggiero and Collins, 2006)	Southern boundary of the Porcupine Seabight, North-east Atlantic Ocean	Northern Rockall Trough and lower Malin-Hebrides slope; 54°–58° N; 1,496–2,020m.	North-east Atlantic, Irish and Scottish waters from 49°N – 59°N, 1,400m–2,520m (Collins *et al.* 2001; Allcock *et al.* 2006).
Muusoctopus normani (Massy, 1907)	South-west Ireland, north-west boundary of the Celtic Sea, North-east Atlantic Ocean.	Hebrides slope and western slope of Rockall Bank; 56°–58° N; 988–1,843m.	North-east Atlantic, Porcupine Seabight to Wyville Thomson ridge, 500m–1,800m (Massy 1907; Allcock *et al.* 2006).
Muusoctopus sp.		Northern Wyville Thomson Ridge and eastern Faroe-Shetland Channel slope; 60°–61° N; 704–1,198m.	N/A

Nova Scotia, Canada, North-west Atlantic Ocean (Table 2), which would indicate that the western specimen (AF000029) is the true *B. bairdii*.

The size of the ligula and number of laminae vary geographically in *B. bairdii* (Muus 2002). The total number of laminae ranges from 7–13 but a geographical variation is apparent along the western Atlantic from America to western Greenland. The mean number of laminae decreases with increased latitude northward along the western Atlantic from a mean

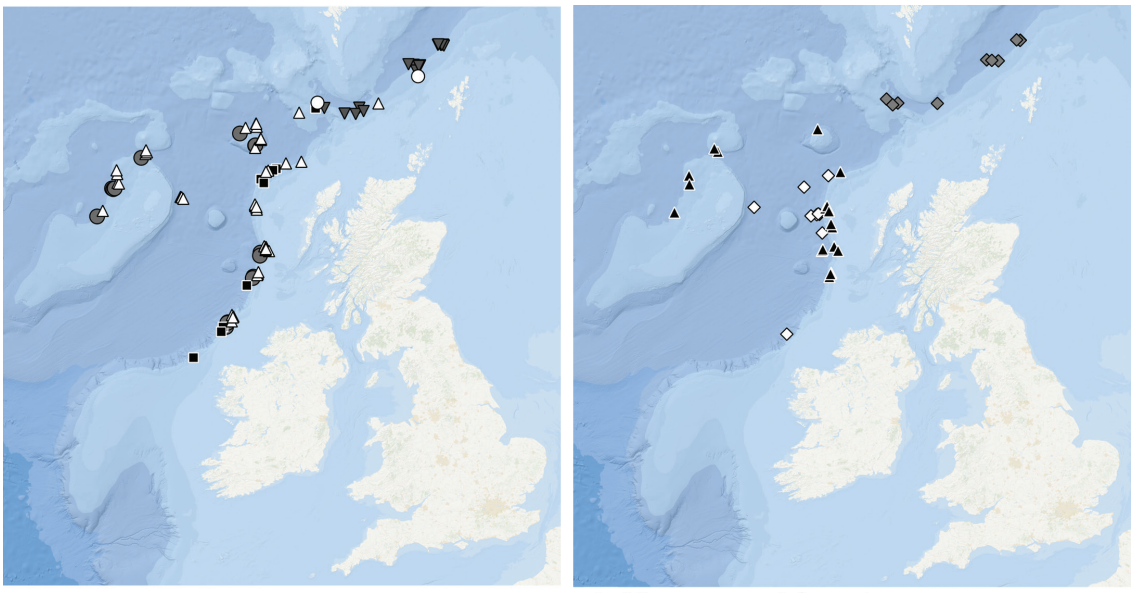

Fig. 3—Capture locations of octopuses in the North-east Atlantic.

of 10.2 in the American population to a mean of 8.38. The size of the ligula varies between the eastern American population (ML > 30, ligula length 24–44) and the western Greenland and the rest of the North Atlantic population (ML > 30, ligula length 18–38) (Muus 2002). Again, due to the lack of dispersal associated with crawl-away young, these taxa may not be able to maintain panmixia over long distances. Genetic analyses such as barcoding or population genetics could allow us to investigate whether these differences represent inter or intra species differences.

Haplotypes from the *B. pugniger* network (H25-H27, OK489787; network D, Fig. 1) also did not form a unique clade in the phylogenetic analysis (Fig. 2A). This, again, suggests deep-water octopods associated with slope environments may not able to maintain panmixia across the Atlantic Ocean, although these sequences were recovered as a single species by the species delimitation analysis. Additional sampling could determine species boundaries. Overall, though, this suggests that slope species may remain to be discovered in areas that have been less explored.

MUUSOCTOPUS

Prior to this study, two *Muusoctopus* species were known to occur in the North-east Atlantic, *M. johnsonianus* (type locality North-east Atlantic Ocean, southern boundary of Porcupine Seabight (Allcock *et al.* 2006)) and *M. normani* (type locality North-east Atlantic, north-western boundary of the Celtic Sea (Massy 1907)). The unidentified species of *Muusoctopus* is the most northerly (Fig. 3). Two additional *Muusoctopus* species occur in the Eastern Atlantic: *Muusoctopus berryi* (Robson, 1924) and *Muusoctopus pseudonymus* (Grimpe, 1922). Each is only known from its type locality: South Africa for *M. berryi*, and the Azores for *M. pseudonymus.* It is unlikely that our unidentified *Muusoctopus* specimens represent either of these species because of the large distances separating the collection locations, in particular the very deep waters surrounding the Azores, and the fact that *Muusoctopus* species produce large eggs, with crawl-away young, and thus lack a dispersal stage. Thus, we tentatively conclude that our unidentified species of *Muusoctopus* represents an undescribed species. An undescribed species of *Muusoctopus* from slightly more northerly waters is being researched by others (Alexey Golikov, pers. comm.) and our specimens may pertain to that species.

Gleadall (2013) noted that there was a 'close resemblance between *M. normani* and *M. januarii'*. No studies to date have included material of *M. januarii* from close to the type locality, although Ibanez *et al.* (2016) appear to report unreferenced sequences of *M. normani* from GenBank in a tree as *M. januarii*, presumably accepting Gleadall's (2013) proposed synonymy. *Muusoctopus normani* eggs have been reported to exceed 2cm in length and thus almost certainly hatch to benthic juveniles (Barratt *et al.* 2007).

As noted above, we find it highly unlikely that a species without a dispersal stage can maintain panmixia over such large distances and continue to treat these species as separate.

DISTRIBUTION

Muusoctopus normani and *M. johnsonianus* have a limited distribution extending from the Porcupine Seabight to the Wyville Thomson ridge and from the Porcupine Seabight to the Rockall Trough, respectively (Table 2, Fig. 3). *Muusoctopus januarii* (Hoyle, 1885) is known from the Gulf of Mexico to Brazil, but more recently has been reported from off Mauritania (Rocha *et al*. 2017), South Morocco, Western Sahara and Guinea Bissau (Luna *et al*. 2021). These recent records postdate Gleadall's (2013) proposed synonymy of *M. normani* with *M. januarii*, and the authors could be embracing that synonymy and the records could, in fact, represent an increase in the range of *M. normani*, which, as stated above, we believe to be a distinct species. Nesis (2001) reported four specimens of *B. piscatorum* from west Svalbard and the northern Kara Sea which, based on the description, morphology of the hectocotylus and the arm length (3x the mantle length), could be *M. johnsonianus* (Allcock *et al*. 2006) but are likely to represent an undescribed species (Xavier *et al*. 2018). The apparently limited distribution of known North Atlantic *Muusoctopus* species further supports our conclusion that our unidentified *Muusoctopus* specimens represent a new species.

Atlantic *Muusoctopus* species occur only on one side of the Atlantic whereas, of the six *Bathypolypus* species known to occur in the Atlantic, three of them, all known from the North Atlantic, *B. bairdii*, *B. sponsalis* and *B. pugniger,* are reported to occur on both eastern and western sides (Jereb *et al*. 2016). The amphi-Atlantic distribution of these specimens may be explained by their northerly distribution, although our data suggest separation between eastern and western populations. The eastern and western Atlantic are connected in the north via the Greenland-Scotland ridge, which could facilitate population connectivity and gene flow of northerly occurring species. Muus (2002) noted that physiochemical barriers could have prevented *Benthoctopus* (sensu *Muusoctopus*) from extending into the Arctic, thus preventing gene flow from east to west Atlantic and vice versa, although evidence for *Muusoctopus* in the Kara Sea contradicts this. It may simply be that the distance (given the lack of larval dispersal), and varying environmental conditions, are not conducive to wide distributions. Tracts of deep ocean (Allcock *et al*. 1997) and suboptimal water temperatures (Allcock *et al*. 2011) are known to prevent dispersal and limit gene flow in benthic octopods which may, in time, lead to speciation. Speciation can also occur between specimens for which there are few apparent barriers to gene flow such as with *Muusoctopus thielei* (Robson, 1932) and *Muusoctopus levis* (Hoyle, 1885). These species are valid (Vecchione *et al*. 2009) despite being known from two geographically adjacent locations, the Kerguelen Islands and Heard Island respectively, situated on the Kerguelen plateau without deep-water separation. Vecchione *et al*. (2009) suggest that the Antarctic Polar Frontal Zone may be a barrier to gene flow between these two species.

DNA BARCODING

DNA barcoding is a quick and easy way to identify species when there is confusion in morphology, however comparator sequences must be available from specimens which have been accurately identified and catalogued for future investigation. As the hectocotylus is one of the main identifying characters in deep sea octopods (Muus 2002; Vecchione *et al*. 2009) males are more easily identified to species level. Females have previously been identified based on body proportion comparison with males (Muus 2002). Identifying females to species level using DNA barcoding may provide us with the ability to identify important morphological characters that have hitherto been overlooked.

DNA barcoding has previously been shown to aid in the identification of new cephalopod species e.g. *Pareledone* cf. *felix* (Allcock *et al*. 2011), *Benthoctopus rigbyae* (Vecchione *et al*. 2009) and *Cistopus chinensis* (Zheng *et al*. 2012), and new deep-sea species e.g. discovery of 20 new polychaete species (Brasier *et al*. 2016) and 133 molecular operational taxonomical units in North-west Pacific deep-sea amphipods, almost all of which had never been sequenced before (Jażdżewska and Mamos 2019). It is evident that the true biodiversity of the deep-sea in particular is yet to be discovered and that DNA barcoding may play an important role in uncovering this biodiversity.

ACKNOWLEDGEMENTS

This work was funded by a Tony Ryan Fellowship and an Irish Research Council postgraduate scholarship (GOIPG/2017/1740) to MT. We thank Martin Collins and two anonymous reviewers for comments on an earlier version of this manuscript.

SUPPLEMENTARY MATERIAL

A summary of the specimens examined in this study is available online here: http://muse.jhu.edu/resolve/184

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