Acta Oceanol. Sin., 2021, Vol. 40, No. 8, P. 160–167
https://doi.org/10.1007/s13131-021-1800-8
http://www.aosocean.com
E-mail: ocean2@hyxb.org.cn

Application of DNA metabarcoding to characterize the diet of the moon jellyfish *Aurelia coerulea* polyps and ephyrae

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Received 16 March 2020; accepted 25 November 2020

Abstract

Dietary studies of polyps and ephyrae are important to understand the formation and magnitude of jellyfish blooms and provide important insights into the marine food web. However, the diet of polyps and ephyrae *in situ* is largely unknown. Here, prey species of the polyps and ephyrae of the moon jellyfish *Aurelia coerulea in situ* were identified using high-throughput DNA sequencing techniques. The results show that *A. coerulea* polyps and ephyrae consume a variety of prey items. The polyps consume both planktonic and benthic prey, including hydromedusae, copepods, ciliates, polychaetes, stauromedusae, and phytoplankton. *A. coerulea* ephyrae mainly feed on copepods and hydromedusae. Gelatinous zooplankton, including *Rathkea octopunctata* and *Sarsia tubulosa*, were frequently found as part of the diet of *A. coerulea* polyps and ephyrae. The utilization of high-throughput sequencing technique is a useful tool for studying the diet of polyps and ephyrae in the field, complementing the traditional techniques towards a better understanding of the complex role of gelatinous animals in marine ecosystems.

Key words: *Aurelia coerulea*, *in situ*, dietary analysis, feeding, high-throughput sequencing

Citation: Sun Tingting, Wang Lei, Zhao Jianmin, Dong Zhijun. 2021. Application of DNA metabarcoding to characterize the diet of the moon jellyfish *Aurelia coerulea* polyps and ephyrae. Acta Oceanologica Sinica, 40(8): 160–167, doi: 10.1007/s13131-021-1800-8

1 Introduction

The moon jellyfish *Aurelia* spp. is widely distributed in harbors, bays and coastal waters throughout the East Asian margin sea, the Mediterranean coast of France, the Atlantic, Australia, and the east coast of North America (Dawson et al., 2005; Dong et al., 2015; Scorrano et al., 2017). As many as 12 *Aurelia* species or subspecies been described previously (Dong, 2019), *Aurelia* sp.1 is now recognized as the previously recorded species *A. coerulea* (Scorrano et al., 2017), which is widely distributed in Chinese coastal waters (Dong et al., 2015). The high abundances reached during their frequent blooms can have negative influences on various coastal industries, including the coastal power plant industry, local fisheries and aquaculture (Purcell et al., 2013; Dong et al., 2014). Seawater temperature increases, eutrophication, and habitat modifications have been suggested as possible factors contributing to the blooms of *A. coerulea* in coastal waters (Dong et al., 2010; Purcell, 2012).

A. coerulea have the typical metagenetic life cycle characteristic of most scyphozoans, comprising alternate generations of asexual benthic polyps and sexual pelagic medusae (Arai, 1997). While the medusae are the "problematic" and conspicuous stage, the tiny, inconspicuous polyps play a key role in determining the times and intensity of blooms by producing and releasing ephyrae into the water column through a process known as strobilation. Therefore, factors controlling growth and mortality of polyps and ephyrae influence the magnitude of medusa blooms (e.g., Purcell et al., 2009; Han and Uye, 2010; Lucas et al., 2012).

Empirical evidence suggests that growth and mortality of polyps and ephyrae are influenced by a combination of factors, among which food plays a major role (Han and Uye, 2010; Kogovšek et al., 2010; Schiariti et al., 2014; Wang and Li, 2015). Production of new polyps significantly increases with increasing food availability in the blooming jellyfish *Aurelia* spp. (Han and Uye, 2010; Schiariti et al., 2014). Results from Wang and Li (2015) showed that abundant food conditions increase ephyrae survival rate and enhance individual development, and thus producing large populations of medusae. Therefore, knowledge of the diet of polyps and ephyrae is key to understanding the factors that affect medusae recruitment, the formation and magnitude of jellyfish blooms.

Adult *Aurelia* spp. have been well studied as a top-down controller within the classical food web, preying on crustacean zooplankton (e.g., Purcell and Sturdevant 2001; Titelman and Hansson, 2006; Uye, 2011; Riisgård and Madsen, 2011), and possibly also microplankton (Båmstedt, 1990; Graham and Kroutil,

Foundation item: The National Key Research and Development Program of China under contract No. 2018YFC1406501; the Strategic Priority Research Program of the Chinese Academy of Sciences under contract No. XDA23050301; the National Natural Science Foundation of China under contract No. 41876138; the Instrument Developing Project of the Chinese Academy of Sciences under contract No. YJKYYQ20180047; the Key Research and Development Program of Yantai under contract No. 2018ZHGY073.
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2001; Purcell and Sturdevant, 2001; Uye and Shimauchi, 2005; Malej et al., 2007; Lo and Chen, 2008). However, studies dealing with the diet of polyps and ephyrae are much scarcer and most describe laboratory observations. It has been shown that polyps of *A. coerulea* can prey on copepods, molluscs, fish larvae, planulae, and phytoplankton (Gröndahl, 1988, 1989; Tsikhon-Lukanina et al., 1996; Östman, 1997; Kamiyama, 2013; Wang et al., 2015). Planktonic ciliates could also be part of the diet of polyps (Kamiyama, 2013). On the other hand, ephyrae of *A. coerulea* prey on mesozooplankton, microzooplankton and phytoplankton (Sullivan et al., 1997; Båmstedt et al., 2001). In addition, it has been demonstrated that *A. labiata* ephyrae possess the ability to assimilate and metabolize dissolved organic matter (Skikne et al., 2009). However, the dietary composition of the polyps and ephyrae in the field are largely unknown.

Traditionally, *in situ* dietary analysis of scyphozoans has been mainly conducted by microscopic examination of gastric contents (Graham and Kroutil, 2001; Purcell and Sturdevant, 2001; Uye and Shimauchi, 2005). However, microscopic examination of gastric contents has limitations, especially in the early life stages (e.g., polyps, ephyrae) due to their small size. Additionally, it is difficult to identify species from semi-digested fragments of marine organisms and ingested prey often lacks diagnostic taxonomic features (Pompanon et al., 2012).

To overcome these difficulties, high-throughput DNA sequencing has been employed extensively in the dietary analyses of bats, penguins, seals and fish (Deagle et al., 2010; Bohmann et al., 2011; Albaina et al., 2016; Su et al., 2018; Brassea-Pérez et al., 2019). In addition, this technique has been used to study the diets of larvae and has greatly enhanced the speed and resolution of the dietary analyses in the early life stages of marine organisms (O'Rorke et al., 2012, 2014; Hirai et al., 2017; Kodama et al., 2017). Therefore, the aim of this study was to reveal the dietary composition of *A. coerulea* polyps and ephyrae using for the first time the high-throughput DNA sequencing techniques.

2 Materials and methods

2.1 *Sample collection*

Polyps and ephyrae of *A. coerulea* were collected from Fenghuang Lake, a coastal marine lake located in Shidao Bay on the southern Yellow Sea, China. It covers an area of 1.39 km^2 and has an average depth of about 5 m. The dam of Fenghuang Lake is made of concrete. One intake valve and two emptying valves are used to exchange seawater with Shidao Bay (Fig. 1).

Polyps attached to the biogenic reefs formed by tubeworms *Hydroides dianthus* were collected by scuba divers in March 2016. Immediately after collection, the polyps were gently removed from the substrates using tweezers. Ephyrae were collected in April 2016 by gently trawling a standard plankton net (31.6 cm mouth diameter, 160 μm mesh size). To prevent change in the gut contents of *A. coerulea* polyps and ephyrae in the sampling progress, the polyp and ephyra samples were preserved on site immediately in sterile neutral Lugol's solution at 2% final concentration (Hu et al., 2015). At the laboratory, 360 polyps and 360 ephyrae of *A. coerulea* were sorted using a wide-bore plastic

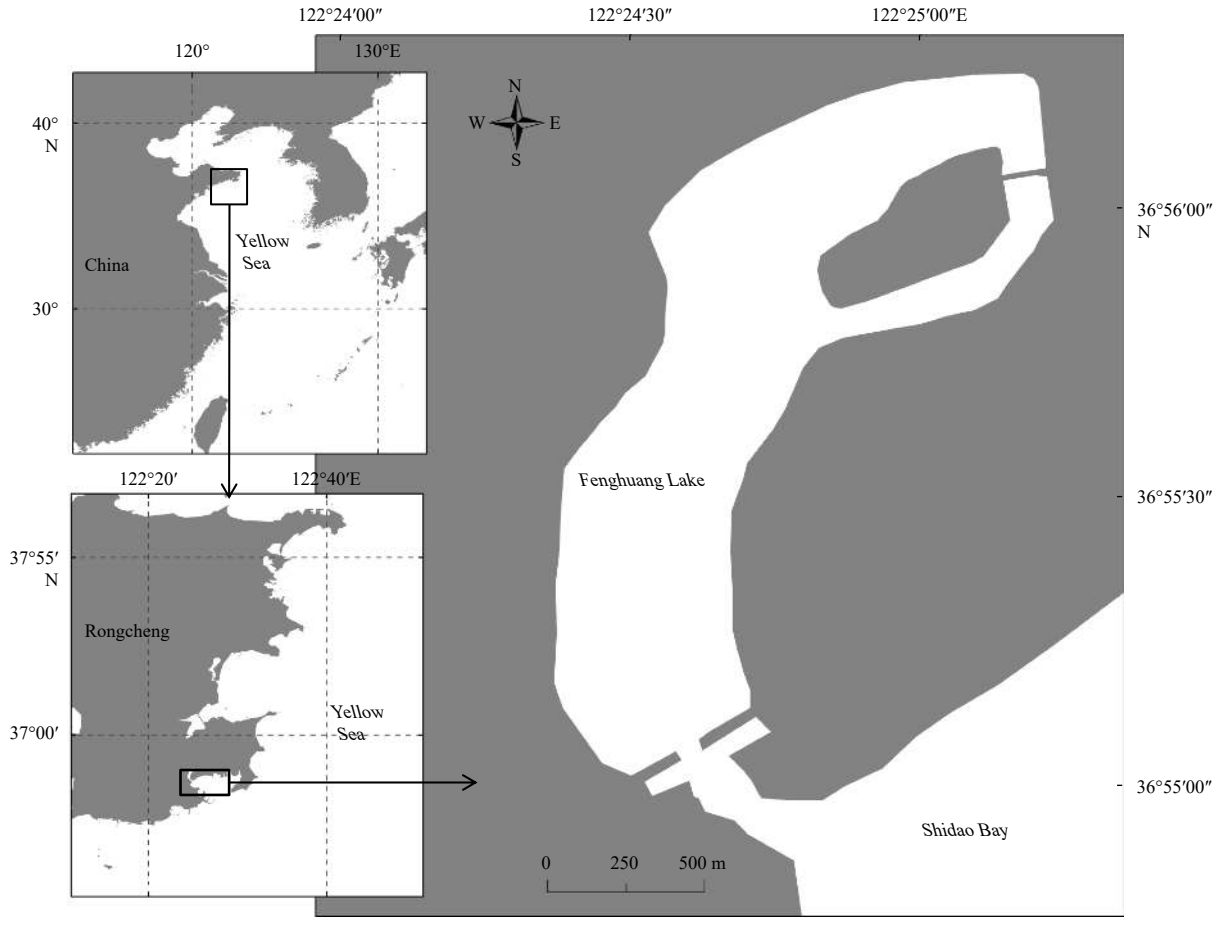

Fig. 1. Sampling site for *Aurelia coerulea* polyps and ephyrae in the Fenghuang Lake, southern Yellow Sea.

pipette under an Olympus SZX10 stereomicroscope (Olympus Corporation, Tokyo, Japan). The sorted samples were carefully and thoroughly rinsed three times with autoclaved 0.22 μm filtered natural seawater and examined under a stereomicroscope to ensure that no other visible organisms were attached to the body. Thirty polyps and 30 ephyrae were preserved in one autoclaved tube each. Thirty individuals were required for each sample for DNA extraction due to the very small size and high water content of the polyps and ephyrae. In total, in order to maximise the number of prey species identified, 12 samples of polyps and 12 samples of ephyrae were analyzed. They were stored at –80°C after seawater was removed.

2.2 *DNA extraction, amplification and sequencing*

The polyp and ephyra samples were homogenized and incubated in lysis buffer for three days at 55°C to allow for thorough cell lysis. DNA was extracted following a modified CTAB protocol, and finally eluted in 50 mL of 10 mmol/L Tris-HCl (pH 8.0), as previously reported. Universal eukaryotic primers were used to target the V4 region of the 18S rDNA (528F: 5′-gcctccctcgcgccatcag-GCGGTAATTCCAGCTCCAA-3′; 706R: 5′-gccttgccagcccgctcag-AATCCRAGAATTTCACCTCT-3′). PCR amplification was carried out in a total volume of 30 μL, which contained 15 μL of Phusion High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA), 0.22 μmol/L of forward and reverse primers, and 10 ng of template DNA. Following the initial denaturation at 98°C for 1 min, the PCR conditions consisted of 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 5 min. The PCR products were purified with GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Waltham, MA, USA) and pooled together at the same concentration prior to sequencing. Sequencing libraries were generated using Illumina Truseq DNA PCR-Free Library Preparation Kit (Illumina, San Diego, CA, USA). Libraries were sequenced on an Illumina HiSeq PE250 platform and 300 bp paired-end reads were generated.

2.3 *Bioinformatic analyses*

Paired-end reads from the original DNA fragments were merged using FLASH version 1.2.9 (Magoč and Salzberg, 2011) and assigned to each sample according to the unique barcodes. Sequence reads were filtered by QILME quality filters (Caporaso et al., 2010). Sequences with ⩾97% similarity were assigned to the same operational taxonomic unit (OTU). Taxonomic assignments of the dominant sequence of each OTU were determined using RDP classifier. Only sequences that were found more than three times in one OTU were kept for further analysis. Sequence homology searches of GenBank were performed with ⩾90% similarity level threshold. A phylogenetic tree was established for prey taxa with the neighbor-joining method in MEGA 5.0 (Ballard and Melvin, 2010), using the Tajima-Nei model with 1 000 bootstrap replications.

3 Results

The total number of reads returned from the Illumina HiSeq PE250 platform was 2 087 983. Of these, 1 951 254 reads remained after initial sequence quality control. The average number of sequencing reads for each sample was 81 302±7 940 (mean±SD). A total of 88 718 sequencing reads were retained for further analysis after the removal of the predator sequences. Analysing the sequences against the GenBank database identified 19 prey species with V4 regions of the 18S rRNA which were 100% identical to sequences obtained from the *A. coerulea* polyps and ephyrae (Table 1).

Based on the next-generation-sequencing analysis (NGS), 44 prey taxa grouped in 10 phyla were recognized as part of the diet of *A. coerulea* polyps and ephyrae (Fig. 2, Table 1). Of these, 19 prey taxa were found in the *A. coerulea* ephyrae and 38 prey taxa were found in the *A. coerulea* polyps, with 13 prey taxa found both in polyps and ephyrae. Cnidaria, Arthropoda and Annelida were the most frequently observed phyla and Hydrozoa, Maxillopoda and Polychaeta were the most frequently observed classes in both the polyps and ephyrae of *A. coerulea* (Fig. 3a, Table 1). Filifera, Capitata and Harpacticoida were the most frequently observed orders in the polyps, while Calanoida, Capitata, Spionida and Filifera were the most frequently observed orders in the ephyrae (Fig. 3b, Table 1).

A. coerulea ephyrae mainly preyed on pelagic zooplankton, including copepods, hydromedusae and polychaetes (Table 1). The dominant prey taxa of *A. coerulea* ephyrae were copepods (*Eurytemora pacifica*), hydromedusae (*Sarsia tubulosa* and *Rathkea octopunctata*), and polychaetes (*Pseudopolydora paucibranchiata*) which accounted for 48%, 18%, 11% and 11% of the dietary contents, respectively. On the other hand, *A. coerulea* polyps fed on both planktonic and benthic prey, including hydromedusae, copepods, ciliates, polychaetes, stauromedusae, and phytoplankton (Table 1). The dominant prey taxa of *A. coerulea* polyps were two hydromedusae, *R. octopunctata* and *S. tubulosa*, which accounted for 60% and 18% of the dietary contents, respectively. The benthic preys including *Thalestridae* sp., *Amphiascoides atopus*, *Amonardia* sp., *Haliclystus* sp. were only found in the diets of *A. coerulea* polyps.

4 Discussion

Traditional microscopic examination of gastric contents has limitations in determining the diet of scyphozoans, especially in the polyps and ephyrae stage due to their small size. Moreover, it is difficult to identify species from semi-digested fragments of marine organisms and ingested prey often lacks diagnostic taxonomic features. In the present study, the results show that high-throughput sequencing techniques can overcome these difficulties and identify individual prey species of *A. coerulea* polyps and ephyrae *in situ* in fine resolution. *A. coerulea* polyps and ephyrae consume a variety of prey items, including both mesozooplankton and micro-plankton.

Previous studies by direct observation of feeding in laboratory have shown that copepods, mollusc and fish larvae, planulae, planktonic ciliates and phytoplankton were preyed upon by the polyps of *A. coerulea* (Tsikhon-Lukanina et al., 1996; Östman, 1997; Kamiyama, 2013; Wang et al., 2015; Zheng et al., 2015). Few studies have examined the natural feeding ecology of polyps (Gröndahl, 1988; Tsikhon-Lukanina et al., 1996; Östman, 1997). In the few studies that report the natural diets of polyps, there appears to be low selectivity, as a great variety of zooplankton prey are consumed (Gröndahl, 1988; Tsikhon-Lukanina et al., 1996; Östman, 1997). Copepods, hydromedusae, polychaetes, phytoplankton and ciliophorans were detected, in the polyps of *A. coerulea in situ*, which is in accordance with the previous studies (e.g., Kamiyama, 2013; Wang et al., 2015). Although information about prey availability in the natural environment was not available, the diversity of prey taxa found in this paper confirms the low selectivity of *Aurelia* polyps. The observations agree with those of Tsikhon-Lukanina et al. (1996), who suggested that the polyps capture both planktonic prey species from the water column and benthic ones from the bottom.

A. coerulea polyps preyed on a more diverse range of species than ephyrae. *A. coerulea* ephyrae mainly prey on pelagic zo-

Table 1. Prey taxa detected in the polyps and ephyrae of the moon jellyfish *Aurelia coerulea*

Phylum	Class	Order	Species	Similarity	Percentage%		Accession numbers
					ephyrae	polyps	
Annelida	Polychaeta	Spionida	*Pseudopolydora paucibranchiata*	100%	11.13	0	MN165825
			Polydora sp.	99%	0.07	0	MN165843
		Sabellida	*Hydroides panamensis*	100%	0	2.49	MN165831
		Terebellida	*Ctenodrilus serratus*	100%	0	0.03	MN165847
		Scolecida	*Arenicola brasiliensis*	100%	0	0.03	MN165854
Arthropoda	Malacostraca	Brachyura	*Hemigrapsus takanoi*	100%	0.69	0	MN165834
	Maxillopoda	Calanoida	*Labidocera* sp.	96%	6.65	0.01	MN165826
			Eurytemora affinis	99%	1.26	0.06	MN165828
			Acartia pacifica	100%	0.12	0	MN165842
			Eurytemora pacifica	99%	48.47	0.33	MN165822
		Cyclopoida	*Cyclopina* sp.	95%	1.64	0.06	MN165829
		Harpacticoida	*Thalestridae* sp.	99%	0	2.21	MN165832
			Stenhelia sp.	92%	0.13	1.85	MN165833
			Amphiascoides atopus	99%	0	1.43	MN165835
			Normanellidae sp.	98%	0	1.00	MN165836
			Mesochra sp.	99%	0	0.42	MN165839
			Tisbe sp.	99%	0	0.01	MN165863
			Amonardia sp.	100%	0	7.72	MN165827
			Uncultured copepod	94%	0	0.43	MN165837
Chordata	Ascidiacea	Stolidobranchiata	*Styela clava*	100%	0	0.01	MN165861
Ciliophora	Oligohymenophorea	Sessilida	*Pseudovorticella* sp.1	98%	<0.01	0.02	MN165851
			Pseudovorticella sp.2	100%	0	0.02	MN165853
	Phyllopharyngea	Endogenida	*Acineta* sp.	99%	0	0.02	MN165848
	Spirotrichea	Choreotrichida	*Rimostrombidium veniliae*	99%	0	0.01	MN165857
		Tintinnina	*Choreotrichia* sp.	100%	0	<0.01	MN165862
			Uncultured clilate	97%	0	<0.01	MN165864
	Telonemea	Telonemida	*Telonema* sp.	99%	0	0.01	MN165852
Cnidaria	Hydrozoa	Filifera	*Rathkea octopunctata*	100%	10.79	60.48	MN165823
		Capitata	*Sarsia tubulosa*	100%	18.40	18.47	MN165824
			Cladonema californicum	100%	0.33	2.47	MN165830
			Ectopleura larynx	99%	<0.01	0.03	MN165845
	Staurozoa	Stauromedusae	*Haliclystus* sp.	100%	0	0.04	MN165849
Nematoda	Adenophorea	Chromadorida	*Neochromadora* sp.	96%	0	0.01	MN165855
		Enoplida	*Oncholaimus* sp.	91%	0	0.01	MN165856
			Anticomidae sp.	100%	0	0.02	MN165858
			Enoplus taipingensis	100%	0	0.01	MN165860
Platyhelminthes	Rhabditophora	Polycladida	*Notoplana australis*	100%	0.16	0	MN165838
		Macrostomida	*Microstomum* sp.	99%	0.05	0.10	MN165840
			Macrostomum pusillum	99%	0.02	0.03	MN165844
		Rhabdocoela	*Promesostoma* sp.	99%	0.07	0.05	MN165841
Ochrophyta	Phaeophyceae	Chordariales	*Halothrix ambigua*	100%	0	0.01	MN165865
Bacillariophyta	Centricae	Naviculales	*Navicula* sp.	99%	0	0.07	MN165846
		Discoidales	*Thalassiosira guillardii*	100%	0	<0.01	MN165859
Deuteromycotina	Hyphomycetes	Hyphomycetales	*Aspergillus* sp.	100%	0.01	0	MN165850

Note: The datasets generated for this study are available for download via GenBank with accession numbers MN165822-MN165865. The classification system used here is Cavalier-Smith's system of classification (Cavalier-Smith, 1998).

oplankton, while *A. coerulea* polyps feed on both planktonic and benthic prey. *Aurelia* polyps are generally attached to shells, rocks or artificial structures (Duarte et al., 2013). Therefore, the polyps of *A. coerulea* could prey on benthic organisms (e.g., benthic copepods, Stauromedusae, Ascidiacea). In addition, zooplankton would be preyed upon when they move close to the polyps due to the characters of diurnal vertical migration or vertical mixing of the water column. Some algae and unidentified particulate organic matter have previously been found to be part of the diet of *Aurelia* polyps (Tsikhon-Lukanina et al., 1996; Östman, 1997). In accordance with these studies, some microalgae and fungi were detected in the diet of *A. coerulea* polyps. The possibility that these microalgae and fungi were ingested by the polyps' prey, such as copepods, cannot be excluded. Once again, further studies are needed in order to comprehend the feeding strategies of *Aurelia* and its variations through the ontogeny. More recently, it has been shown that planktonic ciliates serve as a food source for growth at the ephyra stage of *Aurelia* spp. (Zoccarato et al., 2016; Kamiyama, 2018). Ciliates, *Pseudovorticella* sp.1 were detected in the ephyrae of *A. coerulea in situ*, which is

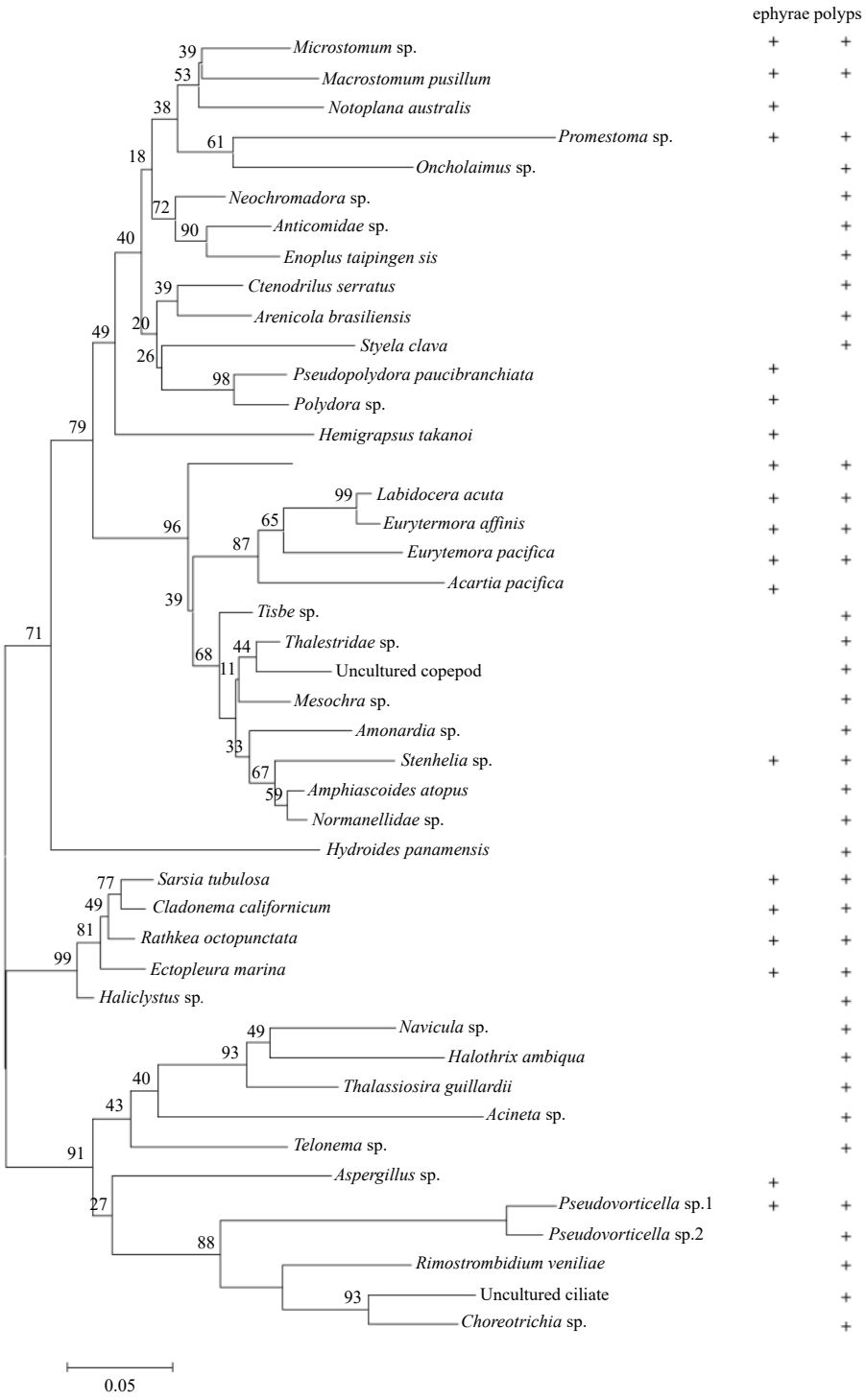

Fig. 2. Neighbor joining tree for prey taxa in the polyps and ephyrae of the moon jellyfish *Aurelia coerulea* using the Tajima-Nei model with 1 000 bootstrap replications. + Presence.

in accordance with the direct observation of feeding in laboratory studies. Little is known about the mechanisms driving prey selectivity in scyphozoan polyps. Prey of scyphozoan polyps is captured and transported to the mouth by the tentacles. In addition, currents also pass up the column carrying mucus and particles to the tips of the tentacles. Meanwhile, the scyphozoan polyps are sessile and non-visual predators, it is an advantage to be able to feed more diverse types of prey they encountered and contacted.

Interestingly, hydromedusae were the most frequently occurring prey items found in *A. coerulea* polyps and ephyrae. Consumption of gelatinous prey by adult scyphozoans is a common phenomenon and is widely described in various taxa including *Aurelia* (Purcell, 1997; Bayha and Dawson, 2010). However, the diet of polyps and ephyrae is probably distinct from that of adults since ontogenetic diet shifts have been described in some species (Graham and Kroutil, 2001), or at least the way that food is captured surely changes according to the morphological changes

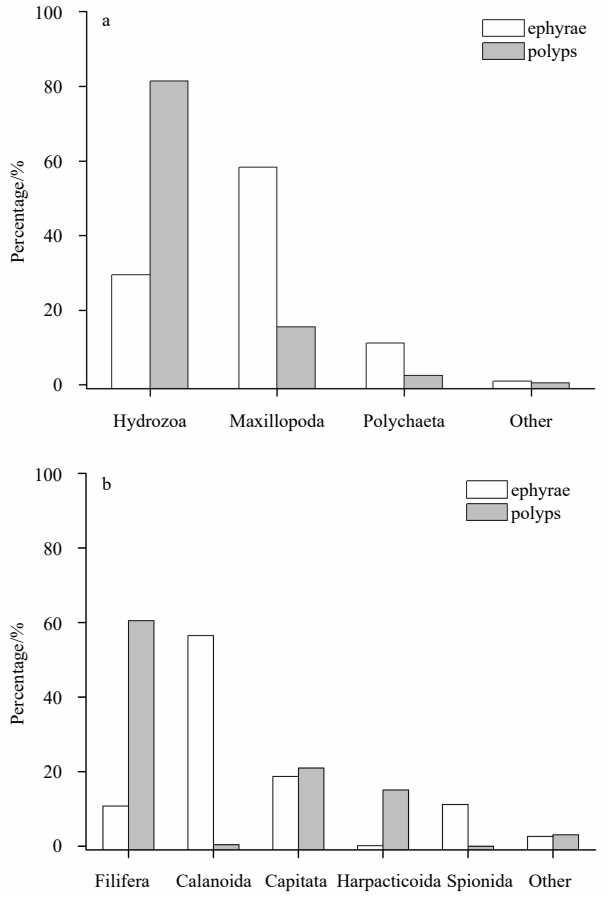

Fig. 3. Rank abundance of phylogenetic class and order of prey organisms detected in the polyps and ephyrae of the moon jellyfish *Aurelia coerulea*. The *y* axis shows percent of total reads that each taxonomic contributes. a. Class and b. order.

that occur during the ontogeny (Costello et al., 2008; Carrizo et al., 2016). Adult medusae utilize bell pulsations to generate vortices that transport fluids and prey toward oral arms. However, small ephyrae live within a fluid regime where transporting prey by feeding currents is inefficient (Higgins III et al., 2008). Therefore, the capacity of an ephyra to feed on very sensitive and fast-moving prey, such as copepods, is limited (Sullivan et al., 1997). Studies of gut contents of field collected *Aurelia* spp. ephyrae have demonstrated that copepods and copepod nauplii are unimportant food sources, even when they are dominant in the plankton. On the other hand, slow-moving prey items (e.g., small ctenophores and hydromedusae) may be easy to catch as they do not attempt to resist capture as energetically as crustacean plankton (Östman, 1997; Sullivan et al., 1997). In addition, high abundance of hydromedusae in the field at the time of collection might explain this high percentage.

Increasing evidence suggests that gelatinous organisms are preyed upon by other marine animals and might also play an important role in the marine pelagic food web (Cardona et al., 2012; Huang, 2013; Jarman et al., 2013; Cardona et al., 2015; Carrizo et al., 2016; McInnes et al., 2017; Thiebot et al., 2017). For example, hydromedusae (*R. octopunctata* and *Clytia hemisphaerica*) were the dominant prey species in the two copepods *Calanus sinicus* and *Acartia pacifica* from the Yellow Sea and Bohai Sea (Huang, 2013). *Cotylorhiza tuberculata* and *Pelagia noctiluca* were the most important species in the diets of swordfish, tuna and turtles from the Western Mediterranean Sea (Cardona et al., 2012). DNA analyses estimated that a substantial portion of the diet of *Adélie penguins* in the Southern Ocean was made up of groups such as hydromedusae, scyphomedusae, and cubomedusae (Jarman et al., 2013). Sardines from the Western Mediterranean Sea fed predominantly on *P. noctiluca* (Cardona et al., 2015). McInnes et al. (2017) showed that scyphozoan jellyfish were a common prey of black-browed and Campbell albatross. Recently, Thiebot et al. (2017) used animal-borne video data loggers to record direct observations of predation and revealed that all four species of penguins across the southern oceans prey on jellyfish.

In the present study, the V4 region of the 18S rRNA was amplified from whole specimens of *A. coerulea* polyps and ephyrae, due to the difficulties in isolating gut contents from the small and fragile polyps and ephyrae. Therefore, the amplified predator sequences accounted for the majority of these fragment data sets. In this study, over 45% of prey sequences were accurately identified to the species level and the majority of prey sequences were identified to the genus level. Therefore, the V4 region of the 18S rRNA was able to identify individual prey species of *A. coerulea* polyps and ephyrae *in situ* to an acceptable level of resolution. The use of this region has limitations and is not as specific as those found in mitochondria. For instance, high-throughput DNA sequencing using short mtCOI fragments was recently able to accurately identify over 95% of prey sequences to the species level (Su et al., 2018). However, potential priming sites of these barcodes would not be conserved enough to cover a broad range of taxa, probably leading to a failure or a low rate of amplification (Ficetola et al., 2010). Therefore, further studies are needed to combine a DNA barcode with a broad taxonomic coverage with other DNA barcodes that resolve some of the higher taxonomic units to species level.

5 Conclusion

This study reveals the dietary composition of *A. coerulea* polyps and ephyrae in the field using the high-throughput DNA sequencing technique. The results show that *A. coerulea* polyps and ephyrae consume a variety of prey items. *A. coerulea* polyps consume both planktonic and benthic prey, including hydromedusae, copepods, ciliates, polychaetes, stauromedusae, and phytoplankton. *A. coerulea* ephyrae mainly feed on plankton, including copepods and hydromedusae. Hydromedusae were the most frequently occurring prey item found in both polyps and ephyrae. The high-throughput sequencing technique is a useful tool for studying the diet of polyps and ephyrae in the field, complementing the traditional techniques to produce a better understanding of the complex role of gelatinous animals in marine ecosystems. Further studies are needed to examine the temporal and geographical variations in the dietary composition of *A. coerulea* and their environmental plankton communities using the high-throughput sequencing technique.

Acknowledgements

We would like to acknowledge the valuable comments and suggestions of Agustin Schiariti in Instituto Nacional de Investigación y Desarrollo Pesquero. We also appreciate all the foundation items that supported this research.

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