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METHODS AND RESOURCES ARTICLE

Demersal fish diversity and molecular taxonomy in the Bering Sea and Chukchi Sea

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Abstract

DNA barcoding by sequencing a standard region of cytochrome c oxidase subunit I (COI) provides an accurate, rapid method for identifying different species. In this study, we provide a molecular taxonomic assessment of demersal fishes in the Bering Sea and Chukchi Sea based on DNA barcoding, and a total of 123 mitochondrial COI partial fragments with a length of 652 bp were obtained. The consensus among all sequences was determined by alignment via a BLAST search in GenBank. Phylogenetic relationships were reconstructed based on neighbor-joining trees and barcoding gaps. The 39 species investigated in this analysis were distributed among 10 families. Five families within Scorpaeniformes including 19 species accounted for almost half of the species. The next largest group was Perciformes, with 9 species, followed by Pleuronectiformes and Gadiformes, with 5 species each, and the smallest number of species belonged to Rajiformes. At the family level, Cottidae was the largest family, followed by Zoarcidae, accounting for 8 species. The other eight families—Gadidae, Pleuronectidae, Psychrolutidae, Agonidae, Liparidae, Ammodytidae, Hexagrammidae, and Rajidae—accounted for a smaller proportion of species. In brief, our study shows that DNA barcodes are an effective tool for studying fish diversity and taxonomy in the Bering Sea and Chukchi Sea. The contribution of DNA barcoding to identifying Arctic fish species may benefit further Arctic fish studies on biodiversity, biogeography and conservation in the future.

Keywords DNA barcoding · Fish diversity · Taxonomy · Arctic Pacific · Demersal fish

Introduction

The correct identification of species is a prerequisite for studying fish diversity. Traditional morphology-based identification systems rely mostly on expert experience and the integrity of samples (Li et al. 2017). Furthermore, some taxa show a variety of complex characteristics, such as sexual dimorphism (Kenchington et al. 2017) or developmental variability of larvae (Batta-Lona et al. 2019; Webb et al. 2006). Therefore, identification based on morphological features is very difficult, complex and error prone. The limitations inherent in traditional identification entail the need for a new approach to species recognition. DNA barcoding is a technology for identifying species based on sequence diversity in cytochrome c oxidase subunit I (COI) (Hebert et al. 2003b). To date, there have been many studies showing the effectiveness of the COI gene for species identification in diverse animals (Hebert et al. 2003a, 2003b), including fishes (McCusker et al. 2013; Ward et al. 2005). This technology is free from excessive dependence on experience and can allow the automation and standardization of specimen identification to be realized. It provides a powerful supplement to traditional taxonomy and species identification methods. DNA barcodes can be used not only to identify whole fish but also to identify fry, roe, fish meat, fish fins,

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fish products or other body fragments that are difficult to identify based on morphology (Smith et al. 2008; Ward et al. 2005). Therefore, the use of DNA barcodes as an accurate and effective method of species identification is currently favored by an increasing number of researchers. Recent studies have indicated that this technology is highly reliable and efficient in many fish groups, including freshwater fishes (Keskin et al. 2013), coral reef fishes (Ward et al. 2005), ocean fishes (McCusker et al. 2013), Antarctic fishes (Li et al. 2018), and Arctic fishes (Mecklenburg et al. 2010). Moreover, it is widely used in a variety of fields, such as biodiversity assessment, fish larva identification and fishery management (Gao 2015; Panprommin et al. 2020).

The Bering Sea is located at the northernmost tip of the Pacific Ocean, while the Chukchi Sea is the marginal sea of the Arctic Ocean. The two seas are connected through the narrow Bering Strait. The seasonal ice-covered Bering and Chukchi Sea shelves are among the largest continental shelves in the world. These high-latitude shelf systems are highly productive during both the ice melt and open-water periods (Huntington et al. 2020). As seawater warms and the extent of sea ice declines, the vulnerability of the ecosystem to environmental change is thought to be high (Grebmeier et al. 2006a). As a key component of the normal operation of marine ecosystems, fish exhibit a series of basic ecological functions and play an important role in determining the carrying status of ecosystems, reflecting changes in the ecological environment (Izzo et al. 2016). There have been many reported studies of fish species and fishery resources in the Bering Sea and Chukchi Sea. For example, analyses of the composition of fish species (Meyer 1997; Norcross et al. 2010), the distribution of fish species (Busby et al. 2005; Mecklenburg et al. 2010; Rand and Logerwell 2011) and the response of fish to changes in the Arctic environment have been performed (Grebmeier et al. 2006b; Mueter et al. 2009; Robertis and Cokelet 2012). However, there have been few studies on fish taxonomy in the Bering Sea and Chukchi Sea via DNA barcoding (Mecklenburg et al. 2010). Here, we examine COI diversity among 123 fish species, with the goal of examining whether DNA barcoding can achieve clear and definite species recognition in fish. Our study provides an important scientific basis for further studies regarding biodiversity, biogeography and conservation in the Bering Sea and Chukchi Sea.

Materials and methods

Specimen collection and morphological identification

The samples were collected during the 6th (2014), 8th (2017) and 9th (2018) Chinese National Arctic Research Expeditions (CHINAREs). The surveyed sea areas were the Bering Sea continental slope, the Bering Sea continental shelf, the Chukchi Sea continental shelf and the Chukchi Sea continental slope area (the boundary of the Bering Strait is located at 65°05'N) (Fig. 1). All specimens were collected with a triangular bottom trawl net (20 mm mesh size; 6.5 m long, 2.2 m wide and 0.65 m high) in surveys conducted on the R/V Xuelong icebreaker. The time of each network operation was 10–60 min, with a speed of 3–4 kn. Specimens were fished from each station, and specimens from the same station were packaged together. Following morphological identification by visual inspection, all fish were classified by taxonomic specialists. The systemic classification and naming procedures were based primarily on "Fishes of the World (4^{th} edition)" and the "Latin-Chinese Dictionary of Fish Names by Classification System". Muscle samples of fish were obtained and preserved in 95% ethanol for DNA extraction after morphological characterization and specimen identification. Then, the whole fish were preserved in a 95% ethanol solution and stored as voucher specimens at the Third Institute of Oceanography, Ministry of Natural Resources.

DNA extraction, amplification and sequencing

A total of 123 genomic DNA samples were extracted, including 16 from the 6th Arctic expedition, 24 from the 8^{th} Arctic expedition, and 83 from the 9^{th} Arctic expedition. Back muscle tissue of each fish was obtained and preserved in a centrifuge tube with 95% ethanol. A TransGen kit (Easy Pure Marine Animal Genomic DNA Kit) was used to extract the genomic DNA of the Arctic fish, which was then stored at 4 °C for later use. The primers used to amplify the COI gene fragment were F1:5′-TCAACCAAC CACAAAGACATTGGCAC-3′ and R1:5′-TAGACTTCT GGGTGGCCAAAGAATCA-3′ according to Ward et al. (2005). The PCR system had a volume of 25 μL, containing 2.5 μL of 10 × PCR buffer (including Mg^{2+}), 2 μL of dNTPs (2 mmol•L^{-1}), 1 μL of each primer, 0.25 μL of *Taq* DNA polymerase, 1 μL of the extracted DNA, and deionized water to the final of 25 μL. The thermal cycling program included an initial denaturation step of 4 min at 95 °C followed by 30 cycles of 0.5 min at 94 °C, annealing for 0.5 min at 52 °C, and extension for 0.5 min at 72 °C, with a final step of 10 min at 72 °C. Negative controls were included in all amplification reactions to confirm the absence of contaminants. The PCR products were visualized on 1.0% agarose stained with gel green (Biotium, Hayward, CA, USA), and successful amplification products were sent to Personalbio for purification and sequencing.

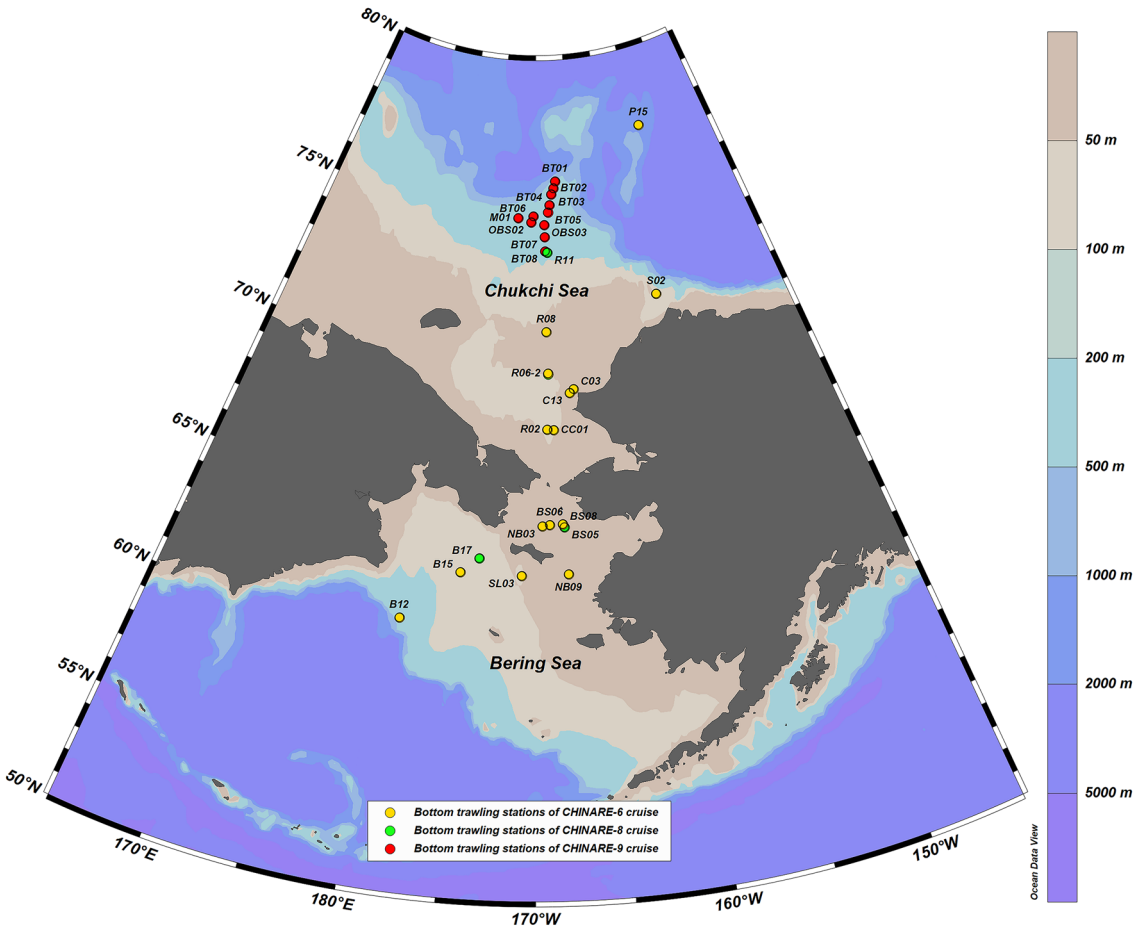

Fig. 1 Map of sampling stations during CHINARE-6, CHINARE-8 and CHINARE-9 cruises

Data analysis

The original data obtained by sequencing were manually compared with the corresponding sequencing peak map to check for errors to ensure the accuracy of the data. The DNASTAR Lasergene software package was used to edit and align the sequences. All high-quality sequences were compared with the NCBI BLAST program to determine the species identity of the samples. Sequence similarity greater than 98% was the criterion for identification at the species level, and a similarity lower than 98% was used for identification at the genus level (Wong and Hanner 2008). Neighbor-joining (NJ) analysis implemented in MEGA 7.0 based on the K2P model with 1000 bootstrap replicates was employed to both calculate the genetic distances and examine the relationships among taxa.

Results

Morphological analysis

A total of 123 specimens were collected during three CHINAREs. Most of them were adults and could be directly distinguished. However, there were also some juvenile and incomplete specimens, which were difficult to identify based on morphological characteristics. These specimens were identified as *Limanda* sp., *Hippoglossoides* sp., *Lycodes* sp., *Ammodytes* sp., *Hemilepidotus* sp. and *Liparis* sp., etc. (Table 1).

Table 1 Information of samples and species identification using morphology and DNA barcode

Sample ID	Cruise	Sampling station	Location	Longitude (°/E)	Latitude (°/S)	Sampling Depth (m)	Morphological taxonomy	Molecular taxonomy	Similarity (%)
R08Z2	CHINARE-6	R08	Chukchi Sea	− 168.9208	71.0246	37	*Ammodytes* sp.	*Ammodytes hexapterus*	100
P151	CHINARE-6	P15	Chukchi Sea	− 154.6817	77.4497	1259	*Amblyraja hyperborea*	*Amblyraja hyperborea*	100
CC01Z	CHINARE-6	CC01	Chukchi Sea	− 168.4819	67.6819	48.6	*Anisarchus macrops*	*Anisarchus medius*	100
R08Z	CHINARE-6	R08	Chukchi Sea	− 168.9208	71.0246	37	*Anisarchus macrops*	*Anisarchus medius*	100
12,359	CHINARE-6	R06-2	Chukchi Sea	− 168.8694	69.6142	53.65	*Boreogadus saida*	*Boreogadus saida*	99
12,281	CHINARE-6	NB09	Bering Sea	− 167.6811	62.5833	25	*Gadus chalcogrammus*	*Gadus chalcogrammus*	100
NB07ZX1	CHINARE-6	NB09	Bering Sea	− 167.6811	62.5833	25	*Hemilepidotus* sp.	*Hemilepidotus papilio*	99
C13Z	CHINARE-6	C13	Chukchi Sea	− 166.9764	68.9233	45.35	*Hexagrammos stelleri*	*Hexagrammos stelleri*	99
NB07ZD	CHINARE-6	NB09	Bering Sea	− 167.6811	62.5833	25	*Hippoglossoides* sp.	*Hippoglossoides dubius*	99
5218	CHINARE-6	B12	Bering Sea	− 178.9133	60.6542	259	*Icelus spatula*	*Icelus spiniger*	100
5220	CHINARE-6	S02	Chukchi Sea	− 157.5067	71.8842	71	*Icelus spatula*	*Icelus spiniger*	100
5217	CHINARE-6	B12	Bering Sea	− 178.9133	60.6542	259	*Leptagonus decagonus*	*Leptagonus decagonus*	100
NB03Z	CHINARE-6	NB03	Bering Sea	− 169.4856	64.3206	39.95	*Limanda* sp.	*Limanda aspera*	99
5178	CHINARE-6	B15	Bering Sea	− 175.2158	62.5633	78	*Liparis tanakae*	*Liparis bathyarticus*	100
5177	CHINARE-6	BS06	Bering Sea	− 168.9492	64.3539	40	*Liparis tanakae*	*Liparis bathyarticus*	100
5176	CHINARE-6	R02	Chukchi Sea	− 169.0022	67.6986	50	*Liparis tanakae*	*Liparis bathyarticus*	100
5215	CHINARE-6	B12	Bering Sea	− 178.9133	60.6542	259	*Lycodes brevipes*	*Lycodes brevipes*	100
5213	CHINARE-6	SL03	Bering Sea	− 170.9483	62.5354	37	*Liparis* sp.	*Liparis gibbus*	99
12,364	CHINARE-6	R06-2	Chukchi Sea	− 168.8694	69.6142	53.65	*Lycodes adolfi*	*Lycodes palearis*	99
P153	CHINARE-6	P15	Chukchi Sea	− 154.6817	77.4497	1259	*Lycodes seminudus*	*Lycodes seminudus*	99
P152	CHINARE-6	P15	Chukchi Sea	− 154.6817	77.4497	1259	*Lycodes seminudus*	*Lycodes seminudus*	99
5180	CHINARE-6	BS08	Bering Sea	− 167.9825	64.3731	36	*Liparis tanakae*	*Liparis tunicatus*	99
5179	CHINARE-6	C03	Chukchi Sea	− 166.5903	69.0522	33	*Liparis tanakae*	*Liparis tunicatus*	99
5335	CHINARE-6	NB09	Bering Sea	− 167.6811	62.5833	25	*Myoxocephalus jaok*	*Myoxocephalus jaok*	99

Table 1 (continued)

Sample ID	Cruise	Sampling station	Location	Longitude (°/E)	Latitude (°/S)	Sampling Depth (m)	Morphological taxonomy	Molecular taxonomy	Similarity (%)
12,275	CHIN-ARE-6	R06-2	Chukchi Sea	− 168.8694	69.6142	53.65	*Artediellus atlanticus*	*Myoxocephalus scorpius*	99
5284	CHIN-ARE-6	BS08	Bering Sea	− 167.9825	64.3731	36	*Podothecus veternus*	*Podothecus veternus*	99
12,365	CHIN-ARE-6	R06-2	Chukchi Sea	− 168.8694	69.6142	53.65	*Triglops pingelii*	*Triglops pingelii*	98
12,331	CHIN-ARE-8	B17	Bering Sea	− 173.9575	63.1153	78.65	*Anisarchus macrops*	*Anisarchus medius*	100
12,330	CHIN-ARE-8	B17	Bering Sea	− 173.9575	63.1153	78.65	*Anisarchus macrops*	*Anisarchus medius*	100
12,304	CHIN-ARE-8	BS05	Bering Sea	− 167.8625	64.2664	35.5	*Aspidophoroides olrikii*	*Aspidophoroides olrikii*	99
12,315	CHIN-ARE-8	R11	Chukchi Sea	− 168.6575	73.6878	149.1	*Boreogadus saida*	*Boreogadus saida*	99
12,306	CHIN-ARE-8	BS05	Bering Sea	− 167.8625	64.2664	35.5	*Eleginus gracilis*	*Eleginus gracilis*	99
12,305	CHIN-ARE-8	BS05	Bering Sea	− 167.8625	64.2664	35.5	*Eleginus gracilis*	*Eleginus gracilis*	99
12,280	CHIN-ARE-8	BS05	Bering Sea	− 167.8625	64.2664	35.5	*Gadus chalcogrammus*	*Gadus chalcogrammus*	100
12,308	CHIN-ARE-8	BS05	Bering Sea	− 167.8625	64.2664	35.5	*Gadus chalcogrammus*	*Gadus chalcogrammus*	100
12,274	CHIN-ARE-8	R06	Chukchi Sea	− 168.8344	69.5944	53.47	*Gymnocanthus detrisus*	*Gymnocanthus tricuspis*	100
12,303	CHIN-ARE-8	BS05	Bering Sea	− 167.8625	64.2664	35.5	*Gymnocanthus detrisus*	*Gymnocanthus tricuspis*	100
12,278	CHIN-ARE-8	B17	Bering Sea	− 173.9575	63.1153	78.65	*Hippoglossoides* sp.	*Hippoglossoides dubius*	99
12,276	CHIN-ARE-8	R11	Chukchi Sea	− 168.6575	73.6878	149.1	*Hippoglossoides robustus*	*Hippoglossoides elassodon*	100
12,279	CHIN-ARE-8	B17	Bering Sea	− 173.9575	63.1153	78.65	*Hippoglossoides robustus*	*Hippoglossoides elassodon*	100
12,277	CHIN-ARE-8	B17	Bering Sea	− 173.9575	63.1153	78.65	*Hippoglossoides robustus*	*Hippoglossoides robustus*	100
12,338	CHIN-ARE-8	B17	Bering Sea	− 173.9575	63.1153	78.65	*Icelus spatula*	*Icelus spatula*	99
12,337	CHIN-ARE-8	B17	Bering Sea	− 173.9575	63.1153	78.65	*Icelus spatula*	*Icelus spatula*	99
12,312	CHIN-ARE-8	BS05	Bering Sea	− 167.8625	64.2664	35.5	*Limanda sakhalinensis*	*Limanda sakhalinensis*	100
12,273	CHIN-ARE-8	R06	Chukchi Sea	− 168.8344	69.5944	53.47	*Lycodes adolfi*	*Lycodes palearis*	99
12,313	CHIN-ARE-8	R11	Chukchi Sea	− 168.6575	73.6878	149.1	*Zoarcidae* sp.	*Lycodes polaris*	99

Table 1 (continued)

Sample ID	Cruise	Sampling station	Location	Longitude (°/E)	Latitude (°/S)	Sampling Depth (m)	Morphological taxonomy	Molecular taxonomy	Similarity (%)
12,353	CHIN-ARE-8	R06	Chukchi Sea	− 168.8344	69.5944	53.47	*Artediellus atlanticus*	*Myoxocephalus scorpius*	99
BT0114	CHIN-ARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Arctogadus glacialis*	*Arctogadus glacialis*	100
BT016	CHIN-ARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Arctogadus glacialis*	*Arctogadus glacialis*	100
BT019	CHIN-ARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Arctogadus glacialis*	*Arctogadus glacialis*	100
BT0118	CHIN-ARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Arctogadus glacialis*	*Arctogadus glacialis*	100
BT0119	CHIN-ARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Arctogadus glacialis*	*Arctogadus glacialis*	100
BT017	CHIN-ARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Arctogadus glacialis*	*Arctogadus glacialis*	100
BT065	CHIN-ARE-9	BT06	Chukchi Sea	− 170.2581	74.9044	251	*Arctogadus glacialis*	*Arctogadus glacialis*	100
M012	CHIN-ARE-9	M01	Chukchi Sea	− 172.0981	74.8286	340	*Arctogadus glacialis*	*Arctogadus glacialis*	100
BT0110	CHIN-ARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Arctogadus glacialis*	*Arctogadus glacialis*	100
M013	CHIN-ARE-9	M01	Chukchi Sea	− 172.0981	74.8286	340	*Arctogadus glacialis*	*Arctogadus glacialis*	100
BT0122	CHIN-ARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Arctogadus glacialis*	*Arctogadus glacialis*	100
BT0115	CHIN-ARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Arctogadus glacialis*	*Arctogadus glacialis*	100
BT0117	CHIN-ARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Arctogadus glacialis*	*Arctogadus glacialis*	100
BT0113	CHIN-ARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Arctogadus glacialis*	*Arctogadus glacialis*	100
BT0111	CHIN-ARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Arctogadus glacialis*	*Arctogadus glacialis*	100
BT0112	CHIN-ARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Arctogadus glacialis*	*Arctogadus glacialis*	100
BT0123	CHIN-ARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Arctogadus glacialis*	*Arctogadus glacialis*	100
OBS032X	CHIN-ARE-9	OBS03	Chukchi Sea	− 168.9556	74.6033	181	*Artediellus atlanticus*	*Artediellus atlanticus*	100
BT034	CHIN-ARE-9	BT03	Chukchi Sea	− 167.985	75.6147	170	*Artediellus atlanticus*	*Artediellus atlanticus*	100
BT072	CHIN-ARE-9	BT07	Chukchi Sea	− 168.9189	74.2089	178	*Artediellus atlanticus*	*Artediellus atlanticus*	100
OBS032D	CHIN-ARE-9	OBS03	Chukchi Sea	− 168.9556	74.6033	181	*Artediellus atlanticus*	*Artediellus atlanticus*	100
BT033	CHIN-ARE-9	BT03	Chukchi Sea	− 167.985	75.6147	170	*Artediellus atlanticus*	*Artediellus atlanticus*	100
BT042	CHIN-ARE-9	BT04	Chukchi Sea	− 168.245	75.2569	167	*Artediellus atlanticus*	*Artediellus atlanticus*	100
BT014	CHIN-ARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Artediellus atlanticus*	*Artediellus atlanticus*	100
BT061	CHIN-ARE-9	BT06	Chukchi Sea	− 170.2581	74.9044	251	*Artediellus atlanticus*	*Artediellus atlanticus*	100

Table 1 (continued)

Sample ID	Cruise	Sampling station	Location	Longitude (°/E)	Latitude (°/S)	Sampling Depth (m)	Morphological taxonomy	Molecular taxonomy	Similarity (%)
BT0214	CHINARE-9	BT02	Chukchi Sea	− 167.6922	75.8169	228	*Artediellus atlanticus*	*Artediellus atlanticus*	100
BT063	CHINARE-9	BT06	Chukchi Sea	− 170.2581	74.9044	251	*Boreogadus saida*	*Boreogadus saida*	99
BT082	CHINARE-9	BT08	Chukchi Sea	− 168.9231	73.7411	155	*Boreogadus saida*	*Boreogadus saida*	99
BT052	CHINARE-9	BT05	Chukchi Sea	− 168.4794	75.0181	167	*Boreogadus saida*	*Boreogadus saida*	99
BT035	CHINARE-9	BT03	Chukchi Sea	− 167.985	75.6147	170	*Boreogadus saida*	*Boreogadus saida*	99
BT0212	CHINARE-9	BT02	Chukchi Sea	− 167.6922	75.8169	228	*Boreogadus saida*	*Boreogadus saida*	99
BT0125	CHINARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Boreogadus saida*	*Boreogadus saida*	99
BT064	CHINARE-9	BT06	Chukchi Sea	− 170.2581	74.9044	251	*Boreogadus saida*	*Boreogadus saida*	99
BT0126	CHINARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Boreogadus saida*	*Boreogadus saida*	99
BT053	CHINARE-9	BT05	Chukchi Sea	− 168.4794	75.0181	167	*Boreogadus saida*	*Boreogadus saida*	99
OBS021	CHINARE-9	OBS02	Chukchi Sea	− 170.5383	74.6944	233	*Boreogadus saida*	*Boreogadus saida*	99
OBS034	CHINARE-9	OBS03	Chukchi Sea	− 168.9556	74.6033	181	*Boreogadus saida*	*Boreogadus saida*	99
BT028	CHINARE-9	BT02	Chukchi Sea	− 167.6922	75.8169	228	*Boreogadus saida*	*Boreogadus saida*	99
BT027	CHINARE-9	BT02	Chukchi Sea	− 167.6922	75.8169	228	*Boreogadus saida*	*Boreogadus saida*	99
BT0213	CHINARE-9	BT02	Chukchi Sea	− 167.6922	75.8169	228	*Boreogadus saida*	*Boreogadus saida*	99
BT0210	CHINARE-9	BT02	Chukchi Sea	− 167.6922	75.8169	228	*Boreogadus saida*	*Boreogadus saida*	99
BT054	CHINARE-9	BT05	Chukchi Sea	− 168.4794	75.0181	167	*Boreogadus saida*	*Boreogadus saida*	99
BT0120	CHINARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Boreogadus saida*	*Boreogadus saida*	99
BT036	CHINARE-9	BT03	Chukchi Sea	− 167.985	75.6147	170	*Boreogadus saida*	*Boreogadus saida*	99
BT0121	CHINARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Boreogadus saida*	*Boreogadus saida*	99
BT018	CHINARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Boreogadus saida*	*Boreogadus saida*	99
BT0211	CHINARE-9	BT02	Chukchi Sea	− 167.6922	75.8169	228	*Boreogadus saida*	*Boreogadus saida*	99
BT029	CHINARE-9	BT02	Chukchi Sea	− 167.6922	75.8169	228	*Boreogadus saida*	*Boreogadus saida*	99
BT0124	CHINARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Boreogadus saida*	*Boreogadus saida*	99
BT037	CHINARE-9	BT03	Chukchi Sea	− 167.985	75.6147	170	*Boreogadus saida*	*Boreogadus saida*	99
BT0116	CHINARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Boreogadus saida*	*Boreogadus saida*	99

Table 1 (continued)

Sample ID	Cruise	Sampling station	Location	Longitude (°/E)	Latitude (°/S)	Sampling Depth (m)	Morphological taxonomy	Molecular taxonomy	Similarity (%)
R08Z1	CHINARE-9	BT08	Chukchi Sea	− 168.9231	73.7411	155	*Boreogadus saida*	*Boreogadus saida*	99
M014	CHINARE-9	M01	Chukchi Sea	− 172.0981	74.8286	340	*Boreogadus saida*	*Boreogadus saida*	99
BT071	CHINARE-9	BT07	Chukchi Sea	− 168.9189	74.2089	178	*Careproctus reinhardti*	*Careproctus reinhardti*	100
BT011	CHINARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Cottunculus microps*	*Cottunculus microps*	100
BT012	CHINARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Cottunculus microps*	*Cottunculus microps*	100
BT051	CHINARE-9	BT05	Chukchi Sea	− 168.4794	75.0181	167	*Gadus chalcogrammus*	*Gadus chalcogrammus*	100
OBS035	CHINARE-9	OBS03	Chukchi Sea	− 168.9556	74.6033	181	*Gadus chalcogrammus*	*Gadus chalcogrammus*	100
BT081	CHINARE-9	BT08	Chukchi Sea	− 168.9231	73.7411	155	*Hippoglossoides robustus*	*Hippoglossoides elassodon*	100
BT031	CHINARE-9	BT03	Chukchi Sea	− 167.985	75.6147	170	*Liparis fabricii*	*Liparis fabricii*	100
BT021	CHINARE-9	BT02	Chukchi Sea	− 167.6922	75.8169	228	*Liparis fabricii*	*Liparis fabricii*	100
BT022	CHINARE-9	BT02	Chukchi Sea	− 167.6922	75.8169	228	*Liparis fabricii*	*Liparis fabricii*	100
OBS033	CHINARE-9	OBS03	Chukchi Sea	− 168.9556	74.6033	181	*Liparis fabricii*	*Liparis fabricii*	100
BT032	CHINARE-9	BT03	Chukchi Sea	− 167.985	75.6147	170	*Liparis fabricii*	*Liparis fabricii*	100
OBS022	CHINARE-9	OBS02	Chukchi Sea	− 170.5383	74.6944	233	*Lycodes adolfi*	*Lycodes adolfi*	99
OBS031	CHINARE-9	OBS03	Chukchi Sea	− 168.9556	74.6033	181	*Lycodes lavalaei*	*Lycodes lavalaei*	99
BT041	CHINARE-9	BT04	Chukchi Sea	− 168.245	75.2569	167	*Lycodes lavalaei*	*Lycodes lavalaei*	99
BT013	CHINARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Lycodes pallidus*	*Lycodes pallidus*	98
BT025	CHINARE-9	BT02	Chukchi Sea	− 167.6922	75.8169	228	*Lycodes pallidus*	*Lycodes pallidus*	98
BT062	CHINARE-9	BT06	Chukchi Sea	− 170.2581	74.9044	251	*Lycodes pallidus*	*Lycodes pallidus*	98
M011	CHINARE-9	M01	Chukchi Sea	− 172.0981	74.8286	340	*Lycodes pallidus*	*Lycodes pallidus*	98
BT024	CHINARE-9	BT02	Chukchi Sea	− 167.6922	75.8169	228	*Lycodes pallidus*	*Lycodes pallidus*	98
BT023	CHINARE-9	BT02	Chukchi Sea	− 167.6922	75.8169	228	*Lycodes pallidus*	*Lycodes pallidus*	98
OBS023	CHINARE-9	OBS02	Chukchi Sea	− 170.5383	74.6944	233	*Lycodes pallidus*	*Lycodes pallidus*	98
BT015	CHINARE-9	BT01	Chukchi Sea	− 167.4278	76.0389	284	*Triglops nybelini*	*Triglops nybelini*	100
BT026	CHINARE-9	BT02	Chukchi Sea	− 167.6922	75.8169	228	*Triglops nybelini*	*Triglops nybelini*	100

Amplification and sequencing

A total of 123 mitochondrial COI gene DNA fragments were successfully amplified using primers. No stop codons, deletions or insertions were observed in any of the sequences after alignment. The length of the amplified COI gene was 652 bp. The number of haplotypes identified in each species ranged from 1 to 6. Overall nucleotide frequencies were C (27.7%), T (30.8%), A (23.4%), and G (18.2%). The 123 COI sequences were deposited in the GenBank database under the accession number MW435025—MW435147.

Species identification by phylogenetic analysis of COI sequences

The phylogenetic tree constructed by the NJ method is shown in Fig. 2. The same morphological species of fish formed cohesive units. All high-quality sequences were identified by BLAST searches and comparisons

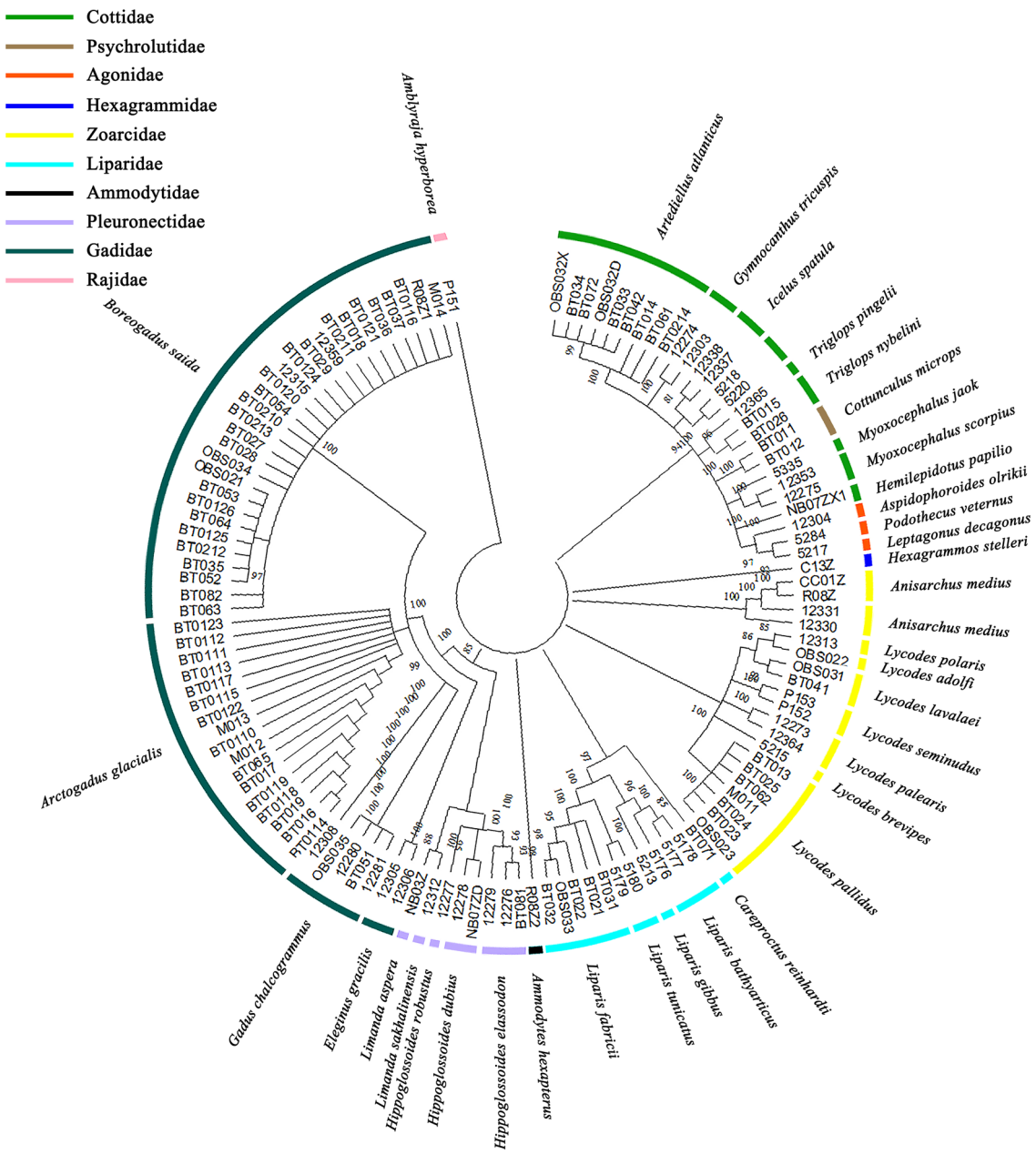

Fig. 2 Neighbour-joining (NJ) tree constructed using COI gene sequence. Bootstrap values higher than 70 are indicated along the branches. Different-colored bands indicate different families

in GenBank, and the similarity was higher than 98% (Table 1). A total of 39 fish species belonging to 5 orders, 10 families and 23 genera were identified through DNA barcoding analysis in this survey. Among these species, 19 species of Scorpaeniformes accounted for 48.72% of the total species. Additionally, 9 species of Perciformes accounted for 23.08% of the total number of species, and 5 species from each of Pleuronectiformes and Gadiformes accounted for 12.82% of the total number of species. The smallest number of species was found in Rajiformes, which included only one species. At the family level, the number of Cottidae species was largest, at 9, accounting for 23.08% of the total number of species, followed by Zoarcidae, with 8 species, accounting for 20.51%. The other eight families—Gadidae, Pleuronectidae, Psychrolutidae, Agonidae, Liparidae, Ammodytidae, Hexagrammidae, and Rajidae—accounted for smaller proportions. At the genus level, the number of species from the genus *Lycodes* was greatest, at 8. Based on the NJ tree, all species from the same family were clustered together, indicating that the families were all monophyletic except for Cottidae, in which *Hemilepidotus papilio* was sister to other genera, thus, the NJ analysis recovered the family Cottidae as polyphyletic. Besides, Psychrolutidae was nested within Cottidae, indicating the complicated relationship between the two families.

The NJ tree showed that the barcoding results were not all consistent with the conventional taxonomy. However, 61.54% of species were identified successfully, showing at least 98% similarity. *Icelus spiniger* was distinctly different from *I. spatula*, with specimens identified as *I. spatula* morphologically sharing 100% nucleotide sequence similarity with the *I. spiniger* reference. *Hippoglossoides elassodon* was identified as *H. robustus* morphologically but presented 100% similarity to the *H. elassodon* reference. In similar cases, *Lycodes palearis* was identified as *L. adolfi*, *Anisarchus medius* was identified as *A. macrops*, *Myoxocephalus Scorpius* was identified as *Artediellus atlanticus*, and *Gymnocanthus tricuspis* was identified as *G. detrisus*. The sequences of *Liparis bathyarcticus* and *L. tunicatus* were distinctly different from the *L. tanakae* references. Some juvenile fish and incomplete individuals that were initially recorded as unidentified "sp." were identified effectively based on barcoding. *Limanda* sp. shared 99% nucleotide sequence similarity with the reference *L. aspera*, indicating that it was *L. aspera*. *Hippoglossoides* sp. was identified as *H. dubius* with 99% nucleotide sequence similarity. Zoarcidae sp. was identified as *L. polaris* with 99% nucleotide sequence similarity. *Ammodytes* sp. was identified as *A. hexapterus* with 100% nucleotide sequence similarity. *Hemilepidotus* sp. was identified as *H. papilio* with 99% nucleotide sequence similarity. *Liparis* sp. was identified as *L. gibbus* with 99% nucleotide sequence similarity.

Genetic distance and barcoding gaps

The intraspecific distances ranged from 0 to 0.35%, and the minimum interspecific distances of the species were greater than 2% except for *Liparis tunicatus* vs *L. fabricii* (1.43%), *Hippoglossoides elassodon* vs *H. robustus* (0.62%), *H. elassodon* vs *H. dubius* (0.54%), *H. robustus* vs *H. dubius* (0.54%), and *Icelus spatula* vs *I. spiniger* (0.69%). Nevertheless, the minimum interspecific distance of all species was still greater than the maximum intraspecific distances. Thus, it was obvious that there were barcode gaps in the genetic distance between intraspecific distances and interspecific distance (Fig. 3).

Discussion

Correct species identification is the foundation for revealing fish diversity. Traditional morphological identification methods require the experience of high-level classification experts and sample preservation integrity. Among the samples utilized in this study, there were some juveniles and damaged individuals, and it was difficult to carry out morphological identification. Thus, many samples could only be identified to the family or genus level, such as *Limanda* sp., *Hippoglossoides* sp., *Lycodes* sp., *Ammodytes* sp., *Hemilepidotus* sp. and *Liparis* sp. At the same time, only a few species with similar morphological features could not be clearly distinguished. However, after we conducted a molecular evaluation of the fish in the Bering Sea and the Chukchi Sea, COI-based DNA barcoding was proven to be effective for identifying Arctic fish species, with 100% of species exhibiting monophyletic DNA clusters. Among the 39 species investigated in this study, only 24 species (61.54%) were identified correctly by morphological examination. However, all species were accurately identified with COI sequences. The effectiveness (number of species exhibiting monophyletic clusters) of the DNA barcoding analysis in our study was demonstrated to be higher than those found in other barcoding studies of fishes (Hubert et al. 2008; Steinke et al. 2009; Ward et al. 2005).

The existence of barcode gaps increases the effectiveness of DNA barcodes for identifying species. In this study, all the obtained minimum interspecific distances were greater than the maximum intraspecific genetic distances. A value of 2% has been suggested as a threshold value between species and genus divergence (Ward 2009). However, the genetic distances between species of *Hippoglossoides, Icelus* and *Liparis* were lower than 2% (0.54% to 1.43%), which was probably associated with recent speciation (McCusker et al. 2013). The NJ tree illustrated short genetic distances between congeneric species of the genera *Hippoglossoides*, *Icelus* and *Liparis*. The members of these three genera

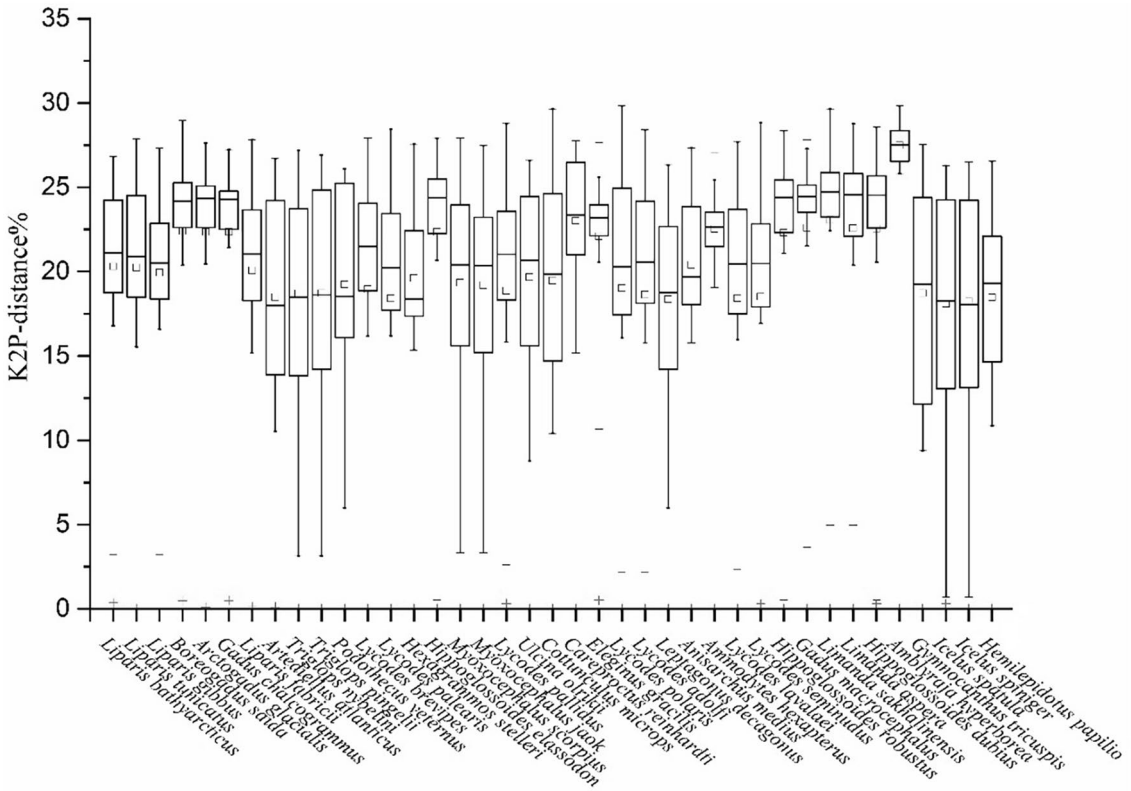

Fig. 3 DNA barcoding gaps for fish species based on the K2P model, species that represented by single individual are removed. Maximum and minimum interspecific distance values are represented by the upper and lower bars, respectively. Red cross: maximum intraspecific distance; quadrate: mean value

exhibited the shortest interspecific distances, which was consistent with recent molecular phylogenies (McCusker et al. 2013). Despite the low interspecific distance between these congeneric species, based on the phylogenetic tree, the species within each genus clustered closely together, formed their own branches and showed a high support rate (97–100%). McCusker et al. (2013) reported that barcoding was still effective (species were monophyletic) under conditions of low genetic divergence. Nevertheless, it is recommended that other molecular markers be combined in barcodes in future efforts aimed at the molecular identification of these species (Qu et al. 2018). Overall, 123 fish collected from the Bering Sea and the Chukchi Sea could be identified to the species level using COI sequences.

The phylogenetic tree constructed based on the obtained sequences showed cluster formation; clustering in the phylogenetic tree can help detect problems and is a valuable tool, especially for closely related species without obvious morphological differences (Dettai et al. 2011). Although barcode analysis is mostly used to delimit species boundaries, there are obvious phylogenetic signals within COI sequence information (Hebert et al. 2003a; Ward et al. 2005). In the phylogenetic tree based on the NJ method obtained in this study, different individuals of each species were clustered together. However, it should be noted that at the family level, *H. papilio* of Cottidae and Agonidae formed a separate branch while Psychrolutidae was nested within Cottidae. This may be the reason why Mecklenburg et al. (Mecklenburg et al. 2010) indicated that the internal relationships of the Cottidae are obscure and not well defined. However, *H. papilio* was represented in the specimens by only one specimen, and additional specimens will certainly be necessary to further clarify the relationship between *H. papilio* and Cottidae.

Conclusions

This study shows that DNA barcoding is an accurate and efficient method of species identification. A total of 123 fish collected from the Bering Sea and the Chukchi Sea were identified by DNA barcoding. Thirty-nine species from ten families were characterized; all species were identified correctly. We also observed low interspecific divergence (< 2%), probably associated with recent speciation. It is recommended that other molecular markers be included to develop unique DNA barcodes that are suitable for Arctic fish. In

follow-up studies, it is necessary to combine morphology-based identification systems with DNA barcoding to identify species because morphological identification alone may not be sufficiently robust. In addition, our work provides important information for further studies regarding the biodiversity, biogeography and conservation of Arctic fishes.

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Author contributions HL designed experiments and wrote the manuscript, FY performed data analyses, XW wrote the manuscript, YL, NZ, RZ, CL and HZ performed experiments, and, LL and PS conceived experiments. All the authors discussed the results and gave their opinions on the manuscript.

Data availability All data generated in this study are included in this published article [and its supplementary information] and in GenBank (NCBI) [https://www.ncbi.nlm.nih.gov/genbank] where sequences with accession numbers MW435025—MW435147 were deposited.

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