DNA Barcoding Provides Insights Into Fish Diversity and Molecular Taxonomy of the Amundsen Sea

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Abstract

The Southern Ocean is experiencing complex climate change, and the Amundsen Sea is one of the regions that responds most rapidly to climate change. Due to their role in ecosystems, environmental sensitivity and high endemism, Antarctic demersal fish are a favourable group that can act as an indicator to indicate the response of Antarctic organisms to climate change. However, our knowledge of Antarctic fish fauna is insufficient, with knowledge gaps even in their taxonomy. This situation is greatly influenced by the limitations of traditional taxonomy and thus calls for alternative solutions such as DNA barcoding. In this study, DNA barcoding analyses of 69 fish samples obtained from the Amundsen Sea were conducted using the mitochondrial COI gene. Based on molecular species delimitation results, 13 fish species belonged to two orders, six families, and 12 genera. Both the maximum likelihood and Bayesian inference methods showed that the phylogenetic relationships of Bathydraconidae were paraphyletic, which was consistent with previous phylogenetic research. Our research showed that the COI gene, as a DNA barcode, not only is suitable for the identification of Antarctic fish species but also reflects some phylogenetic characteristics that might provide important evidence and support for studies of Antarctic fish phylogenetic relationships. In summary, our study provides an important reference for fish diversity and taxonomy in the Amundsen Sea, which may further enhance our understanding of the biodiversity, taxonomy and biogeography of fish in this area.

1 Introduction

The Southern Ocean occupies almost 10% of the ocean area on Earth (Joyner 1998). It is the only ocean that surrounds Earth and is not divided by continents. This gives it a unique ocean current system. The Antarctic Circumpolar Current (ACC) travels around Antarctica in a clockwise direction, driven by sustained westerly winds (Allison et al. 2010). It prevents warm water from flowing from lower latitudes to higher latitudes, making the Southern Ocean one of the coldest oceans on Earth (Tynan 1998). Organisms in the Southern Ocean have adapted to the polar climate after millions of years of evolution (Clarke & Johnston 1996). However, complex climate change occurs in Antarctica, especially in West Antarctica, such as the Amundsen Sea (Jun et al. 2020). Changes in the marine environment, especially temperature (McGlone et al. 2010), salinity (Haumann et al. 2016), and dissolved oxygen(Keller et al. 2016), may have important effects on the marine ecosystem and biological community structure (La et al. 2019). As one of the most widely distributed and richest species groups in the ocean, fish are a key component of the marine ecosystem that maintains their balance. They not only serve a basic ecological function but also play an important role in indicating the operating status of the ecosystem (Hunt Jr et al. 2002; Vander Zanden et al. 2011). Modern Antarctic fish fauna, whether in terms of biodiversity, abundance, or biomass, are mainly dominated by Notothenioidei, including Artedidraconidae, Bathydraconidae, Channichthyidae, Harpagiferidae, and Nototheniidae (Mintenbeck et al. 2012). These fish live in cold, oxygen-rich, and stable ocean environments and are highly endemic (Mintenbeck & Torres 2017). These characteristics, along with the roles the fish play in the ecosystem, make Antarctic fishes a favourable group that can act as an indicator of environmental change in the Southern Ocean.

Compared to the vast ocean area of the Southern Ocean, there are only approximately 370 species of fish described which account for ~2% of all fish species worldwide, nevertheless, this number is underestimated (Eastman 2000). Ice cover, lack of deep-sea samples, low sampling frequency and insufficient traditional taxonomy may also be the reasons for the underestimation of the number (Alt et al. 2021). Unfortunately, the situation of the fish fauna of the Amundsen Sea is even worse, because the Amundsen Sea is located in a remote location relative to scientific research stations and routes (Griffiths et al. 2011). There were only limited observation records and an underwater observation survey report (Eastman et al. 2012), while studies based on molecular taxonomy have not yet been reported. Currently, the Amundsen Sea is among the places where the sea temperature in the Southern Ocean rises most obviously (Kim et al. 2021). The rapid rise in sea temperature has led to a decrease in sea ice cover and a sustained decline in the ice shelf (Haumann et al. 2016). Meanwhile, the benthic ecosystem in Antarctica is vulnerable (Pineda-Metz et al. 2020), glacier retreat (Sahade et al. 2015) and associated iceberg scouring (Gutt & Piepenburg 2003; Barnes & Souster 2011) have a huge impact on benthic communities, including Antarctic fish, which mostly belong to demersal fish (Mintenbeck et al. 2012). Moreover, the decline in salinity and dissolved oxygen (Yager et al. 2012; Randall-Goodwin et al. 2015) also brings challenges to fish survival that cannot be ignored. As one of the important indicator groups of climate change, the lack of information on the composition of fish communities in the Amundsen Sea will seriously affect the evaluation of the structure and function of its marine ecosystem. Therefore, a fish diversity baseline inventory is urgently needed, and clarifying the characteristics of Amundsen Sea fish diversity patterns can help us better understand the impacts of climate change on Amundsen Sea marine ecosystems.

Traditional fish classification is based on morphological identification, which is time-consuming and depends on the experience of the taxonomist (Steinke et al. 2009). However, the morphologies of sibling species are similar, which can easily lead to misidentification. In particular, the amazing diversity of sizes, colours, and shapes in different life stages of fish is a challenge to taxonomists (Zhang & Hanner 2012). At the same time, the taxonomic division of some fish in the Southern Ocean is controversial (De Broyer et al. 2014). All these problems require new solutions. The emergence of species identification methods based on molecular biology has given taxonomists more choices and has the potential to become a universal method. This method is expected to become one of the most convincing types of classification evidence (Hebert et al. 2003a). DNA barcoding is increasingly being advocated for in the identification of species. DNA barcoding based on the cytochrome c oxidase subunit I (COI) mitochondrial gene was applied to the identification of species (Hebert et al. 2003b). A COI fragment of 650 bp has enough sequence diversity to reflect significant species-level differences and has demonstrated high efficiency and accuracy in species identification on a global scale, such as in Japanese marine fish (Zhang & Hanner 2011), Indian marine fish (Lakra et al. 2011), Cuban freshwater fish (Lara et al. 2010), Indo-Pacific coral reef fish (Hubert et al. 2012), and even birds (Hebert et al. 2004), mammals (Francis et al. 2010), and bivalves (Mikkelsen et al. 2007), among others. In this paper, the COI-based molecular identification method is applied to Antarctic fish of the Amundsen Sea. Our research aims to provide fundamental taxonomic information for fish species of the Amundsen Sea, and thus provide a solid scientific basis for the ecological assessment and biological conservation of the Southern Ocean.

2 Materials And Methods

2.1 Specimen collections

All specimens were collected at Xuelong icebreaker research vessels during the 36th Chinese National Antarctic Research Expedition (CHINARE) in 2020. Specimens were caught by a bottom trawling net (2.2 m wide, 0.65 m high, and 6.5 m long, 20 mm mesh diameter). Every net was employed for approximately 10~15 minutes at speeds of 2~3 kn. All samples were collected from 4 stations (Fig. 1) in the Amundsen Sea. All caught fish were sorted at -20°C and provisionally identified. Muscle samples were stored in 95% ethanol for DNA extraction. Finally, all fish were fixed in 10% formaldehyde and stored as voucher samples at the Third Institute of Oceanography, Ministry of Natural Resources.

2.2 DNA preparation, PCR and sequencing

DNA extraction was carried out with muscle tissue by using a DNeasy Blood and Tissue Kit [Qiagen, Hilden, Germany]. Some steps followed those of Hellberg et al (2014). Microtubes of 1.5 mL [Axygen, New York, American] and ethanol (99.7%) [XILONG SCIENTIFIC, Guangdong, China] were prepared in advance. Muscle samples (approximately 30 mg) were weighed into 1.5 mL microtubes, and then the steps in the manufacturer's instructions were followed. Finally, DNA was stored at -20 ℃ until PCR amplification. The primers in this study were designed by Ward (2005) and were used for COI amplification.

All PCRs had a total volume of 25 μL and included 17.25 μL of ultrapure water, 2 μL of dNTPs (2.5 mM), 2.5 μL of 10 × PCR buffer (including Mg^{2+}) (20 mM), 1 μL of each primer, 0.25 μL of Taq polymerase [TaKaRa, Kusatsu, Japan] (5 U/μL), and 1 μL of DNA template. Amplifications were performed using a SensoQuest LabCycler [SensoQuest, Germany] gradient thermal cycler. PCR cycling consisted of an initial step of 4 min at 95 ℃ and 35 cycles of 30 s at 94 ℃, 30 s at 50 ℃, and 30 s at 72 ℃, followed by a final extension at 72 ℃ for 10 min. PCR products were loaded onto 1% agarose gels and selected for sequencing, and all the PCR products were purified and sequenced by Personal Biotechnology Co., Ltd.

2.3 DNA identification and phylogenetic analysis

All COI sequences were edited using DNASTAR Lasergene SeqMan Pro 7.1 and aligned manually using Sequencher 4.1 (Gene Codes 2000). All the COI sequences (> 650 bp) were BLAST searched against the NCBI database and matches with more than 98% similarity were considered the same species(Murphy et al. 2016). To facilitate the calculation of the genetic distance, two additional data points from the NCBI database were added for each species with fewer than three fish. The newly isolated nucleotide sequences were deposited in GenBank under Accession Numbers (Table 1).

Table 1
Information of samples and species identification

Sample No.	Sample site	Longitude (°/W)	Latitude (°/S)	Sample Depth(m)	Molecular identification	Morphological identification	Genbank voucher No.	Similarity (%)	Genbank No.
AN1	A11-1	113.35	73.52	627	*Dacodraco hunteri*	*Dacodraco hunteri*	HQ712963.1	99.85	OK493632
AN2	A11-4	117.32	72.25	523	*Lycenchelys* sp.	Ophthalmolycus amberensis	EU326372.1	99.35	OK493633
AN3	A11-4	117.32	72.25	523	*Trematomus scotti*	*Trematomus scotti*	KX676179.1	99.85	OK493645
AN4	A11-4	117.32	72.25	523	*Trematomus scotti*	*Trematomus scotti*	HQ713279.1	99.69	OK493646
AN5	A11-4	117.32	72.25	523	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	99.54	OK493647
AN6	A11-4	117.32	72.25	523	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	99.69	OK493648
AN7	A11-4	117.32	72.25	523	*Trematomus scotti*	*Trematomus scotti*	EU326433.1	100.00	OK493649
AN8	A11-4	117.32	72.25	523	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	99.85	OK493650
AN9	A11-4	117.32	72.25	523	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	100.00	OK493651
AN10	A11-4	117.32	72.25	523	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	100.00	OK493652
AN11	A11-4	117.32	72.25	523	*Trematomus scotti*	*Trematomus scotti*	KX676181.1	99.85	OK493653
AN12	A11-4	117.32	72.25	523	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	100.00	OK493654
AN13	A11-4	117.32	72.25	523	*Trematomus scotti*	*Trematomus scotti*	KX676179.1	99.54	OK493655
AN14	A11-1	113.35	73.52	627	*Vomeridens infuscipinnis*	*Vomeridens infuscipinnis*	HQ713358.1	100.00	OK493677
AN15	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	100.00	OK493681
AN16	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	HQ713279.1	99.85	OK493682
AN17	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	99.85	OK493683
AN18	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	99.85	OK493684
AN19	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	100.00	OK493685
AN20	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	99.85	OK493686
AN21	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676177.1	100.00	OK493687
AN22	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676177.1	99.85	OK493688
AN23	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676177.1	99.85	OK493689
AN24	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676181.1	99.85	OK493690
AN25	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	99.39	OK493691

Note: Morphological names in bold are misidentified samples using morphological taxonomy.

Sample No.	Sample site	Longitude (°/W)	Latitude (°/S)	Sample Depth(m)	Molecular identification	Morphological identification	Genbank voucher No.	Similarity (%)	Genbank No.
AN26	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	EU326433.1	99.85	OK493692
AN27	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676171.1	99.69	OK493693
AN28	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	HQ713279.1	99.54	OK493694
AN29	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	EU326433.1	100.00	OK493695
AN30	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	99.85	OK493696
AN31	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676175.1	100.00	OK493697
AN32	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	100.00	OK493698
AN33	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	100.00	OK493699
AN34	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	HQ713279.1	99.54	OK493700
AN35	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	99.85	OK493701
AN36	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676177.1	100.00	OK493702
AN37	A11-3	113.58	73.41	423	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	100.00	OK493703
AN38	A11-1	113.35	73.52	627	*Trematomus scotti*	*Trematomus scotti*	KX676171.1	99.69	OK493704
AN39	A11-1	113.35	73.52	627	*Trematomus scotti*	*Trematomus scotti*	KX676177.1	100.00	OK493705
AN40	A11-1	113.35	73.52	627	*Trematomus scotti*	*Trematomus scotti*	KX676176.1	99.69	OK493706
AN41	A11-1	113.35	73.52	627	*Trematomus scotti*	*Trematomus scotti*	KX676173.1	99.85	OK493707
AN42	A11-1	113.35	73.52	627	*Trematomus scotti*	*Trematomus scotti*	KX676173.1	100.00	OK493708
AN43	A4-3	112.99	72.91	438	*Chaenodraco wilsoni*	*Chaenodraco wilsoni*	NC_039158.1	99.09	OK493709
AN44	A11-1	113.35	73.52	627	*Chionodraco myersi*	*Chionodraco myersi*	DQ526430.1	99.70	OK493710
AN45	A11-1	113.35	73.52	627	*Chionodraco myersi*	*Chionodraco myersi*	DQ526430.1	99.56	OK493711
AN46	A11-1	113.35	73.52	627	*Macrourus whitsoni*	*Macrourus whitsoni*	MT157320.1	97.99	OK493712
AN47	A11-1	113.35	73.52	627	*Macrourus whitsoni*	*Macrourus whitsoni*	MT157320.1	100.00	OK493713
AN48	A4-3	112.99	72.91	438	*Chaenodraco wilsoni*	*Chaenodraco wilsoni*	NC_039158.1	99.24	OK493714
AN49	A11-1	113.35	73.52	627	*Dolloidraco longedorsalis*	*Dolloidraco longedorsalis*	NC_057667.1	99.56	OK493715
AN50	A11-1	113.35	73.52	627	*Dolloidraco longedorsalis*	*Dolloidraco longedorsalis*	NC_057667.1	99.56	OK493716
AN51	A11-1	113.35	73.52	627	*Dolloidraco longedorsalis*	*Dolloidraco longedorsalis*	NC_057667.1	99.56	OK493717

Note: Morphological names in bold are misidentified samples using morphological taxonomy.

Sample No.	Sample site	Longitude (°/W)	Latitude (°/S)	Sample Depth(m)	Molecular identification	Morphological identification	Genbank voucher No.	Similarity (%)	Genbank No.
AN52	A11-2	115.10	73.02	693	*Dolloidraco longedorsalis*	*Dolloidraco longedorsalis*	NC_057667.1	99.71	OK493718
AN53	A4-3	112.99	72.91	438	*Artedidraco lonnbergi*	*Artedidraco lonnbergi*	HQ712823.1	100.00	OK493719
AN54	A11-1	113.35	73.52	627	*Trematomus cf. lepidorhinus/loennbergi*	*Trematomus loennbergii*	NC_048965.1	99.27	OK493720
AN55	A4-3	112.99	72.91	438	*Trematomus cf. lepidorhinus/loennbergi*	*Trematomus loennbergii*	NC_048965.1	99.56	OK493721
AN56	A11-1	113.35	73.52	627	*Akarotaxis nudiceps*	*Akarotaxis nudiceps*	NC_057664.1	99.09	OK493722
AN57	A11-1	113.35	73.52	627	*Akarotaxis nudiceps*	*Akarotaxis nudiceps*	NC_057664.1	99.41	OK493723
AN58	A4-3	112.99	72.91	438	*Ophthalmolycus amberensis*	*Ophthalmolycus amberensis*	JN641043.1	100.00	OK493724
AN59	A11-4	117.32	72.25	523	*Gerlachea australis*	*Gerlachea australis*	NC_057668.1	99.56	OK493725
AN60	A4-3	112.99	72.91	438	*Macrourus whitsoni*	*Macrourus whitsoni*	MT157320.1	99.56	OK493726
AN61	A11-1	113.35	73.52	627	*Dacodraco hunteri*	*Dacodraco hunteri*	HQ712963.1	99.85	OK493727
AN62	A4-3	112.99	72.91	438	*Chaenodraco wilsoni*	*Chaenodraco wilsoni*	NC_039158.1	98.69	OK493728
AN63	A4-3	112.99	72.91	438	*Trematomus cf. lepidorhinus/loennbergi*	*Trematomus loennbergii*	NC_048965.1	99.41	OK493730
AN64	A4-3	112.99	72.91	438	*Trematomus cf. lepidorhinus/loennbergi*	*Trematomus loennbergii*	HQ713304.1	99.85	OK493731
AN65	A11-1	113.35	73.52	627	*Chionodraco myersi*	*Chionodraco myersi*	DQ526430.1	99.56	OK493732
AN66	A11-1	113.35	73.52	627	*Vomeridens infuscipinnis*	*Vomeridens infuscipinnis*	HQ713358.1	100.00	OK493740
AN67	A11-1	113.35	73.52	627	*Macrourus whitsoni*	*Macrourus whitsoni*	MT157320.1	99.70	OK493741
AN68	A11-1	113.35	73.52	627	*Akarotaxis nudiceps*	*Akarotaxis nudiceps*	NC_057664.1	99.70	OK493743
AN69	A4-3	112.99	72.91	438	*Gerlachea australis*	*Gerlachea australis*	NC_057668.1	99.70	OK493745

Note: Morphological names in bold are misidentified samples using morphological taxonomy.

We used two DNA identification methods to access taxonomic units: assemble species by automatic partitioning (ASAP) (Puillandre et al. 2021)and Bayesian phylogenetics and phylogeography (BPP)(Yang et al. 2014) to infer putative species boundaries based on the COI gene. ASAP uses single locus sequence alignments to create species partitions; it is based on the implementation of a hierarchical clustering algorithm and compares only pairwise genetic distances. All aligned COI sequences were calculated by the ASAP (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html), with JC69 (Jukes-Cantor) model to compute the distance and default settings (split groups below probability 0.01, keep 10 best scores). BPP is a Bayesian Markov chain Monte Carlo (MCMC) tool for analysing DNA sequences under the multispecies coalescent (MSC) model. The ultra-metric tree with haplotypes was reconstructed using BEAST v1.10.4 (Drummond et al. 2012). The parameters in BEAUti use the GTR model and gamma shape site model. The number of gamma categories is 4, uncorrelated relaxed clock, and a chain length of 30000000 iterations for MCMC.

Genetic distances were calculated using the Kimura two-parameter (K2P) distance model (Kimura 1980) with 1000 bootstrap replicates and uniform rates using MEGA X (Kumar et al. 2018). Including intra- and interspecies genetic distances and pairwise distance. We used the online tool SMS to find suitable models of nucleotide substitution under the Akaike information criterion (AIC). BI tree and ML tree were used to construct the phylogenetic relationships. BI tree was constructed using MrBayes v3.1.2 (Huelsenbeck et al. 2001), and MCMC analysis was run with 10000000 generations, sampling every 1000 generations. We used PhyML3.0 (Guindon et al. 2010) to build an ML tree with GTR and 0.186 gamma shape parameters as substitution models, NII for tree improvement, aLRT SH-like fast likelihood method. Finally, the majority-rule consensus tree was reconstructed and displayed using Figtree v1.4.4.

3 Results

3.1 Morphological and DNA identification

A total of 69 fish samples were collected in this study. Most of them were adults and well preserved, but some individuals were small or damaged during preservation and thus difficult to identify. We followed Gon's classification method (Graeme et al. 1992). Limited by the poor Antarctic fish classification literature, the probability of misidentification is greatly increased. In this study, 12 fish species were identified by morphological characters and keys, but 1 species was identified incorrectly as a sister species (Table 1).

All COI fragments were successfully amplified and sequenced. The sequences of the COI gene with high quality (no double peaks, short fragments or background noise) were aligned and contained no insertions, deletions,or stop codons. The length of the COI sequences was 652 bp after alignment, including 237 polymorphic sites (223 parsimony-informative sites, 14 singleton variable sites). The average base composition was A = 21.03%, C = 27.90%, G = 19.71%, and T = 31.36% on average, with a slight bias against G and C. The best classification result in ASAP (second-best model) supported 69 sequences that represent 11 taxonomic units. *Artedidraco lonnbergi* and *Dolloidraco longedorsalis* suggested being one taxonomic unit. *Lycenchelys* sp. and *Ophthalmolycus amberensis* were also in the same situation. However, BPP shows a different result with ASAP (Fig. 2). BPP confirmed that 69 COI sequences belonged to 13 taxonomic units, and this result is basically consistent with the result of traditional morphological identification. Altogether, molecular methods proved that 69 sequences belonged to 13 species of fish, 12 genera, 6 families, and 2 orders (Table 2).

Table 2
Fish fauna of the Amundsen Sea in 36th CHINARE

Order	Family	Species	Amundsen Sea	Record Amundsen Sea
Gadiformes	Macrouridae	*Macrourus whitsoni*	+	+
Perciformes	Nototheniidae	*Trematomus cf. lepidorhinus/loennbergi*	+	+
		Trematomus scotti	+	+
	Artedidraconidae	*Dolloidraco longedorsalis*	+	+
		Artedidraco lonnbergi	+	
	Bathydraconidae	*Vomeridens infuscipinnis*	+	
		Akarotaxis nudiceps	+	
		Gerlachea australis	+	+
	Channichthyidae	*Chaenodraco wilsoni*	+	+
		Chionodraco myersi	+	+
		Dacodraco hunteri	+	
	Zoarcidae	*Ophthalmolycus amberensis*	+	
		Lycenchelys sp.		

Note: Species that have been described in this area were marked with a cross (+).

3.2 Genetic distance and phylogeny analysis

The uncorrected K2P pairwise distance within species was below 1%, averaged 0.31%, and ranged from 0~1.01%. The genetic distance between species varied between 1.84%~29.9% (Fig. 3). The best-fitting model was GTR +G and gamma distribution shape parameter 0.186. Two phylogenetic trees, BI tree and ML tree, showed a similar topology, and the majority-rule consensus tree was used to show the phylogenetic relationship of fish. The tree supported a branch of Bathydracinidae nested within Channichthyidae. Most individuals in the tree clustered together in groups of the same species.

4. Discussion

4.1 Effectiveness of COI barcoding and species delimitation

The accuracy of DNA barcoding is the key to species identification, which depends on the degree of intra- and interspecific variation of the selected gene fragments. The less intra- and interspecific overlap there is, the more effective the barcoding. Intraspecific variations are generally similar among species (Waugh 2007). However, the range of interspecific differences varies depending on the size of the selected group and geographic populations. The use of means for intraspecific and interspecific genetic distance comparisons does not allow the detection of problematic cases. Therefore, we compared the minimum interspecific distance with the maximum intraspecific genetic distance (Meier et al. 2008). In this study, the minimum interspecific distance was 1.84%, the maximum intraspecific genetic distance was 1.01%, and the barcoding gap was between 1.01% and 1.84%.

With the two different methods we used to infer the putative species boundaries, namely ASAP and BPP. ASAP is based on single-marker pairwise genetic distance and avoids the heavy computational burden of phylogenetic reconstruction. It does not require any biological priori insights and can quickly come up with relevant species hypotheses (Puillandre et al. 2021). BPP can accurately assign species identity at the species level without knowing species boundaries in advance, even when analysis rare taxa with only one locus available(Yang & Rannala 2017). The classification of most species is consistent. BPP and morphology have obtained similar results, while ASAP has some differences. As the BPP results were consistent with the BLAST results against the Genbank database, BPP was likely to show more accurate species identification results. However, it is worth noting that there are ten results displayed by ASAP. We only consider the classification results of the first- and second-best scores. If barcoding gaps or other prior conditions are considered, ASAP can achieve the same results as BPP. Overall, DNA identification can provide simple and reliable species classification results and shows the uniqueness of the method when morphology is difficult to perform.

4.2 Phylogenetic relationships

The COI gene is a short nucleotide fragment from mitochondria and is not the best choice for phylogenetic analysis; however, the topology of its phylogenetic tree might still have reference value (Steinke et al. 2009). The tree topology based on COI barcoding is usually related to the delineation of clusters. Although the ML tree was based on a priori inference and Bayesian inference was based on a posteriori inference, the topology supported by the results was basically the same (Fig. 4). In particular, they both supported that Bathydracinidae were paraphyletic. Previous studies also reported similar results (Derome et al. 2002; Bargelloni et al. 2004). Multiple nuclear markers and multiple studies also confirmed that Bathydracinidae were paraphyletic (Near et al. 2004; Rock et al. 2008). In terms of the phylogenetic relationship, our COI-based phylogenetic signal further verifies the topological structure revealed by other studies.

4.3 The demersal fish fauna in the Amundsen Sea

In recent decades, with the deepening of research and the emergence of commercial fishing, increasing information about the community structure and classification of fish in the Southern Ocean has been discovered. In general, Notothenioidei, including Artedidraconidae, Bathydraconidae, Channichthyidae, Harpagiferidae, and Nototheniidae, has an absolute advantage in terms of number, accounting for most of the total species biodiversity (Eastman & McCune 2000; Eastman 2004). Additionally, there are some typical deep-sea fish groups, such as Liparidae and Zoarcidae. Some Antarctic fish diversity studies based on molecular taxonomy have been applied in the Ross Sea (Smith et al. 2012), Prydz Bay (Li et al. 2018), Scotia Sea (Rock et al. 2008), The Dumont d'Urville Sea (Dettai et al. 2011), and Antarctic Peninsula (Mabragaña et al. 2016) and verified the aforementioned Antarctic fish diversity pattern.

In this study, 13 species of fish were identified in the surveyed seas, most of which belonged to Artedidraconidae, Bathydraconidae, Channichthyidae, and Nototheniidae in addition to Liparidae and Zoarcidae. Harpagiferiade did not appear in our study because these species are usually distributed in the sub-Antarctic region (Navarro et al. 2019), but the Amundsen Sea is located at high latitudes. Relatively speaking, there were only a few sampling stations with shallow sampling depths, which may be the reason why we missed those typical deep-sea groups. At present, the fish fauna of the Amundsen Sea area have been studied by underwater observations. Our results supported that Notothenioidei dominates both in abundance and biomass. This is consistent with the aforementioned general pattern of the Southern Ocean fish fauna. The fish we caught were also roughly similar to the fauna observed by Eastman et al(2012), however, our study provided more detailed species assignment at the species level, with some additional exclusive species recorded. In particular, *Ophthalmolycus amberensis, Chaenodraco wilsoni, Dacodraco hunteri*, *Akarotaxis nudiceps*, *Artedidraco lonnbergi* and *Vomeridens infuscipinnis* might be recorded for the first time in the Amundsen Sea. It should also be noted that Eastman's data came from underwater photography, and some species are difficult to identify by morphology, in contrast, our results are based on molecular taxonomy analysis of fish catches. From this perspective, our identification results are undoubtedly more credible.

To the best of our knowledge, our study is the first on the molecular taxonomy of fish in the Amundsen Sea. Our results provide important taxonomic information on the demersal fish fauna in the Amundsen Sea. This is of great significance for understanding the biodiversity, taxonomy and biogeography of fish in the Amundsen Sea. However, we believe there are still many unknowns about the diversity of demersal fish in this area that need to be explored. Broader sampling of latitudes, deeper sampling depths, and higher sampling densities are all necessary for future research. Finally, the integration of molecular identification and morphological identification is suggested to ensure precise taxonomy in future studies of Antarctic fishes.

5 Conclusions

Based on fish samples collected during the 36th CHINARE, DNA barcoding analysis revealed 13 species belonging to six families (mainly composed of Notothenioidei). We used two different DNA identification methods (ASAP and BPP), combined with barcode gap analysis to determine the species boundary. At the same time, phylogenetic trees of two different algorithms were reconstructed to determine the phylogenetic relationship of Antarctic fish. The evolutionary relationships of Antarctic fish shown in the ML tree and BI tree based on COI in this paper are also consistent with the results of previous phylogenetic studies to some extent. In general, our research provides a more comprehensive description of the molecular taxonomy and diversity of demersal fish in the Amundsen Sea and confirms that DNA barcoding is an effective supplementary method for the identification of most species and can be applied to biodiversity surveys of Antarctic fish. Our study is also the first report to identify the fish fauna in the Amundsen Sea using molecular methods. To fully understand the fish diversity in the Amundsen Sea, more specimens from the broader sampling of latitudes, deeper sampling depths, and higher sampling densities should be collected. In future studies, molecular identifications are suggested to be integrated to and morphological identification of Antarctic fishes to obtain more accurate identification results.

Declarations

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Credit author statement

Shuai Cao: Conceptualization, Data curation, Writing-original draft preparation. Yuan Li: Data curation, Software, Writing- reviewing and editing. Ran Zhang Investigation, Visualization. Xing Miao Investigation, Writing- reviewing and editing. Longshan Lin: Supervision, Conceptualization, Writing-reviewing and editing, Resources. Hai Li: Supervision, Conceptualization, Writing-original draft preparation, Methodology, Data curation, Validation.

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Figures

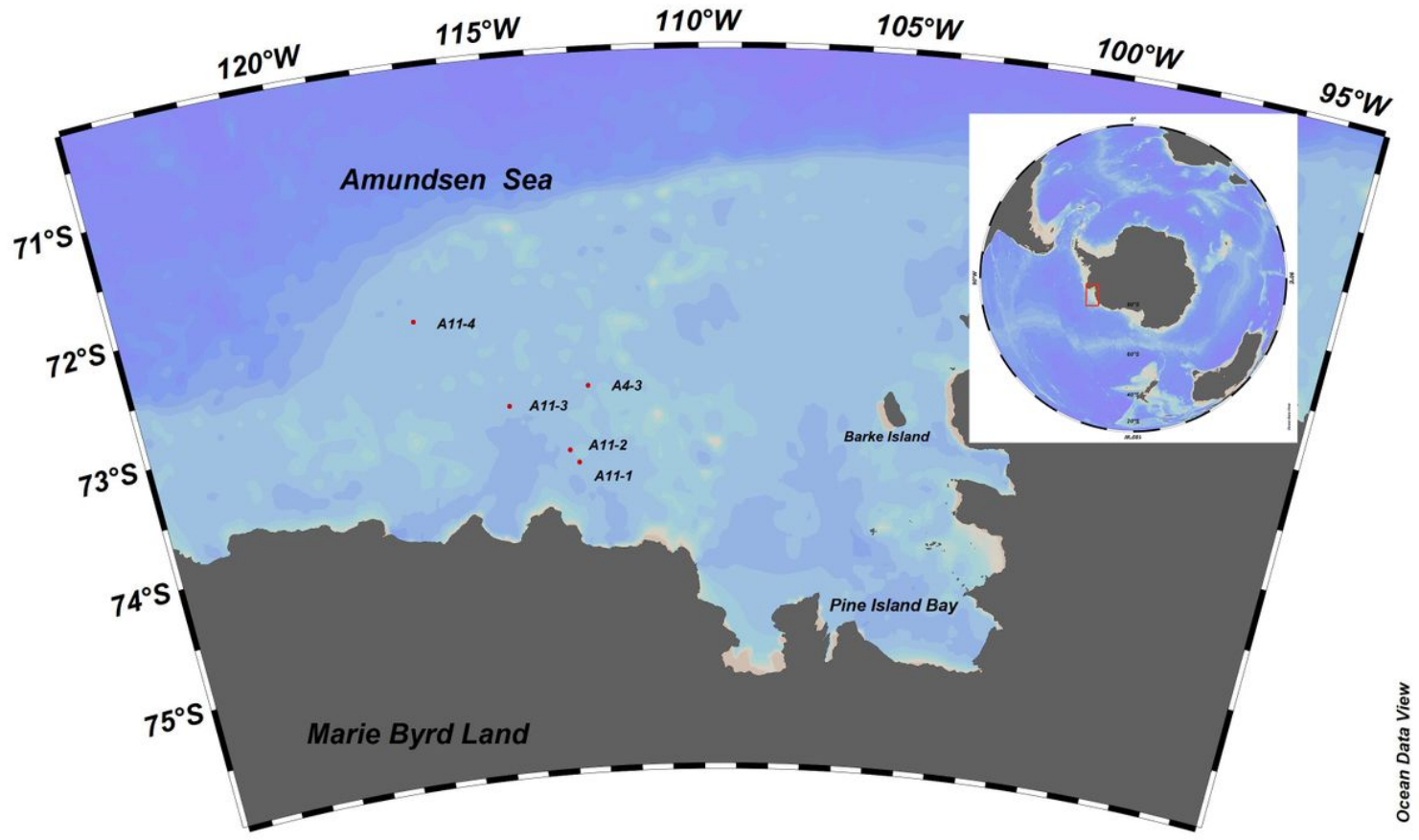

Figure 1

Map of bottom trawl stations of CHINARE-36 cruise in the Amundsen Sea.

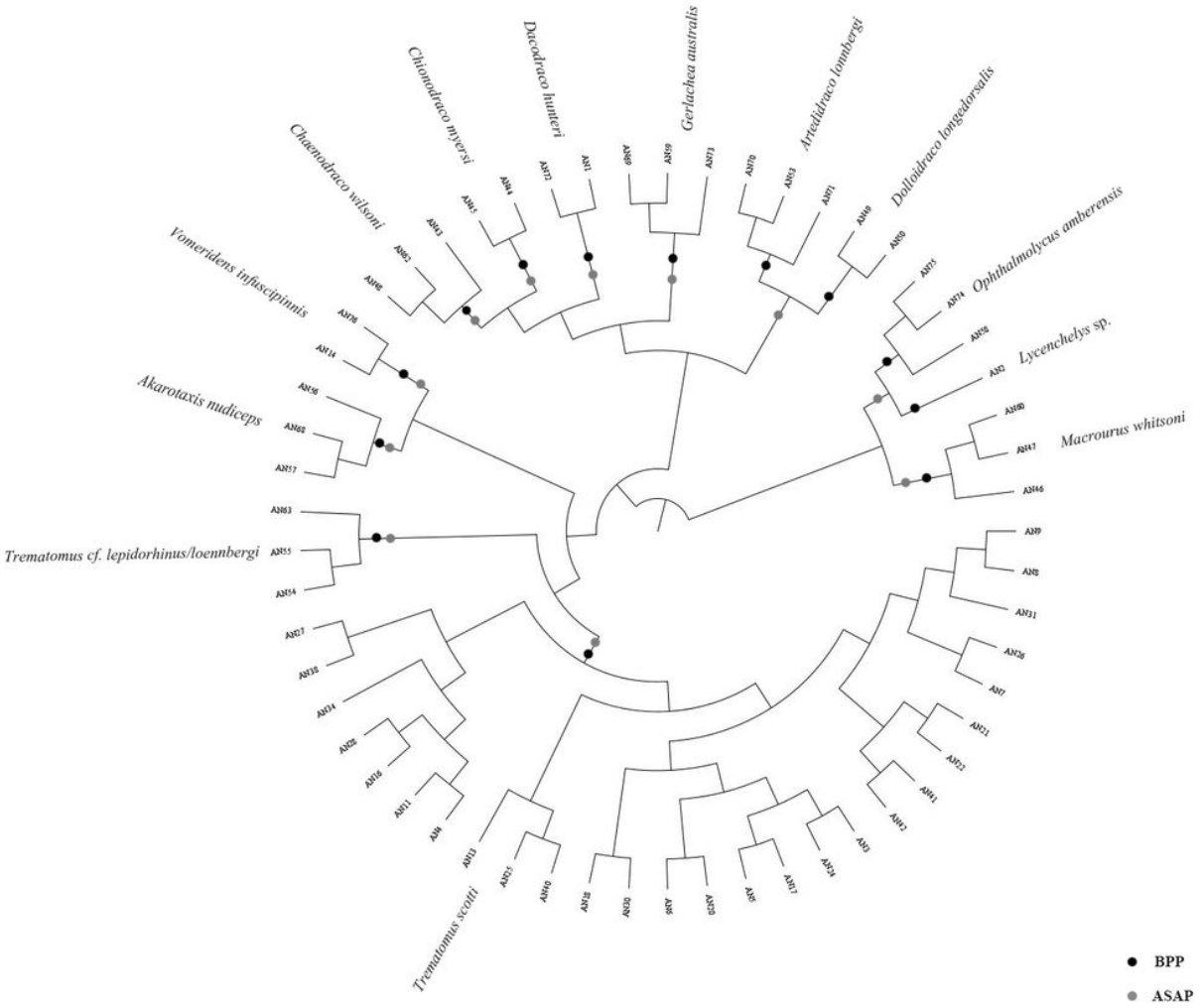

Figure 2

Results of DNA-based classification from ASAP and BPP on COI. The ultra-metric tree with haplotypes was obtained from BEAST.

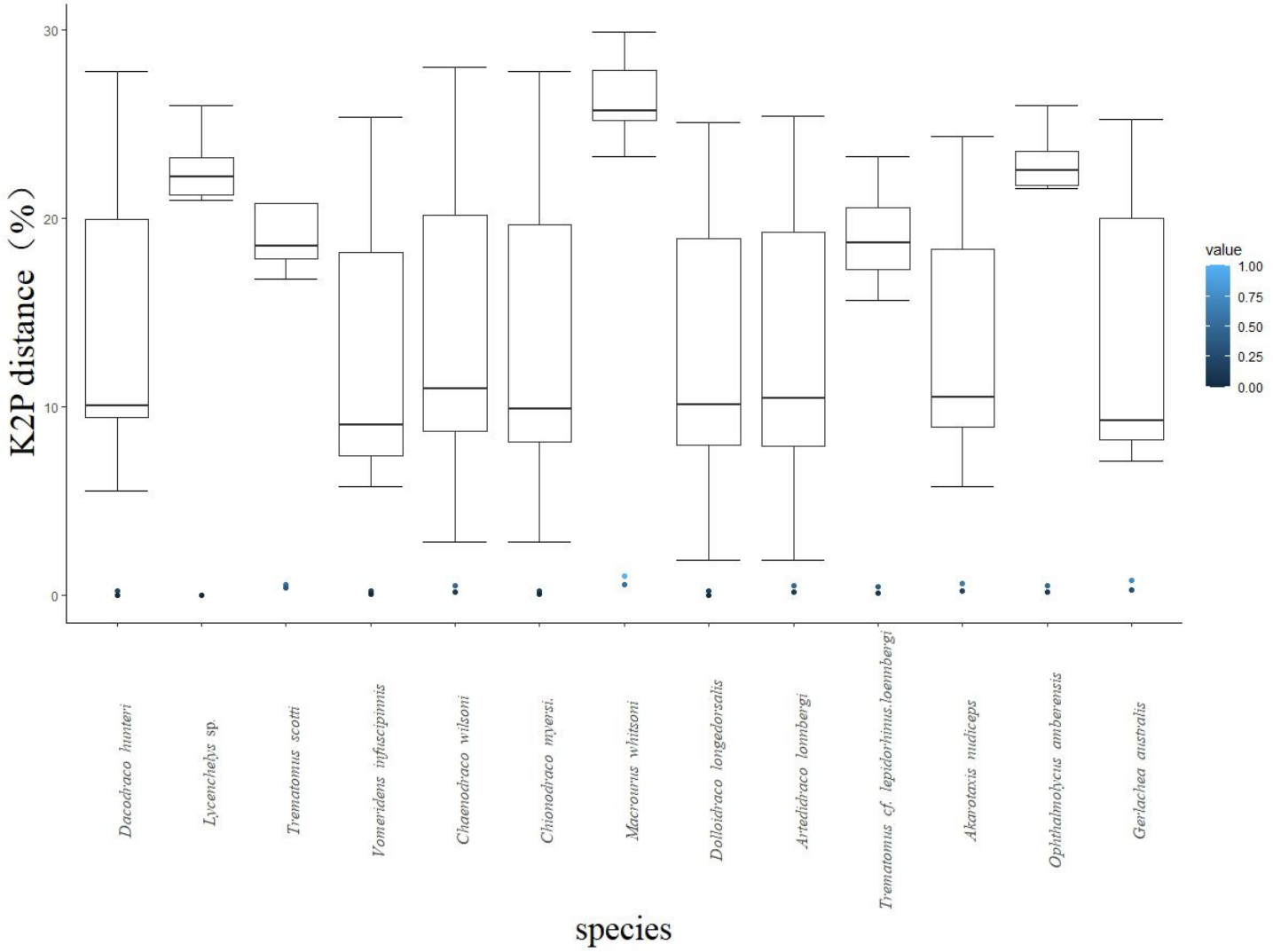

Figure 3

DNA barcoding gaps for all species based on the K2P model. Median interspecific distances with maximum and minimum values are represented by the upper and lower bars, respectively. The maximum and the minimum intraspecific genetic distance are represented by blue dots with different color depths.

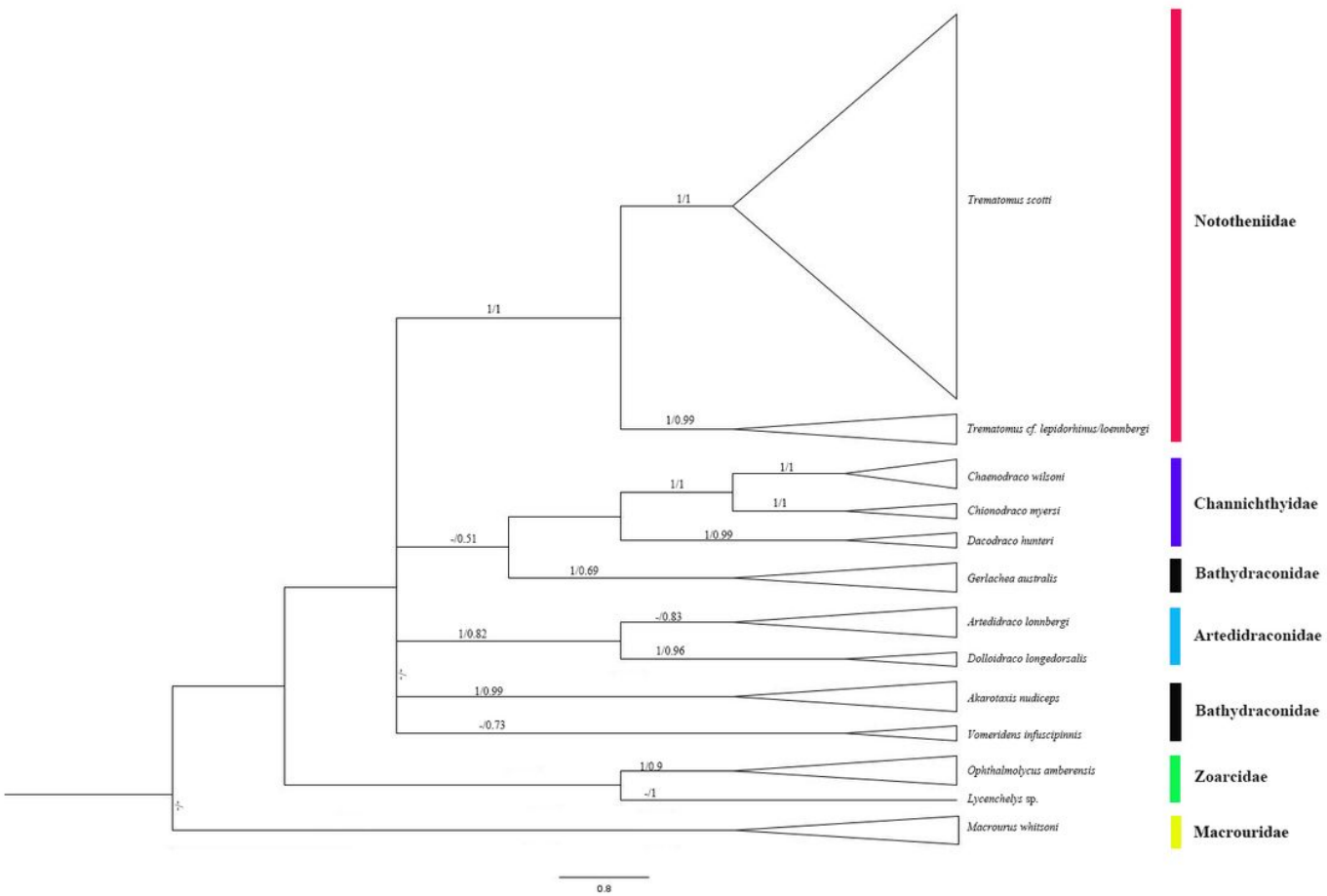

Figure 4

The Bayesian inference COI phylogenetic tree for 69 Antarctic fish in the Amundsen Sea was obtained from MrBayes, with the scale bars proportional to substitution rates; support values are ML Probabilities support/ Bayesian Posterior; ML supports for the clades are also present in the ML trees.