Fisheries Research 225 (2020) 105504

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

DNA barcoding for identification of marine gastropod species from Hainan island, China

Ke Ran[a], Qi Li[a,b,*], Lu Qi[a], Weidong Li[c], Lingfeng Kong[a]

[a] Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, 5 Yushan Road, Qingdao 266003, China
[b] Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China
[c] College of Life Sciences and Ecology, Hainan Tropical Ocean University, 1 Yucai Road, Sanya 572022, China

ARTICLE INFO

Handled by J. Viñas

Keywords:
DNA barcoding
Gastropods
Hainan island
Species identification

ABSTRACT

Morphological identification of gastropods can be difficult considering the larva of species and high similarity among the same genera. DNA barcoding has been widely used in species identification and biodiversity research. The purpose of this study was to explore the feasibility of using the cytochrome c oxidase subunit I (COI) gene for the identification of gastropod species and to construct a reliable DNA barcoding reference database of gastropods in Hainan island, China. A total of 306 mitochondrial COI barcode sequences were obtained from 120 species, 35 families and 7 orders of gastropods. The average length of the sequence was 640 base pairs. The average genetic distances based on Kimura two parameter (K2P) within species, genera, families, orders and classes were 0.9 %, 14.7 %, 18.9 %, 24.5 % and 28.6 %, respectively. Most of the gastropod species could be identified using COI sequences. Our results confirmed that the identification method combining morphology and DNA barcode greatly improved the efficiency of species identification. In this study, we found three new record species in China, namely *Semiricinula tissoti* (Petit de la Saussaye, 1852), *Engina alveolata* (Kiener, 1836) and *Wallaconchis ater* (Lesson, 1831). Overall, this study revealed that the identification of gastropods by DNA barcoding is efficient, and COI sequencing technology can be used for the identification of gastropod species and thereby can be used to manage fisheries and assess biodiversity.

1. Introduction

The gastropod is the most abundant groups of mollusks and one of the few animals that inhabit marine, freshwater and terrestrial communities (Loker, 2010). There are approximately 80,000 species of gastropod exist worldwide, accounting for more than four-fifths of all mollusks (Rüdiger, 1992). In addition to being a component of biodiversity (Puillandre et al., 2012; Modica et al., 2014), gastropods are also important aquatic animal protein sources for human and have great economic value (Germán and Castilla, 2002). Marine gastropods are among the most important invertebrate fisheries all over the world. Gastropod fisheries play an important role in the national economy of many countries (Keegan et al., 2003; Cob et al., 2009). Some species of gastropods, such as *Haliotis* spp., *Glossaulax* spp., *Strombus* spp., *Rapana* spp., have high economic value in international markets and play important social roles in small-scale artisanal fisheries (Leiva et al., 2002; Culha at al., 2009; Li et al., 2014). Due to their high economic value and excessive collection, however, many marine gastropods have been overexploited. (Schmidt et al., 2002).

The classification and identification of gastropod is not only an important part of taxonomic research, but also a reference basis for fishery resource survey and natural resource assessment. At present, the identification of gastropod mainly depends on the traditional morphological characteristics. Gastropods have significant diversity of morphological features and most gastropods have different morphological characteristics at different growth stages. Convergent evolution and phenotypic plasticity can also result in morphological changes of gastropod species, which poses great challenges to morphological classification (Wilke and Falniowski, 2001). Thus, it has been questioned for the classification of many gastropod species. Due to the shortage of traditional morphological methods and the reduction of taxonomic experts, it is necessary to find a molecular method to identify species.

In recent years, with the development of molecular analysis technology, DNA barcoding technology has become a focus of attention (Köhler, 2007). The COI gene sequences was used as a barcoding for species identification with expectation of barcoding all species for the aim of species identification (Hebert et al., 2003a). Some studies have shown that interspecific difference of the COI gene in animals was

* Corresponding author at: Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China.
E-mail address: qili66@ouc.edu.cn (Q. Li).

https://doi.org/10.1016/j.fishres.2020.105504
Received 30 August 2019; Received in revised form 10 January 2020; Accepted 16 January 2020
Available online 21 January 2020

significantly higher than intraspecific difference, using the COI gene as a barcode was feasible for the classification and identification of invertebrates and vertebrates (Dona et al., 2015; Nzelu et al., 2015; Barco et al., 2016; Xing et al., 2018). Compared with traditional morphology, DNA barcoding can obtain the molecular data of specimens and identify a mass of samples rapidly (Frezal and Leblois, 2008). It can help accurately distinguish some species that have extremely similar external morphological features (Ratnasingham and Hebert, 2007; Xing et al., 2018), and is useful for the rapid identification of damaged specimens (Yang et al., 2010) and species of different growth stages (Azmir et al., 2017). DNA barcoding has also facilitated the discovery of cryptic species and new species (Puillandre et al., 2009). To date, DNA barcoding technology has been successfully used in the identification of marine mollusks (Sun et al., 2012; Barco et al., 2016). In order to better protect and manage fishery resources, there has been a trend to establish DNA barcoding reference database of local species.

Hainan island is part of the south China sea and belongs to the shallow sea of the tropical continental shelf in China (Fang and Bailey, 1998). The coastal habitats of Hainan island are rock reefs, coral reefs, gravel, sand and mud, which is particularly suitable for the survival and reproduction of gastropod species (Quan et al., 1988; Zuschin and Hohenegger, 1998). According to the intertidal survey data, there are a large number of shellfish resources in the Hainan island (Bai et al., 2016). In recent years, due to overfishing by local fishermen and environmental pollution, the shellfish resources in the Hainan island have been decreasing. It is necessary to effectively protect and manage the shellfish resources. The accurate and clear identification and classification of gastropod species is still important issue in fisheries management. In this study, we sequenced COI from 306 specimens of 120 species of Gastropod to test the utility of DNA barcoding as a tool to identify gastropod species, and established a comprehensive barcoding reference database of gastropods for the first time in Hainan island, which will serve as resource for national and international barcoding reference database.

2. Materials and methods

2.1. Specimen collection

The samples of gastropod used in this study were collected from the coast of Hainan island, China from March to December 2018 (Fig. 1). A total of 27 sites of the Hainan island were surveyed for gastropod species. All specimens were preserved in 95 % alcohol and transported to laboratory for identification. A total of 306 gastropod samples was chosen for the research, and one to eight individual specimens were collected for each gastropod species. Specimens were identified based on morphological features. Several uncertain specimens were showed as sp. The specimens of Cerithiidae used in this study belonged to the individuals used our previous study. The detailed information of specimens was showed in Table S1.

Table 1
Details of primer sequences used in this study.

Primer name	Primer sequences (5'-3')	References
LCO1490	GGT CAA CAA ATC ATA AAG ATA TTG G	Folmer et al. (1994)
HCO2198	TAA ACT TCA GGG TGA CCA AAA AAT CA	
LDAN	CTT TAA CTT TAC TCC TGG CAT CCT	Zhao et al. (2017)
RDAN	CGG TGA AAT AAG CAC GGG T	
COXAF	CWA ATC AYA AAG ATA TTG GAA C	Colgan et al. (2001)
COXAR	AAT ATA WAC TTC WGG GTG ACC	
dgLCO	GGT CAA CAA ATC ATA AAG AYA TYG G	Meyer et al. (2005)
dgHCO	TAA ACT TCA GGG TGA CCA AAR AAY CA	

2.2. DNA isolation, amplification and sequencing

A small piece of tissue (about 100 mg) was removed from the foot of each sample or from the entire animal. DNA was extracted by CTAB method (Winnepenninckx et al., 1993). The extracted DNA were preserved in TE solution and frozen at − 20 ℃ until used.

Approximately 640 bp COI fragment was amplified using universal primers (Folmer et al., 1994). For the species that were not successfully amplified by universal COI primers, other primer pairs previously described for gastropods were used (Table 1). The total polymerase chain reaction (PCR) volume was 25 μL, including 2.5 μL 10 × PCR Buffer (Mg^{2+} Plus), 0.5 μL 2.5 mM dNTP, 1 μL of each 10 μmol/L prime, 0.2 μL 5U/μL *Taq* DNA Polymerase, 1 μL 100 ng/μL template DNA and 18.8 μL sterile distilled water. The PCR reaction procedure was performed under the following conditions: an initial denaturation for 3 min at 94 ℃, 35 cycles or 30 cycles (primers of Zhao et al., 2017) of 94 ℃ for 30 s, 42–52 ℃ for 1 min and 72 ℃ for 1 min, with final extension of 10 min at 72 ℃, and then preserved 4 ℃ refrigerator. PCR products were detected by 1.5 % agarose gel, and the qualified products were sent to Sangon Biotech for purification and bidirectional sequencing.

2.3. Data analyses

All COI gene sequences were entered into the SeqMan program of DNASTAR software (Pettengill and Neel, 2010) to edit sequences and manually delete primer sequences. The corrected COI gene sequences were aligned using the Clustal W in BioEdit v.7.2.6.1 (Hall, 1999), and then uploaded to Genbank (Table 2). DnaSp 5.0 (Librado and Rozas, 2009) software was used to calculate the number of different

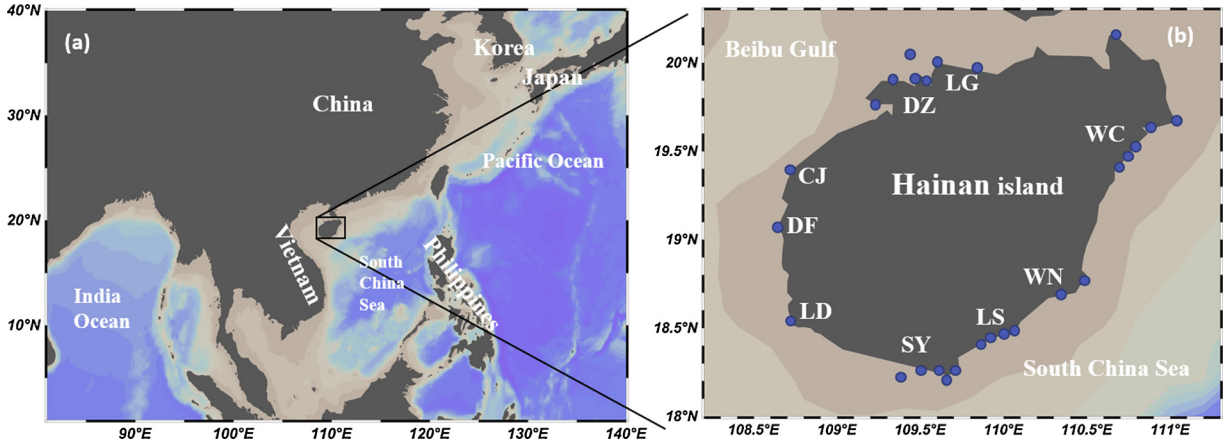

Fig. 1. Distribution of the sampling localities for the specimens collected in this study. Locations of Hainan island (a) and sampling sites (b). SY = Sanya; LS = Lingshui; WN = Wanning; WC = Wenchang; LG = Lingao; DZ = Danzhou; CJ = Changjiang; DF = Dongfang; LD = Ledong.

Table 2
Classification of gastropod species collected from Hainan island and details of the number of haplotypes and GenBank accession number.

Order	Family	Species	N	Number of haplotypes	GenBank accession numbers					
Archaeogastropoda	Fissurellidae	*Scutus sinensis*	5	4	MN388943	MN388944	MN388945	MN388946	MN388947	
	Patellidae	*Cellana toreuma*	8	5	MN388948	MN388949	MN388950	MN388951	MN388952	MN388953
					MN388954	MN388955				
		Cellana grata	3	1	MN388956	MN388957	MN388958			
		Scutellastra flexuosa	2	1	MN388959	MN388960				
		Scutellastra optima	1	1	MN388961					
	Lottiidae	*Patelloida saccharina lanx*	5	4	MN388962	MN388963	MN388964	MN388965	MN388966	
		Lottia luchuana	3	3	MN388967	MN388968	MN388969			
	Trochidae	*Trochus maculatus*	4	2	MN388970	MN388971	MN388972	MN388973		
		Trochus stellatus	3	2	MN388974	MN388975	MN388976			
		Monodonta labio	3	3	MN388977	MN388978	MN388979			
		Umbonium vestiarium	2	1	MN388980	MN388981				
		Trochidae sp	1	1	MN388982					
	Tegulidae	*Tectus pyramis*	2	2	MN388983	MN388984				
	Angariidae	*Angaria delphinus*	3	1	MN388985	MN388986	MN388987			
	Turbinidae	*Turbo bruneus*	4	2	MN388988	MN388989	MN388990	MN388991		
		Turbo chrysostomus	1	1	MN388992					
		Lunella correensis granulate	4	4	MN388993	MN388994	MN388995	MN388996		
	Neritidae	*Nerita albicilla*	3	3	MN388997	MN388998	MN388999			
		Nerita histrio	4	4	MN389000	MN389001	MN389002	MN389003		
		Nerita signata	2	2	MN389004	MN389005				
		Nerita litterata	1	1	MN389006					
		Nerita ocellata	2	2	MN389007	MN389008				
		Nerita chamaeleon	2	2	MN389009	MN389010				
		Nerita costata	1	1	MN389011					
		Nerita plicata	4	3	MN389012	MN389013	MN389014	MN389015		
		Clithon oualaniense	3	2	MN389016	MN389017	MN389018			
Mesogastropoda	Littorinidae	*Echinolittorina radiata*	2	1	MN389019	MN389020				
		Echinolittorina tricincta	2	1	MN389021	MN389022				
		Nodilittorina pyramidalis	4	3	MN389023	MN389024	MN389025	MN389026		
		Littoraria undulata	1	1	MN389027					
		Littoraria melanostoma	1	1	MN389028					
		Littoraria sinensis	1	1	MN389029					
	Vermetidae	*Ceraesignum maximum*	2	2	MN389030	MN389031				
	Planaxidae	*Planaxis sulcatus*	1	1	MN389032					
	Cerithiidae	*Cerithium zonatum*	6	6	MN249981	MN249982	MN249983	MN249984	MN249985	MN249986
		Cerithium traillii	8	8	MN249972	MN249958	MN249973	MN249974	MN249975	MN249976
					MN249977	MN249978				
		Cerithium coralium	5	4	MN249953	MN249954	MN249955	MN249956	MN249957	
		Cerithium punctatum	2	1	MN249950	MN249979				
		Cerithium mangrovum	2	2	MN249987	MN249949				
		Clypeomorus petrosa	4	3	MN249965	MN249966	MN249967	MN249968		
		Clypeomorus subbrevicula	4	3	MN249962	MN249963	MN249952	MN249964		
		Clypeomorus pellucida	5	2	MN249959	MN249960	MN249971	MN249961	MN249969	
		Clypeomorus batillariaeformis	2	1	MN249970	MN249971				
		Rhinoclavis sinensis	1	1	MN249980					
	Potamodidae	*Pirenella cingulata*	1	1	MN389033					
		Cerithideopsilla djadjariensis	3	3	MN389034	MN389035	MN389036			
		Terebralia sulcata	2	2	MN389037	MN389038				
	Batillarriidae	*Batillaria zonalis*	5	3	MN389039	MN389040	MN389041	MN389042	MN389043	
		Batillaria cumingi	3	3	MN389044	MN389045	MN389046			
		Batillaria sordida	2	2	MN389047	MN389048				
	Strombidae	*Canarium urceus*	3	1	MN389049	MN389050	MN389051			
		Canarium mutabile	1	1	MN389052					
		Dolomena swainsoni	2	2	MN389053	MN389054				
		Doxander vittatus	4	2	MN389055	MN389056	MN389057	MN389058		
		Conomurex luhuanus	2	2	MN389059	MN389060				
	Cypraeidae	*Erronea errones*	5	3	MN389061	MN389062	MN389063	MN389064	MN389065	
		Lyncina vitellus	1	1	MN389066					
		Mauritia arabica asiatica	5	2	MN389067	MN389068	MN389069	MN389070	MN389071	
		Monetaria moneta	3	2	MN389072	MN389073	MN389074			
		Monetaria annulus	1	1	MN389075					
	Naticidae	*Mammilla melanostomoides*	3	3	MN389076	MN389077	MN389078			
		Mammilla sebae	1	1	MN389079					
		Neverita didyma	1	1	MN389080					
		Notocochlis gualtieriana	2	2	MN389081	MN389082				
	Ranellidae	*Monoplex pilearis*	2	1	MN389083	MN389084				
	Bursidae	*Bufonaria rana*	2	2	MN389085	MN389086				
	Tonnidae	*Tonna sulcosa*	1	1	MN389087					
		Tonna galea	1	1	MN389088					
	Ficidae	*Ficus varicgata*	1	1	MN389089					
		Ficus gracilis	1	1	MN389090					
		Ficus ficus	2	2	MN389091	MN389092				

(*continued on next page*)

Table 2 (*continued*)

Order	Family	Species	N	Number of haplotypes	GenBank accession numbers					
Neogastropoda	Muricidae	*Drupella margariticola*	7	7	MN389093	MN389094	MN389095	MN389096	MN389097	MN389098
					MN389099					
		Drupella rugosa	3	3	MN389100	MN389101	MN389102			
		Drupella minuta	2	2	MN389103	MN389104				
		Ergalatax contracta	4	4	MN389105	MN389106	MN389107	MN389108		
		Muricodrupa anaxares	3	1	MN389109	MN389110	MN389111			
		Tenguella granulata	2	2	MN389112	MN389113				
		Tenguella musiva	4	4	MN389114	MN389115	MN389116	MN389117		
		Oppomorus purpureocinctus	1	1	MN389118					
		Purpura bufo	4	1	MN389119	MN389120	MN389121	MN389122		
		Purpura panama	2	2	MN389123	MN389124				
		Neothais marginatra	1	1	MN389125					
		Semiricinula tissoti	1	1	MN389126					
		Reishia luteostoma	6	6	MN389127	MN389128	MN389129	MN389130	MN389131	MN389132
		Indothais javanica	2	2	MN389133	MN389134				
		Indothais sacellum	3	2	MN389135	MN389136	MN389137			
		Rapana bezoar	1	1	MN389138					
		Tylothais aculeata	4	3	MN389139	MN389140	MN389141	MN389142		
		Chicoreus brunneus	3	2	MN389143	MN389144	MN389145			
		Chicoreus torrefactus	2	2	MN389146	MN389147				
		Rapana rapiformis	2	1	MN389148	MN389149				
	Columbellidae	*Euplica scripta*	5	4	MN389150	MN389151	MN389152	MN389153	MN389154	
	Buccinidae	*Babylonia areolata*	2	2	MN389155	MN389156				
		Babylonia spirata	1	1	MN389157					
		Engina mendicaria	1	1	MN389158					
		Engina alveolata	1	1	MN389159					
		Pollia undosa	5	1	MN389160	MN389161	MN389162	MN389163	MN389164	
	Nassariidae	*Nassarius teretiusculus*	2	2	MN389165	MN389166				
		Nassarius acuminatus	1	1	MN389167					
		Nassarius glans	2	2	MN389168	MN389169				
		Nassarius dorsatus	1	1	MN389170					
		Nassarius conoidalis	2	2	MN389171	MN389172				
		Nassarius hepaticus	2	2	MN389173	MN389174				
		Nassarius siquijorensis	2	2	MN389175	MN389176				
	Fasciolariidae	*Peristernia nassatula*	1	1	MN389177					
	Olividae	*Miniaceoliva miniacea*	1	1	MN389178					
	Mitridae	*Strigatella scutulata*	5	4	MN389179	MN389180	MN389181	MN389182	MN389183	
		Strigatella aurantia	1	1	MN389184					
	Volutidae	*Melo melo*	1	1	MN389185					
	Conidae	*Conus caracteristicus*	1	1	MN389186					
		Conus miles	1	1	MN389187					
		Conus quercinus	1	1	MN389188					
Entomotaeniata	Pyramidellidae	*Otopleura auriscati*	2	2	MN389189	MN389190				
Nudibranchia	Bornellidae	*Bornella stellifera*	1	1	MN389191					
Ellobiida	Ellobiidae	*Cassidula nucleus*	2	2	MN389192	MN389193				
	Siphonariidae	*Siphonaria atra*	1	1	MN389194					
Stylommatophora	Oncidiidae	*Wallaconchis graniferus*	7	2	MN389195	MN389196	MN389197	MN389198	MN389199	MN389200
					MN389201					
		Wallaconchis ater	2	1	MN389202	MN389203				
		Peronia sp	2	2	MN389208	MN389209				
		Peronia verruculata	4	3	MN389204	MN389205	MN389206	MN389207		

Table 3
Genetic divergence (percentage, K2P distance) within various taxonomic levels.

Comparisons within	Distance		
	Mean (%)	Minimum (%)	Maximum (%)
Species	0.9	0	11.4
Genus	14.7	0.4	24.2
Family	18.9	4.8	32.1
Order	24.5	1.3	69.2
Class	28.6	11.8	73.3

haplotypes detected for each species. Some parameters were calculated using the software MEGA 6.0 (Tamura et al., 2013). The genetic distance and the neighbor-joining (NJ) tree of COI gene sequences were analyzed. The Kimura 2-parameter (K2P) model (Kimura, 1980) was used to calculate genetic distance of intraspecific and intergeneric and create NJ tree. Tree topology and branch lengths were optimized artificially. Node support was assessed by performing bootstrapping analysis with 1000 replicates (Felsenstein, 1985).

3. Results

Using morphological method and molecular identification, 120 species were identified from 306 gastropod specimens. In this study, we discovered three new record species, *E. alveolata*, *S. tissoti* and *W. ater* not reported previously from China Seas.

A total of 306 COI sequences were obtained from 120 species, 35 families and 7 orders of gastropods (Table 2). All sequences were deposited in GenBank (Accession Number: MN388943-MN389209). After editing, the sequence lengths were between 530 bp to 658 bp, with an average of 640 bp. No stop codons, insertion, or deletions were found in any of the sequences. The average K2P genetic distances within each taxonomic level were shown in the Table 3. The intraspecific K2P genetic distances of COI gene ranged from 0 to 11.4 %, with an average distance of 0.9 %; the species with a maximum genetic distance of 11.4 % was *Euplica scripta.* The interspecific genetic distances ranged from 0.4 to 24.2 %, with an average distance of 14.7 %, which is 16 times of

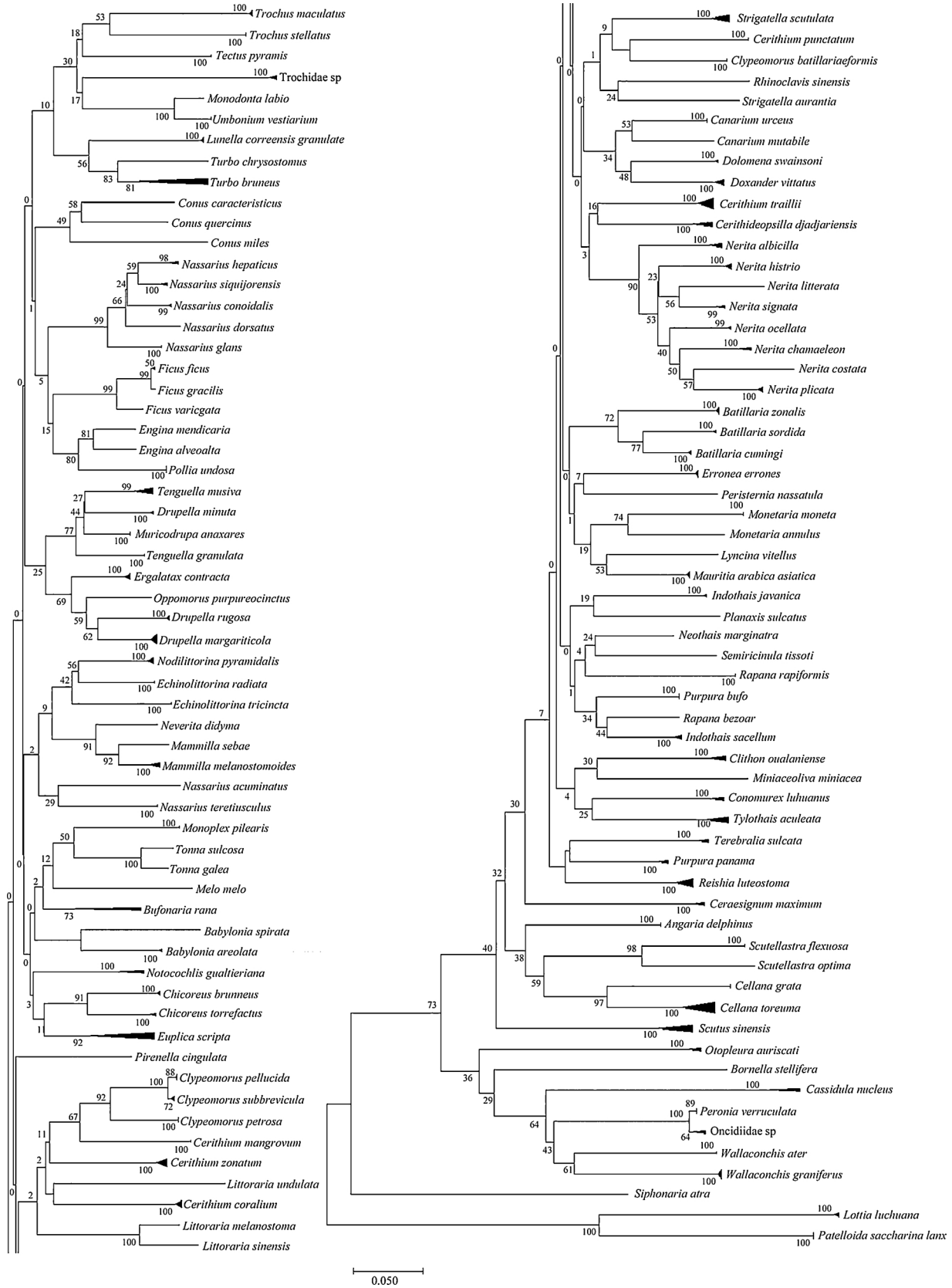

Fig. 2. Neighbor-joining (NJ) tree of 120 gastropod species based on 306 COI sequences using K2P distances. Branches leading to conspecific individuals are collapsed. Detailed intraspecific distances are showed in the tree provided as Fig. S1.

intraspecific genetic distance. The genetic distance within families was 4.8–32.1 %, with an average of 18.9 %, the genetic distance within orders was 1.3–69.2 %, with an average of 24.5 %, and the genetic distance within classes was 11.8–73.3 %, with an average of 28.6 %. *Ficus ficus* and *F. gracilis* contributed to the lowest interspecific genetic distance (0.4 %), while the largest K2P distance was founded between *Lottia luchuana* and *Littoraria undulata* (73.3 %). Except that the average genetic distance within species is less than 1 %, the average genetic distance within genera, families and orders were all higher than 10 %, which were much higher than intraspecific genetic distance. Although the genetic variation was increased at rising taxonomic levels, the rate of increase declined at higher taxonomic levels (Table 3).

The phylogenetic NJ tree was constructed based on 306 individuals' DNA barcode sequences (Fig. 2). The NJ tree including 120 species with 241 haplotypes is provided in Fig. S1. All individuals from each species belonged to single monophyletic clusters and most of individuals of the same species formed a branch. Some of the specimens of the same species formed multiple separate branches, respectively, such as *Scutus sinensis, Cellana toreuma, E. scripta* and *Turbo bruneus*. The intraspecific K2P divergence of *S. sinensis* ranged from 0 to 5.8 %, with an average of 3.2 %, the value within *C. toreuma* ranged from 0 to 4.4 %, with an average of 1.2 %, the value within *E. scripta* ranged from 0 to 11.4 %, with an average of 5.7 % and the value within *T. bruneus* ranged from 0 to 10.1 %, with an average of 5.1 % (Table 4). In this study, 35 families of gastropod were characterized by DNA barcoding. The species of most families formed distinct clusters in the tree (Fig. 1). However, 9 families including Neritidae, Littorinidae, Cerithiidae, Potamodidae, Strombidae, Naticidae, Muricidae, Buccinidae and Nassariidae of gastropod were not clustered together in the NJ tree.

4. Discussion

In this study, we found three new record species in China using a morphology-based approach and molecular technology. The species of *E. alveolata* has previously been recorded from Philippines and Japan (Cernohorsky, 1975; Okutani, 2017). *S. tissoti*, a muricid gastropod is being reported for the first time from Hainan island, China. A genus of onchidiid slugs, *Wallaconchis*, was discovered by Goulding et al. (2018) and the *W. ater* was first discovered in China, which was originally classified in *Onchidium*. In addition, we also found the species of *W. graniferus*, which Goulding et al. (2018) described for the first time in China. The *Echinolittorina tricincta* was first discovered in Hainan island, which was only previously recorded from Taiwan in China (Su et al., 2011).

Traditional morphological identification methods require rich experience and knowledge, and the phenotypic plasticity of taxa may lead to wrong identification (Wang et al., 2018). DNA barcoding technology does not rely too heavily on an individual's abilities and experience and can effectively identify species, especially for some individuals that are damaged, incomplete or distinct at different growth stages (Xing et al., 2018). However, because of the introgression and incomplete lineage sorting, DNA barcoding may be not applicable for some organisms (Toffoli et al., 2008). Therefore, DNA barcoding can be used as a complementary method for species identification, and it cannot replace the method of morphological classification (Peɛnikar and Buzan, 2014). The mitochondrial COI gene is usually used as a species barcode, because it is highly conserved within species and typical pattern of genetic variability between different species (Hebert et al., 2003a, b). DNA barcoding method has been testified to be a valid tool for species identification and was triumphantly used to identify the marine mollusk in other regions (for example, the North Sea, Barco et al., 2016). In our study, DNA barcoding technology based on COI gene was able to identify most marine gastropod shellfish from Hainan island, and the identification results were consistent with morphological identification. Furthermore, this study constructed a reliable DNA barcoding reference library for the gastropod from Hainan island, which can be used for better monitoring, protection and management of shellfish in this area.

The method for species identification using DNA barcoding is based on both interspecific difference and intraspecific difference. DNA barcoding technology attempts to find the boundaries to describe species, which corresponds to the difference between the nearest neighbors within a group (Hebert et al., 2003a; Chakraborty and Ghosh, 2014). However, there is no uniform threshold for species division. Minimum congeneric and maximum conspecific differences have recently been used to define the barcoding gap, which are more effective than the average of intraspecific and interspecific sequence variability (Meier et al., 2008). Although it is much debatable (Srivathsan and Meier, 2012), distance-based technique remains as the standard method in DNA barcoding (Reid et al., 2011). In order to guarantee the consistency and comparability with other barcode researches, the K2P model is also adopted in this study. The average interspecific genetic distance (about 15 %) was greatly higher than the average intraspecific genetic distance (0.9 %) in Hainan island. The mean interspecific difference was 10 times of intraspecific difference, which was considered as the threshold of animal species identification (Hebert et al., 2004). These results showed that it is feasible to use COI gene sequence as DNA barcode to identify gastropod from Hainan island. The average genetic distance was increased at rising taxonomic levels in this study. The average K2P distances within species, genera, families, orders and classes were 0.9 %, 14.7 %, 18.9 %, 24.5 % and 28.6 %, respectively, which were consistent with other barcoding studies in aquatic animals. For instance, the average K2P distances of Caenogastropoda along coast of China within species, genera and families were 0.44 %, 13.96 % and 22.27 %, respectively (Sun et al., 2012); the distances of Taiwan Strait fishes were 0.21 % within species, 6.50 % within genera, 23.70 % within families and 25.6 % within orders (Xing et al., 2018). The average genetic distance increased slightly within the higher taxonomic levels of families and orders, with values of 18.9 % and 24.5 %, respectively. The decrease in the rate of increase in higher taxa may be due to substitutional saturation (Iyiola et al., 2018).

In this study, the same species of gastropod were clustered together in monophyletic groups by high bootstrap values supported, indicating that DNA barcoding technology based on COI sequence could distinguish and identify the gastropod species within the studied taxa efficiently and accurately. However, the species of *Ficus*, which formed a cohesive cluster, showed low interspecific divergence with a value of 0.4 % and could not be fully differentiated by DNA barcoding. This failure may be due to recent and rapid speciation, and the specimens of *F. ficus* and *F. gracilis* are genetically similar in the region of DNA barcoding (Xing et al., 2018). Fortunately, these species can be distinguished by traditional morphological methods. In addition, high intraspecific distances were found in *S. sinensis, C. toreuma E. scripta* and *T. bruneus*, whose maximum value of K2P distance was over 11 %. These species had multiple haplotypes and formed multiple separate branches in NJ tree, respectively, which may be due to the complex genetic structure and intraspecific variability. Further sampling of these less-studied species may help resolve the incongruence between taxonomic boundaries and genetic structure. Furthermore, some species of same families did not exhibit cohesive clustering in the NJ tree. This indicated that the current classification system of gastropod may not be

Table 4
Genetic distance (percentage, K2P distance) within species of *S. sinensis, C. toreuma, E. scripta* and *T. bruneus*.

Species	Distance		
	Mean (%)	Minimum (%)	Maximum (%)
S. sinensis	3.2	0	5.8
C. toreuma	1.2	0	4.4
E. scripta	5.7	0	11.4
T. bruneus	5.1	0	10.1

able to effectively reflect a natural subdivision, which requires further study.

Because of overfishing by local fishermen, gastropod diversity in the Hainan island faces great challenge. With the obvious decline of biodiversity, the extinction of species promotes the need for the protection and management of marine biodiversity (Kress et al., 2015). Our results indicated that DNA barcoding could be used as an effective method for rapid and accurate identification of gastropods in Hainan island. Species identification based on DNA barcoding can be used to manage fisheries and assess biodiversity (Weigt et al., 2012).

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

This study was supported by research grants from Fundamental Research Funds for the Central Universities (201762014), National Natural Science Foundation of China (31772414), and National Natural Science Foundation of Guangxi Province (AA17204080-4).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fishres.2020.105504.

References

Azmir, I.A., Esa, Y., Amin, S.M.N., Md Yasin, I.S., Md Yusof, F.Z., 2017. Identification of larval fish in mangrove areas of Peninsular Malaysia using morphology and DNA barcoding methods. J. Appl. Ichthyol. 33, 998–1006.
Bai, L., Zhao, Z., Liu, Q., Cui, J., 2016. Investigation on shellfish species living in the intertidal zone to subtidal zone of Fenjiezhou Island of Hainan. Agric. Sci. Technol. 17, 1204–1210.
Barco, A., Raupach, M.J., Laakmann, S., Neumann, H., Knebelsberger, T., 2016. Identification of North Sea molluscs with DNA barcoding. Mol. Ecol. Resour. 16, 288–297.
Cernohorsky, W.O., 1975. Supplementary notes on the taxonomy of buccinid species of the subfamily Pisaniinae (Mollusca: gastropoda). Rec. Auckland Inst. Mus. 12, 175–211.
Chakraborty, M., Ghosh, S.K., 2014. An assessment of the DNA barcodes of Indian freshwater fishes. Gene 537, 20–28.
Cob, Z.C., Arshad, A., Bujang, J.S., Ghaffar, M.A., 2009. Age, growth, mortality and population structure of *Strombus canarium* (Gastropoda: Strombidae): variations in male and female sub-populations. J. Appl. Sci. 9, 3287–3297.
Colgan, D.J., Hutchings, P.A., Brown, S., 2001. Phylogenetic relationships within the Terebellomorpha. J. Mar. Biol. Assoc. U. K. 81, 765–773.
Culha, M., Bat, L., Dogan, A., Dagli, E., 2009. Ecology and distribution of the veined rapa whelk *Rapana venosa* (Valenciennes, 1846) in Sinop peninsula (Southern Central Black Sea), Turkey. J. Anim. Vet. Adv. 8, 51–58.
Dona, J., Diaz-Real, J., Mironov, S., Bazaga, P., Serrano, D., Jovani, R., 2015. DNA barcoding and minibarcoding as a powerful tool for feather mite studies. Mol. Ecol. Resour. 15, 1216–1225.
Fang, Z., Bailey, R.L., 1998. Height-diameter models for tropical forests on Hainan Island in Southern China. For. Ecol. Manage. 110, 0–327.
Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.
Folmer, O., Black, M., Hoeh, W., Lute, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.
Frezal, L., Leblois, R., 2008. Four years of DNA barcoding: current advances and prospects. Infect. Genet. Evol. 8, 727–736.
Germán, E.L., Castilla, J.C., 2002. A review of the world marine gastropod fishery: evolution of catces, management and the Chilean experience. Rev. Fish Biol. Fish. 11, 283–300.
Goulding, T.C., Khalil, M., Tan, S.H., Benoît, D., 2018. Integrative taxonomy of a new and highly-diverse genus of onchidiid slugs from the Coral Triangle (Gastropoda, Pulmonata, Onchidiidae). ZooKeys 763, 1–111.
Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.
Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard, J.R., 2003a. Biological identifications through DNA barcodes. Proc. R. Soc. B 270, 313–321.
Hebert, P.D.N., Ratnasingham, S., de Waard, J.R., 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc. R. Soc. B 270, S96–S99.
Hebert, P.D.N., Stoeckle, M.Y., Zemlak, Y.S., Francis, C.M., 2004. Identification of birds through DNA barcodes. PLoS Biol. 2, 1657–1663.
Iyiola, O.A., Nneji, L.M., Mustapha, M.K., Nzeh, C.G., Oladipo, S.O., Nneji, I.C., Okeyoyin, A.O., Nwani, C.D., Ugwumba, O.A., Ugwumba, A.A., Faturoti, E.O., Wang, W., Chen, J., Wang, W., Adeola, A.C., 2018. DNA barcoding of economically important freshwater fish species from north-central Nigeria uncovers cryptic diversity. Ecol. Evol. 8, 6932–6951.
Keegan, W.F., Portell, R.W., Slapcinsky, J., 2003. Changes in invertebrate taxa at two pre-Columbian sites in southwestern Jamaica, AD 800-1500. J. Arch. Sci. 30, 1607–1617.
Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.
Köhler, F., 2007. From DNA taxonomy to barcoding: how a vague idea evolved into a biosystematic tool. Zoosyst. Evol. 83, 44–51.
Kress, W.J., Garcia-Robledo, C., Uriarte, M., Erickson, D.L., 2015. DNA barcodes for ecology, evolution, and conservation. Trends Ecol. Evol. 30, 25–35.
Leiva, G.E., Castilla, J.C., 2002. A review of the world marine gastropod fishery: evolution of catches, management and the Chilean experience. Rev. Fish Biol. 11, 283–300.
Li, X., Zhao, L., Zhang, Q., Xiong, Q., Jiang, C., 2014. Purification, characterization and bioactivity of polysaccharides from *Glossaulax didyma*. Carbohydr. Polym. 102, 912–919.
Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.
Loker, E.S., 2010. Gastropod immunobiology. Adv. Exp. Med. Biol. 708, 17–43.
Meier, R., Zhang, G.Y., Ali, F., 2008. The use of mean instead of smallest interspecific distances exaggerates the size of the “Barcoding gap” and leads to misidentification. Syst. Biol. 57, 809–813.
Meyer, C.P., Geller, J.B., Paulay, G., 2005. Fine scale endemism on coral reefs: archipelagic diff ;erentiation in turbinid gastropods. Evolution 59, 113–125.
Modica, M.V., Puillandre, N., Castelin, M., Zhang, Y., Holford, M., 2014. A good compromise: rapid and robust species proxies for inventorying biodiversity hotspots using the Terebridae (Gastropoda: Conoidea). PLoS One 9, e102160.
Nzelu, C.O., Caceres, A.G., Arrunategui-Jimenez, M.J., Lanas-Rosas, M.F., Yanez-Trujillano, H.H., Luna-Caipo, D.V., Holguín-Mauricci, C.E., Katakura, K., Hashiguchi, Y., Kato, H., 2015. DNA barcoding for identification of sand fly species (Diptera: Psychodidae) from leishmaniasis-endemic areas of Peru. Acta Trop. 145, 45–51.
Okutani, T., 2017. Marine Mollusks in Japan. Tokai University Press, Tokyo.
Peɛnikar, Ž.F., Buzan, E.V., 2014. 20 years since the introduction of DNA barcoding: from theory to application. J. Appl. Genet. 55, 43–52.
Pettengill, J.B., Neel, M.C., 2010. An evaluation of candidate plant DNA barcodes and assignment methods in diagnosing 29 species in the genus Agalinis (Orobanchaceae). Am. J. Bot. 97, 1391–1406.
Puillandre, N., Cruaud, C., Kantor, Y.I., 2009. Cryptic species in Gemmuloborsonia (Gastropoda: Conoidea). J. Mollus. Stud. 76, 11–23.
Puillandre, N., Modica, M.V., Zhang, Y., Sirovich, L., Boisselier, M.C., Cruaud, C., Holford, M., Samadi, S., 2012. Large-scale species delimitation method for hyperdiverse groups. Mol. Ecol. 21, 2671–2691.
Quan, S., Wang, G., Lu, B., 1988. The recent carbonate sedimentary facies and sedimentation of the muddy water in the paipu fringing coral reef, Hainan Island. Oceanol. Limnol. Sin. 19, 179–187.
Ratnasingham, S., Hebert, P.D.N., 2007. BOLD: the barcode of life data system (http://www.barcodinglife.org). Mol. Ecol. Notes 7, 355–364.
Reid, B.N., Le, M., McCord, W.P., Iverson, J.B., Georges, A., Bergmann, T., Amato, G., Desalle, R., Naro-Maciel, E., 2011. Comparing and combining distance-based and characterbased approaches for barcoding turtles. Mol. Ecol. Resour. 11, 956–967.
Rüdiger, B., 1992. Gastropod phylogeny and systematics. Annu. Rev. Ecol. Evol. Syst. 23, 311–338.
Schmidt, S., Wolff, M., Vargas, J.A., 2002. Population ecology and fishery of *Cittarium pica* (Gastropoda: Trochidae) on the Caribbean coast of Costa Rica. Rev. Biol. Trop. 50, 1079–1090.
Srivathsan, A., Meier, R., 2012. On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. Cladistics 28, 190–194.
Su, C., Chiu, Y., Yang, C., Chen, M., Li, J., 2011. The marine mollusks of Siaolanyu Isle, Taiwan. Platax 8, 13–24.
Sun, Y., Li, Q., Kong, L., Zheng, X., 2012. DNA barcoding of Caenogastropoda along coast of China based on the COI gene. Mol. Ecol. Resour. 12, 209–218.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular Evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.
Toffoli, D., Hrbek, T., Araújo, M.L.G., de Almeida, M.P., Charvet-Almeida, P., Farias, I.P., 2008. A test of the utility of DNA barcoding in the radiation of the freshwater stingray genus Potamotrygon (Potamotrygonidae, Myliobatiformes). Genet. Mol. Biol. 31, 324–336.
Wang, L., Wu, Z., Liu, M., Liu, W., Zhao, W., Liu, H., You, F., 2018. DNA barcoding of marine fish species from Rongcheng Bay, China. PeerJ 6, e5013.
Weigt, L.A., Baldwin, C.C., Driskell, A., Smith, D.G., Ormos, A., Reyier, E.A., 2012. Using DNA barcoding to assess caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PLoS One 7, e41059.
Wilke, T., Falniowski, A., 2001. The genus Adriohydrobia (Hydrobiidae: Gastropoda): polytypic species or polymorphic populations? J. Zoolog. Syst. Evol. Res. 39, 227–234.
Winnepenninckx, B., Backeljau, T., De Wachter, R., 1993. Extraction of high molecular weight DNA from molluscs. Trends Genet. 9, 407.
Xing, B., Lin, H., Zhang, Z., Wang, C., Wang, Y., Wang, J., 2018. DNA barcoding for identification of fish species in the Taiwan Strait. PLoS One 13, e0198109.
Yang, R., Wu, X.B., Yan, P., Li, X.Q., 2010. Using DNA barcodes to identify a bird involved in a bird strike at a Chinese airport. Mol. Biol. Rep. 37, 3517–3523.
Zhao, D., Li, Q., Kong, L., Yu, H., 2017. Cryptic diversity of marine gastropod Monodonta labio (Trochidae): did the early Pleistocene glacial isolation and sea surface temperature gradient jointly drive diversification of sister species and/or subspecies in the Northwestern Pacific? Mar. Ecol. 38, e12443.
Zuschin, M., Hohenegger, J., 1998. Subtropical coral-reef associated sedimentary faciès characterized by molluscs (Northern Bay of Safaga, Red Sea, Egypt). Facies 38, 229–254.