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REVIEW

# Role of DNA barcoding in marine biodiversity assessment and conservation: An update



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**Abstract** More than two third area of our planet is covered by oceans and assessment of marine biodiversity is a challenging task. With the increasing global population, there is a tendency to exploit marine resources for food, energy and other requirements. This puts pressure on the fragile marine environment and necessitates sustainable conservation efforts. Marine species identification using traditional taxonomical methods is often burdened with taxonomic controversies. Here we discuss the comparatively new concept of DNA barcoding and its significance in marine perspective. This molecular technique can be useful in the assessment of cryptic species which is widespread in marine environment and linking the different life cycle stages to the adult which is difficult to accomplish in the marine ecosystem. Other advantages of DNA barcoding include authentication and safety assessment of seafood, wildlife forensics, conservation genetics and detection of invasive alien species (IAS). Global DNA barcoding efforts in the marine habitat include MarBOL, CeDA-Mar, CMarZ, SHARK-BOL, etc. An overview on DNA barcoding of different marine groups ranging from the microbes to mammals is revealed. In conjunction with newer and faster techniques like high-throughput sequencing, DNA barcoding can serve as an effective modern tool in marine biodiversity assessment and conservation.

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## Contents

1. An introduction to DNA barcoding . . . . .	162
2. Advantages of DNA barcoding in marine perspective . . . . .	162
3. Worldwide DNA barcoding initiative for marine organisms . . . . .	163
4. DNA barcoding of marine microbes . . . . .	163
5. DNA barcoding of seagrasses, mangroves and marine phytoplanktons . . . . .	163
6. DNA barcoding of marine algae . . . . .	163
7. DNA barcoding of marine zooplanktons . . . . .	164
8. DNA barcoding of marine invertebrates . . . . .	165
9. DNA barcoding of lower chordates . . . . .	165
10. DNA barcoding of marine fishes . . . . .	166
11. DNA barcoding of marine reptiles . . . . .	166
12. DNA barcoding of sea birds . . . . .	166
13. DNA barcoding of marine mammals . . . . .	168
14. Criticisms of DNA barcoding . . . . .	168
15. Future of marine DNA barcoding . . . . .	168
16. Conclusions . . . . .	168
Acknowledgements . . . . .	168
References . . . . .	168

### 1. An introduction to DNA barcoding

The concept of DNA barcoding has become one of the most important and significant scientific visions in the last decade. As an emerging and effective tool for species identification, the concept of DNA barcoding has gained worldwide popularity. The ground-breaking concept of DNA barcoding was put forward in the year 2003 by Professor Paul Hebert and collaborators serving at University of Guelph, Canada. Mitochondrial *cytochrome c oxidase* subunit 1 (COI) gene was suggested as unique barcode region for animals (Hebert et al., 2003). This sequence was validated at the 1st International Conference on DNA Barcode of Life. Henceforth, several studies have shown that the sequence diversity in a ~650 bp region near the 5' region of the COI gene provides strong species level resolution for different animal groups like birds (Yoo et al., 2006; Tavares and Baker, 2008; Schindel et al., 2011), springtails (Hogg and Hebert, 2004), shrimps (Trivedi et al., 2011), fishes (Ward et al., 2005; Yancy et al., 2008; Bhattacharjee et al., 2012; Laskar et al., 2013; Trivedi et al., 2014), tortoise (Kundu et al., 2013), oysters (Trivedi et al., 2012), mammals (Lim, 2012), spiders (Greenstone et al., 2005), mosquitoes (Cywinska et al., 2006), ticks (Zhang and Zhang, 2014) etc.

The Consortium for the Barcode of Life (CBOL) was established to support worldwide DNA barcoding and subsequently an international online data management system – the Barcode of Life Data Systems (<http://www.barcodinglife.org>) came into effect. Survey and assessment of genetically diverse organisms of the earth through DNA barcoding is led by CBOL. A milestone in the field of DNA barcoding was achieved by launching of International Barcode of Life Project (iBOL). Canada was the first country to establish national network for DNA barcoding as The Canadian Barcode of Life Network (BOL-NET.ca). Subsequently, several countries and regions have also established barcoding networks as part of the iBOL like Europe (ECBOL; <http://www.ecbol.org/>), Norway (NorBOL; <http://dnabarcoding.no/en/>), Mexico (MexBOL; <http://www.mexbol.org/>) and Japan (JBOLI; <http://www.jboli.org/>).

Besides this, thematic programs like human health (HealthBOL), polar life (PolarBOL) and quarantine and plant pathogens (QBOL, as a part of the ECBOL) are also in place.

### 2. Advantages of DNA barcoding in marine perspective

More than 70% of our planet is covered by oceans that have higher biodiversity compared to terrestrial or freshwater ecosystems. The massive marine ecosystem is the habitat for a large number of flora and fauna, both macro and micro. Among the 35 animal phyla, 34 phyla have marine representatives while 14 include exclusively marine animals (Briggs, 1994; Gray, 1997). The occurrence of cryptic species is relatively common in marine ecosystems. Cryptic species are those species that are morphologically similar but genetically distinct. DNA barcoding can be a very effective tool in assessment of these cryptic species. Another problem that persists in the marine and estuarine habitat is the linking of the larval stages with the adult forms. DNA barcoding can accurately link the larval stages of a species in order to unravel the life cycle of different marine species, which is usually difficult and in some cases not possible using the morphological approach. The threat of invasive species to marine biodiversity can be globally assessed through DNA barcoding (Molnar et al., 2008).

The invasive alien species (IAS) poses severe threat and is capable of inflicting huge economic losses. DNA barcoding can be used to quickly and accurately identify the invasive alien species and prompt preventive measures with subsequent regulatory control can be initiated. Barcoding of indicator species can be fruitful in the monitoring and abatement of marine pollution including coastal pollution. One main aim of DNA barcoding initiative is the discovery of new species. DNA barcoding can be used as an important tool for identification, authentication and safety assessment of sea food, particularly for processed, cooked or smoked products. This molecular identification can even allow us to trace the origin of certain products (Galimberti et al., 2013). A study conducted on the Japanese delicacy tuna sushi from different restaurants in

USA, revealed the presence of endangered species, fraud and also a health hazard (Lowenstein et al., 2009). An analysis of 254 Canadian seafood samples revealed that 41% of the samples were mislabeled (Hanner et al., 2011).

DNA barcoding is an important tool in wildlife forensics and conservation. It can be used to identify endangered sea turtles by assessing turtle meat, carcasses or eggs that are illegally traded (Vargas et al., 2009). One important requirement of DNA barcoding is the collection and maintenance of samples as voucher specimens, which allows reliable means of corroborating the identification of the species from which data is accumulated. The voucher specimens provide permanent documentation for investigation of marine biodiversity. DNA barcoding has a great utility in the field of taxonomy (Ali et al., 2014).

DNA barcoding can be very effective for molecular phylogenetic studies, geographical distribution and conservation of marine biodiversity. DNA barcoding can be used for pest and disease control as well. With the recent developments in deep sea research and the revelation that several deep sea organisms possess extraordinary pharmaceutical properties, DNA barcoding of deep sea organisms has gained global attention. Census of the Diversity of Abyssal Marine Life (CeDAMar) is devoted to the barcoding of deep sea organisms. The user-friendliness of DNA barcodes is also an added advantage and can be effectively used for marine biodiversity assessment, fisheries management and conservation (Pérez-Huete and Quezada, 2013).

### 3. Worldwide DNA barcoding initiative for marine organisms

MarBOL, the Marine Barcode of Life, is an international campaign to barcode marine species. MarBOL (<http://www.marinebarcoding.org>) is led by an International Steering Committee and an affiliated project of the Census of Marine Life (CoML). CoML is involved in several Ocean Realm Field Projects (Table 1). Already five International Barcode of Life Conferences have been held and the 6th International Barcode of Life Conference is scheduled to be held in Guelph, Ontario, Canada during August 18–22, 2015. Some important marine DNA barcoding conferences are shown in Table 2.

### 4. DNA barcoding of marine microbes

Assessment of biodiversity in the microbial world has always been a challenging task. Rapid and accurate identification of the microbes is frequently necessary to prevent the spread of diseases caused by microbes. Protists are eukaryotic microbes which have short generation time and asexual reproductive capability. An ecologically significant group of protists are the dinoflagellates which serve as primary producers, coral symbionts and cause red tides. DNA barcoding of marine environmental samples revealed massive dinoflagellate diversity (Stern et al., 2010).

### 5. DNA barcoding of seagrasses, mangroves and marine phytoplanktons

Seagrasses are important submerged flowering plants that have very noticeable ecological influence on the coastal environment

due to their nutrient recycling ability and high primary productivity. Besides this, they contain valuable secondary compounds like phenolic acids which are used in traditional medicines. Rosmarinic acid and zosteric acid obtained from seagrasses are widely used as an antioxidant and effective anti-fouling agent respectively. Although these marine plants have wide geographical distribution worldwide there is rapid decline in sea grass species and cover globally. It is reported that seagrasses are disappearing at the rate of 110 km<sup>2</sup> per year, since 1980 (Waycott et al., 2009). Hence, there is urgent need for assessment and conservation of seagrasses. Seagrasses perform both, sexual and asexual reproduction, but vegetative reproduction is more common and sexual progenies are short lived. Species identification becomes difficult because the flower as a distinct morphological trait is often unavailable. In such a situation, DNA barcoding can serve as a useful identification tool. Different markers have been used for identification of seagrasses like nuclear ITS for *Halophila* (Waycott et al., 2002), trnK introns and rbcL for *Zostera* (Les et al., 2002), ITS1, 5.8S rDNA and ITS2 for *Halophila* (Uchimura et al., 2008). By using rbcL and matK sequences it was revealed that it is possible to develop DNA barcoding for seagrasses (Lucas et al., 2012).

Mangroves at the intersection of terrestrial, estuarine and near shore marine ecosystem have immense ecological and economic significance. The ecosystem services provided by mangrove forests are worth at least US\$1.6 billion per year worldwide (Field et al., 1998; Costanza et al., 1997). This dynamic and unique ecosystem is increasingly threatened and depleted. The conservation of mangroves is of utmost importance in order to maintain the health of this fragile environment. Loss of evolutionary unique species in the mangrove ecosystem has been reported and DNA barcoding provided phylogenetic information for developing unified mangrove management plan worldwide (Daru et al., 2013). The Sunderbans is the single largest block of tidal halophytic mangrove forest listed in the UNESCO world heritage list (<http://whc.unesco.org/en/list>). It is regarded as the world's largest natural nursery where a large number of marine and estuarine species come to breed and the juveniles stay back to exploit its rich natural resources (Trivedi et al., 2013). In a study conducted in the Sunderbans mangrove ecosystem, molecular methods based on rbcL subunit of RuBisCO enzyme were used for identification of phytoplankton groups lesser than 10 µm size (Bhattacharjee et al., 2013).

### 6. DNA barcoding of marine algae

Different species of red marine macro algae are often difficult to identify by using morphological techniques. Two molecular markers namely mitochondrial COI gene and UPA (Universal Plastid Amplicon) domain V of the 23S rRNA gene were used for identification of different species of red alga belonging to the family Kallymeniaceae. Results showed that COI was a more sensitive marker and led to the discovery of a new species *Euthora timburtonii* (Clarkston and Saunders, 2010). A similar study was conducted involving inter tidal red macro algae in China with three molecular markers – COI, UPA and ITS (nuclear internal transcribed spacer). Although COI was effective to identify species but not all species gave successful amplicons due to lack of universal primers. UPA had effective

**Table 1** Involvement of Census of Marine Life (CoML) in various Ocean Realm Field Projects.

S. No.	Ocean Realm Field Projects of CoML	Abbreviations
1	Arctic Ocean Diversity	ArcOD
2	Biogeography of Chemosynthetic Ecosystems	ChEss
3	Census of Antarctic Marine Life	CAML
4	Census of Diversity of Abyssal Marine Life	CeDAMar
5	Census of Marine Zooplankton	CMarZ
6	Continental Margin Ecosystems on a Worldwide Scale	CoMargE
7	Global Census of Coral Reef Ecosystems	CREEFS
8	Global Census of Marine Life on Seamounts	CenSeam
9	Gulf of Maine Area Program	GOMA
10	International Census of Marine Microbes	ICOMM
11	Natural Geography in Shore Areas	NaGISA
12	Pacific Ocean Shelf Tracking	POST
13	Tagging of Pacific Pelagics	TOPP

**Table 2** Important international conferences on DNA barcoding.

S. No.	Conferences	Place	Date
1	6th International Barcode of Life Conference	Ontario, Canada	August 18–22, 2015
2	5th International Barcode of Life Conference	Kunming, China	October 27–31, 2013
3	Training cum workshop on DNA barcoding of fish and marine life	Tiruchirappalli, India	September 12–14, 2012
4	4th International Barcode of Life Conference	Adelaide, Australia	November 29–December 3, 2011
5	Science Symposium on the Census of Marine Life	London, United Kingdom	October 5–6, 2010
6	Census of Marine Life News Conference and Panel Presentations	London, United Kingdom	October 4, 2010
7	DNA Barcoding Planning Meeting at Coastal Marine Biolabs, Ventura Harbor	California, USA	August 2–3, 2010
8	2nd Conference of the European Consortium for the Barcode of Life (ECBOL2)	Braga, Portugal	June 2–4, 2010
9	3rd International Barcode of Life Conference	Mexico City, Mexico	November 7–12, 2009
10	ICES Annual Science Conference	Berlin, Germany	September 21–26, 2009
11	MarBOL workshop at Ocean Research Institute	Tokyo, Japan	May 21–22, 2009
12	MarBOL workshop at Woods Hole Oceanographic Institution	Woods Hole, USA	April 30–May 1, 2009
13	MarBOL workshop at Alfred Wegener Institute for Polar and Marine Research	Bremerhaven, Germany	April 16–17, 2009
14	World Conference on Marine Biodiversity	Valencia, Spain	November 11–15, 2008
15	10th International Conference on Copepoda	Pattaya, Thailand	July 14–18, 2008
16	2nd International Barcode of Life Conference	Taipei, Taiwan	September 17–21, 2007
17	4th International Zooplankton Production Symposium	Hiroshima, Japan	May 28–June 1, 2007
18	Third Regional Barcoding Meeting	Campinas, Brazil	March 19–21, 2007
19	ABBI-FISHBOL Meeting	Buenos Aires, Argentina	March 14–16, 2007
20	7th Asia Pacific Marine Biotechnology Conference	Kochi, India	November 2–5, 2006
21	Second Regional Barcoding Meeting	Nairobi, Kenya	October 16–17, 2006
22	Census for Marine Life Workshop	Amsterdam, Netherlands	May 15–17, 2006
23	First Regional Barcoding Meeting	Cape Town, South Africa	April 7–8, 2006
24	BOLNET Fish Meeting at Biodiversity Institute of Ontario	Ontario, Canada	February 3, 2006
25	1st International Conference on DNA Barcode of Life	London, UK	February 5–8, 2005

universal primers but showed problems with closely related species, while ITS was the least effective (Xiaobo et al., 2013).

Gracilariaceae is a red algal family which is commercially important for its use in biotechnology and microbiology research as a phycocolloid agar. *Gracilaria* species are difficult to identify morphologically and DNA barcoding holds promise in species level identification (Kim et al., 2010). Recently, a novel microalga was isolated and characterized from Indian Ocean which has biofuel potential. In this study 16S rRNA and 23S rRNA were used as barcode (Ahmad et al., 2013). DNA barcoding can be useful as a rapid, sensitive and reliable

method for monitoring programs of marine and coastal ecosystems for detecting Harmful Algal Bloom (HAB) species.

## 7. DNA barcoding of marine zooplanktons

Zooplanktons have great ecological significance and represent 15 animal groups (phyla). Therefore, DNA barcoding of zooplanktons is an important aspect of modern ecological studies. Census for Marine Zooplanktons (CMarZ) is devoted to the study of global zooplankton assemblages. The DNA Barcoding Centers of CMarZ are located in UConn (USA), Bremer-

haven (Germany), ORI (Japan), Qingdao (China) and Goa (India). Fig. 1 shows the five CMarZ barcoding centers of the world. Barcode analysis using COI gene involving 52 specimens of 14 species of chaetognaths could successfully discriminate different species of chaetognaths across the phylum. The average K2P distance within species was 0.0145. Among the marine zooplanktons the copepods are one of the most systematically complex and ecologically significant groups with more than 2500 species. Several studies have been conducted on this diverse group. The occurrence of cryptic species is widespread among the copepods which necessitates more DNA barcoding studies. Some important publications on DNA barcoding of marine copepods are shown in Table 4.

Since it is difficult to identify the different chaetognath species based on morphological characters, especially with those preserved in alcohol, DNA barcoding can be very effective to resolve this problem (Jennings et al., 2010b). A study was conducted with *Neocalanus* copepods involving four marker genes namely COI, 12S, nuclear ITS, and 28S. The results showed that although all the four markers could identify distinctly all the species but distinction of the form variants was only confirmed by the COI sequences (Machida and Tsuda, 2010). DNA sequence variation of a 575 base-pair region of 28S rDNA, from North and South Atlantic regions could accurately and reliably identify the three species of *Oithona*, an ecologically important copepod species (Cepeda et al., 2012).

### 8. DNA barcoding of marine invertebrates

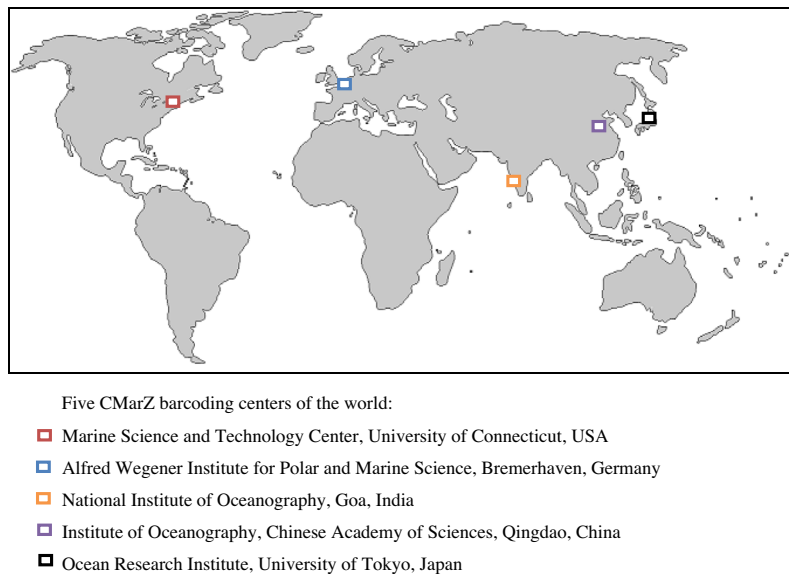
The pteropods which belong to the phylum Mollusca and class Gastropoda are of unique research interest due to their vulnerability to ocean acidification. Barcoding of *Diacavolinia* pteropods indicated that the Atlantic specimens comprise a single monophyletic species and show probable species-level divergence between Atlantic and Pacific populations (Maas et al., 2013). DNA barcoding comprising 227 species of Canadian marine mollusks indicated possible cases of overlooked species (Layton et al., 2014). DNA barcoding projects should be

developed for megadiverse groups such as mollusks to facilitate species discovery and conservation (Puillandre et al., 2009). A study involving 315 specimens from around 60 venerid species showed that DNA barcoding can be very effective in species delimitation (Chen et al., 2011). Marine oysters are bivalves that have great economic significance. Identification of oysters largely based on phenotypic characters like shell morphology is problematic due to the taxonomic controversies. Shell morphology, used as a primary distinguishing feature is greatly affected by habitat (Tack et al., 1992). In such cases, molecular identification proves to be useful (Table 4).

Echinoderms are exclusively marine animals. DNA barcoding of 191 echinoderm species belonging to five classes was undertaken. Based on shallow intraspecific versus deep congeneric divergences 97.9% specimens were assigned to known species (Ward et al., 2008a). Sponges have canal system inside the body and possess pharmaceutical properties. Sponge Barcoding Project, <http://www.spongebarcoding.org> is a global initiative. A DNA barcoding workflow capable of analyzing large sponge collections has been developed through this project (Vargas et al., 2012). Nematodes are known for their role as indicator of anthropogenic stress in the marine ecosystems. In the nematodes, 18S gene was able to amplify across several taxa and showed identification success rate of 97% (Bhadury et al. (2006)). Universal primers for diverse group of marine metazoan invertebrates are available (Folmer et al., 1994; Lobo et al., 2013) (Table 4).

### 9. DNA barcoding of lower chordates

Ascidians are filter-feeding marine urochordates which are regarded as model organisms used to study complex biological processes. They are used to study the transcriptional control of embryonic development, mechanism of metal accumulation, evolution of the immune system, conservation of gene regulatory networks in chordates, development of heart, etc. (Holland and Gibson-Brown, 2003; Trivedi et al., 2003; Satoh et al., 2003; Stolfi and Christiaen, 2012; Tolkin and Christiaen, 2012; Razy-Krajka et al., 2014). The genome of



**Figure 1** Five CMarZ barcoding centers of the world.

an ascidian species *Ciona intestinalis* is the smallest of any experimentally manipulable chordate, as a consequence it is used in genome analysis studies. COI gene analysis of *Ciona* specimens from New Zealand revealed for the first time, the existence of solitary ascidian *Ciona savignyi* in the Southern Hemisphere (Smith et al., 2012). A new ascidian species belonging to the genus *Diplosoma* has been revealed through DNA barcoding in the Ryukyu Archipelago of Japan (Hirose and Hirose, 2009).

## 10. DNA barcoding of marine fishes

Marine fish is an important source of protein, vitamin D, vitamin B<sub>12</sub>, iodine, selenium and omega-3 fatty acids. Marine fisheries sector has a very significant contribution in food security and economic welfare. Proper identification of fish species is important for management of fisheries and authentication of food products. DNA barcoding allows fast and efficient means of fish identification. Two main global barcoding initiatives for fish are FISH-BOL (<http://www.fishbol.org>) and SHARK-BOL (<http://www.sharkbol.org>). DNA barcoding is useful not only for the identification of whole fish but also for the identification of larvae, eggs, fillets, fins or other fragments of the body which are difficult to identify based on morphology. This molecular technique was used to identify shark fins that were confiscated from illegal fishers in Australia (Holmes et al., 2009). Demand for ornamental fish is rapidly increasing globally. COI gene analysis of 391 ornamental fish species from 8 coral reef locations revealed that most (98%) of these species belonged to distinct barcode clusters (Steinke et al., 2009a,b). Some important publications on DNA barcoding of marine fishes are depicted in Table 3.

## 11. DNA barcoding of marine reptiles

As compared to fishes there is less information on the DNA barcoding of reptiles. First large scale DNA barcoding of

reptiles (including Squamata and Testudines) was conducted with 468 specimens from biodiversity hotspot of Madagascar. In this study 41–48 new (undescribed) species were identified thereby indicating the utility of DNA barcoding in biodiversity assessment. This study also revealed that the average interspecific genetic distance within families was 13.4% in Boidae and 29.8% in Gekkonidae (Nagy et al., 2012). A study conducted on the Brazilian sea turtles revealed that species-specific COI barcode tags can be used for identifying each of the marine turtle species that were investigated (Vargas et al., 2009). In a separate study DNA barcoding was done for globally threatened marine turtles. This study showed that DNA barcoding is not only a powerful tool for species identification but also can play a vital role in wildlife forensics and conservation genetics (Naro-Maciel et al., 2009).

## 12. DNA barcoding of sea birds

The All Birds Barcoding Initiative (ABBI) was launched in September 2005 with the aim of barcoding approximately 10,000 known species of world birds. DNA barcoding studies revealed that there are hundreds of undescribed avian species. Seabirds inhabit the marine environment either directly or indirectly and spend most of their life in the sea but cannot be considered as sea creatures because they do not have the ability to use the dissolved oxygen in sea water. Sea birds include Gulls, Terns, Brown Booby, Carp Plover, Swans, Spoonbill, Ardeidae, etc. The seabirds depend on the marine environment for food or use the sandy beaches, tidal flats or mangrove environment for food and nesting. DNA barcoding analysis was conducted with 387 individuals of 147 species of birds (including seabirds) from the Netherlands, which is ornithologically one of the best covered countries (Aliabadian et al., 2013). Due to depletion of mangrove forests, ocean acidification, rising salinity and sea level elevation, more efforts are needed for DNA barcoding of sea birds and their conservation.

**Table 3** Some important publications on DNA barcoding of marine fishes.

Serial No.	Topics	References
1	Red Sea fishes	Trivedi et al. (2014)
2	Mediterranean Sea and Cantabric Sea fishes	Ardura et al. (2013)
3	Caribbean and western central Atlantic fishes	Weigt et al., 2012
4	Antarctic fishes	Dettai et al. (2011)
5	Arctic marine fishes	Mecklenburg et al. (2011)
6	Marine and brackish water fishes from Argentina	Mabragan-a et al. (2011)
7	Marine fishes from Japan	(Zhang and Hanner, 2011)
8	Marine fishes of China	(Zhang, 2011)
9	European marine fishes	(Kochzius et al., 2010)
10	Marine fishes of India	(Lakra et al., 2010)
11	Campaign to barcode all fishes	(Ward et al., 2009)
12	Coral reef fishes	(Steinke et al., 2009a)
13	Indo-Pacific, Australian and South African Marine Fishes	(Zemlak et al., 2009)
14	North American marine fishes	(Steinke et al., 2009b)
15	Salmon and trout species from North America	(Rasmussen et al., 2009)
16	North-east Atlantic deep-water sharks	(Moura et al., 2008)
17	Sharks and rays of Australia	(Ward et al., 2008b)
18	Fish larvae in Great barrier Reef, Australia	(Pegg et al., 2006)
19	Marine fishes of Australia	(Ward et al., 2005)

**Table 4** Some important publications on DNA barcoding of marine invertebrates.

Group	Article	References
Arthropoda (Copepoda)	DNA Barcoding of Marine Copepods: Assessment of Analytical Approaches to Species Identification	Blanco-Bercial et al. (2014)
	DNA barcoding of Arctic Ocean holozooplankton for species identification and recognition.	Bucklin et al.(2010a)
	A “Rosetta Stone” for metazoan zooplankton: DNA barcode analysis of species diversity of the Sargasso Sea (Northwest Atlantic Ocean)	Bucklin et al.(2010b)
	Zooplankton diversity analysis through single-gene sequencing of a community sample	Machida et al. (2009)
	Comparison of molecular species identification for North Sea calanoid copepods (Crustacea) using proteome fingerprints and DNA sequences	Laakmann et al. (2013)
	Comparison of morphological and molecular traits for species identification and taxonomic grouping of oncaeid copepods	Böttger-Schnack and Machida (2011)
	Morphological and molecular phylogenetic analysis of evolutionary lineages within <i>Clausocalanus</i> (Copepoda: Calanoida)	Bucklin and Frost (2009)
	Dissimilarity of species and forms of planktonic Neocalanus copepods using mitochondrial COI, 12S, nuclear ITS, and 28S gene sequences	Machida and Tsuda (2010)
	Speciation of two salinity associated size forms of <i>Oithona dissimilis</i> (Copepoda: Cyclopoida) in estuaries	Ueda et al. (2011)
	Evolution in the deep sea: Biological traits, ecology and phylogenetics of pelagic copepods	Laakmann et al. (2012)
	Morphological and genetic variation in the North Atlantic copepod, <i>Centropages typicus</i>	Castellani et al. (2012)
	Multi-Gene analysis reveals a lack of genetic divergence between <i>Calanus agulhensis</i> and <i>C. sinicus</i> (Copepoda; Calanoida)	Kozol et al. (2012)
	Comparative phylogeography and connectivity of sibling species of the marine copepod <i>Clausocalanus</i> (Calanoida)	Blanco-Bercial et al. (2011)
	Probing marine <i>Gammarus</i> (Amphipoda) taxonomy with DNA barcodes	Costa et al. (2009)
Arthropoda (Amphipoda)	DNA Barcoding of marine Metazoa	Bucklin et al. (2011)
	DNA Barcodes for Marine Biodiversity: Moving Fast Forward?	Radulovici et al. (2010)
Metazoa	Complete lack of mitochondrial divergence between two species of NE Atlantic marine intertidal gastropods	Kemppainen et al. (2009)
	Species diversity of planktonic gastropods (Pteropoda and Heteropoda) from six ocean basins based on DNA barcode analysis	Jennings et al. (2010a)
Mollusca (Gastropods)	A new <i>Poecilogonous</i> species of sea slug (Opisthobranchia: Sacoglossa) from California: comparison with the planktotrophic congener <i>Alderiamodesta</i> Loven, 1844	Krug et al. (2007)
	Patterns of DNA Barcode Variation in Canadian Marine Molluscs	Layton et al. (2014)
Mollusca (Bivalves)	Local scale DNA barcoding of bivalves (Mollusca): a case study	Mikkelsen et al. (2007)
	Molecular phylogeny of oysters belonging to the genus <i>Crassostrea</i> through DNA barcoding	Trivedi et al. (2015)
	Four genes, morphology and ecology: distinguishing a new species of <i>Acesta</i> (Mollusca; Bivalvia) from the Gulf of Mexico	Järnegren et al. (2007)
	Phylogeny of venus clams (Bivalvia: Venerinae) as inferred from nuclear and mitochondrial gene sequences	Kappner and Bieler (2006)
Chaetognatha	Barcoding of arrow worms (Phylum Chaetognatha) from three oceans: genetic diversity and evolution within an enigmatic phylum	Jennings et al. (2010b)
Platyhelminthes	DNA taxonomy of Swedish <i>Catenulida</i> (Platyhelminthes) and a phylogenetic framework for catenulid classification	Larsson et al. (2008)
Nemathelminthes	Disentangling taxonomy within the <i>Rhabditis (Pellioiditis) marina</i> (Nematoda, Rhabditidae) species complex using molecular and morphological tools	Derycke et al. (2008)
Annelida	Development and evaluation of a DNA-barcoding approach for the rapid identification of nematodes	Bhadury et al. (2006)
	<i>Grania</i> (Annelida: Clitellata: Enchytraeidae) of the Great Barrier Reef, Australia, including four new species and a re-description of <i>Grania trichaeta</i> Jamieson, 1977.	De Wit et al. (2009)
Porifera	Morphological description and DNA barcodes of shallow-water <i>Tetractinellida</i> (Porifera: Demospongiae) from Bocas del Toro, Panama, with description of a new species	Cardenas et al. (2009)
Cnidaria	DNA barcoding reveals cryptic diversity in marine hydroids (Cnidaria, Hydrozoa) from coastal and deep-sea environments	Moura et al. (2007)
Bryozoa	Mating trials validate the use of DNA barcoding to reveal cryptic speciation of a marine bryozoan taxon	Gómez et al. (2007)
Echinodermata	DNA barcoding discriminates echinoderm species	Ward et al. (2008a)
	Genetic barcoding of commercial Bêche-de-mer species (Echinodermata: Holothuroidea)	Uthicke et al. (2010)

### 13. DNA barcoding of marine mammals

Mammalia Barcode of Life (<http://www.mammaliabol.org>) is devoted to barcoding of mammals including the marine mammals. A study conducted along the French Atlantic coast demonstrated that DNA barcoding in conjunction with a stranding network can be used in monitoring marine mammal diversity (Alfonsi et al., 2013).

### 14. Criticisms of DNA barcoding

Initially the concept of DNA barcoding invoked criticisms from traditional taxonomists (Will and Rubinoff, 2004; DeSalle et al., 2005; Will et al., 2005; Rubinoff et al., 2006). DNA barcoding has some limitations, like low resolutions in the cases of recently diverged species, species complexes and hybrids. The presence of pseudogenes and mitochondrial introgression is highlighted by some researchers (Song et al., 2008; Kempainen et al., 2009). Reproductive isolation, an important aspect for the biological species concept is difficult to investigate in the marine ecosystems. A study involving cosmopolitan marine bryozoan revealed that divergent barcode clusters corresponded to reproductively isolated groups, thereby establishing a link between biological species concept and DNA barcoding (Gómez et al., 2007). The integration of morphological, ecological and physiological data with DNA barcode data will improve species discovery and identification process (Waugh, 2007; Padial et al., 2010). Some shortcomings of integrating DNA barcode data were revealed (Goldstein and DeSalle, 2011). Collins and Cruickshank (2013) assessed seven deficiencies and outlined potential improvements on each of them. Seven shortcomings in the experimental design addressed by these workers are as follows:

1. Failure to test clear hypotheses.
2. Inadequate a prior identification of specimens.
3. The use of the term 'species identification'.
4. Inappropriate use of neighbor-joining trees.
5. Inappropriate use of bootstrap resampling.
6. Inappropriate use of fixed distance thresholds.
7. Incorrectly interpreting the barcoding gap.

During DNA barcoding, care has to be taken to address these issues. Several studies have shown that finding a universal barcode for all life forms is more difficult than initially supposed.

### 15. Future of marine DNA barcoding

Genomic studies in conjugation with DNA barcoding can be very effective in assessment of global biodiversity. Recently, Centre for Biodiversity Genomics (CBG) was established at University of Guelph, Canada, as a global hub for DNA barcoding. With the advancement of high-throughput sequencing, it can be useful in DNA barcoding of bulk environmental samples. Environmental barcoding was very effective in analyzing zooplankton samples collected from Equatorial Pacific Ocean (Machida et al., 2009). Screening of cDNA libraries can reduce artifacts caused by pseudogenes or mispriming. Real Time Quantitative PCR (qPCR) can be very useful in assessing the

species diversity and abundance particularly for unsorted and bulk samples. A taxonomic approach of integrating DNA sequences with morphological characters will achieve higher efficiency in species identification. With the development of newer and faster techniques, DNA barcoding holds great promise in the assessment, analysis and conservation of marine biodiversity.

### 16. Conclusions

Most DNA barcodings are focused on animals and more effort is needed on the barcoding of plants and protists. One main reason may be the lack of universal barcode gene in plants that makes the situation comparatively tricky. Despite of some limitations, DNA barcoding approach can be used for survey of marine biodiversity and prioritizing conservation strategies. In conclusion it can be said that DNA barcoding can play a very significant role in assessment and conservation of biodiversity in the massive and diverse marine ecosystem.

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