DNA Barcoding of Calanoid Copepods from the Gulf of California

Juan Ramon Beltrán-Castro and Sergio Hernández-Trujillo

Abstract The diversity of pelagic copepods was studied in different sites around the Gulf of California, to confirm their morphological identification with of CO1 gen analysis. This is the first study of its kind for the Gulf of California and we report the results of 101 barcode sequences for 27 species of copepods. The separation of species based on morphological characters was very clear for most species and consistent with the formation of genetic groups obtained with the CO1 gene and its corresponding barcode without overlap between the sequences, thus becoming the initial records a database of genetic sequences for the area.

Keywords Gulf of California · Calanoid copepods · Barcoding · Cytochrome Oxidase 1 · Marine zooplankton

1 Introduction

The crustaceans are one of the most diverse marine groups, and have a significant role in the food web and be represented in all ecosystems; These and others features make the group present ambiguous morphological characters from the earliest stages of development to adulthood, so the DNA barcode becomes a powerful tool for reliable identification of species (Radulovici et al. 2010).

Pelagic copepods are maxillopod crustaceans that its abundance and frequency of occurrence in the epi and mesopelagic zone are one of the most important taxa in the marine food chain. More than 2500 species of marine planktonic copepods have been described, with distributions ranging from shallow waters to abyss to hadopelagic depths (Razouls et al. 2011). Its great diversity needs accuracy on species identification because, in some cases, share morphometric and meristic characteristics that tend to confuse the identity of the species. To help solve cryptic species

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S. Trivedi et al. (eds.), *DNA Barcoding in Marine Perspectives*,
DOI 10.1007/978-3-319-41840-7_9

occurrence and other taxonomic issues, molecular tools are used since the late 20th century, specifically through the use of a DNA fragment known as cytochrome C Oxidase 1 (Hebert et al. 2003).

Recent research of DNA barcode on marine copepods have been focused on the exploration of species diversity mostly in ocean regions, the problem of taxa, biogeographic analysis and finding cryptic species (Blanco-Bercial et al. 2014).

In Central and Southern Gulf of California, have been identified between 53 (Hernández-Trujillo and Esquivel-Herrera 1989) and 84 (Brinton et al. 1986) copepods pelagic species; at the Bay and Ensenada of La Paz have been recorded 129 species (Palomares 1996; González-Navarro and Saldierna-Martínez 1997; Lavaniegos-Espejo and González-Navarro 1999; Aceves-Medina et al. 2007); these figures, compared with 197 species identified at national level (Hernández-Trujillo and Esqueda-Escárcega 2002) indicate the importance of the Gulf of California from the standpoint of not only the holoplankton biodiversity, but also the meroplankton.

The Cytochrome c oxidase 1 (CO1), such as has been previously mentioned, it has been widely used in studies of diversity of fungi (Kurtzman 1985), bacteria (Wilson et al. 1995), on marine copepods (Bucklin et al. 1998), lepidoptera (Brown et al. 1999), onychophora (Trewick 2000), annelidae, arthropoda, chordata, cnidarians, echinoderms and flatworms (Hebert et al. 2003), birds (Hebert et al. 2004), fishes (Trivedi et al. 2014) euphausiids (Bucklin et al. 2007) Branchiopoda resistance eggs, Copepoda, Rotifera, Bryozoa and Ascidia in ballast water (Briski et al. 2011), among other metazoarians.

Bucklin et al. (2010a, b) estimated at 230,000 the number of marine animals of known species and one million which have not yet been described, so consider that the process of description of new species will be continued in the coming decades.

For copepods, studies of DNA barcode has focused to reviewing the copepod morphological descriptions which by their level of uncertainty may result in sibling or cryptic species, and CO1 is a gene that has shown very useful for separating copepods species of genus *Calanus* (Hill et al. 2001; Unal et al. 2006), *Clausocalanus* (Bucklin et al. 2003), *Neocalanus* (Machida et al. 2009), *Pseudocalanus* (Bucklin et al. 2003), *Oncaea* and *Triconia* (Böttger-Schnack and and Machida 2011).

In order to document the biodiversity of pelagic copepods from Gulf of California, we identified by gene cytochrome C Oxidase 1 (CO1) several of the species that inhabit the study area, and contribute to mainstreaming a barcode library.

2 Materials and Methods

Specimens were collected with a plankton net (300 μm mesh size) from the Gulf of California mostly. The sampling polygon extended from 24 to 29° N and 110 to 112° W (Fig. 1) while specific geographic coordinates and collection dates for all

localities are recorded in the project files Copepods of La Paz Bay, BCS, and Copepods of the NW of Mexico in the Barcode of Life Data System (Ratnasingham and Hebert 2007).

Whenever possible, we barcoded at least five adults of each species. Individuals were photographed and are kept as vouchers in Copepoda Collection at Centro Interdisciplinario de Ciencias Marinas of Instituto Politécnico Nacional. All identifications were based on specialized literature and direct comparison with previously deposited material in the same collection, as well as a validation by the curator of the collection.

Extraction and amplification of CO1 gene was carried out according to protocol proposed by Hajibabaei et al. (2007) and modified by Elías-Gutiérrez and Valdez-Moreno (2008a, b) and sequencing as described in Hajibabaei et al. (2006).

Having obtained the sequences were aligned using the MEGA 5 software, afterwards manually alignment was made to 550 bp with GeneDoc software; MEGA 5 software 5 similarity tree was constructed using statistical Maximun Likelihood method, with bootstrap 1000, genetic differences were calculated using the distance model of two parameters or K2P Kimura (Tamura et al. 2011). The sequences are deposited in www.boldsystems.org.

3 Results

483 individuals belonging to 101 species of copepods were morphologically identified, of which 27 were subjected to the process of DNA extraction and amplification of mitochondrial CO1 gene (Table 1); 93 sequences, of which 89 % had more than 500 base pairs (Fig. 2), were obtained. The largest number of specimens sequenced came from the area of the Islas Marias, whereas the rest come from the central Gulf of California (Fig. 1).

The tree of similarity shows the clustering of specimens of Calanoida and Poecilostomatoida orders (Fig. 3). The average distance (K2P) among the species was 0.36 % and the divergence between genus 28.6 % (Table 2); the highest frequency of interspecific divergence was between 0 and 0.5 %, and in the case of the genera all had more than 20 %.

In the Calanoida order 4 families were pooled: Pontellidae, Paracalanidae, Eucalanidae and Candaciidae with bootstrap values of 20.2, 17.5, 23.7 and 54.9 % respectively (Fig. 2); for Poecilostomatoida there were only representatives of one family which was grouped (52.7 % bootstrap).

In order Calanoida, for example, Pontellidae family had a similarity value of 77 %; *Pontellina plumata*, *Pontellopsis armata*, *Calanopia elliptica*, *Labidocera acuta* and *Labidocera johnsoni* were the most divergent with 22 %. The outside group was genus *Candacia* and *Candacia catula, Candacia curta* and *Candacia simplex* were similar to each other by approximately 41 %.

Table 1 Copepod species identified

Order	Family	Species
Calanoida	Acartidae	*Acartia danae* (Giesbrecht 1889)
	Aetideidae	*Aetideus armatus* (Boeck 1872)
	Calanidae	*Undinula vulgaris* (Dana 1852)
		Canthocalanus pauper (Giesbrecht 1888)
	Paracalanidae	*Acrocalanus gibber* (Giesbrecht 1888)
		Paracalanus parvus (Claus 1863)
	Calocalanidae	*Calocalanus pavo* (Dana 1849)
	Candaciidae	*Candacia curta* (Dana 1849)
		Candacia simplex (Giesbrecht 1889)
	Centropagidae	*Centropages furcatus* (Dana 1849)
	Eucalanidae	*Pareucalanus sewelli* (Fleminger 1973)
		Rhincalanus nasutus (Giesbrecht 1888)
		Subeucalanus mucronatus (Giesbrecht 1888)
		Subeucalanus subcrassus (Giesbrecht 1888)
	Clausocalanidae	*Clausocalanus furcatus* (Brady 1883)
	Pontellidae	*Calanopia elliptica* (Dana 1849)
		Labidocera johnsoni (Fleminger 1964)
		Labidocera acutifrons (Dana 1849)
		Labidocera acuta (Dana 1849)
		Pontellopsis armata (Giesbrecht 1889)
		Pontellopsis occidentalis (Esterly 1906)
		Pontellina plumata (Dana 1849)
	Euchaetidae	*Euchaeta indica* (Wolfenden 1905)
	Temoridae	*Temora discaudata* (Giesbrecht 1889)
Poecilostomatoida	Sapphirinidae	*Copilia mirabilis* (Dana 1849)
		Sapphirina intestinata (Giesbrecht 1891)
		Sapphirina scarlata (Giesbrecht 1892)

4 Discussion

The separation of species based on morphological characters was clear for most species, although in the case of *Labidocera diandra* male the existence of two morphotypes hampered identification (Beltrán-Castro 2014). In the other species studied so far, no cryptic genotypes were found; on the other hand, for the morphological identification of the species in this study proved characteristics of sufficient quality for proper taxonomic identification, coinciding with findings in the Mediterranean with Oncaeidae (Böttger-Schnack and Machida 2011) family, hydrothermal vents with Dirivultidae (Gollner et al. 2011) family and various areas of the world with families Clausocalanidae and Calanidae (Bucklin et al. 2003).

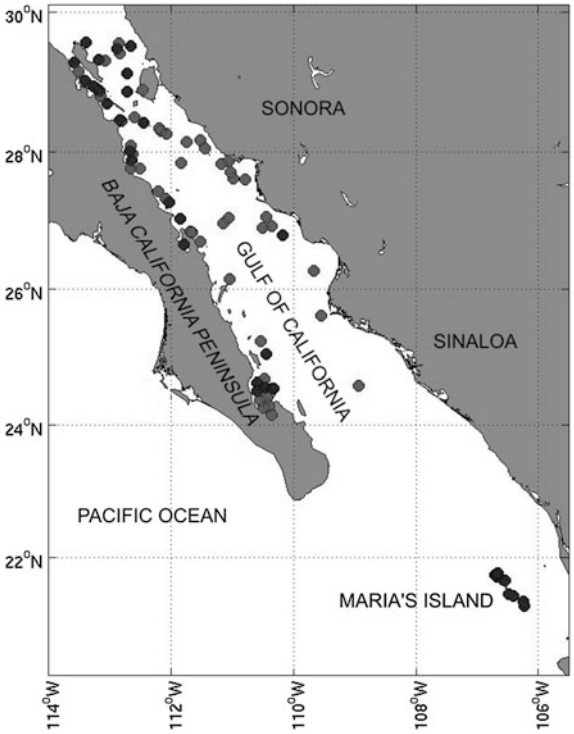

Fig. 1 Sampling stations in the study area

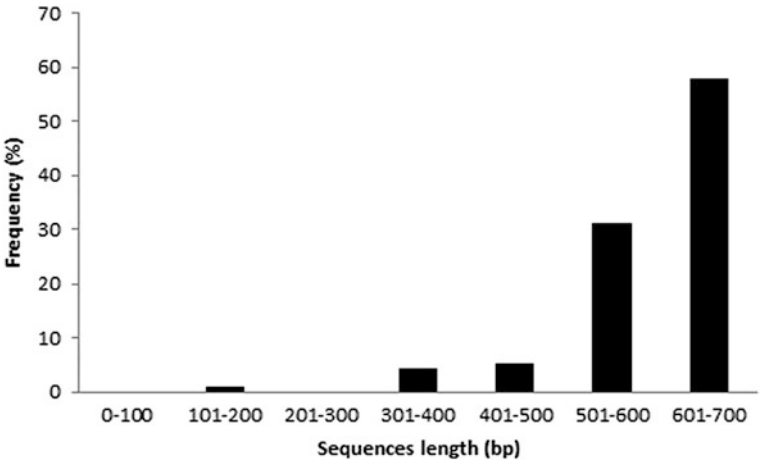

Fig. 2 Sequence length distribution of pelagic copepods from Gulf of California

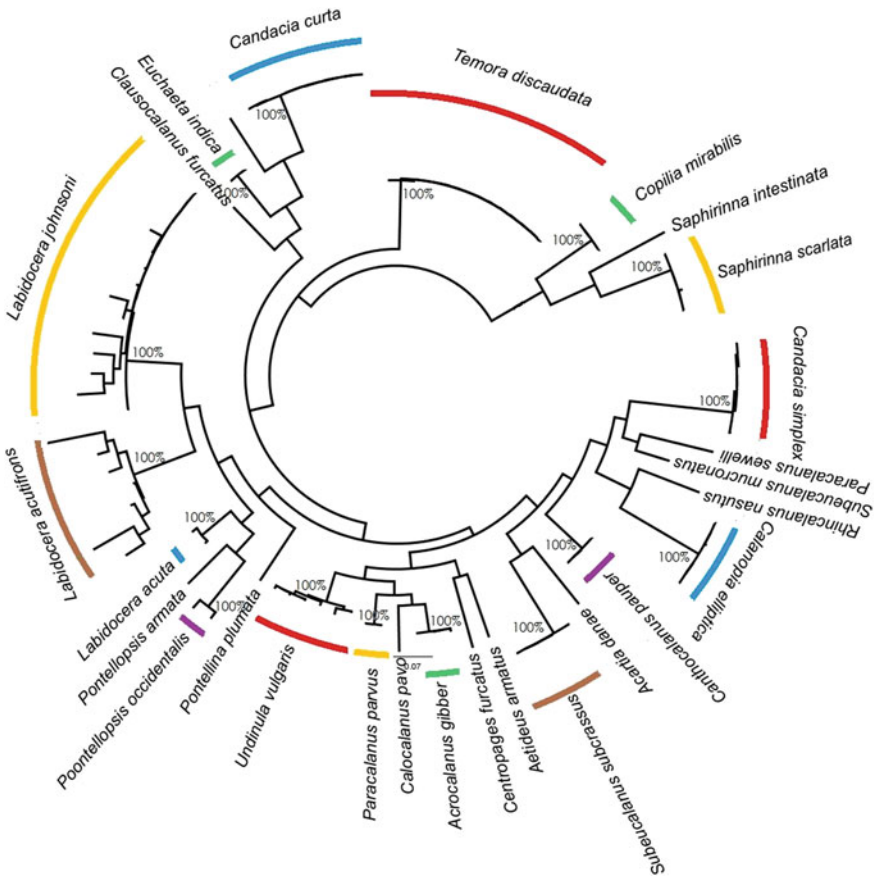

Fig. 3 Neighbor-joining tree for 27 pelagic copepod species (Maximum Likelihood, bootstrap = 1000), using K2P distances

Taking into consideration that the genetic sequences of the species alone do not identify species, but is an indispensable requirement a detailed morphological revision to get to the correct identification, has to be put into perspective how relevant is the taxonomists work for allocating bar codes based on the CO1 or 12S genes by comparing sequence similarities (Bucklin et al. 2010a, b).

Comparison of sequences obtained from the Gulf of California copepods allowed to differentiate species, as has happened in other marine regions (Folmer et al. 1994; Hill et al. 2001; Hebert et al. 2003; Davolos and Maclean 2005; Matz and Nielsen 2005; Hajibabaei et al. 2006; Costa et al. 2007; Elías-Gutiérrez et al. 2008; Elías-Gutiérrez and Valdez-Moreno 2008a, b; Machida et al. 2009; Bucklin et al. 2010a, b; Camacho et al. 2011; Raupach et al. 2010; Blanco-Bercial et al. 2011); in most species variation lower than 2 % of CO1, confirms as a gene with systematic feature (Unal et al. 2006) for reliable identification, although there are

Table 2 Genetic divergences (K2P) at different taxonomic levels

Comparisons within	Taxa	Number of comparisons	Minimum (%)	Distance mean (%)	Maximum (%)	SE dist (%)
Species	15	216	0	0.364	2.823	0.033
Genus	13	61	21.276	28.593	35.257	0.458
Family	8	104	13.159	25.204	33.896	0.454
Order	2	2132	13.393	28.508	38.565	0.092
Class	1	568	31.745	37.354	43.468	0.106

families like Oncaeidae in which the gene 12S better performance (Böttger-Schnack and Machida 2011).

Morphological identification of species was consistent with the formation of genetic groups obtained with CO1 gene and its corresponding barcode without overlap between the sequences. This morphological-genetic match is the start of a library of barcodes copepods in the Gulf of California.

Because of the relatively rapid evolution of CO1 gene, its variation may help solve taxonomic problems associated with geographic variation in distribution of some species of copepods; for instance *Calanus pacificus* have three subspecies which differ geographically as well as their CO1 sequences almost 3 % (Hill et al. 2001). In this study, were found haplotype of *Undinula vulgaris, Calanus pacificus, Canthocalanus pauper, Euchaeta indica, Candacia simplex, Temora discaudata, Paracalanus parvus, Clausocalanus furcatus, Acartia danae, Subeucalanus subcrassus, Rhincalanus nasutus, Aetideus armatus* and *Centropages furcatus*, which are located at various sampling sites and are representing different habitat types, as has been observed with family Dirivultidae (Gollner et al. 2011).

This tool opens an option for the study of biodiversity in the study area since it is distribution area of numerous planktonic species, not just of copepods, so a larger sampling effort has to be carried out to reveal the presence of more species, to correct misidentification that lead to a reclassification and/or description of the "problem" species (Elías-Gutiérrez and Valdez-Moreno 2008a, b; Camacho et al. 2011), revitalize the biological collections, accelerate inventory biodiversity, performing phylogenetic and biogeographic analysis and learn more about the process of speciation.

Molecular analysis of copepods in the Gulf of California will surely help you discover the taxonomic significance of intraspecific genetic separation discovering cryptic species (Bucklin et al. 1998), especially in the calanoid copepods in which numerous examples of sibling species have been discriminated with few morphological characters. The ability to understand the dynamics of plankton community depends on the ability to accurately measure the diversity of species and to accurately identify individuals' species morphologically similar.

This work confirms that the molecular and morphological methods can be considered complementary and when applied in combination, constitute a powerful

tool for identification with minimal errors not only of copepods in the Gulf of California, but in the adjoining marine areas; the results are the first step in building databases of sequences and update morphological identification keys.

Acknowledgments To the Instituto Politécnico Nacional and Centro Interdisciplinario de Ciencias Marinas by funding SIP20110224, 20120950 and 20130752 projects. Thank to Dr. Jaime Gómez by providing access to zooplankton samples from Islas Marias and Gulf of California. Thank the node Chetumal of Laboratorio Nacional de Código de Barras, especially Dr. Manuel Elias Gutierrez and MC Areli M. Arce for support in the processing of DNA extraction. To mexican Barcode iniciative by financially supporting the authors in obtaining results and their disclosure. Authors are thankful to BEIFI Programme, EDI and COFAA-IPN by grants received.

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