

Ecology

Diversity of planktonic gastropods from western Baja California Peninsula assessed by 18S rDNA sequences

Diversidad de gasterópodos planctónicos del oeste de la Península de Baja California evaluada mediante secuencias de 18S ADNr

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Abstract

The study of planktonic mollusks is a relevant element to understand the dynamics of the benthic communities under present and future environmental conditions. We present the description of the biodiversity of planktonic gastropods at 2 sampling sites on the Pacific coast of the Baja California Peninsula, Mexico. Organisms collected from plankton tows at 2 locations (Cabo Tosco, n = 89 and La Bocana, n = 213) were sequenced for a portion of the 18S rDNA gene. High diversity was registered, with 71 Molecular Operational Taxonomic Units (MOTUs), which contrasts with the low phenotypic diversity of stereoscopic images. Differences in community composition between and within sampling sites indicate that planktonic gastropod distribution is not random but probably modulated by micro-environmental processes such as currents or biological events. The presence of non-gastropod sequences within some shells (n = 6) suggests their use as carriers of eggs or larvae of other taxa.

Keywords: Larval dispersal; Mollusca; Meroplankton; GenBank

Resumen

El estudio de moluscos planctónicos es un elemento relevante para la comprensión de la dinámica de la comunidad bentónica bajo las condiciones ambientales actuales y futuras. Se presenta la descripción de la biodiversidad de gasterópodos planctónicos en 2 sitios de la costa del Pacífico de la península de Baja California, México. Organismos recolectados en arrastres planctónicos en 2 localidades (Cabo Tosco, n = 89 y La Bocana, n = 213) se secuenciaron para

una fracción del gen 18S ADNr. Se registró una alta diversidad con 71 unidades taxonómicas moleculares operativas (MOTU), que contrasta con la baja diversidad fenotípica observada en imágenes de estereoscopio. Las diferencias en la composición de la comunidad entre y dentro de los sitios de muestreo, indican que la distribución de gasterópodos planctónicos no es aleatoria, sino que se encuentra, probablemente, modulada por procesos microambientales tales como corrientes o eventos biológicos. La presencia de secuencias de organismos que no son gasterópodos dentro de algunas conchas ($n = 6$) sugiere que son utilizadas por organismos de otros taxones como portadores de huevos o larvas.

Palabras clave: Dispersión larvaria; Mollusca; Meroplankton; GenBank

Introduction

Planktonic dispersal in the marine environment is one of the leading connectivity processes among populations of meroplanktonic and holoplanktonic gastropods (Crocetta et al., 2020; Roegner, 2000), and is a relevant element of the trophic dynamics of ecosystems and indirect indicators of the health of adult populations (Campos & Landaeta, 2016; Chávez-Villegas et al., 2014). Planktonic dispersal study will aid in a better understanding of the molluscan community's behavior under present and future environmental conditions (e.g., Aceves-Medina et al., 2020; Molina-González et al., 2018). For this purpose, baseline studies on the species composition that rely on the use of genetic markers are a starting point.

Planktonic gastropod identification is usually made using their morphology through microscope observation. The use of genetic markers (the Cytochrome Oxidase Subunit I of the mitochondrial DNA, COI) for this purpose is more recent. It has been used to study the genetic connectivity of the marine caenogastropod *Bursa scrobilator* (Crocetta et al., 2020). Due to its high conservation, the 18S ribosomal nuclear DNA (18S rDNA) gene has been proposed for biodiversity studies (Ranjithkumar et al., 2018).

Planktonic gastropods have been quite extensively studied worldwide (Bandel et al., 1997; Campos & Landaeta, 2016; Chávez-Villegas et al., 2014; Oliva-Rivera & Navarrete, 2007). However, in northwestern Mexico, the main efforts have been in the study of the species composition of holoplanktonic mollusks in the Gulf of California (Angulo-Campillo et al., 2011) and the western coast of the Baja California Peninsula (BCP) (Aceves-Medina et al., 2020; Molina-González et al., 2018; Moreno-Alcántara et al., 2020; Sánchez-Hidalgo y Anda, 1989), characterizing these regions as highly diverse. However, there is no available information about meroplanktonic gastropods in the region. Therefore, this work presents the first record of the planktonic gastropod biodiversity at 2 sites of the western coast of the BCP obtained by genetic markers.

Materials and methods

The sampling design originally intended to evaluate the presence of abalone larvae at 2 sites on the western coast of the Baja California Peninsula: La Bocana (LB: 26°45'1.9" N, 113°42'20.9" W) and Cabo Tosco (CT: 24°18'26.2" N, 111°42'36.6" W) (Fig. 1). However, we focused our study on other mollusks, as abalone larvae were not found. Sampling was done on December 1st, and 3rd, 2009 for LB and CT, respectively. At LB, the sampling area was located about 4.5 km southward, with an average depth of 10 m (Fig. 1). The rocky bottom floor is mostly flat with several small stone promontories composed of boulders, crevices, and a high abundance of macroalgae, mainly *Eisenia arborea* (Areschoug, 1876). CT is located at the southernmost part of Isla Margarita, and the sampling site was just 150 m off the coastline (Fig. 1), with a depth of approximately 3 m. The bottom is composed of large rocks with abundant crevices, surrounded by soft bottom but low macroalgae coverage. These sites were selected because they represent a high abalone productive area (LB) and the southern extreme of abalone species distribution (CT) (León-Carballo & Muciño-Díaz, 1996). The 8-day mean surface temperature in the first week of December 2009 at each site, which includes the sampling period and was obtained from the Aqua MODIS satellite —NPP, 0.0125°, West U.S., day time (11 microns), Simons (2022)— was 21 °C and 24 °C, respectively (Fig. 1).

At LB, there were 3 towing stations: 1) surface and 2) at 3-4 m depth at the same geographical position (LB-01 and LB-10, respectively), and 3) at the surface, approximately 1 km to the west (LB-07) of the previous position. At CT, there was one towing station (CT-01) at the surface, in a semi-protected area on the southwestern side of Isla Margarita. Tows followed a circular trajectory at a speed of 2.5 knots for 5 min. The plankton net was conical (0.45 m mouth diameter and 180 µm mesh size), with a calibrated flowmeter and a collection cup also with a 180 µm mesh size. Samples were transferred to a 500 mL plastic flask and preserved in 80% ethanol.

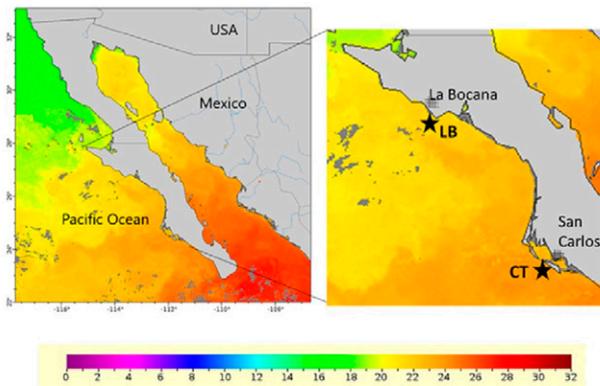


Figure 1. Sampling sites at La Bocana (LB) and Cabo Tosco (CT) on the Baja California Peninsula. Color scale represents the 8-day sea surface temperature (°C) data from Aqua MODIS satellite (Simons, 2022).

The biomass from the plankton tows was estimated by displaced volume (Beers, 1976) and standardized to mL of biovolume per 1,000 m³ of filtered seawater (Smith & Richardson, 1979). Due to high biomass at each towing station, the samples were fractionated to ¼ with a Folsom splitter. Using a stereoscope at 35×, following the description of Courtois de Viçose et al. (2007) for *Haliotis tuberculata* abalone larvae, gastropod mollusks with egg or globular shapes were separated (Bandel et al., 1997). A total of 384 individuals were separated, set in slides with wells (1 individual per well), and photo-documented (Leica Application Suite EZ, ver. 1.5.0). Individuals were transferred to 96-well PCR plates containing 70% ethanol.

DNA extraction was done following the protocol of Selvamani et al. (2001), with slight modifications. Before extraction, individuals were crushed with a plastic pestle and dried at ambient temperature. Samples were incubated in 18 µL of lysis buffer (Tris-HCl 10 mM, pH 8.3, KCl 50 mM, Tween-20 0.05%) and 2 µL of Proteinase-K (Sigma®, 2.5 mg/mL) at 55 °C for 24 h. After this time, the proteinase was inactivated by incubating the samples at 95 °C for 10 min. The plates were preserved at -20 °C until the PCR was performed.

A fragment of the 18S rDNA gene was amplified with primers 18SHal2F (5'-TTGGATAACTGTGGTAATTCTAGAGC) and 18SHal2R (5'-CCGGAATCGAACCCCTGAT) (Aranceta-Garza et al., 2011). The reactions contained 0.2 mM of dNTPs (Invitrogen), 0.5 µM of each primer, PCR buffer 1× (Invitrogen), 1.5 mM MgCl₂, 0.05 U Platinum® *Taq* DNA polymerase (Invitrogen), 2 µL of DNA and completed to 30 µL with MilliQ® water. PCR

conditions were: 1 cycle at 94 °C for 4 min, 30 cycles at 94 °C for 45 s, 57 °C for 45 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min in a thermal cycler (C1000, Bio-Rad). PCR products were verified in a 1.5% agarose gel stained with SYBR gold (Promega), visualized in a UV transilluminator (UVP BioDoc-it Imaging System).

PCR products of approximately 220 bp were individually sequenced either in forward and reverse (LB-07) or in forward only (LB-01, LB-10, CT-01) (Macrogen, Korea). The sequences were manually revised for quality and edited with ChromasPro v. 2.1.9 (Technelysium Pty Ltd). The edited sequences were analyzed to determine the number of similar genotypes with the software DnaSP v. 6.12.03 (Rozas et al., 2017). Identical sequences (100% match) were organized into Molecular Operational Taxonomical Units (MOTUs), for which the putative identity was obtained by a local alignment analysis with BLAST (Altschul et al., 1990). MOTUs were identified either to family, genus, or species level based on the hits with the highest similarity percentage (> 95%) and lowest E value (probability of the sequence match being random < 1 × 10⁻⁹⁰). Nomenclature follows the NCBI (National Center for Biotechnology Information) Taxonomy database (Schoch, 2011).

The MOTUs sequences were analyzed as haplotypes (most sequences were homozygotes) to construct a minimum spanning network (Bandelt et al., 1999). The software PopArt (Leigh & Bryant, 2015) was used to visualize genealogical relationships among towing stations and obtain genetic diversity estimators (nucleotide diversity and number of segregating and parsimony-informative sites). The MOTUs' diversity among towing stations was estimated by the Shannon-Weaver index using the natural logarithm with the vegan package (Oksanen et al., 2020) in R ver. 4.1.1 (R Core Team, 2013), using MOTUs as putatively non-identified species.

Results

At LB, the plankton biomass at the mid-water tow was more than twice as high (LB-10, 1,222 mL 1,000 m⁻³) as at the 2 surface stations (LB-01 and LB-07, 491 and 557 mL 1,000 m⁻³, respectively). Surface biomass at CT (CT-01) was 883 mL 1,000 m⁻³. Planktonic gastropods, similar in shape, varied between 200 - 500 µm in width. As shells were translucent, it was possible to assess the presence of tissue within (Fig. 2).

Of a total of 384 individuals analyzed, 302 resulted in reliable DNA sequences matching gastropod mollusks (phylum Mollusca, class Gastropoda). The rest of the sequences were of low quality (n = 75) or matched other

Table 1

Number of MOTUs and individuals per taxonomic group of the Class Gastropoda* at the 2 sampling sites.

Subclass	Order	MOTU	Individuals
Caenogastropoda	Caenogastropoda <i>incertae sedis</i>	2	3
	Littorinimorpha	16	126
	Neogastropoda	5	8
	Ptenoglossa (suborder)	1	2
Heterobranchia	Aplysiida	3	16
	Cephalaspidea	6	11
	Ellobiida	1	2
	Euthyneura (clade)	1	1
	Nudibranchia	9	16
	Pleurobranchida	1	4
	Pteropoda	5	14
	Heterostropha	1	1
	Sacoglossa	9	34
	Neritimorpha	Cycloneritida	5
Patellogastropoda	Patellogastropoda	1	1
Vetigastropoda	Lepetellida	2	2
	Trochida	2	5
Unclassified gastropod		1	21
	Total	71	302

* Nomenclature from the Taxonomy browser of the NCBI database (Schoch, 2011).

taxonomic groups (phylum Cnidaria, order Bivalvulida, and phylum Arthropoda, orders Euphausiacea and Calanoidea, $n = 6$) or showed no significant match to any species ($n = 1$). A total of 71 MOTUs were distinguished with matching identities higher than 95% and E-values lower than 1×10^{-100} in most sequences, putatively belonging to several families and orders of 5 subclasses (Tables 1, 2).

From the similarity analysis to GenBank sequences by BLAST, 23 MOTUs gave 100% identity. Identification to the species level was likely for 7 MOTUs (i.e., MOTUs 02, 05, 13, 33, 48, 61, and 62; Table 2). The remaining 16 are not certain as some of these MOTUs showed matching hits either to several genera (i.e., MOTUs 01, 03, 08, 10, 12, 15, 20, 22, 25, 28, 64, and 68; Table 2) or several species of a certain genus (i.e., MOTUs 29, 65, 66, and 67; Table 2).

When individual images were organized per MOTU (Supplementary material: Fig. S1[<https://doi.org/10.5281/zenodo.7685921>]), it was observed that there are many

similarities among individuals at distinct taxonomic levels, including subclasses. In contrast, a high genetic diversity was found (nucleotide diversity $\pi = 0.045$, with 72 segregating sites and 55 parsimony-informative sites; Supplementary material: Table S1). The phylogenetic analysis inferred from the Minimum Spanning Network indicated that most identified subclasses were represented in all locations (Fig. 3). One exception was Neritimorpha, which was mainly observed in CT.

The MOTUs' diversity (Shannon index) varied among towing stations with the highest at LB-10 and the lowest at (LB-07) (Fig. 4). This large difference can be inferred by the presence of many MOTU 04 (family Cypraeidae) individuals at LB-07 (Table 2). Even though the diversity was rather similar among CT-01, LB-01, and LB-10 (Fig. 4), the MOTU composition was different with several MOTUs found only in a single towing station (CT-01: 17; LB-01: 16; LB-10: 14; LB-07: 7), i.e., 54 of the 71 MOTUs found were unique (Table 2).

Table 2

Individuals per sampling station, organized in Molecular Operational Taxonomic Units (MOTU) from the genetic identity analysis (BLAST) of the 18S rDNA gene sequences in the GenBank database. Genbank accession numbers are shown only for the MOTUs of highest identity percentage and lowest E-value hits. CT-01: Cabo Tosco; LB: La Bocana. BCP: Baja California Peninsula. Proporcionar tabla.

MOTU	Sampling stations					Taxonomy of hits			GenBank hits characteristics		Notes on possible species in the study region (the Pacific coast of the BCP) based on the Global Biodiversity Information Facility web [https://www.gbif.org-Consulted: Jan, 14 2022]	
	CT-01	LB-01	LB-10	LB-07	Subclass	Order	Superfamily	Family	Genera and species (GenBank accession number)	E		% Identity
01	2	-	-	-	Caenogastropoda <i>incertae sedis</i>		Abyssochrysoidea	Abyssochrysidae	<i>Abyssochrysos melanioides</i> (AB930376) <i>Rubyspira osteovora</i> (GQ290530)	5×10^{-99}	99.1	There are no records of the genera <i>Abyssochrysos</i> or <i>Rubyspira</i> in the BCP
02	-	-	1	-			Epitoniioidea	Epitoniidae	<i>Alexania inawazai</i> (AB930380)	4×10^{-106}	100	There are no records of <i>Alexania</i> or other species of the genus in the BCP
03	5	-	-	-			Littorinoidea	Lacunidae	<i>Lacuna pallidula</i> (AJ488686)	3×10^{-102}	100	The species <i>Lacuna unifasciata</i> (One-band lacuna) has been registered in the BCP. Several species of the genus <i>Crepidula</i> (American slipper limpet) have been registered in the BCP. <i>Thylacodes adamsii</i> (Scaly worm shell) has been registered in the north of the BCP
							Calyptraeidea	Calyptraeidae	<i>Crepidula fornicata</i> (AY377660)			
							Vermetoidea	Vermetidae	<i>Thylacodes adamsii</i> (HQ833992)			
04	1	5	8	51	Caenogastropoda	Littorinimorpha	Cypraeoidea	Cypraeidae	<i>Erosaria erosa</i> (KT753625) <i>Erronea erones</i> (HQ833998)	5×10^{-125}	100	<i>Erosaria erosa</i> and <i>Erronea erones</i> (Mistaken cowrie) are not registered in the BCP
05	-	1	-	-			Pterotracheoidea	Atlantidae	<i>Atlanta</i> sp. (MW203647)	3×10^{-101}	100	Several species of the genus <i>Atlanta</i> have been registered in the Gulf of California (Angulo-Campillo et al., 2011), and the BCP (Aceves-Medina et al., 2020; Molina-González et al., 2018; Moreno-Alcántara et al., 2020).
06	-	-	-	1			<i>Atlanta</i> sp. (MW204226)	2×10^{-123}	99.6			
07	-	1	-	-	Pterotracheoidea	Pterotracheidae	<i>Firoloida desmarestia</i> (MW204031)	4×10^{-88}	95.3	The species <i>Firoloida desmarestia</i> have been registered in the Gulf of California (Angulo-Campillo et al., 2011), and the BCP ((Molina-González et al., 2018)		
08	2	-	-	-	Rissooidea	Rissoinidae	<i>Phosinella clathrata</i> (AB930392) <i>Rissoina fasciata</i> (DQ916528)	1×10^{-106}	100	No genera of families Rissoinidae or Hydrobiidae are registered in the BCP		
09	-	11	2	-	Truncatelloidea	Tateidae	<i>Trochidrobia punicea</i> (KT313223) <i>Novacaledonia numee</i> (KT313210) <i>Kanakyella gentilsiana</i> (KT313209) and others	5×10^{-99}	99.1	No genera of family Tateidae are registered in the BCP		
10	-	1	-	-			<i>Trochidrobia punicea</i> (KT313223) <i>Novacaledonia numee</i> (KT313210) <i>Kanakyella gentilsiana</i> (KT313209) and others	9×10^{-102}	100	No genera of family Tateidae are registered in the BCP		

Table 2. Continued

MOTU	Sampling stations					Taxonomy of hits				GenBank hits characteristics		Notes on possible species in the study region (the Pacific coast of the BCP) based on the Global Biodiversity Information Facility web [https://www.gbif.org-Consulted: Jan, 14 2022]	
	CT-01	LB-01	LB-10	LB-07	Subclass	Order	Superfamily	Family	Genera and species (GenBank accession number)	E	% Identity		
16	-	-	1	-	Caenogastropoda	Littorinimorpha			<i>Phrantela daveyensis</i> (KT313215)	3×10^{-97}	97.7	No genus of Truncatelloidea is registered in the BCP	
11	-	-	1	-				Tateidae	Hydrobiidae	<i>Phrantela daveyensis</i> (KT313215)	1×10^{-93}	97.7	No genera of family Tateidae or Hydrobiidae are registered in the BCP
12	1	-	-	-				Vanikoroidea	Eulimidae	<i>Pyramidelloides angustus</i> (AB930386)	6×10^{-104}	100	No species of Pyramidelloides or Hemiliostraca have been registered in the BCP
13	-	8	6	-						<i>Balcis eburnea</i> (AF120519)	3×10^{-108}	100	The genus <i>Balcis</i> has been registered in the Gulf of California
14	-	1	6	-						<i>Melanella acicula</i> (AB930381)	1×10^{-101}	98.2	Several species of the genus <i>Melanella</i> have been registered in the Gulf of California, and the BCP
15	5	-	-	-			Hipponicidae	<i>Cheilea pileopsis</i> (AB930397)	3×10^{-102}	100	The species <i>Cheilea cepacea</i> has been registered in the BCP		
18	7	-	-	-		Littorinimorpha	Vanikoridae	<i>Vanikoro helicoidea</i> (AB930395)	2×10^{-100}	97.7	The species <i>Vanikoro aperta</i> has been registered in the Gulf of California but not in the BCP		
70	-	1	-	-		Littorinimorpha	Naticoidea	Naticidae	<i>Sinum halioideum</i> (FJ623466)	8×10^{-99}	98.1	Several species of the genus <i>Sinum</i> have been registered in the BCP	
17	-	-	-	1	Caenogastropoda	Neogastropoda	Conoidea	Several families	Several species	2×10^{-125}	99.6	No information available	
19	-	1	-	-			Buccinoidea	Several families	Several species	4×10^{-100}	99.5	No information available	
20	-	-	2	-				Several families	Several species	5×10^{-105}	100	No information available	
21	2	-	-	-			Muricoidea	Muricidae	<i>Coralliophila caribaea</i> (MW204229)	2×10^{-103}	99.5	Several species of the genus <i>Coralliophila</i> have been registered in the Gulf of California, and the BCP	
22	-	-	-	2			Muricoidea	Muricidae	Several species	6×10^{-124}	100	Several families of the genus <i>Coralliophila</i> have been registered in the Gulf of California, and the BCP	
23	-	-	-	2	Caenogastropoda	Ptenoglossa	Triphoroidea	Cerithiopsidae	Unclassified Cerithiopsidae	3×10^{-122}	99.6	Several genera of family Cerithiopsidae are found in the BCP	
24	7	3	1	-		Akeroidea	Akeridae	<i>Akera bullata</i> (AY427502)	3×10^{-113}	99.6	The species <i>Akera maga</i> has been registered in the BCP		

Table 2. Continued

MOTU	Sampling stations				Taxonomy of hits					GenBank hits characteristics		Notes on possible species in the study region (the Pacific coast of the BCP) based on the Global Biodiversity Information Facility web [https://www.gbif.org-Consulted: Jan, 14 2022]
	CT-01	LB-01	LB-10	LB-07	Subclass	Order	Superfamily	Family	Genera and species (GenBank accession number)	E	% Identity	
25	3	-	-	-	Heterobranchia	Aplysiida	Aplysioidea	Aplysiidae	<i>Stylocheilus longicauda</i> (DQ093439) <i>Dolabrifera dolabrifera</i> (DQ237960)	3×10 ⁻¹¹²	100	The species <i>Stylocheilus ricketsii</i> has been registered in the BCP and the Gulf of California. The species <i>Dolabrifera dolabrifera</i> has been registered in the Gulf of California
26	-	1	1	-			Aplysia			<i>Aplysia californica</i> (XR_004859253)	6×10 ⁻¹¹⁵	99.6
27	2	-	1	-		Cephalaspidea	Bulloidea	Bullidae	<i>Bulla vernicosa</i> (DQ923452)	6×10 ⁻¹⁰⁵	97.8	Several species of the genus <i>Bulla</i> are registered in the BCP
28	-	-	1	-					Haminoeidae	<i>Liloa mongii</i> (MH933263) <i>Atys curta</i> (DQ923459)	9×10 ⁻¹¹³	100
29	-	-	1	-		Cephalaspidea	Diaphanoidea	Diaphanidae	<i>Diaphana globosa</i> (MH933321)	1×10 ⁻¹⁰⁷	98.2	<i>Diaphana californica</i> has been registered in the BCP
30	1	-	-	-			Philinoidea	Cylichnidae	<i>Acteocina lepta</i> (MH933295)	6×10 ⁻¹¹⁰	99.1	Several species of the genus <i>Acteocina</i> have been registered in the BCP
31	3	-	-	-		Cephalaspidea	Philinoidea	Cylichnidae	<i>Acteocina lepta</i> (MH933295)	7×10 ⁻⁹⁰	99.5	Several species of the genus <i>Acteocina</i> have been registered in the BCP
32	1	-	1	-					Laonidae	<i>Laona confusa</i> (MH933303)	6×10 ⁻⁹⁰	94.3
33	-	1	1	-		Ellobiida	Ellobioidea	Ellobiidae	<i>Melampus bullaoides</i> (KM280980)	7×10 ⁻¹¹⁴	100	Several species of the genus <i>Melampus</i> have been registered in the BCP
35	-	1	-	-		Euthyneura	Pyramide- lloidea	Pyramidellidae	<i>Turbonilla elegantissima</i> (GU331941)	3×10 ⁻⁹²	96.7	Several species of the genus <i>Turbonilla</i> have been registered in the BCP
36	-	2	-	-	Heterobranchia	Anadoridoidea	Polyceridae	<i>Plocamopherus aurantinodulosa</i> (EF534011)	8×10 ⁻⁹⁴	93.7	There are no records of <i>Plocamopherus</i> for the BCP	
37	-	-	1	-		Fionoidea	Trinchesiidae	<i>Phestilla</i> sp. (MK088224)	2×10 ⁻¹⁰⁴	97.4	<i>Phestilla lugubris</i> has been registered in the Gulf of California	
43	-	-	1	-		Fionoidea	Eubranichidae	<i>Eubranichus sanjuanensis</i> (GQ326909)	2×10 ⁻¹⁰⁰	96.5	Several species of the genus <i>Eubranichus</i> have been registered in the BCP	
38	-	1	-	-		Dendrono-toidea	Dotidae	<i>Doto columbiana</i> (GQ326881)	1×10 ⁻¹²²	98.8	<i>Doto columbiana</i> has been registered in a northern region of the BCP	
39	-	-	1	-				<i>Doto columbiana</i> (GQ326881)	3×10 ⁻¹¹⁸	97.6	<i>Doto columbiana</i> have been registered in a northern region of the BCP	
40	-	1	-	-		Anadoridoidea	Onchidorididae	<i>Corambe pacifica</i> (KP340341)	1×10 ⁻¹⁰⁶	97.8	<i>Corambe pacifica</i> has been registered in the BCP	
41	-	-	1	-		Nudibranchia	Eudoridoidea	Chromodorididae	<i>Mexichromis porterae</i> (EF534014)	3×10 ⁻¹⁰⁸	97.8	Two species of <i>Mexichromis</i> (<i>M. antonii</i> , <i>M. tura</i>) have been registered in the Gulf of California

Table 2. Continued

MOTU	Sampling stations				Taxonomy of hits					GenBank hits characteristics		Notes on possible species in the study region (the Pacific coast of the BCP) based on the Global Biodiversity Information Facility web [https://www.gbif.org-Consulted: Jan, 14 2022]
	CT-01	LB-01	LB-10	LB-07	Subclass	Order	Superfamily	Family	Genera and species (GenBank accession number)	E	% Identity	
42	-	-	1	2		Nudibranchia	Eudoridoidea	Chromodorididae	<i>Diversidoris aurantinodulosa</i> (EF534011)	5×10^{-111}	94.1	There are no records of <i>Diversidoris</i> for the BCP
44	1	-	4	-		Nudibranchia			<i>Diversidoris aurantinodulosa</i> (EF534011)	4×10^{-92}	94	There are no records of <i>Diversidoris</i> for the BCP
45	-	2	2	-		Pleurobranchida	Pleurobranchioidea	Pleurobranchidae	<i>Berthella stellata</i> (AY427495)	9×10^{-73}	90.5	<i>Berthella idiomorpha</i> has been registered in the BCP
46	-	2	-	-			Thecosomata (Suborder)	Desmopteridae	<i>Desmopterus papilio</i> (GU969171)	3×10^{-112}	99.6	<i>Desmopterus papilio</i> and <i>D. pacificus</i> have been registered in the Gulf of California (Angulo-Campillo et al., 2011) and the BCP (Molina-González et al., 2018; Sánchez-Hidalgo y Anda, 1994).
47	-	2	-	1					<i>Desmopterus papilio</i> (GU969171)	2×10^{-135}	99.6	
48	-	3	-	-					<i>Desmopterus</i> sp. (MW203406)	2×10^{-115}	100	
49	-	2	3	-					<i>Desmopterus</i> sp. (MW203406)	2×10^{-103}	99.1	
50	-	-	-	1		Pteropoda			<i>Desmopterus papilio</i> (GU969171)	9×10^{-134}	99.3	
51	-	1	-	-	Heterobranchia	Heterostrophia	Pyramide-lloidea	Pyramidellidae	<i>Hinemoa</i> sp. (GU331936)	2×10^{-105}	97.8	The genus <i>Hinemoa</i> has not been registered in the BCP
52	1	-	-	-			Limapontioidea	Caliphyllidae	<i>Polybranchia</i> sp. (MH375070)	1×10^{-111}	99.6	<i>Polybranchia mexicana</i> has been registered in the BCP
53	-	1	-	-					<i>Polybranchia</i> sp. (MH375067)	2×10^{-109}	99.1	<i>Polybranchia mexicana</i> has been registered in the BCP
54	1	-	1	-					<i>Polybranchia</i> sp. (MH375070)	4×10^{-96}	96	<i>Polybranchia mexicana</i> has been registered in the BCP
55	-	1	-	-					<i>Mourgona</i> sp. (MH375064)	1×10^{-96}	96.4	The genus <i>Mourgona</i> has not been registered in the BCP
56	-	-	1	-					<i>Mourgona</i> sp. (MH375064)	3×10^{-97}	96.4	The genus <i>Mourgona</i> has not been registered in the BCP
57	1	7	10	2				Limapontiidae	<i>Aplysiopsis minor</i> (AB501328)	1×10^{-127}	98.9	<i>Aplysiopsis enteromorphae</i> has been registered in the BCP
58	-	-	1	-					<i>Aplysiopsis minor</i> (AB501328)	1×10^{-127}	98.9	<i>Aplysiopsis enteromorphae</i> has been registered in the BCP

Table 2. Continued

MOTU	Sampling stations				Taxonomy of hits			GenBank hits characteristics		Notes on possible species in the study region (the Pacific coast of the BCP) based on the Global Biodiversity Information Facility web [https://www.gbif.org-Consulted: Jan, 14 2022]		
	CT-01	LB-01	LB-10	LB-07	Subclass	Order	Superfamily	Family	Genera and species (GenBank accession number)		E	% Identity
34	5	-	1	-	Heterobranchia	Sacoglossa			<i>Stiliger smaragdinus</i> (AB501324)	3×10^{-97}	96.4	<i>Stiliger fuscovittatus</i> have been registered in the BCP
59	1	-	-	-			Oxynooidea	Oxynoeidae	<i>Oxynoe viridis</i> (AB501318) <i>Oxynoe antillarum</i> (FJ917441)	1×10^{-121}	100	<i>Oxynoe aliciae</i> , <i>O. viridis</i> and <i>O. panamensis</i> have been registered in the Gulf of California
60	17	-	-	-	Neritimorpha	Cycloneritida	Neritoidea	Neritidae	<i>Nerita tessellata</i> (FJ977654) <i>Nerita funiculata</i> (DQ093429)	7×10^{-104}	99.1	<i>Nerita funiculata</i> and <i>Nerita tessellata</i> have not been registered in the BCP
61	13	-	-	-			Neritidae	<i>Nerita funiculata</i> (DQ093429)	4×10^{-106}	100	<i>Nerita funiculata</i> and <i>Nerita tessellata</i> have not been registered in the BCP	
62	2	-	-	-	Neritimorpha	Cycloneritida	Neritoidea	Neritidae	<i>Nerita tessellata</i> (FJ977654)	4×10^{-106}	100	<i>Nerita funiculata</i> and <i>Nerita tessellata</i> have not been registered in the BCP
63	1	-	-	-			Neritidae	<i>Nerita funiculata</i> (DQ093429)	5×10^{-105}	99.5	<i>Nerita funiculata</i> and <i>Nerita tessellata</i> have not been registered in the BCP	
64	-	-	2	-	Patellogastropoda	Cycloneritida		Phenacolepadidae	<i>Phenacolepas osculans</i> (AY923890)	1×10^{-105}	100	<i>Phenacolepas osculans</i> has been registered in the Gulf of California
65	-	-	-	1			Lottioidea	Lottiidae	<i>Lottia gigantea</i> (KP274858) <i>Lottia scabra</i> (GQ160769) <i>Lottia jamaicensis</i> (FJ977633)	6×10^{-151}	100	Several species of the genus <i>Lottia</i> have been registered in the BCP
66	-	-	-	1			Fissurelloidea	Fissurellidae	<i>Fissurella</i> sp. (MK331687) <i>Fissurella virescens</i> (MK322194) <i>Fissurella volcano</i> (HM775293)	2×10^{-129}	100	<i>Fissurella volcano</i> and other <i>Fissurella</i> species have been registered in the BCP
67	-	1	-	-	Vetigastropoda	Lepetellida	Haliotoidea	Haliotidae	<i>Haliotis corrugata</i> (HM775288) <i>Haliotis fulgens</i> (HM775289)	3×10^{-102}	100	<i>Haliotis corrugata</i> and <i>Haliotis fulgens</i> have been registered in the BCP
68	4	-	-	-			Trochoidea	Turbinidae	<i>Megastrea undosa</i> (KY766259) <i>Turbo</i> sp. (KY766259) <i>Lunella</i> sp. (EU530105)	1×10^{-95}	100	Several genera of family Turbinidae are found in the BCP
69	-	1	-	-	Vetigastropoda	Trochida	Trochoidea	Phasianellidae	<i>Tricolia pullus</i> (AM048661)	6×10^{-85}	94.1	There are no species of the genus <i>Tricolia</i> registered in the BCP
71	-	15	6	-			Gastropoda unclassified (MW203299)			3×10^{-101}	99.1	
Σ	89	78	70	65								

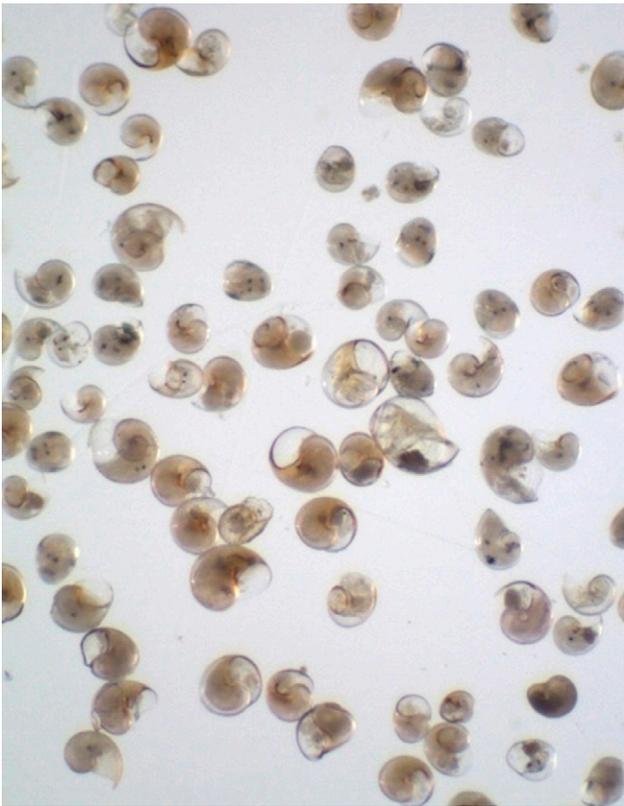


Figure 2. Gastropod mollusks obtained from a plankton tow from La Bocana. Scale = approx. 200 μ m.

Discussion

A relatively high planktonic gastropod diversity was observed in the present study as compared to that found in 2 sites of the Caribbean Sea: 31 species (Oliva-Rivera & Navarrete, 2007) and 34 species (Chávez-Villegas et al., 2014), but lower than the 62 holoplanktonic species from the Gulf of California (Angulo-Campillo et al., 2011), the 6 species of the order Thecosomata (Sánchez-Hidalgo y Anda, 1989), and the 18 species of the family Atlantidae off the BCP (Aceves-Medina et al., 2020; Moreno-Alcántara et al., 2020). Nevertheless, according to a preliminary rarefaction analysis (results not shown due to reduced sample size) the actual species diversity in our study region is probably under-represented. To overcome the sample size issue, which is mainly due to budget constraints, new research technologies such as environmental DNA (eDNA) metabarcoding are broadening the sampling possibilities (Mychek-Londer et al., 2020).

Differences in community composition between the sampling sites of CT and LB (Fig. 1) would be expected as environmental conditions are usually distinct. Personal observations indicated that both sites are mainly composed of rocky bottoms and a few sandy flats, with a higher abundance of macroalgae at LB than at CT, and clearer waters at CT than LB. These differences, which are probably due to a higher productivity at LB, are explained by the position of the sites relative to the Magdalena

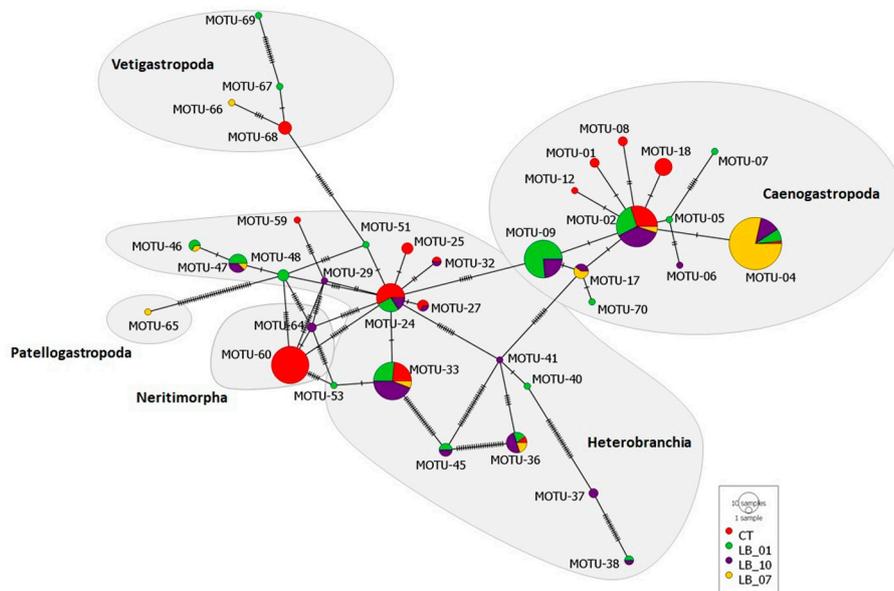


Figure 3. Minimum spanning network from 18S rDNA sequences per MOTUs. Background shades represent gastropod subclasses.

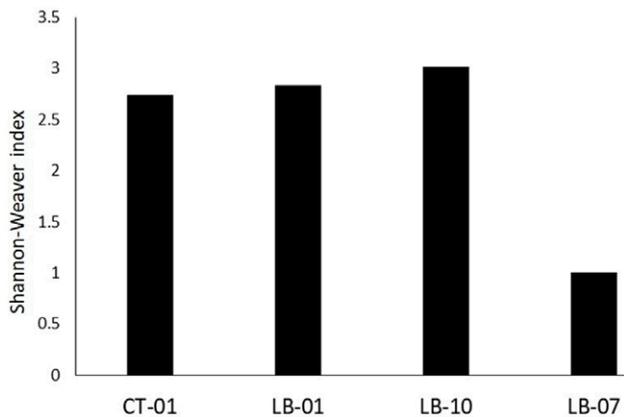


Figure 4. MOTUs' Shannon-Weaver diversity index, using the natural logarithm, per towing station.

Biological Activity Center (MBAC) (Lluch-Belda, 2000), with year-round cooler waters at LB (north of MBAC) than at CT (south of MBAC) (Jerónimo & Gómez-Valdés, 2006). In contrast, the differences in MOTUs composition among stations within LB let us hypothesize that planktonic gastropod distribution within a specific area is probably modulated by micro-environmental processes such as currents and biological events (Hernández-Trujillo et al., 2001). For example, many Cypraeidae individuals at LB-07 points to a massive spawning event in that area.

Even though 18S rDNA has been proposed for biodiversity studies (Ranjithkumar et al., 2018), it generally fails to separate species (Aranceta-Garza et al., 2011; Wu et al., 2015). In addition, the lack of a comprehensive database of gastropod 18S rDNA sequences from the western coast of the Baja California Peninsula limits the capacity of proper species identification. For example, the report of Angulo-Campillo et al. (2011) indicates the presence of several Atlantidae or Desmopteridae species, which in our case could not be distinguished (Table 2). Thus, future studies will require additional genetic markers, such as the 16S rRNA or COI genes (e.g., Crocetta et al., 2020).

The putative presence of molluscan MOTUs not recorded in the study region can be explained by either the low number of 18S rDNA sequences in the public databases, the lack of differences among species in this gene sequence of the same genus or family, and the lack of physical records of those specimens in the study region. In any case, efforts should be devoted to increase the number of gastropod 18S rDNA sequences and other genes in the databases and to promote frequent survey campaigns to detect previously undescribed species in this region. This is particularly relevant because mollusks are

gaining attention within climate change research, not only to study phenotypic plasticity and genetic variation (Matoo & Neiman, 2021), but also because of their utility to act as indicators of climatic variability (Molina-González et al., 2018). Also, eDNA metabarcoding, which can be used to detect diverse ecological phenomena such as potential species invasions (e.g., Mychek-Londer et al., 2020), requires solid supporting information.

The presence of non-gastropod sequences within the analyzed individuals is a phenomenon that requires further attention. Even though we cannot rule out the possibility of cross-contamination, these individuals were visualized as planktonic gastropods (Supplementary material: Fig. S1), so it is unlikely that individuals of Cnidaria (order Bivalvulida), and Arthropoda (orders Euphausiacea, and Calanoida) were mistakenly put into the PCR tubes. We also consider that their presence as stomach content or as epibionts was unlikely because when mixed DNA is individually sequenced, it is not possible to separate the signature of each species. Rather, we hypothesize that these sequences might come from eggs or larvae of those other taxa that use gastropod shells as a substratum.

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