SYSTEMATICS AND MOLECULAR PHYLOGENY OF MARINE PRAWNS AND SHRIMPS OF GUJARAT

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In

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List of abbreviations used throughout the text

M	:	Meter
Km	:	Kilometer
Mm	:	Millimeter
v/v	:	Volume/Volume
e.g.,	:	For example
i.e.	:	That is
viz.	:	that is to say; namely
GoK	:	Gulf of Kachchh
Gokh	:	Gulf of Khambhat
WoRMS	:	Word Register of Marine Sciences
Coll.	:	Collector
Ovi. female	:	Ovigerous female
P	:	pereiopod (P1 to P5: First to fifth pereiopod)
Plp	:	Pleopod (P1 to P5: First to fifth pleopods)
Mxp1& Mxp2	:	First and Second maxilliped
Mxp3	:	Third maxilliped
CMFRI	:	Central Marine Fisheries Research Institute
DNA	:	Deoxyribonucleic acid
RNA	:	Ribonucleic acid

Mt DNA	:	Mitochondrial DNA
COI gene	:	Mitochondrial cytochrome oxidase subunit 1
16S rRNA	:	16S ribosomal RNA
NCBI	:	National Center for Biotechnology Information
BoLD	:	Barcode of Life Datasystem

SUMMURY

India is one of the 12 megadiverse countries of the world and supports 4 terrestrial biodiversity hotspots, highly biodiversity-rich, but endangered eco-regions (Myers et al., 2000). The coastline of India is about 7516.6 km long, with the mainland contributing 5422.6 km and the offshore islands contributing 2094 km (Andaman and Nicobar Islands: 1962 km; Lakshadweep Islands: 132 km) (Ahmad 1972; Kumar et al., 2006). The coastal area is divided into the west coast and the east coast. Both the coasts are significantly different in their geo-morphology. The Western coast of India is dominated by rocky shore habitat, while the east coast of India mostly has sandy beaches, mudflats, lagoons, and marshes. Crustaceans exist to the fourth-largest diversity, and they are the second most abundant diverse animal group on the planet. They abundantly inhabit the coastal marine environment. Gujarat is the western proximity of India and harbors the longest coastline of approximately 1650 km. The state's coastline is divided primarily into three coastal areas, *i.e.,* the Gulf of Kachchh, Saurashtra coast, and the Gulf of Khambhat. The coastline of Gujarat encompasses almost all types of intertidal habitat, from hyper-saline estuaries, salt marsh, mudflats to sandy and rocky shores with every degree of exposure, and widely different profile. The subtidal habitats are equally diverse and rich.

Prawns and shrimps belong to Order Decapoda, infraorders Dendrobranchiata and Pleocyemata, respectively, and they are one of the most diverse and important groups of crustaceans. Prawns are the most significant food source with great economic importance as both capture and culture fisheries. Shrimps also have been attractive due to their great diversity throughout their evolutionary history and ornamental values. Some species may not have commercial value but are important to form an integral part of the food web of the tropical marine system. The commercial marine species are generally found in shallow or moderately deep-water regions along the continental shelves at less than 100m depth, and some

are found even at nearly 5700 m depth. Many shrimp species are pelagic, but most of the species are benthic, living on a variety of hard and soft substrates like rock, mud, sand, shell particles, or a mixture of these fragments, and some species are symbiotically associated with others marine organisms.

Prawns and shrimps are a highly important group of marine decapods, and so far, 4048 species belonging to 471 genera are reported globally (Grave and Fransen, 2011). In India, so far, 364 species belonging to 128 genera are reported (Samuel et al., 2016). The maximum number of species are reported from the East coast of India compared to the West coast. As compared to the other coastal states of India's western coast, the prawn and shrimp fauna of Gujarat is less studied in terms of the intertidal species. In 2015, Trivedi et al. reviewed the literature available on the crustacean fauna of Gujarat and compiled a checklist. They have reported 30 species of prawns and shrimps belonging to 12 genera. It is noteworthy that all the earlier studies on the prawn fauna of Gujarat were focused on the commercial species (e.g., population study, stock assessment) as compared to the intertidal species. The landing centers of the Gulf of Kachchh and Saurashtra region are maximally explored compared to the other area. Only a few studies are carried out on the systematics of this group.

The present study was initiated with the following objectives.

1. Study the Diversity, morphological taxonomy and distribution of marine prawns and shrimps.
   I. Systematics of marine prawns and shrimps: A taxonomical approach
   II. Distribution pattern and habitat preferences of prawns and shrimps in different coastal regions of Gujarat

2. Establish the phylogenetic relationship among prawn and shrimp species.
   I. Morphological phylogeny of prawns and shrimps: A cladistic analysis.

II. A comprehensive phylogenetic analysis of prawn and shrimp based on mitochondrial COI sequence data.

Research Methodology:

Study Area-Gujarat

The current research work has been conducted in the coastal region of Gujarat province. It is situated on India's western coastline bounded by the vast Arabian Sea on its three sides. Gujarat state is located between 20°06' N to 24°42' N latitude and 68°10' E to 74°28' E longitude, shares a North-Western border with Pakistan and Rajasthan, a North-Eastern border with Madhya Pradesh, and a South-Eastern border with Maharashtra. It has a 1,96,024 Km^2 total surface area (including Daman and Diu UT) and the longest coastline (about 1,650 km) of India. That accounts for 22% of the total coastline available to the country, with 164,200 km^2 of the continental shelf (35.3% of the country) and 214,000 km^2 of Exclusive Economic Zone (EEZ) (9.9% of the country).

Gujarat's total coastline consists of different marine habitats, including 29% of muddy flats followed by 28% sandy beach, 22% marshy coast, and 21% rocky coast. Gujarat's coastal area extends from the Western Ghats in Valsad (Umargam) to Kori creek (Kachchh) on the North-Western coast. The Gujarat state's coastal area is very different in terms of geo-morphology from the rest of the West coast of India. The coastal zone of Gujarat state is divided into three major geographical parts, two major gulfs; namely Gulf of Kachchh and Gulf of Khambhat, and the Saurashtra coastline (fig. 1), each of which has its distinctive character, climate variation, and diverse geo-environmental features, which embrace diverse coastal habitats as well as ecological significant biota. The three regions vary a lot regarding tidal variation and marine habitat diversity.

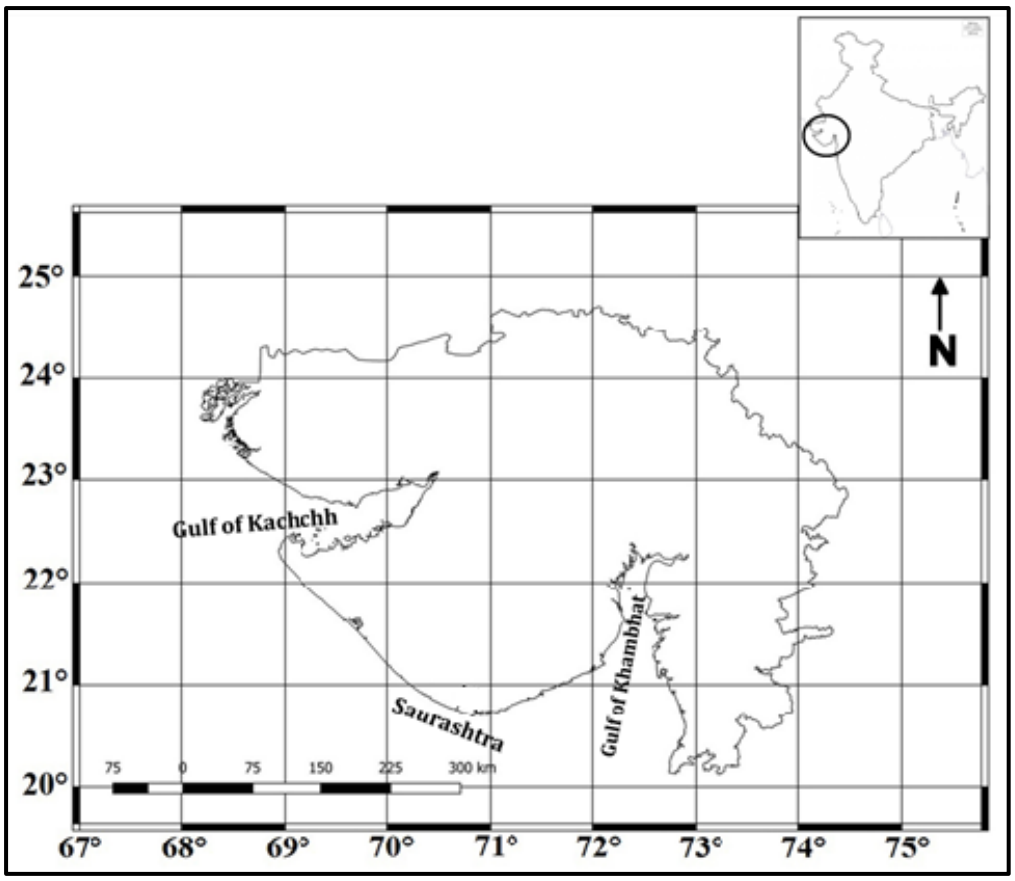


Figure 1 Map showing the division of study area into three major regions.

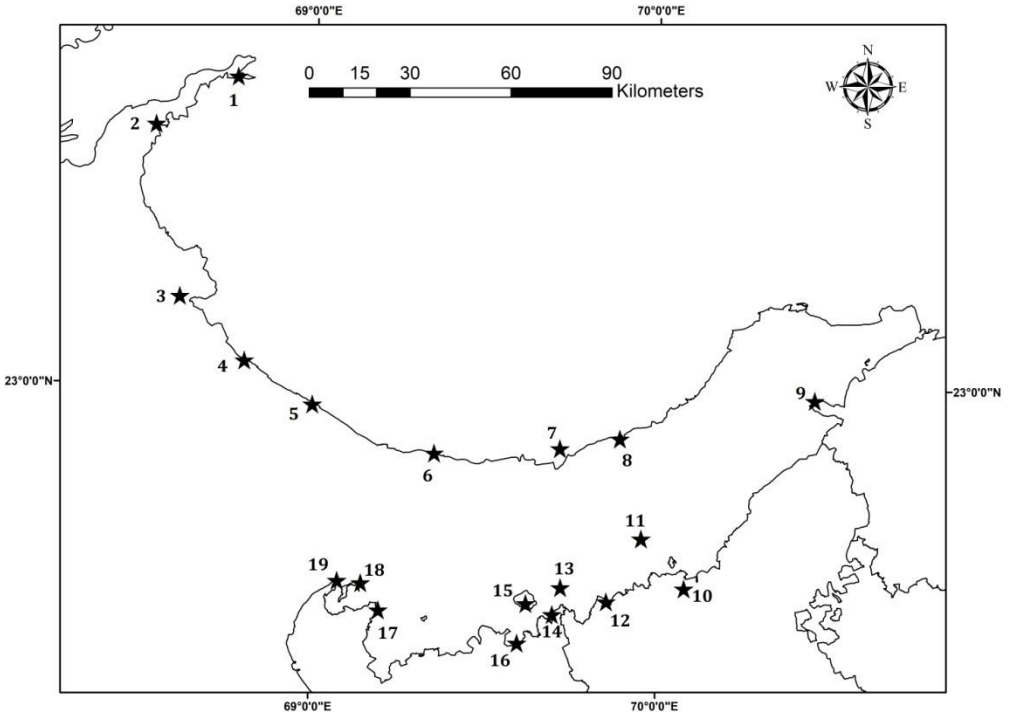
Selection of study sites

On the coast of Gujarat, a comprehensive field survey was conducted. The coastal region is divided into three major regions, and each coastal region is split further into the coastal districts. A total of 15 coastal districts were surveyed, and based on the survey, 70 sites were selected from three coastal regions of Gujarat for the samplings. However, due to repeated seasonal accessibility, permissions from Government authorities in Marine National Park and Defense Services Authorities in Kachchh district, 59 sites were finally selected for regular studies. Therefore, some of the critical island sites of Marine National Park and North Western creek habitats of Kachchh could not be surveyed. During the present study, the selections of the study sites from all the three major coastal regions have been based on the three parameters: one was habitat type (e.g., sandy shore, rocky shore, muddy shore, mangrove mudflats, and coral reef); the second was the

accessibility of the area, and the third one was the sample accessibility or availability.

Gulf of Kachchh

The Gulf of Kachchh is the region of peninsular Saurashtra-Kachchh in the western state of Gujarat and located between 22°15' N and 23°40' N Latitudes and 68°20' E and 70°40' E Longitudes. It is approximately 75 km wide, and 125 km long, narrow mouth funnel-shaped, East-West oriented indentation of Gujarat coast. On the northern sides, the Gulf starts with Kachchh point and lies between the beaches of Devbhumi Dwarka and Jamnagar districts in Saurashtra regions. The tides in the Gulf of Kachchh are mixed, predominantly semi-diurnal types with massive diurnal inequality. On the northern sides of the Gulf, the tides vary from 3 to 8 m, and on the southern sides, it's 3-5 m. The shape and orientation of the Gulf is the main reason for tide amplification. The high tidal range and tidal currents are important geological agents that play a significant role in sedimentation and shaping the land of the Gulf. The gulf comprises 4 coastal districts, namely, Kachchh, Rajkot, Jamnagar, and Devbhumi Dwarka. The narrow mouth funnel-shaped Gulf is covering an area of 7350 km^2, has channel depths varying from 20 m at the head to 60 m at the outer Gulf. The average depth is approximately 30 m, and the minimum is up to 5 m (Gupta and Deshmukh, 2000). It is a semi-enclosed coastal area with diverse coral reefs and rocky ecosystem. The geophysical parameters suggest that the Gulf of Kachchh is quite different in many aspects than the Gulf of Khambhat. There are many major and minor islands in the Gulf of Kachchh; they support rich habitat diversity and biodiversity. According to official records, 42 islands in the GoK cover a total area of about 410.6 km^2.



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A total of 19 sites from 4 coastal districts (Kachchh, Rajkot, Jamnagar, and Devbhumi Dwarka) situated along the Gulf of Kachchh were surveyed from the collection of samples (fig. 1.1). Vast patches of mangroves also exist along the northern shore of the Gulf. In the Gulf, the intertidal zone is rocky-sandy supra-tidal zone, muddy-rocky middle intertidal zone with boulders, and lower zone with diverse coral reef zone. The Kori creek to Jakhau and Mundra to Kandla Port are irregular and exclusively muddy with mangroves patches. Navlakhi to Jodiya has mudflats, while from Jamnagar to Okha (trending E-W), the coast has rocky shoreline (like rock mounds), the subtidal zone with water channels, submerged islands, sand bars, coral reefs, and mangroves. The GoK, Mithapur, Sivrajpur, and Dwarka are the only Gujarat areas where coral reefs exist. The GoK provides various kinds of habitats like coral reefs, mangroves, creeks, open mudflats (hard mud,

soft mud and soup mud), islands, rocky shore, sandy shore, etc., which provide a suitable environment for a large range of fauna and flora.

Saurashtra Coast

The Saurashtra Coast is situated in the South-Western part of Gujarat and located between 20°72' N and 22°42' N Latitudes and 68°99' E and 70°98' E Longitudes. It is approximately 865 km^2 long, East-West oriented indentation of the Gujarat coast. It lies between the Gulf of Kutch on the north-west side and the GoKh on the south-east side and surrounded by the Arabian Sea on the south and south-west. The Saurashtra coastline is very distinctive from the Gulf of Kachchh and the Gulf of Khambhat. The high tide surges maximum up to 5 m at the Saurashtra coast. The Saurashtra peninsular comprises 6 coastal districts, namely, Devbhumi Dwarka, Porbandar, Junagadh, Gir-Somnath, and Amreli. The minimum depth is about 3 m, and the average depth is up to 5 m. The topography of the coast changes all along the Saurashtra coast. The majority of the coastline of the Saurashtra coast is rocky and sandy with randomly distributed patches of muddy regions with heavy sediment load.

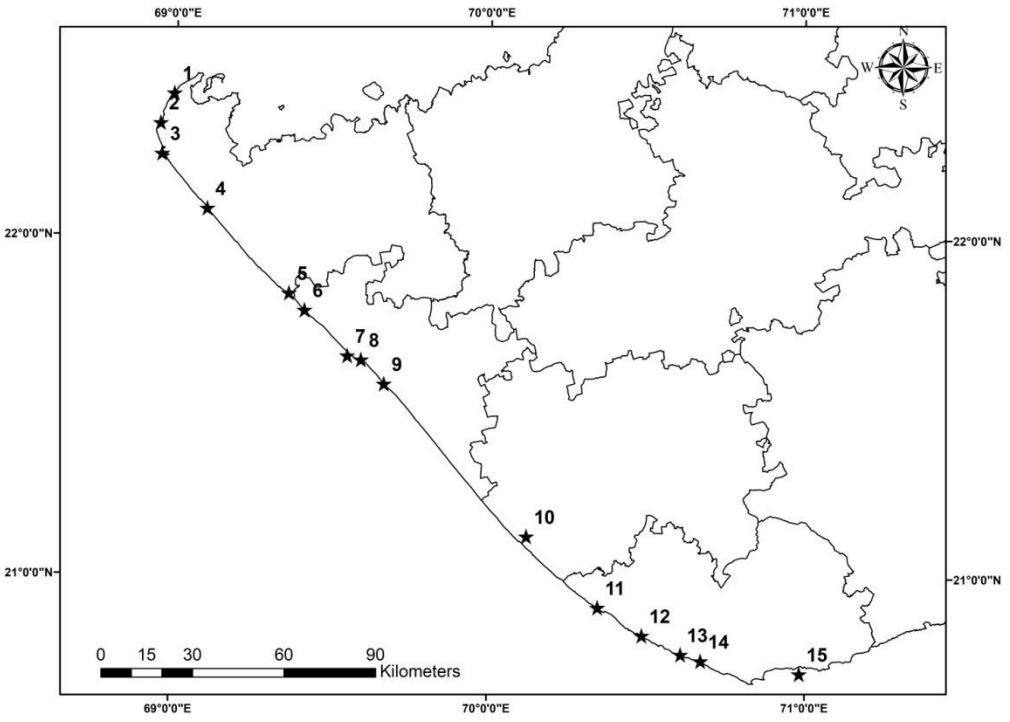

Figure 1.2 Map showing the sampling sites along the coastline of the

Saurashtra coast; 1-Okha;2-Mithapur; 3-Shivrajpur4-Okhamadh; 5-Harshad; 6-Miyani; 7-Kuchhadi Reef; 8-Porbandar; 9-Odadar; 10- Mangrol; 11-Veraval; 12-Sutrapada; 13-Dhamlej; 14-Kodinar; 15-Diu.

A total of 15 sites from 4 coastal districts (Devbhumi Dwarka, Porbandar, Junagadh, and Gir-Somnath) were surveyed for the collection of samples (fig.1.2). The intertidal zone of the Saurashtra coastline is unique in that the upper intertidal zone is rocky, somewhere sandy with mud, the middle intertidal zone is mostly rocky, with crevices, and the lower intertidal zone is mainly rocky with sparse sand. In the mid intertidal zone, tide pools are commonly found. The south coast of Saurashtra from Dwarka- Kodinar is about 250 km patch, with straight and smooth sandy or rocky-sandy beaches. The Saurashtra coastline is rocky-sandy, the east and west (E-W) with Continuous beach with projecting rock mounds, wave-cut platforms, occasional cliffs, the central part is sandy, and more rocky-muddy beaches are found in the eastern part. The formation of milliolite limestone along the coast is remarkable. Due to its unique geomorphologic properties and hydrodynamic processes, it provides a suitable environment for a wide range of marine fauna and flora. The intertidal zone of Saurashtra coasts is not very wide; generally, dynamic wave action is seen due to this reason.

Gulf of Khambhat

The Gulf of Khambhat is situated between the Saurashtra peninsula and the mainland of Gujarat state and located between 20°30' N and 22°20' N Latitudes and 71°45' E and 72°53' E Longitudes. It is approximately 131 km long and 70 km wide, wide inverted mouth, narrow end funnel-shaped North-South oriented indentation of Gujarat coast. On the western side, the Gulf begins from Amreli Point and lies between Surat and Valsad districts on the eastern side. The Gulf is best known for its extreme tides (maximum 12 m), which vary significantly in height and run into excellent speed 3 m/sec. On the western sides of the Gulf, the tides vary from 3 to 12 m, and on the eastern sides, it is 6-10 m, and the shape of the Gulf is the main reason for tide amplification. The Gulf of Khambhat comprises 8 coastal

districts Amreli, Bhavnagar, Ahmadabad, Anand, Bharuch, Surat, Navsari, and Valsad. The funnel-shaped Gulf, which covers an area of 3,120 km^2 comprised mainly of mudflats with some rocky area, is shallow with depths varying from 5 m at the head to 40 m in the channels. The average depth is about 20 m, and the minimum is up to 5m (Gupta and Deshmukh, 2000). There are a few sandy patches that are also observed intermittently.

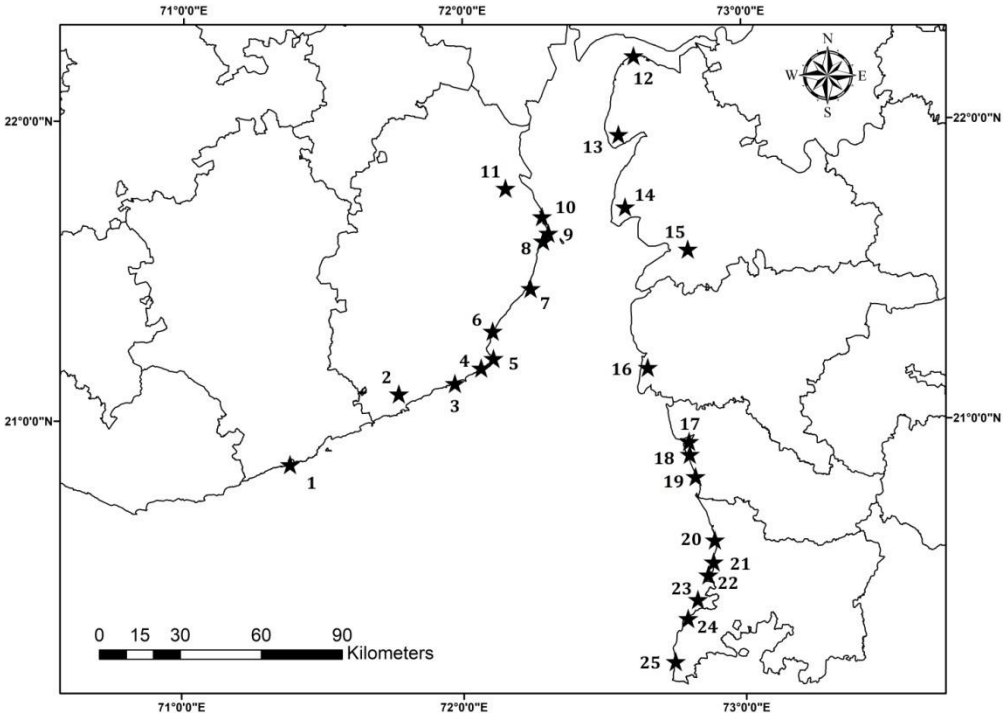

Figure 1.3 Map showing the sampling sites along the coastline of the Gulf of Khambhat; 1-Jafrabad; 2-Mahuva; 3-Unchakotda; 4-Jhanjhmer; 5-Sartanpar; 6-7-Gopnath; 8-Alang; 9-Koliyak; 10-Kuda; 11-Gogha; 12-Bhavnagar; 13-Kamboi; 14-Nada; 15-Dahej; 16-Hansot; 17-Hazira; 18-Vansi-Borsi; 19-Dandi; 20-Onjal; 21-Thithal; 22-Umersadi; 23-Udwada;24-Daman; 25-Fansa; 26- Umargam.

A total of 26 sites from 6 coastal districts (Amreli, Bhavnagar, Bharuch, Surat, Navsari, and Valsad) situated along the Gulf of Khambhat have surveyed for the collection of samples (fig. 1.3). The rocky beaches (some rocky patches) are commonly found from Mahuva to Gopnath, reducing towards Ghogha and Bhavnagar. The Gulf receives freshwater by several inlets like major rivers, especially in monsoons such as Sabarmati, Mahi,

Narmada, Tapi, Shetrunji, and many minor rivers. All the rivers from estuaries and their inflow carry solid suspended sediments into the Gulf. A medium-sized delta is present near Sartanpur, known as the Shetrunji River delta between Gopnath and Ghogha. The excessive turbulence churns the seabed and produces enormous quantities of silt and clay, making the seawater turbid and brownish, so that the light rays are impermeable. The Gulf's marine ecosystems, comprising mangroves, estuaries, creeks, and vast intertidal mudflats, are known to have rich biodiversity and several endemic flora and fauna.

Sample collection

The sampling was done from 59 sites along the Gujarat coastal area from 2015 to 2019 (figs. 1.1, 1.2, & 1.3). Various methods were employed for the collection of prawn and shrimps. Hand-picking and the handheld net method were adopted to collect intertidal shrimp species during the low tides. The fish landing center and local fish markets were also visited for the collections, where the trawler catch was examined for commercial species of prawn. The fish landing centers location, type of fishing trawlers, approximate depth of sample collected, gears used, etc. all this information was collected from the fishermen. The fresh specimen's photographs were taken immediately after the collection for bright and fresh coloration, and then the samples were preserved and brought to the laboratory.

Relaxation of specimens

All the live samples were narcotized first to avoid dissection and diagnosis difficulties during the morphological identification. Narcotization was obtained with menthol crystal and by chilling and freezing the live specimens. For a few minutes, the specimen was submerged in the solution. After this, the samples were ready for preservation or morphological analysis.

Preservation for Morphological Analysis

Samples for the morphological examination were immersed in 4% formalin for 4-6 hours and later transferred in 10% formalin (v/v). Formalin denatures DNA by rendering it unavailable by binding the surrounding histone proteins so that the isolation of DNA for genetic research is more complicated and, in some cases, impossible.

Morphological Identification and Deposition

In order to identify each specimen up to the species level using morphological characters (Annexure 1), the following steps were considered. The coloration was observed immediately after the collection of samples. Detailed morphometry and sex determination were carried out for each specimen. The taxonomically important body parts were dissected and examined under a stereomicroscope. All the specimens were identified up to the species level using various identification keys, monograms, and taxonomic literature. The detailed sketches of body parts were prepared for different species, and photographic identification plates were prepared. The total length (TL- tip of the rostrum to tip of telson) and carapace length (CL-tip of the rostrum to the posterior end of the carapace) were measured for all the specimens of every species using the digital vernier caliper (± 0.1 mm accuracy). Smaller samples, mostly caridean, were measured using the stereomicroscope equipped with the measuring tool. For the validation of taxonomic classification, the species detailed were compared with the details available on Marine Species Identification Portal Website (www.speciesidentification.org), and the classification was adopted from the world Registered of Marine Species website (www.marinespecies.org). All the identified species were deposited into the Museum at the Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Gujarat, India, with a unique museum accession code (*e.g.*, ZL-AR-PR-1: *Metapenaeus affinis*) assigned for each species. The clear label was given to the specimen bottles with species accession number, scientific name, family name, collection site, and collector's name. All the families, genera as well as the species, and their synonyms are listed

following the criteria of WoRMS, and the taxa within the families are ordered alphabetically.

Since the coast of Gujarat is very long and has distinct and widely variable habitats, the species distribution was recorded in two different ways:

1. Geographical region-wise distribution of the species in the Gulf of Kachchh, Saurashtra, and Gulf of Khambhat.

2. Habitat wise distribution to understand the ecological role of the shrimp species.

1 Region-wise distribution

The coastline of Gujarat is divided into three major regions, namely the Gulf of Kachchh, the Saurashtra coast, and the Gulf of Kachchh. All three areas have distinctive characters and habitat variations. There is a total of 15 coastal districts of Gujarat, where the Kachchh has the longest coastline. Gulf of Kachchh is bound by 4 districts, Saurashtra coast by 6 districts, and the Gulf of Khambhat by 8 districts, including the lower south Gujarat extension. During the sample collection at each sampling site, the prawn and shrimp species present were listed with latitude and longitude information. All the data were maintained in the excel sheets for the analysis.

2 Habitat-wise distribution

During 2015 to 2019 field surveys, the coastal regions of Gujarat were conducted to study the habitat preference and distribution of the species. In the present study, the habitats are divided mainly into types of the shore (macro-habitat) as rocky, sandy, mudflats, and sub-littoral or pelagic regions of the coastal ecosystem (fig. 2). At each sampling site, the prawn and shrimp species present were listed, dominant species of animal and type of shore were notated. The zonation pattern is the most important phenomenon observed in the intertidal area. Here in the present study, different zones have been identified based on the presence of the dominant

animal community. The microhabitat preference by the intertidal species was also studied. On the sighting of animals, micro-habitat type and zone type were recorded.

Micro-habitat classification- Macro-habitats (types of shore) were further divided into six different micro-habitats (figs. 3 & 4).

EnZ (Endozoic): Always recorded in an internal association with a particular species of animal.

EZ (Epizoic)**:** Always recorded in an external association with a particular animal species (e.g., coral reef and sea anemone)***.***

H (Hard Substrate): Recorded associated with hard substrates (e.g., rock).

M (Mangrove): Recorded amongst mangroves.

OM (Open Mudflats): Recorded in the areas of open mudflat coast.

SS (Soft Substrate)**:** Recorded associated with soft substrates, sand, or mud tide pools (e.g., sand and mud).

Figure 2 Macro-habitats or intertidal habitats: a) Rocky shoreline at Harshad (Saurashtra); b). Muddy shoreline at Kambhoi (Gokh); Sandy shoreline at Mandvi (GoK).

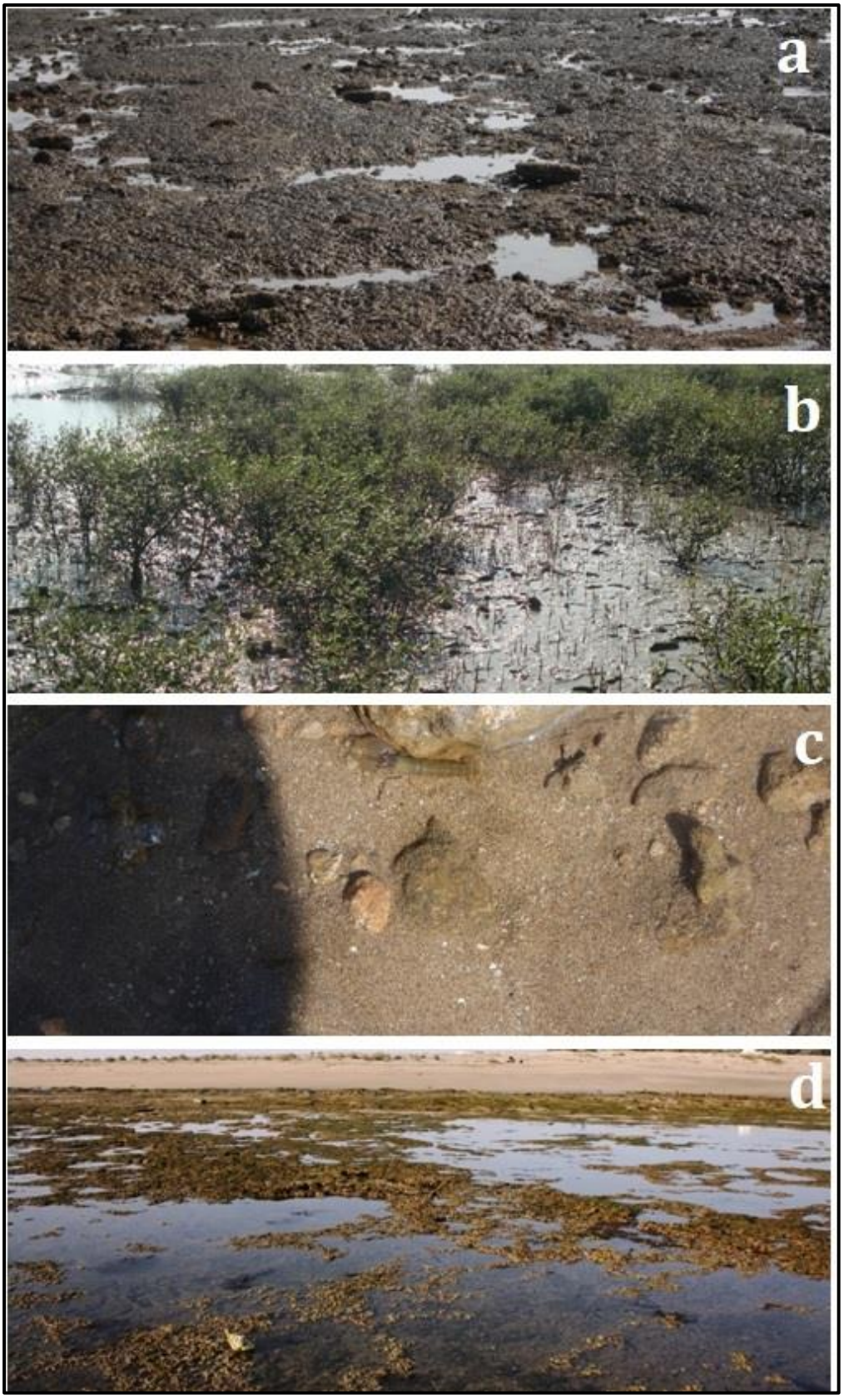

Figure 3 Type of micro-habitats or intertidal habitats: a) Open mudflat; b). Mangrove mudflat; c) Soft substrate; d) Hard substrate.

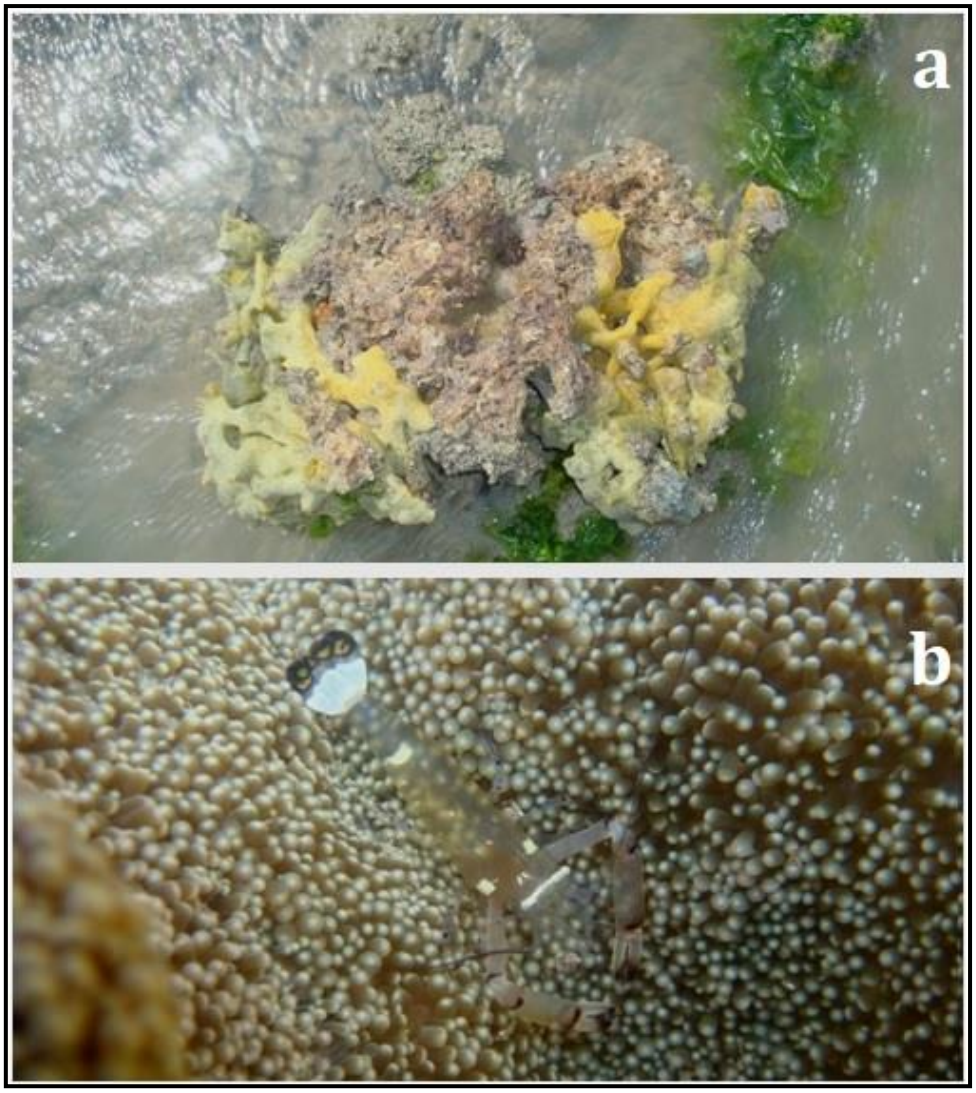

Figure 4 Types of micro-habitats or intertidal habitats: a) Endozoic; b) Epizoic.

Specimen Information

The morphometric analysis includes 152 specimens belonging to 39 valid species (Table 1). For the morphometric analysis, 4 families viz. are Callianassidae Alpheidae, Upogebiidae, and Spongicolidae were omitted because the morphological structure is different. Family Pandaloidea was also not considered because, in this species, the only ovigerous females were reported during the present study. After identifying species belonging to suborder dendrobranchiate and pleocyemata shrimp, from each species of Penaeidae, three male samples were taken, and the female omitted due to the size variation. In the case of caridean shrimps (in few

species), female specimens were also considered due to a smaller number of samples were reported.

Multivariate Analysis of Morphometric characters

The morphometric measurements were taken based on the truss network system shows in fig 5 (Aktas et al., 2006). In this system total of eighteen landmarks and forty variables were measured. The total length (TL) was also calculated from the tip of the rostrum to the posterior end of the telson, and forty-one variables (Table 1) were measured using a digital vernier caliper (accuracy ±0.1 mm). Small specimens were measured using a stereomicroscope equipped with a measuring tool. All the measurements were taken from thawed samples.

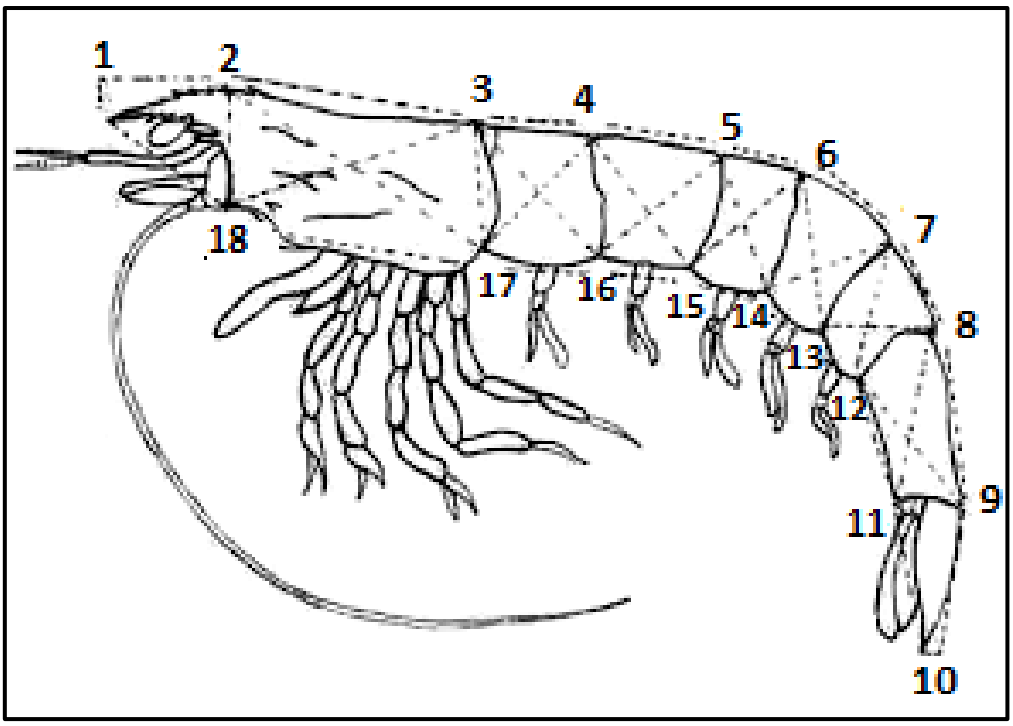

Figure 5 Truss network system used for morphometric analysis among prawn and shrimp species.

The average value was calculated. In this chapter, a significant correlation observed the average measurement of each variable and the total length. Therefore, it was necessary to remove the effect of size variation on species. To eliminate the variation resulting from each morphometric variable, they were standardized, according to Elliott et al. (1995).

$$Madj = M(Ls/Lo)^b$$

M is the original (primary) measurement; Madj is the size adjusted measurement of each variable. Ls is the overall mean of the standard length of all samples, and Lo is the standard length of the model or sample. Parameter b was estimated for each character from the observed data as the slope of the regression of log M on log Lo, using all prawn and shrimp group. The Ls/Lo values computed in the MS excel sheet and detecting the b parameter was carried out using the PAST (Paleontological Statistics Software) package version 3.25. The cluster analysis was performed UPGMA multivariate method to evaluate the morphometric relationship among all species in each order. In this chapter, hierarchical clustering analysis was represented as a dendrogram, where a joint of the tree illustrated each step in the clustering formation. The single linkage method was used with the Euclidean distance (statistic tool that quantifies the extent to which species within-cluster are similar) with 1000 bootstrapped.

Table 1 List of the morphometric variables used for the study of the taxonomical relationship of the prawn and shrimp species

S. No.	No Variables*	Variable Name
1	var1-2	Rostrum length
2	var2-3	Carapace dorsal
3	var3-4	First Abdominal segment dorsal
4	var4-5	Second Abdominal segment dorsal
5	var5-6	Third Abdominal segment dorsal
6	var6-7	Fourth Abdominal segment dorsal
7	var7-8	Fifth Abdominal segment dorsal
8	var8-9	Sixth Abdominal segment dorsal
9	var9-10	Telson dorsal
10	var10-11	Telson ventral
11	var11-12	Sixth Abdominal segment ventral

12	vae12-13	Fifth Abdominal segment ventral
13	var13-14	Fourth Abdominal segment ventral
14	var14-15	Third Abdominal segment ventral
15	var15-16	Second Abdominal segment ventral
16	var16-17	First Abdominal segment ventral
17	var17-18	Carapace ventral
18	var1-18	Diagonal of anterior end carapace to rostrum end
19	var2-18	Perpendicular Carapace 1
20	var2-17	Diagonal of Carapace 1
21	var3-18	Diagonal of Carapace 2
22	var3-17	Perpendicular Carapace 2
23	var3-16	Diagonal of first Abdominal segment 1
24	var4-17	Diagonal of first Abdominal segment 2
25	var4-16	Perpendicular of first Abdominal segment
26	var4-15	Diagonal of second Abdominal segment 1
27	var5-16	Diagonal of second Abdominal segment 2
28	var5-15	Perpendicular of second Abdominal segment
29	var5-14	Diagonal of third Abdominal segment 1
30	var6-15	Diagonal of third Abdominal segment 2
31	var6-14	Perpendicular of third Abdominal segment
32	var6-13	Diagonal of fourth Abdominal segment 1
33	var7-14	Diagonal of fourth Abdominal segment 2
34	var7-13	Perpendicular of the fourth segment
35	var7-12	Diagonal of fifth Abdominal segment 1
36	var8-13	Diagonal of fifth Abdominal segment 2
37	var8-12	Perpendicular fifth Abdominal segment
38	var8-11	Diagonal of sixth Abdominal segment 1
39	var9-12	Diagonal of sixth Abdominal segment 2
40	var9-11	Perpendicular of fifth Abdominal segment

41	TL	Tip of the rostrum to the posterior margin of telson

Sampling of Molecular data

A total of 112 specimens of marine prawn and shrimps were collected for DNA examination. The samples represented 52 species of shrimps and prawns from different habitat and localities along the coastal area of Gujarat, including Union territory Daman and Diu.

DNA extraction

Genomic DNA was extracted from abdominal tissue or pleopods of prawns and shrimps. The initial weight was approximately 20 mg, and extraction was carried out using DNeasy Blood and Tissue kit (Qiagen) (Annexure 2).

Quantification of genomic DNA

After extraction of genomic DNA, quantification was carried out using QIAxpert (QIAGEN). Purity and concentration of DNA were measured using gel electrophoresis (Annexure 3).

PCR amplification

The region of the mtCOI gene was amplified using primers given in (Table 2). The final volume of 20 µl containing 10 µl Taq PCR master mix (HiMedia), 10 pmol forward primer, 10 pmol reverse primer, 2 µl template DNA and nuclease-free water (to make up the final volume). The gene amplification was carried out in a thermal cycler (Applied Biosystems Veriti®) using the PCR condition given in table 3 and 4.

Table 2 Oligonucleotide primers used in the present study to amplify the COI gene.

Primer	Primer sequence 5'-3'	Reference
LCO 1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al., 1994
HCO 2198	TAAACTTCAGGGTGACCAAAAAATCA	

C1-J-1718	GGAGGATTTGGAAATTGATTAG	Simon et al., 1994
C1-N-2191	CCCGGTAAAATTAAAATATAAACTTC	

Table 3 Thermal cycling conditions used in the present study to amplify the COI gene using LCO 1490 and HCO2198.

Stage 1	**Stage 2 (35 Cycles)**			**Stage 3 (5 Cycles)**			**Stage 4**	
94°C 1 min.	94°C 1min.	45°C 1 min. 30 sec.	68°C 1 min. 30 sec.	94°C 1 min.	50°C 1 min. 30 sec.	68°C 1 min. 30 sec.	68°C 10 min.	4°C ∞

Table 4 Thermal cycling conditions used in the present study to amplify the COI gene using C1-J-1718 and C1-N-2191.

Stage 1	**Stage 2 (35 Cycles)**			**Stage 3 (1cycle)**	**Stage 4**
95°C 3 min.	95°C 30 sec.	50°C 30 sec.	72°C 1 min. 30 sec.	72 °C 7 min	4°C ∞

Qualification and Quantification of PCR

After PCR amplification, the amplified regions were observed using gel electrophoresis (Annexure 3).

PCR Purification

Positive PCR amplified were purified, suing EXOSAP IT (affymetrix) according to the manufacturer's instructions. In a new PCR tube, 10 µl of positive PCR product and 4 µl of EXOSAP were added. A thermal cycler was run using PCR condition given in table 5.

Table 5 PCR purification condition used in the present study.

Stage I	**Stage II**	**Stage III**
37˚C for 15 minutes	80˚C for 15 minutes	4˚C

Sequencing

The PCR amplified product was bi-directionally sequenced using the same set of the universal primers (LCO 1490 HCO 2198 and COI 1718F COI 2191R) on the ABI 3730x196 capillary DNA analyzer using Big Dye Terminator v 3.1 sequencing kit at Eurofins, Bangalore.

Sequence and Phylogenetic analysis

The qualities of the bi-directional chromatogram of the generated sequences were checked, and noisy parts were trimmed at both ends to avoid the noisy part using sequences nucleotide sequence DNA baser assembler 5.15 version. Further, each sequence was a check for sequence similarity through BLASTn analysis with Gene bank sequences (NCBI database). All the sequences were submitted on the NCBI portal with a unique accession code. The multiple sequences alignment was done for all the species of order Dendrobranchiata and Pleocyemata by multiple sequences alignment was opting using the MEGA X version. All missing data and gaps were eliminated from the data set. The phylogenetic was performed using the ML (Maximum Likelihood) method in the Kimura 2-parameter model with 1000 bootstrap replicates using the MEGA X version (Kumar et al., 2018).

Key findings

- The present study of marine prawns and shrimps' taxonomy and systematics revealed 52 species belonging to 27 genera and subgenera, 11 families, and 4 superfamilies.
- Among all the families recorded, the family Penaeidae and Alpheidae were dominant in the study area.
- Three species of shrimps of suborder Pleocyemata like *Gilvossius rotundicaudatus, Alpheus chiragricus,* and *Athanas parvus* were recorded for the first time from India.
- *Microprosthema validum* know as a rock lobster, was the first time reported from India's West Coast.

- Three species of caridean shrimp *viz. Synalpheus coutierei*, *Thor amboinensis,* and *Procletes levicarina* were reported first time from the western coast of India
- In the present study, species like *Megokris granulosus, Megokris sedili, Parapenaeus fissuroides indicus, Solenocera choprai, Latreutes anoplonyx, Alpheus lobidens, Alpheus malabaricus, Alpheus edwadrsii, Alpheus pacificus, Lysmata vittata , Palaemon pacificus, Palaemon serrifer,* and *Cuapetes grandis* were first time reported from Gujarat.
- So, the present study adds three more species to the list of India's prawns and shrimps, 4 more species to the checklist of India's western coast, and 22 more species to the checklist of prawns and shrimp of Gujarat.
- The study is divided into three major regions based on the distinctive character and habitat variation.
- The maximum diversity was observed from the Saurashtra coast, followed by the Gulf of Kachchh and the Gulf of Khambhat. The Gulf of Kachchh supports marine organisms' growth, and it is considered one of the biological most productive marine habitats.
- The habitat preferences and association of intertidal shrimp species were recorded and studied.
- *Alpheus lobidens* is the single species, which was reported from all the microhabitats except mangroves.
- *A. malabaricus* is a single species that was exclusively reported only from the mangrove habitat.
- The variation in the distribution pattern and abundance of organisms in different microhabitats of the intertidal area has provided the basis for so many ecological aspects, especially for the rocky intertidal organisms.
- During the present study, detailed cladistic analyses of prawn and shrimp species were studies based on the 41 morphometric variables. For this study, a total of 39 species (28 Dendrobranchiata and 11

Pleocyemata) of prawns and shrimps were used, and its morphometric measurements.

- Dendrobranchiata species had low bootstrap support values for half of the branches and lineages (BS <50%).
- Pleocyemata species had a high bootstrap support value for most branches and lineages (BS>50%).
- In the morphometric analysis species like, *P. merguensis*, *P. japonicus*, and *P. penicillatus* are arranged in the same clade. It also supported the molecular phylogeny data.
- DNA was extracted from 47 different prawn and shrimps collected from other locations and habitats along the Gujarat coast. It is also the first molecular phylogenetic study of prawns and shrimps in Gujarat.
- Most of the generated or developed sequences show the 90-100% similarity with the conspecies database sequences in GeneBank.
- In the present study, a total of 42 sequences (27 sequences of Dendrobranchiata and 15 sequences of Pleocyemata) were developed, and 30 sequences were successfully uploaded on NCBI.
- During this study, 4 sequences were first time submitted on the NCBI database.
- Our molecular phylogeny proposes a close relationship (sister position) between *A. lobidens* and *A. pacificus*

Conclusion

The morphological examination of all the specimens collected from the Gujarat coast of India resulted in identifying 52 different species of prawn and shrimps belonging to 27 genera, 11 families, and 4 superfamilies. Under Suborder Dendrobranchiata and Pleocyemata, 2 families, 10 genera and 9 families, and 17 genera were identified. Family Penaeidae comprised the maximum number of species diversity (25 species, 9 genera), followed by the family Alpheidae (8 species, 3 genera), Plaemonidae (6 species, 4 genera), Hippolytidae (3 species, 3 genera), Solenoceridae (3 species, 1 genus), Callianassidae (2 species, 2 genera), whereas families Lysmatidae,

Thoridae, Pandaloidea, Upogebiidae and Spongicolidae each comprised with a single species. Among the infraorder Caridea, the superfamily Alpheoidea dominated the number of species (13), representing 25% of the total species. Following the family-wise contribution. The analysis reveals 48% of the species are contributed by the family Penaeidae, followed by the family Alpheidae (15%), Plaemonidae (11%). Families Hippolytidae and Solenoceridae have contributed 6% each of the total species diversity.

Information on species distribution and habitat preference of marine invertebrates is the fundamental requirement to understand the presence of different species in benthic communities, providing baseline information for successful conservation of the habitat and benthic fauna. Studies on the distribution and diversity of local fauna are of great importance because they lead to the best understanding of the local animal community's structure, function, and problems (Fransozo et al., 1992; Hebling et al., 1994). The major highlights and recommendations for future studies are listed below.

The morphometrical analysis was conducted using a truss network system on 28 Dendrobranchiata and 11 Pleocyemata shrimp species collected from the Gujarat coast during the present study. Fifty-one morphometric characters representing the carapace, thoracic appendages, and abdominal segments were used to derive the truss network system's (morphometric matrix). In the taxonomical relationship of 28 shrimp of Dendrobranchiata based on morphometrical characters, the standardized size values of species were closely related to respective genera such as *Penaeus*, *Metapenaeus, Parapenaeopsis*, *Parapenaeus,* and *Solenocera* clearly explained the species relationship.

UPGMA cluster analysis showed *Metapenaeus* and *Parapenaeopsis* species came closely, whereas *Penaeus monodon* formed a separated clade. Morphometric relationships of penaeidae were congruent with the molecular and morphological classification of previous studies. Based on the results, it has been concluded that discriminant function analysis and

cluster analysis proved to be an effective procedure for distinguishing and classifying species and describing the taxonomical relationship of penaeidae species. *P. penicillatus, P. japonicus, P. merguiensis,* and *P. cancaliculatus* came closely. Species belonging to the family solenoceridae are close to each other, and they are not arranged in a single clade except one species *viz. S. choprai*. *P. indicus*, *P. merguiensis,* and *P. penicillatus* are morphologically similar, which was also supported by the cluster analysis. All the species belonging to the genus *Metapenaeus* came closely except three viz *M. kutchensis*, *M. moyebi,* and *M. dobsoni.* In the suborder pleocymeta, the UPGMA cluster analysis based on the morphometric traits does not work effectively. For this, we need a greater number of specimens of all the families that come under this group.

The phylogenetic study of Dendrobranchiata and Pleocymeta species was investigated based on COI gene sequencing analysis collected from the Gujarat coast during the present. DNA was successfully extracted from the 47 samples, and 42 samples were successfully amplified using the two different primers. The positive amplicon was sequenced, and a total 42 species sequences were developed. The BLASTn analysis of all 42 sequences was performed and 70% of species clearly matched with respective species available on the NCBI database. In addition, 6 sequences were obtained from the NCBI for the phylogenetic analysis. The phylogenetic analysis of Dendrobranchiata involved 28 nucleotide sequences (27 developed and 1 obtained). It is well supported. All the species level grouping within the genus *Alpheus* came under a subsequent clade that was strongly supported with high bootstrap value. The position of the family Alpheidae closer to Hippolytidae. Based on the cladistic analysis, the taxonomy of Hippolytidae is not clear.

The standardized usage of mt COI gene sequences as DNA barcoding has emerged as an accurate tool for the rapid identification of various organisms providing high species resolution (e.g., Costa et al., 2007; Burns et al., 2008) and is increasingly used for the prawn identification (Mamatha

et al., 2016; Subbaiya et al., 2017; Kundu et al., 2018). The use of the mitochondrial COI gene proves to be a useful technique in resolving long-standing problems in the identification of prawns. Morphological examination of cryptic species can lead to incorrect identification (e.g., genus *Alpheus*; on the other and no misidentification occurs using DNA barcoding. The morphological examination approach overlooks morphologically cryptic species. The standard method described in this chapter can easily be applied and used anywhere else to identify prawn and shrimp. Our study presents the most comprehensive and robust molecular phylogenetic survey of Dendrobranchiata and Pleocymeta species to date from the Gujarat coast. It is also the first molecular phylogenetic study.

In the present study, the phylogenetic relationship of different genera *Penaeus*, *Metapenaeus*, *Parapenaeopsis*, *Penaeopsis,* and the other genera reported based on the molecular tool is congruent with cluster analysis. Thus, it could be concluded that molecular phylogeny using the mtCOI gene is more useful for phylogenic studies of Dendrobranchiata and Pleocymeta species than traditional morphometric study.

Recommendation

- The present study showed that the coastal area of the Gujarat state supports a huge diversity of prawn and shrimps, and detail taxonomic studies are still required to carve a clear picture of the intertidal shrimp fauna of Gujarat.
- The detailed studies on intertidal zonation patterns of marine invertebrates are still required for intertidal shrimps' symbiotic study.
- The coastal areas of Gujarat state support different kinds of marine habitats like the rocky shore, muddy with a rocky bottom, and mudflat was classified as a macro-habitat. In the present study, the micro-habitat pattern was established only based on the dominant animal community present. Future studies can be carried out based on the zonation pattern of shores.

- The rocky shore is the most productive shoreline in terms of diversity, and a detailed study of rocky shore habitat in terms of ecology is still required.
- In the case of caridean shrimps' study of specialized habitats *e.g.,* benthic ground, uniform vegetated canopy and color of host species are required. These habitats can promote the subsequent selection of several adaptive traits, such as camouflage involving remarkable shape and color changes.
- In the morphometric study, we have examined the smaller number of species from Gujarat state. Additional data needed to revise some caridean families' taxonomy like Palaemonidae, Hippolytidae s.l. and Pasiphaeidae, and such taxonomic revision needs additional taxonomic sampling (worldwide) to resolve the taxonomic relationship between these families.
- A limited number of species were examined (each genus and family) in the present study. A complete mitochondrial genome of these groups must understand the comprehensive phylogeny of the Dendrobranchiata and Pleycometa.

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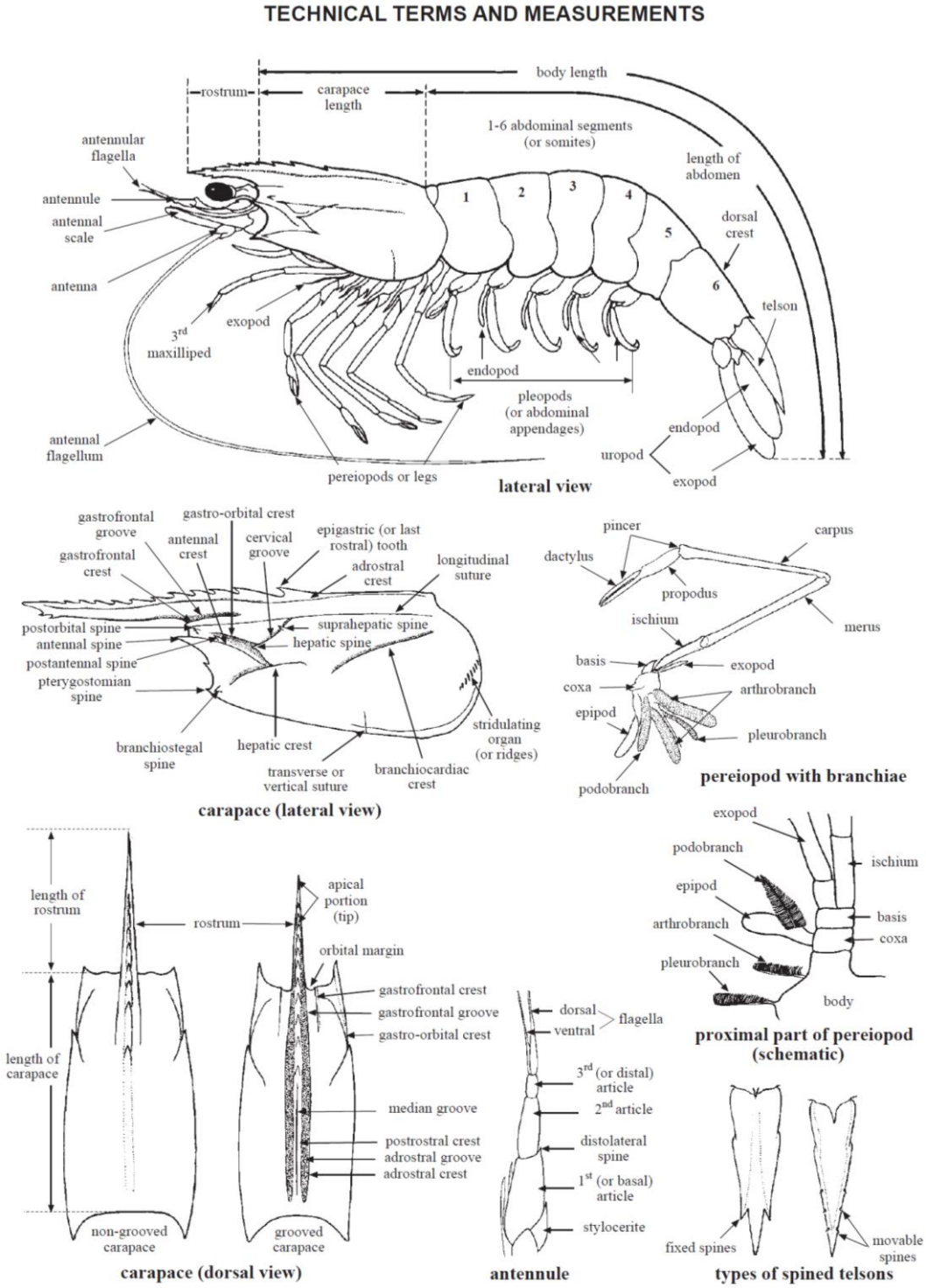
Annexure

Annexure 1 Technical terms and Measurements use for Prawns and shrimps identification (modified from Holthius, 1980).



***Annexure 2*: DNeasy Blood and Tissue Kit (Qiagen) Protocol**

- The animal was washed before proceeding for DNA isolation using 100% ethanol and then rinsed with sterile distilled water.
- Approximately 20 mg of tissue was dissected and chopped into small pieces. Tissue was transferred into a 1.5 ml sterile microcentrifuge tube, and 180 µl ATL buffer was added.
- 20 µl of proteinase K was added and mixed thoroughly by vortexing. The samples were incubated at 56°C until the tissue was completely lysed.
- To clear lysate 200 µl buffer AL was added and mixed thoroughly by vortexing than added 200 µl of chilled ethanol (96-100%) to the above solution and mixed again by vortexing.
- A mixture was transferred to the DNeasy minispin column placed into a 2 ml collection tube. Columns centrifuged at 8000 rpm for 1 minute. Flow-through was discarded.
- DNeasyminispin column was placed in a new 2ml collection tube, and 500 µl buffer AW1 was added. Colum was centrifuged at 8000 rpm for 1 minute. Flow-through was discarded.
- DNeasy minispin column was placed in a new 2ml collection tube, and 500 µl buffer AW1 was added. Colum was centrifuged at 8000 rpm for 1 minute. Flow-through was discarded.
- DNeasy minispin column was placed in a new 2ml collection tube, and 500 µl buffer AW2 was added. Colum was centrifuged at 14,000 rpm for 3 minutes to dry the DNeasy membrane. Flow-through and the collection tube were discarded.
- Columns were placed in 1.5 ml microcentrifuge tubes. 35 µl buffer AE was added directly onto the DNeasy membrane and incubated at room temperature (25º C) for 5 minutes.
- DNA was eluted by spinning the DNeasy columns at 8000 rpm for 1 minute.
- The step mentioned above was repeated for the yield concentration of DNA. DNA was stored at - 20° C.

Annexure 3: **Agarose gel electrophoresis**

Chemicals for agarose gel electrophoresis

Running buffer- 5X Tris borate EDTA (TBE) buffer

Preparation of 5X TBE buffer

Chemical	**Amount added**
Tris base	54 g
Boric acid	27.5 g
0.5 M EDTA (pH 8)	20 ml

Above mentioned chemicals were mixed in a volumetric flask and the solution was made up to 1 liter by adding distilled water. Buffer was subjected to autoclave treatment, as discussed above. 5X TBE was diluted to 0.5X before to use.

Preparation of Tris HCl EDTA buffer

Chemical	**Required Strength in the buffer**	**Amount added in 200 ml**
Tris-HCl	10 mM	0.24g
EDTA	1 mM	0.06g

Two hundred milliliter buffer solution of pH 8.0 was prepared by dissolving above mentioned chemicals.

Ethidium bromide (10 mg/l), 10 ml

0.1 g of Ethidium Bromide was added to 10 ml of distilled water and kept on a magnetic stirrer to make sure that the dye has dissolved completely. This was transferred to an amber-colored reagent bottle and stored at 4 °C.

Plate preparation and casting the gels

The cleaned agarose gel casting cassette and comb were wiped with methanol, and open sides of the tray were sealed with gel sealing tape. The

comb was placed in the given slits of the plate. The calculated amount of agarose in TBE buffer was mixed to prepare a 2 % solution. The agarose was dissolved completely in the buffer by heating the mixture at 80-85°C in the microwave oven and was cooled to 50°C. Ethidium bromide was added in a final concentration of 0.6 mg/ml and mixed well. The liquid was gently poured into the casting tray before it gets solidified. The combs and sealed tape were removed slowly after complete solidification of the agarose gel.

Qualification and quantification DNA and PCR products

After DNA isolation and PCR amplification, the amplicons were observed on 0.8 and 2% agarose gel. The DNA and PCR product were mixed loading dye and carefully loaded in the wells using gel-loading tips. 100 bp Marker Electrophoresis was carried out at 150V. The gel images were recorded in JPEG or TIF formats using a gel documentation system (Biorad, USA). The gel was examined using the software Image lab version 3.0 (Biorad, USA).