

**MOLLUSCANS SPECIES DIVERSITY AND PROTEIN
EXPRESSION ANALYSIS IN *LUNELLA CORONATA* SPP OF
FAMILY TURBINIDAE FROM THE MANORA CHANNEL
KARACHI, PAKISTAN**

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ABSTRACT: Manora rocky shore characterize as a shore having moderate to strong wave exposure, zonation pattern characterize by intertidal habit, different communities of invertebrates and algae bands dominated by molluscan diversity and associated fauna. The current study contribute the species diversity of molluscs from the Manora rocky ledge, coast of Pakistan. A total of 11 species were recorded pertaining to 9 families and 11 genera including family Turbinidae: *Turbo bruneus*, *Lunella coronata*, family Cerithiidae: *Cerithium caeruleum*, family Babyloniidae: *Babylonia spirata*, family Neritidae: *Nerita albicilla*, family: Chilodontidae *Euchelus asper*, family Veneridae: *Circenita callipyga*, family Arcidae: *Barbatia obliquata*. Family Pectinidae, *Mimachlamys townsendi*, family Chamidae: *Chama* sp, family Littorinidae and Sub family Trachycardiinae *V. assimile*. In addition, Protein expression analysis of *L. coronata* was also observed through Polyacrylamide gel electrophoretic. *L. coronata* was previously reported from the coastal water of Pakistan with some morphological variation in the shell shape and the results reveled that electrophoretic patterns of proteins in *L. coronata* spp 1 and *L. coronata* spp2 differentiate from each as seen in morphological characters.

KEYWORDS: molluscans, diversity, electrophoresis, Turbinidae, *Lunella coronata*, Pakistan.

INTRODUCTION

The phylum Mollusca respect as the most diverse phylum, with approximately 50,000-55,000 species been evaluated and characterized Taylor and Lewis, (2007), that increasing yearly increment of 443 new species, mostly 51.5 % from the tropical Indo-Pacific (Bouchet *et al.*, 2016). They are highly diverse, and identified by the morphological characters i.e. shell, mantle, the presence of a radula, nervous system configuration (Herbert *et al.*, 2018). They live in variable habitats in marine environment inhabiting from the intertidal and splash zones to the bottoms of marine trenches such as the Puerto Rico Trench 8595 m deep, as deep as Mount Everest height (Leal and Harasewych, 1999). Mollusc's body is divided into a head, a visceral mass incorporating the body organs, and a muscular foot. The body normally enclosed within a hard shell made of

calcium carbonate (Appleton and Miranda, 2015). Molluscs act as an important food of many marine fish, mammals and humans. They also act as bio-indicators Herbert *et al.*, (2018), medicinal value in the form of carbohydrates, proteins, minerals, lipids, sterols and nucleosides. Some published literature available on the taxonomy, distribution, abundance, species composition of molluscan fauna from the coastal water of Pakistan including Khan and Dastagir, (1971, 1972), Ahmed *et al.*, (1982), Tirmizi and Zehra, (1982, 1984), Kazmi, (1995), Moazzam and Ahmed, (1995), Kazmi *et al.*, (1996), Siddiqui and Ahmed, (2002), Kazmi and Naushaba, (2003, 2004), Barkati and Rehman, (2005), Afsar *et al.*, (2012), Jahangir *et al.*, (2012), Rehman and Barkati, (2012), Kazmi and Moazzam, (2014), Psomadakis *et al.*, (2015), Ullah *et al.*, (2015), Naz *et al.*, (2015), Ghani *et al.*, (2017), Ghani *et al.*, (2018), Ghani and Afsar, (2017).

Some recent morphological taxonomy of molluscan fauna Kazmi, (2019) and taxonomic assessment of mollusks was described by Bibi, (2020, 2021) found in coastal waters of Pakistan. However initial phylogenies of mollusks based on anatomy and shell characters provide a measure of differentiating taxonomic groups. In the morphological taxonomy boundary of classification overlap between closely related groups because of selection and evolutionary progression. Therefore, morphological characteristics were indistinct and revealed the deviation over classification. To understand the situation molecular genetics and biochemical technique were used to understand the genetics of organisms and clear the reservations. Phylogenies based on molecular techniques revolutionized the research on Mollusks evolution and interrelationships. Electrophoresis molecular genetics technique has been used to compare the electrophoretic banding pattern of species or genera to resolve the taxonomic problem and genetic variation. By using the elliptic Fourier descriptors EFDs Williams *et al.*, (2012) evaluated the morphological variation in shell shape of Genus *Lunella* Turbinidae in two species complexes *Coronata* and *Cinerea* groups. The *Lunella coronata* species a group of morphologically similar species along with *L. aff. coronata ophiolite*, *L. coronata* Gmelin, (1791), *L. aff. coronata* Oman. The *L. coronata* was previously reported from the coastal water of Pakistan with some morphological variation in the shell shape. However, no previous work was carried out on the electrophoresis of molluscan species. Therefore, the current study was initiated to estimate the genetic variation of morphologically identical species. The aim of the current study is to observe mollusks species diversity and status of the *L. coronata spp* through Polyacrylamide gel electrophoresis and investigate the protein expression and confirm the protein markers locus differences.

MATERIALS AND METHODS

The Molluscs samples were randomly collected by handpicked at Manora Channel from the coastal waters of Pakistan (Fig. 1A and 1B). The sample was sorted in the Institute of the Marine Science University of Karachi. Each specimen was identified to species level by using the key of (Kazmi *et al.*, 2018). Morphometric measurements were made using calipers accurate to 0.01mm. Each sample was weighed on a digital balance resolution of (0.001g). Each sample's length and width were measured and mean and standard deviation values of sample length, width, and weight were also calculated.



Fig. 1. A, Sampling site Manora rocky Shore from the coastal waters of Pakistan;
B, *Lunella coronata* spp.

Polyacrylamide Gel Electrophoresis: The fresh specimens of Family Turbinidae: *Lunella coronata* spp were randomly collected by handpicked at Manora Channel from the coastal waters of Pakistan. Capture live specimens transferred to the laboratory killed by freezing, before tissue extraction. Specimens initially preserved in 4°C for protein variations, approximately 250-300 mg muscle was removed and homogenized in extraction Tris-Citrate buffer according to (Naz *et al.*, 2017; Naz *et al.*, 2019). For the General protein *Gp* non-specific electrophoresis was performed in vertical gel electrophoresis; SDS polyacrylamide gel and native polyacrylamide gels electrophoresis as described by Laemmli, (1970) under the reducing conditions in the discontinuous buffer system at room temperature. Gels stained for General protein by *Coomassie Brilliant Blue* whereas the standardized genetic nomenclature used to the designated the loci and alleles.

RESULTS AND DISCUSSION

Diversity and distribution of Molluscan species from Manora Rocky shore: Molluscan samples was collected from the rocky ledge of Manora Channel, coastal waters of Pakistan. The identification of specimens was based on the morphological characteristics of shells, shape, size and color. A total of 11 species were recorded pertaining to 9 families and 11 genera including family Turbinidae: *Turbo bruneus*, *Lunella coronata*, family Cerithiidae: *Cerithium caeruleum*, family Babyloniidae: *Babylonia spirata*, family Neritidae: *Nerita albicilla*, family: Chilodontidae *Euchelus asper*, family Veneridae: *Circenita callipyga*, family Arcidae: *Barbatia obliquata*. Family Pectinidae, *Mimachlamys townsendi*, family Chamidae: *Chama* sp., family Littorinidae and Sub family Trachycardiinae *V. assimile*. *C. caeruleum* was observed as the most dominant species (20.29%), followed by *P. viridis* (.39%), *P. viridis* with barnacles were observed as third most abundant species with (11.6%) abundance, *V. assimile* and *C. callipyga* and *L. coronata* abundance was (1.4%). Total length, width, and weight was measured, the maximum length was observed in *V. assimile* species (57mm), second most abundant species was *P. viridis* mean length was (32 mm) followed by *Chama* sp. (29 mm), *B. obliquat* (29 mm), *L. littore* (28.417 mm), whereas minimum length was observed in *E. asper* (17.50 mm). The maximum width was observed in *P. viridis* mean length was (62 mm) and second ranked species was *V. assimile* species (52 mm), third ranked species was *B. obliquata* mean width (47 mm) *L. coronata* and *C. callipyga* ranked as fourth mean width was (35 mm) followed by *Chama* sp. (29 mm), *B. obliquata* (29 mm), *L. littorea* (28.417 mm), whereas minimum length was observed in *E. asper* (17.50 mm). The maximum weight was observed in *L. coronata* mean weight was (26.33g) and second ranked species was *V. assimile* species (25g), third ranked species was *P. viridis* mean weight (13.50g), whereas minimum length was observed in *E. asper* (5.41g) (Table 1).

Polyacrylamide Gel Electrophoresis of General Protein Expression: The Protein expression was observed in the fresh specimens of molluscs of family Turbinidae: *Lunella coronata* spp1, *Lunella coronata* spp2 from the coastal waters of Pakistan. The SDS PAGE Electrophoresis was performed between two species *Lunella coronata* spp1, *Lunella coronata* spp2 and results indicated that two no of loci out of three was observed

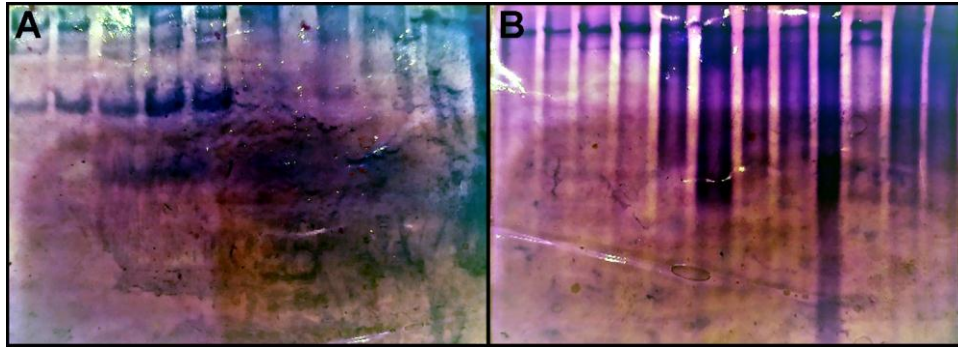


Fig. 2. A, SDS PAGE Electrophoresis pattern of *L. coronata* spp1, *L. coronata* spp2; B, Native PAGE Electrophoresis pattern of *L. coronata* spp1, *L. coronata* spp2. B collected from coastal waters of Pakistan.

Table 1. Total no of mollscan species and their percent abundance collected from Manora Rocky shore from the coastal waters of Pakistan.

No	Species	n	% Abundance	Length		Width		Weight	
				Mean	St. dev	Mean	St. dev	Mean	St. dev
1	<i>P. viridis</i>	8	11.6	28.00	0.00	52.00	8.77	7.83	3.54
2	<i>P. viridis</i> with barnacles	2	2.9	32.00	0.00	62.00	7.07	13.50	5.66
3	<i>V. assimile</i>	1	1.4	61.0	21.2	52.5	21.9	13.00	11.31
4	<i>C. caeruleum</i>	14	20.29	57.00	*	48.00	*	25.10	*
5	<i>C. callipyga</i>	12	17.39	29.00	*	30.00	*	12.10	*
6	<i>L. litorea</i>	12	2.90	29.75	4.16	35.33	4.01	7.62	6.19
7	<i>M. townsendi</i>	2	2.90	24.42	1.785	13.28	0.82	2.786	0.41
8	<i>Chama</i> sp.	1	1.45	46.25	1.815	35.25	1.76	26.73	2.48
9	<i>L. coronata</i>	12	17.39	28.41	2.90	22.66	3.28	10.311	2.14
10	<i>B. spirata</i>	3	4.35	29.00	0.00	47.7	24.1	9.41	11.55
11	<i>E. asper</i>	2	2.90	17.50	6.36	24.00	1.41	5.410	1.00

as polymorphic GP-1, GP-2 in *L. coronate* spp1 whereas single no of loci GP-1 was observed as polymorphic out of two loci in *L. coronate* spp2. Whereas the Native PAGE Electrophoresis was performed between three species *Lunella coronata* spp1, *Lunella coronata* spp 2 results indicated that single loci was observed as polymorphic GP-1 in three specs of family Turbinidae: *L. coronate* spp1, *L. coronate* spp2. According to the results of SDS PAGE Electrophoresis and Native PAGE Electrophoresis indicated that the genetic variation was observed among three species. The genetic relationship

Table 2. SDS PAGE Electrophoresis pattern of *L. coronata* spp1, *L. coronata* spp2. (A) Native PAGE Electrophoresis pattern of *L. coronata* spp1, *L. coronata* spp2. (B) collected from coastal waters of Pakistan.

SDS PAGE Electrophoresis					Native PAGE Electrophoresis		
No	Species	Loci			Species	Loci	
		Gp1	Gp2	Gp3		Gp1	Gp2
1	<i>L. coronata</i> spp1	AA	AB	AA	<i>L. coronata</i> spp1	AA	AA
2		AA	AB	AA		AA	AA
3		AA	AB	AA		AA	AA
4		AB	AB	AA		AA	AA
5		AB	AA	AA		AA	AA
6		AB	AA	AA		AA	CC
7	<i>L. coronata</i> spp2	AB	AA	AA	<i>L. coronata</i> spp2	AA	AA
8		AA	AA	AA		AA	CC
9		AA	AA	AA		AA	AA
10		AB	AA	AA		AA	CC
11		AB	AA	AA		AA	CC

among the species of *Lunella coronata* spp similar from those inferred from morphological features. In electrophoretic studies comparing the electrophoretic patterns of species, to solve taxonomic problems (Table 2, Fig. 2).

Discussion: The Mollusca's species contribute ecological status of the marine environment. As an herbivores, mode of nutrition they stand at lower layers of aquatic trophic level and perform multiple ecological activities. Studies relating to their distribution, diversity, and ecology become imperative, Gastropoda and Bivalvia are the most diverse and dominant classes of Molluscs. The present study contributes the species diversity of molluscs and Protein expression analysis of *L. coronata* found in the Manora rocky shore coastal waters of Pakistan. Manora rocky shore characterize as a shore having moderate to strong wave exposure, vertical zonation pattern characterize intertidal habitat, communities of invertebrates and algae bands dominated by molluscan diversity and associated fauna.

Over the course of our study, 11 species of molluscs were collected from the Manora that includes species of gastropods and bivalves. Gastropods are typically one of the most dominant groups in marine ecosystems than the bivalve A total of 11 species were pertaining to 9 families and 11 genera including family Turbinidae: *T. bruneus*, *L. coronata*, family Cerithiidae: *C. caeruleum*, family Babyloniidae: *B. spirata*, family Neritidae: *N. albicilla*, family: Chilodontidae *E. asper*, family Veneridae: *C. callipyga*, family Arcidae: *B. obliquata*. Family Pectinidae, *M. townsendi*, family Chamidae: *Chama* sp., family Littorinidae and Sub family Trachycardiinae *V. assimile*. *C. caeruleum*.

C. caeruleum was observed as highest abundant species, whereas *V. assimile* and *Chama* sp observed as lowest abundant species. *C. callipyga* and *T. coronatus* was observed as second most abundant species, whereas *P. viridis* was observed as third most abundant species. The maximum length was observed in *V. assimile* species, followed by *P. viridis*, *Chama* spp, *B. obliquata* and *L. littorea* whereas minimum length was observed in *E. asper*. The maximum width was observed in *P. viridis* and second ranked species was *V. assimile*, third ranked species was *B. obliquata*. The maximum weight was observed in *T. coronatus* and second ranked species was *V. assimile* species, third ranked species was *P. viridis*. George, (1995) collected 51 species of gastropods from nine Hong Kong Mangals, with a median of 25 species for all the sites. Tirmizi and Barkati, (1983a, 1983b) reported 30 species of molluscs from the mangroves along the Karachi coast, Pakistan. According to Rehman and Barkati, (2012) a total of 63 molluscan species were recorded in Manora Channel including 49 species of Gastropods dominated the ledge, followed by 12 bivalve's species, the average number of species decreased with an increase in the tidal heights, corresponding to 22.6 species during low tide, 19.6 during mid-tide, and 16.0 during high tide. the representative species are exclusively mangrove associated; five are common to Ullah *et al.*, (2015) describe the species diversity, distribution and seasonal abundance in mangrove associated molluscs along the Karachi Coast, Pakistan and revealed that seven of mangrove and non-mangrove areas, while two are non-mangrove dwellers. The low species diversity in current study attributed to the study techniques, a small number of study sites or possibly to the geographical locations of the study sites.

According to Beasley *et al.*, (2005) a total of 30 species of bivalves in 18 families and 19 species of gastropods in 15 families were recorded in northern and north-eastern coast of Brazil. Seasonal variation in molluscan density might be linked to rainfall and its effect on salinity as well as the effects of desiccation during the dry season. Molluscs have important ecological roles as well as economic importance in the region but habitat deterioration along with overexploitation may threaten this natural resource.

Kurhe *et al.*, (2014) was conducted their research in Ratnagiri Maharashtra India, and the results revealed that the comparative species composition indicated that the significance and diversity of gastropods molluscs was higher within the intertidal region. They categorized rocky intertidal habitat in to three types i.e I Exposed rocky shore high to moderate wave category II Exposed wave cut platforms consisting of wave cut high to moderate wave energy III Sheltered, rocky shores composed of vertical rocky platforms and usually found along the sheltered bays or along the inside of the bay Osorio and Cantuarias, (1989); Benkendrofe and Soares-Gomes, (2009). Kurhe *et al.*, (2014) observed and identified 127 gastropods species. Physiologically these species are tolerated to desiccation and their comparative and predatory interactions with other largely determine their vertical distribution. In the intertidal region which some species were abundantly distributed, and some were very scarce, that suggest a direct relation between specific habitat and population density. Therefore, studies focused on the monitoring of mollusk species, and the knowledge of species wise diversity and distribution pattern are a good scope for the conservation, management and culture of mollusks. During the current study Protein expression analysis of *L. cornata* was also observed through polyacrylamide gel electrophoresis. Some morphological variation was observed in *L. cornata* species from the coastal waters of Pakistan in their outline shape

as also described by Williams et al., (2012) and identified as *L. cornata* sp 1 and *L. cornata* sp 2. The protein expression results was revealed that the *L. cornata* sp 1 and *L. cornata* sp 2 differentiated to each other and the genetic variation was observed. The genetic variation among the species of *L. coronate* spp similar from those inferred from morphological features. In electrophoretic studies comparing the electrophoretic patterns of species, to solve taxonomic problems.

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