# REPORT ON SPECIES IDENTIFICATION THROUGH PARTIAL GENE SEQUENCING

**[1]M. Thilagavathi*, and [2]Dr. A. Christy Ponni**

1. *Research scholar P.G and Research Department of Zoology, TBML College, Porayar - 609 307, Tamil Nadu, India.*
2. *Head of the Department P.G and Research Department of Zoology, TBML College, Porayar - 609 307, Tamil Nadu, India.*

***ABSTRACT* :** Good quality sequence was obtained from all the samples and the sequences were used for alignment and editing using sequence alignment software MEGA 6. Similar sequence was obtained from all the 04 samples which confirm that all the samples are same species. Similarity search was carried out using aligned sequence against the sequences submitted in NCBI using NCBI-BLAST. The sequence shows 99% similarity with the genus *Donax*. The result mentioned above is only based on the BLAST search of aligned sequences. We could confirm the genus as *Donax*. Even though the sequences showing 99% similarity with *Donax deltoides* and *D. faba*. The literature search for related genus shows that it may be *Donax variabilis*. The researchers need to confirm the species before submitting in NCBI using other relevant documents.

## I. INTRODUCTION

*Donax variabilis* (Coquina clams) are tiny, wedge-shaped clams that dominate the intertidal zone by filter feeding on unicellular algae and detritus. Coquina clams are found along the Gulf Coast and along most of the East Coast of the United States[1]. In fact, coquinas have been reported to constitute up to 95% of the macrofaunal biomass of sandy beaches[2], however, they use their foot (a fleshy muscle found in molluscs for movement) to burrow into the sand where they often remain unseen[3&4]. Coquina clams have also been shown to be an important food resource for both sublittoral and supralittoral predators, including surf fish such as *Florida pompano* (*Trachinotus carolinus*)[5] stingrays[6], Moon snails (*Polinices duplicatus*)[7] and shore birds[8&6]. Given their importance in the food web of sandy beach ecosystems, coquinas are considered an indicator species for the ecosystem health, especially given their prevalence and widespread distribution.

*Donax spp*. are dioecious (separate sexes)[9] and have spawning events between January and May, which produces a planktonic, veliger larva that inhabits the pelagic zone of the water column[10]. In the present study, comparing the genetic variation of populations throughout the distribution of *D. variabilis* using the mitochondrial COI (cytochrome c oxidase I) marker which contributes to the electron transport chain, as well as the mitochondrial 16S rRNA marker which is part of the ribosomal RNA subunit. Both of these mitochondrial markers will be used to help determine if speciation has occurred in *D. variabilis* throughout the geographic distribution. These roughly 600 base-pair regions are present in nearly every animal due to their importance within cell functions and are, therefore, ideal genes to use in

DNA barcoding to deduce the level of speciation between and within species. It is unlikely that throughout the natural distribution of *D. variabilis* gene flow is present between every population; instead, it is far more plausible that oceanic or geographic barriers and natural selection have led to phylogeographic breaks and structure.

DNA Extraction is very important step for reliability, feasibility and reproducibility of molecular genetics studies are often limited by the preliminary step of DNA isolation. The obtainment of great amounts of high quality DNA from small quantities of tissue is often a laborious task.

## REPORT ON SPECIES IDENTIFICATION THROUGH PARTIAL GENE SEQUENCING

Present study, four molluscs samples were received from Smt. Thilagavathi, Assistant Professor, TBML College, Porayar for partial gene sequencing. The samples were re-preserved in 95% ethanol until the test commenced.

**Sample received for sequencing:**

# II. MATERIALS AND METHODS

### a. Extraction of Total Genomic DNA

The samples were used for the extraction of total genomic DNA using Phenol Chloroform method standardized by CAGL. Quality of the genomic DNA was assessed using 0.7 % agarose gel along with 1kb DNA ladder as size standard (**Figure 1)** and the quantity of the genomic DNA was assessed in Biophotometer (Eppendorf). Genomic DNA was observed in all the samples with degradation.

**Genomic DNA**

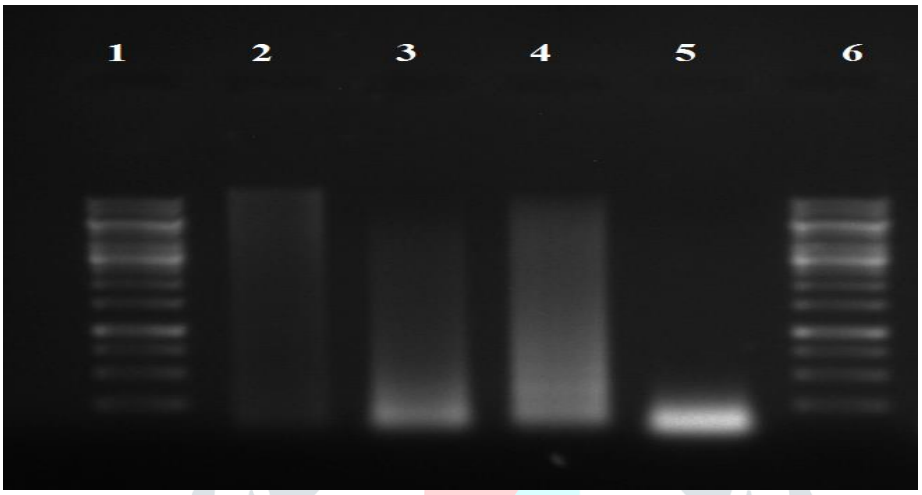


**Figure 1:** Lane 1&6-1Kb DNA marker, Lane 2–P (Pink), Lane 3–W (White), Lane 4– B (Brown), Lane 5–BL (Black)

### *a.* PCR Testing- *18SrRNA* amplification

Amplification of *18S rRNA* was carried out using universal Forward & Reverse primers for all the samples. PCR-generated amplicons **(Figure 2)** were confirmed by running the samples on 2% agarose gel along with 100bp DNA ladder. PCR products were subjected for purification using GeneJET PCR purification kit (Thermo Scientific, EU-Lithuania) to remove the primer dimer and other carryover contaminations. The quality of the purified PCR product was assessed using 2% agarose gel and was found to be good for sequencing.

| Primers used | Sequence (5' to 3') |
|---|---|
| Universal 18S F (Forward) | CAGCAGCCGCGGTAATTCC |
| Universal 18S R (Reverse) | CCCGTGTTGAGTCAAATTAAGC |

**Gel image for PCR product-*18srRNA***

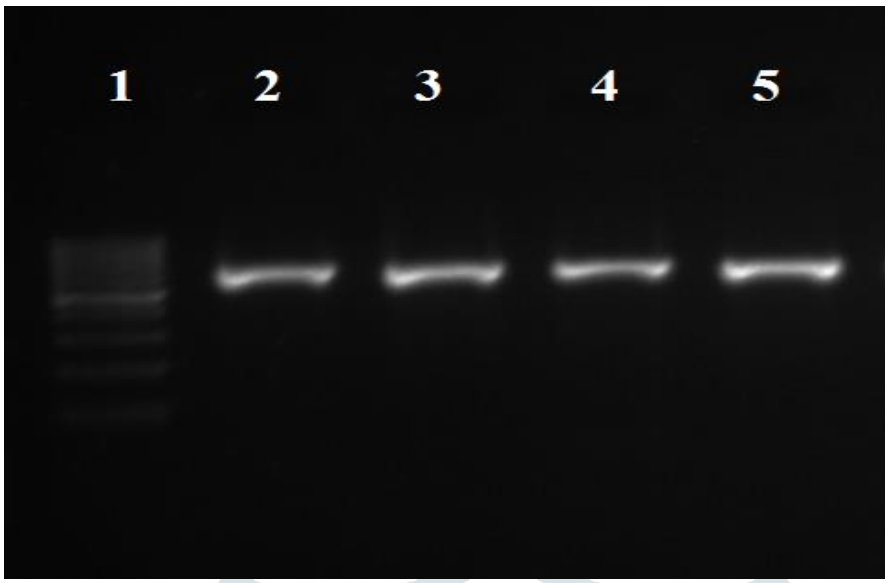


**Figure 2:** Lane 1-100bp DNA ladder, Lane 2–P (Pink), Lane 3–W (White), Lane 4– B (Brown), Lane 5–BL (Black)

**b. Sequencing**

Amplified PCR products were purified and prepared for Cycle sequencing using the Big Dye Terminator 3.1 sequence kit (Applied Biosystems, Foster City, California, USA). After cycle sequencing, the products were purified using Ethanol-EDTA purification protocol to remove the un-incorporated dNTP's, ddNTP's and primer dimer**.** The purified cycle sequencing products were dissolved in 12µl Hi-Di formammide and the sample were subjected for denaturation at $95^0$C for 5mins. Denatured products were subjected for sequencing in forward directions using Genetic Analyzer 3500 (Life Technologies Corporation, Applied Biosystems, California 94404, USA) as per manufacture's instruction. Sequences were aligned and edited using Mega software version 6 to confirm the species.

## III. RESULTS

Good quality sequence was obtained from all the samples and the sequences were used for alignment and editing using sequence alignment software MEGA 6. Similar sequence was obtained from all the 04 samples which confirm that all the samples are same species. Similarity search was carried out using aligned sequence against the sequences submitted in NCBI using NCBI-BLAST. The sequence shows 99% similarity with the genus *Donax*.

**Remarks:**

The result mentioned above is only based on the BLAST search of aligned sequences. We could confirm the genus as *Donax*. Even though the sequences showing 99% similarity with *Donax deltoides* and *D. faba*; the literature search for related genus shows that it may be *Donax variabilis*. The researchers need to confirm the species before submitting in NCBI using other relevant documents.

**The FASTA sequence provided below (after alignment and editing) of *18srRNA* may be submitted to NCBI.**

>N18S P

CAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCGTTTAAAAAGCTCG
TAGTTGGATCTCGGTTCCAGGCCTGCGGTCCGCCTCGAGGCGGATACTGCTCGTCCTGCGTTC
GACGTCGTGGTGGTCCCTTGGTGCTCTTGACTGAGTGTCTCGGGCGGTCCCGAATGTTTACTTT
GAAAAAATTAGAGTGCTCAAAGCAGGCGTATCGCCTGAATAATTCCGCATGGAATAATGGAA
TAGGACCTCGGTTCTAGTTTCGTTGGTTTGCGAATCCTTGAGGTAATGATTAATAGGGACTGC
CGGGGGCATACGTATTGCGGCGGGAGAGGTGAAATTCGTGGATCGCCGCAAGACGAACGACA
GCGAAAGCATTTGCCAAGAATGTTCTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGACG
ATCAGATACCGTCGTAGTTCTGACCCTAAACGATGCCGACTGTCGATCCGCCGGAGTTACTAC
CATGACTCGGCGGGCAGCCTCCGGGAAACCAAAGTCTTTGGGTTCCGGGGGGAGTATGGTTG
CAAAACTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGTGGCTTAA
TTTGACTCAACACGGG

>N18S W

CAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCGTTTAAAAAGCTCG
TAGTTGGATCTCGGTTCCAGGCCTGCGGTCCGCCTCGAGGCGGATACTGCTCGTCCTGCGTTC
GACGTCGTGGTGGTCCCTTGGTGCTCTTGACTGAGTGTCTCGGGCGGTCCCGAATGTTTACTTT
GAAAAAATTAGAGTGCTCAAAGCAGGCGTATCGCCTGAATAATTCCGCATGGAATAATGGAA
TAGGACCTCGGTTCTAGTTTCGTTGGTTTGCGAATCCTTGAGGTAATGATTAATAGGGACTGC
CGGGGGCATACGTATTGCGGCGGGAGAGGTGAAATTCGTGGATCGCCGCAAGACGAACGACA
GCGAAAGCATTTGCCAAGAATGTTCTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGACG
ATCAGATACCGTCGTAGTTCTGACCCTAAACGATGCCGACTGTCGATCCGCCGGAGTTACTAC
CATGACTCGGCGGGCAGCCTCCGGGAAACCAAAGTCTTTGGGTTCCGGGGGGAGTATGGTTG
CAAAACTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGTGGCTTAA
TTTGACTCAACACGGG

>N18S B

CAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCGTTTAAAAAGCTCG
TAGTTGGATCTCGGTTCCAGGCCTGCGGTCCGCCTCGAGGCGGATACTGCTCGTCCTGCGTTC
GACGTCGTGGTGGTCCCTTGGTGCTCTTGACTGAGTGTCTCGGGCGGTCCCGAATGTTTACTTT
GAAAAAATTAGAGTGCTCAAAGCAGGCGTATCGCCTGAATAATTCCGCATGGAATAATGGAA
TAGGACCTCGGTTCTAGTTTCGTTGGTTTGCGAATCCTTGAGGTAATGATTAATAGGGACTGC
CGGGGGCATACGTATTGCGGCGGGAGAGGTGAAATTCGTGGATCGCCGCAAGACGAACGACA
GCGAAAGCATTTGCCAAGAATGTTCTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGACG
ATCAGATACCGTCGTAGTTCTGACCCTAAACGATGCCGACTGTCGATCCGCCGGAGTTACTAC
CATGACTCGGCGGGCAGCCTCCGGGAAACCAAAGTCTTTGGGTTCCGGGGGGAGTATGGTTG
CAAAACTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGTGGCTTAA
TTTGACTCAACACGGG

>N18S BL

CAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCGTTTAAAAAGCTCG
TAGTTGGATCTCGGTTCCAGGCCTGCGGTCCGCCTCGAGGCGGATACTGCTCGTCCTGCGTTC
GACGTCGTGGTGGTCCCTTGGTGCTCTTGACTGAGTGTCTCGGGCGGTCCCGAATGTTTACTTT
GAAAAAATTAGAGTGCTCAAAGCAGGCGTATCGCCTGAATAATTCCGCATGGAATAATGGAA
TAGGACCTCGGTTCTAGTTTCGTTGGTTTGCGAATCCTTGAGGTAATGATTAATAGGGACTGC

CGGGGGCATACGTATTGCGGCGGGAGAGGTGAAATTCGTGGATCGCCGCAAGACGAACGACA
GCGAAAGCATTTGCCAAGAATGTTCTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGACG
ATCAGATACCGTCGTAGTTCTGACCCTAAACGATGCCGACTGTCGATCCGCCGGAGTTACTAC
CATGACTCGGCGGGCAGCCTCCGGGAAACCAAAGTCTTTGGGTTCCGGGGGGAGTATGGTTG
CAAAACTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGTGGCTTAA
TTTGACTCAACACGGG

**The raw sequence of *18srRNA* in FASTA format (without alignment and edit)**

>N18S P1F (Forward sequence)

AMMYSSMWWKMMRAARGRRCGGWGGARAGTTGCTGCGTTTAAAAGCTCGTAGTTGGATCT
CGGTTCCAGGCCTGCGGTCCGCCTCGAGGCGGATACTGCTCGTCCTGCGTTCGACGTCGTGGT
GGTCCCTTGGTGCTCTTGACTGAGTGTCTCGGGCGGTCCCGAATGTTTACTTTGAAAAAATTA
GAGTGCTCAAAGCAGGCGTATCGCCTGAATAATTCCGCATGGAATAATGGAATAGGACCTCG
GTTCTAGTTTCGTTGGTTTGCGAATCCTTGAGGTAATGATTAATAGGGACTGCCGGGGGCATA
CGTATTGCGGCGGGAGAGGTGAAATTCGTGGATCGCCGCAAGACGAACGACAGCGAAAGCAT
TTGCCAAGAATGTTCTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGACGATCAGATACC
GTCGTAGTTCTGACCCTAAACGATGCCGACTGTCGATCCGCCGGAGTTACTACCATGACTCGG
CGGGCAGCCTCCGGGAAACCAAAGTCTTTGGGTTCCGGGGGGAGTATGGTTGCAAAACTGAA
ACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGTGGCTTAATTTGACTCCA
ACACGGG

>N18S W1F (Forward sequence)

CGSGSATAGKTTGCSTGCGTTTAAAGCTCGTAGTTGGATCTCGGTTCCAGGCCTGCGGTCCGCC
TCGAGGCGGATACTGCTCGTCCTGCGTTCGACGTCGTGGTGGTCCCTTGGTGCTCTTGACTGA
GTGTCTCGGGCGGTCCCGAATGTTTACTTTGAAAAAATTAGAGTGCTCAAAGCAGGCGTATCG
CCTGAATAATTCCGCATGGAATAATGGAATAGGACCTCGGTTCTAGTTTCGTTGGTTTGCGAA
TCCTTGAGGTAATGATTAATAGGGACTGCCGGGGGCATACGTATTGCGGCGGGAGAGGTGAA
ATTCGTGGATCGCCGCAAGACGAACGACAGCGAAAGCATTTGCCAAGAATGTTCTCATTAATC
AAGAACGAAAGTCAGAGGTTCGAAGACGATCAGATACCGTCGTAGTTCTGACCCTAAACGAT
GCCGACTGTCGATCCGCCGGAGTTACTACCATGACTCGGCGGGCAGCCTCCGGGAAACCAAA
GTCTTTGGGTTCCGGGGGGAGTATGGTTGCAAAACTGAAACTTAAAGGAATTGACGGAAGGG
CACCACCAGGAGTGGAGCCTGTGGCTTAATTTGACTCAACACGGG

>N18S B1F (Forward sequence)

TWKYMWMWATTTAGCAWGTTGAAGTTGCTGCGTTTAAAAAGCTCGTAGTTGGATCTCGGTT
CCAGGCCTGCGGTCCGCCTCGAGGCGGATACTGCTCGTCCTGCGTTCGACGTCGTGGTGGTCC
CTTGGTGCTCTTGACTGAGTGTCTCGGGCGGTCCCGAATGTTTACTTTGAAAAAATTAGAGTG
CTCAAAGCAGGCGTATCGCCTGAATAATTCCGCATGGAATAATGGAATAGGACCTCGGTTCTA
GTTTCGTTGGTTTGCGAATCCTTGAGGTAATGATTAATAGGGACTGCCGGGGGCATACGTATT
GCGGCGGGAGAGGTGAAATTCGTGGATCGCCGCAAGACGAACGACAGCGAAAGCATTTGCCA
AGAATGTTCTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGACGATCAGATACCGTCGTA
GTTCTGACCCTAAACGATGCCGACTGTCGATCCGCCGGAGTTACTACCATGACTCGGCGGGCA
GCCTCCGGGAAACCAAAGTCTTTGGGTTCCGGGGGGAGTATGGTTGCAAAACTGAAACTTAA
AGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGTGGCTTAATTTGACTCCAACACGG
G

>N18S BL1F (Forward sequence)

CGTGCCTGCGGTCCGCCTCGAGGCGGATACTGCTCGTCCTGCGTTCGACGTCGTGGTGGTCCC
TTGGTGCTCTTGACTGAGTGTCTCGGGCGGTCCCGAATGTTTACTTTGAAAAAATTAGAGTGC
TCAAAGCAGGCGTATCGCCTGAATAATTCCGCATGGAATAATGGAATAGGACCTCGGTTCTAG
TTTCGTTGGTTTGCGAATCCTTGAGGTAATGATTAATAGGGACTGCCGGGGGCATACGTATTG
CGGCGGGAGAGGTGAAATTCGTGGATCGCCGCAAGACGAACGACAGCGAAAGCATTTGCCAA
GAATGTTCTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGACGATCAGATACCGTCGTAG
TTCTGACCCTAAACGATGCCGACTGTCGATCCGCCGGAGTTACTACCATGACTCGGCGGGCAG
CCTCCGGGAAACCAAAGTCTTTGGGTTCCGGGGGGAGTATGGTTGCAAAACTGAAACTTAAA
GGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGTGGCTTAATTTGACTCCAACACGGG.

**STEPS INVOLVED IN SEQUENCNG**

| |
|---|
| DNA extraction using Phenol Chloroform method |
| ↓ |
| Polymerase chain reaction(PCR)- *18srRNA* |
| ↓ |
| Purification of *18srRNA* PCR products |
| ↓ |
| Cycle sequencing of purified *18srRNA* PCR products |
| ↓ |
| Clean-up/purification of *18srRNA* Cycle sequencing product |
| ↓ |
| Denaturation and snap chilling of purified Cycle sequencing product |
| ↓ |
| Sequencing *18srRNA* (Forward/Reverse) |

## IV. DISCUSSION

Our results concur with numerous publications demonstrating that shell shape of Donacidae may vary greatly and that it is unreliable as a taxonomic feature[11&12]. This family is well known to span a wide spectrum of shell colour, pattern and shape. High heterogeneity has been documented for *D. variabilis* Extreme diversity of shell characters and the absence of clear diagnostic features for Donacidae have also been reported in other studies[13&14], suggesting that variation in size, colour, shape and sculpture of the shells may be due to phenotypic plasticity rather than an expression of genetic differentiation between the populations. Donn[15] (1990) stressed the influence of locality and population density and documented that a highintertidal population of *D. serra* at a higher density had thicker and heavier shells, whereas low-intertidal or subtidal populations possessed flatter, more rounded shells. Considering the observed specimens, it can be suggested that the D. marincovichi morphotype may be more adapted to intermediate beaches because it has a flatter and less wedge-shaped form (Figure 3), whereas the *D. obesulus*

morphotype from northern Chile and Peru may be more adapted to reflective beaches, because it is shorter and more wedge-shaped[16]. The diversity of synonyms of Donacidae reflects taxonomical confusion that is mainly due to the use of unreliable shell characteristics for species determination. Future research should include faster-evolving nuclear markers (e.g. AFLP, microsatellites) that could extend our findings based on mitochondrial sequences, and resolve more recent evolutionary events and at a finer geographic resolution than the present study[17].

## V. CONCLUSION

In conclusion, as a consequence of environmentally driven phenotypic plasticity, analysis of shell morphology may be unsuitable for the delimitation of Donax species. Phenotypic plasticity in shape, dimensions, sculpture and colour can be considered adaptive for species living in environments that are physically and biologically dynamic, where it can give rise to distinctive ecomorphs. Present result mentioned above is only based on the BLAST search of aligned sequences. We could confirm the genus as *Donax*. Even though the sequences showing 99% similarity with *Donax deltoides* and *D. faba*; the literature search for related genus shows that it may be *Donax variabilis*. The researchers need to confirm the species before submitting in NCBI using other relevant documents.

## VI. ACKNOWLEDGEMENTS

Author is grateful thanks to the Principal, Head of the Department and other staff members of Zoology, TBML. College, Porayar, Tamil Nadu, India for providing necessary facilities.

## REFERENCES

[1] Ruppert, H., and Fox, R. 1988. *Seashore Animals of the Southeast*. Columbia: University of South Carolina Press.

[2] McLachlan, A. 1981. Sand beach energetics: An ecosystem approach towards a high energy interface. Estuar. Coast. Shelf Sci., 13(1): 11-25.

[3] De la Huz, R., Lastra, M., and Lopez, J. 2002. The influence of sediment grain size on burrowing, growth and metabolism of *Donax trunculus* L. (Bivalvia: Donacidae). J. Sea Res., 47(2): 85-95

[4] Nel Ronel., Anton McLachlan., Deo, P.E., and Winter. 2001. The effect of grain size on the burrowing of two *Donax* species. J. Experimen. Mar. Biol. Ecol., 265(2): 219- 238.

[5] Leber, K.M. 1982. Bivalves (Tellinacea, Donacidae) on a North Carolina beach-- contrasting population-size structures and tidal migrations. Mar. Ecol. Prog. Ser. 7(3): 297-301

[6] Jablonski, E., 2001. The population ecology and reproductive biology of coquina clams (*Donax variabilis*) on a Florida beach.

[7] Tiffany, W.J. 1971. The tidal migration of *Donax variabilis* Say (Mollusca: Bivalvia). *Veliger*. 14(1): 82-85.

[8] Schneider, D. 1982. Predation by ruddy turnstones (*Arenaria interpres*) on a polymorphic clam(*Donax variabilis*) at Sanibel Island, Florida. Bull. Mar. Sci., 32(1): 341-344.

[9] Ansell, A.D. 1983. Sandy beaches as ecosystems: the biology of the genus *Donax*. In Developments in Hydrobiol., 19: 608-635.

[10] Cobb, J.C., Stephenson, S.P., and Arnold, W.S. 2011. Reproductive cycle and recruitment patterns of the coquina clam *Donax variabilis* say along the central Gulf coast of Florida. J. Shellfish Res., 30(1): 25-34.

[11] Olsson, A.A. 1961. Mollusks of the tropical eastern Pacific, Panamic Pacific Pelecypoda. Paleontological Research Institute, Ithaca.

[12] Guzman, N., Saa, S., and Ortlieb, L. 1998. Cata´logo descriptivo de los moluscos litorales (Gastropoda y Pelecypoda) de la zona de Antofagasta 238S (Chile). Estudios *Oceanolo´gicos*, Universidad de Antofagasta, 17: 17-86.

[13] Wade, B.A., 1967a. On the taxonomy, morphology, and ecology of the beach clam, *Donax striatus*. Bull. Mar. Sci., 17: 723-740.

[14] Adamkewicz, S.L. 1989. Differences in the frequencies of several characters in the clam *Donax variabilis* around Cape Hatteras, North Carolina. Veliger, 32: 21-28.

[15] Donn, T.E.J. 1990. Morphometrics of *Donax serra* Roding (Bivalvia: Donacidae) populations with contrasting zonation patterns. J. Coastal Res., 6: 893-901.

[16] Defeo, O., and Mclachlan, A. 2005. Patterns, processes and regulatory mechanisms in sandy beach macrofauna: a multi-scale analysis. Mar. Ecol. Prog. Ser., 295: 1-20.

[17] Held, C., and Leese, F. 2007. The utility of fast evolving molecular markers for studying speciation in the Antarctic benthos. Polar Biol., 30: 513-521.