11. SUMMARY

1. Standardization of DNA extraction
   - In the present study two commercial kits, three CTAB (cetyltrimethylammonium bromide) and one salting-out based DNA extraction methods were tested for its efficacy to extract DNA from marine invertebrates namely polychaetes, molluscs and crabs.
   - A simple and efficient CTAB based DNA extraction method from ethanol preserved specimens was standardized.
   - DNA extracted using the standardized method was amenable to RAPD (Random amplified polymorphic DNA) and successful amplification of COI (Cytochrome c Oxidase Subunit I) gene.
   - The OD of the DNA at 260/280 ranged from 1.8 to 2 for all the samples.
   - The standardized protocol will be useful for DNA extraction from other invertebrates exhibiting high polysaccharide and secondary metabolites.

2. DNA Barcoding and phylogenetic analysis of polychaetes using COI gene
   - 14 Polychaete species belonging to eight families were successfully amplified using mitochondrial cytochrome oxidase subunit I gene with universal primers LCO1490 and HCO2198 (Ward et al., 2005).
   - The maximum nucleotide compositions of A+T content was recorded as 62.00% in Diopatra neapolitana and minimum of 55.00% in Laonice cirrata. Similarly, the maximum C+G value of 45.00% was observed in Laonice cirrata and least content of 38.00% was estimated in Capitella capitata and Diopatra neapolitana respectively.
Summary

- The maximum genetic distance (1.459) was found between Lubrineridae and Capitellidae family. Whereas, the minimum distance of 0.270 was observed between Nereididae and Spionidae.

- Within family the maximum genetic distance was observed in Lubrineridae (0.197) and the minimum value of 0.005 was recorded in the within Spionidae family.

- The maximum K2P distance (1.459) was found between the genus Capitella and Lumbrineris whereas the minimum distance (0.230) was observed between Platynereis and Perinereis.

- The mean K2P genetic variation was maximum (0.5846) among inter family followed by inter genus (0.5091). The least mean variation can be observed amid intra genus (0.0560) polychaete individuals.

- Tajima’s statistic (D) value was found to be positive with an average of 2.001 among the genus. The maximum D value of 2.332381 was observed in Marphysa sanguinea and the minimum in Diopatra neapolitana.

- The phylogenetic analysis using maximum likelihood tree showed consistent clade pattern with good bootstrap support in both congeneric and conspecific clades.

- The nucleotide diversity in COI gene sequences was found satisfactory to discriminate all the species of Polychaete.

3. Molecular identification of polychaetes based on 18S rRNA

- 18S rRNA region of ribosomal gene was successfully amplified for 14 Polychaete species belonging to eight families using universal primers 18SA and 18SB (Medlin et al., 1988).
Summary

• The maximum nucleotide compositions of A+T content was recorded as 52.30% in *Perinereis camiguinoides* and minimum 43.80% in *Naineris quadricuspida*. Similarly, the maximum C+G value of 56.20% was observed in *Naineris quadricuspida* and least content of 47.7% was estimated in *Perinereis camiguinoides*.

• The maximum genetic distance (1.602) was found between Orbiniiidae and Capitellidae family. Whereas, the minimum distance of 0.058 was observed between Lumbrineridae and Glyceridae.

• Within family the maximum genetic distance was observed in Nereididae (0.188) and the minimum value of 0.003 was recorded in the within Capitellidae and Glyceridae family family.

• The maximum K2P distance (1.620) was found between the genus *Scoloplos* and *Capitella* whereas the minimum distance (0.026) was observed between *Platynereis* and *Laonice*.

• The mean K2P genetic variation was maximum (0.5360) among inter family followed by inter genus (0.4235). The least mean variation was observed amid intra genus (0.0006) polychaete individuals.

• The phylogenetic analysis using maximum likelihood tree showed consistent clade pattern with good bootstrap support in both congeneric and conspecific clades.

• The 18S-rDNA was found to be a useful molecular marker within the polychaetes to test the monophyly of a “family”.

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4. Phylogeny of polychaetes based on ITS rRNA

- This study presents the first report to infer the phylogenetic relationship of polychaetes from Vellar estuary, Tamil Nadu, India based on ITS region.

- ITS region of ribosomal gene was successfully amplified for 10 Polychaete species belonging to five families (Nereididae, Lumbrineridae, Glyceridae, Orbiniidae and Spionidae) using ITS4 and ITS5 primers (White et al., 1990).

- The maximum genetic distance between species was found as 2.403 among Glycera capitata and Lumbrineris inflate. Whereas the minimum K2P distance (0.003) was observed between Perinereis camiguinoides and Platynereis dumerilii.

- The phylogenetic analysis based on the maximum likelihood method showed two major clades; one clade comprising 10 species belonging to three families (Nereididae, Lumbrineridae, Orbiniidae) and the next clade consisting of only Glyceridae with strong bootstrap value support of more than 90.

- ITS sequences in the present study show that DNA sequence data can resolve systematic relationships and could be potential barcode gene.

5. Extracellular biosynthesis of silver nanoparticles by marine invertebrate (polychaete) and assessment of its efficacy against human pathogens

- The present study reports an ecofriendly and cost effective extracellular synthesis of silver nanoparticles using marine invertebrate (polychaete) extract at room temperature.
Summary

- The Ultra Violet-Visible spectroscopy (UV-Spec) revealed the formation of silver nanoparticles (AgNPs) by exhibiting the typical surface plasmon absorption maximum at 418–420 nm.

- The structure and composition of silver nanoparticles were analyzed by atomic force microscopy (AFM).

- The average particle size of silver nanoparticles was found in the range of 40–90 nm confirmed by scanning electron microscopy (SEM) analysis.

- The energy-dispersive X-ray spectroscopy (EDX) of the nanoparticles dispersion confirmed the presence of elemental silver signal, whereas X-ray diffraction (XRD) substantiated the crystalline nature of synthesized nanoparticle.

- Fourier transform infrared spectroscopy (FTIR) spectral analysis showed the presence of amides phenols, ethers and fatty acids as major biomolecules responsible for the reduction of silver ions.

- This study advocates that, not only plants and microbes, but marine invertebrates do have potential for synthesizing nanoparticles by a cost effective and eco-friendly approach.

6. Polychaete fatty acids as potential inhibitor against human Glioblastoma multiforme

- The fatty acid methyl esters (FAME) of polychaete species, Namalycastis abuima was assessed for the inhibition property against GBM.
Totally 29 fatty acids and previously reported potential compounds namely Anilinoquinazoline, Thalidomide and Tetracenomycin D3, were structurally optimized and docked against the GBM target proteins EphA2, EGFR and EGFRvIII in Arguslab software.

Interestingly all the fatty acids showed better docking energy (-14.128 to -9.224 Kcal/mol) than the previously reported drugs (-10.582 to -5.874 Kcal/mol).

Among the fatty acids, polyunsaturated fatty acids (PUFA) were found to be the most effective as evident by the strong interaction with the target proteins.

The *in silico* ADME results further substantiated the efficacy of fatty acids as a potential natural source which could inhibit the activity of the overexpressed mutant type EGFR.