



Synthesis and characterization of gold nanoparticles using *Brevibacterium casei* (SOSIST-06) isolated from Southern Ocean water samples and their *in vitro* and *in silico* anti-WSSV activity

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Harnessing microbial biosurfactant for agricultural applications

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Eshwarnath, V. S., Thyagarajan, R., Kishorekumar, A., Gopikrishnan, V., Radhakrishnan, M. and Song, J. J. (2024). Harnessing microbial biosurfactant for agricultural applications. International Journal of Agricultural Technology 20(1):87-104.

Abstract Biosurfactant refers to surface-acting substances that can enhance surface-surface interactions by generating micelles that are derived from natural sources including plants, microorganisms, and animals. Biosurfactants are a structurally diverse group of secondary metabolites with lots of potential to serve mankind. The structural and compositional diversity of biosurfactants is unambiguously substrate-dependent. Microbial surfactants are environment-friendly alternatives to synthetic surfactants. Biosurfactants are surface-active agents produced by microorganisms that have higher efficiency and stability, lower toxicity and higher biocompatibility and biodegradability than chemical surfactants. The differences between biosurfactant production can be attributed to the different compositions of the hydrolyzates.

Keywords: Agriculture, Biodegradability, Biosurfactant, Plant defence, Sustainability

Introduction

Investigating antagonistic activity of fish gut bacterial isolates against aquaculture pathogens: Short Communication

Prakash, M. P. K.¹, Padmanaban, D.², Loganathan, C.², Palaniswamy, K.², Krishnamoorthy, K.², Manikkam, R.¹, Vareeket, R.^{3,4} and Venugopal, G.^{1*}

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Prakash, M. P. K., Padmanaban, D., Loganathan, C., Palaniswamy, K., Krishnamoorthy, K., Manikkam, R., Vareeket, R. and Venugopal, G. (2024). Investigating antagonistic activity of fish gut bacterial isolates against aquaculture pathogens: Short Communication. International Journal of Agricultural Technology 20(3):1197-1200.

Abstract Aquaculture worldwide suffers significant economic losses and high fish mortality due to bacterial diseases. Antibiotics have been used extensively to combat these infections owing to the overuse of antibiotics, Drug-resistant bacteria can emerge. Studies on probiotics, which provide improved growth and disease resistance, are becoming more and more popular as an antibiotic substitute, Bacteria derived from a marine setting such as fish gut might be putative candidate for aquaculture probiont. In vitro antagonistic activity against fish pathogens has been used to assess the antimicrobial properties of bacteria isolated from the gut of *Sardinella longiceps* and *Rastrelliger Kanagurta*.

Exploration of fish gut-associated actinobacteria for its antifouling activity

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Abstract

An attempt has been made to screen the fish gut-associated actinobacterial cultures for antifouling compounds. Fifteen morphologically distinct bacterial cultures were recovered from the biofouling samples scraped from the boat surfaces and other maritime structures in the Kovalam beach (Lat. 12.7870°N; Long. 80.2504°E) coastal areas in Tamil Nadu, India. All the bacterial isolates were identified at generic level from which two isolates namely KB6 and KB7 as *Staphylococcus* sp. were found the exhibit strong biofilm formation. Four actinobacterial strains viz., IM20, PYA9, F8, and SQA4 were evaluated for inhibitory properties against biofouling bacteria using the agar plug method. Strain IM20 which strongly inhibited the biofouling bacteria was chosen as prospective strain for further studies. When compared to submerged fermentation, IM20 produced a high amount of antifouling chemicals on the agar surface fermentation. Among the solvents tested, better extraction of antifouling compounds was seen in ethyl acetate extract. Antifouling compound production by the strain IM20 was found to be influenced by a number of variables such as glucose, fructose, glutamine, malt extract, pH 7 and 9, temperature 30 and 40°C and NaCl at 2.5% and 5% concentrations. Gas chromatography-mass spectrometry (GC-MS) analysis of the strain IM20 extract revealed the presence of pyrrolo (1,2-a)pyrazine-1,4-dione, hexahydro in significant amount. In the present study, the fish gut-associated *Streptomyces* sp. IM20 was identified as an unusual and newly added source for the isolation of antifouling compounds.

KEYWORDS

actinobacteria, biofouling, extraction, fish gut, *Streptomyces*

Abbreviations: AF, antifouling; ASF, agar surface fermentation; F8, Fauna fish; IM20, Indian Mackerel fish; KB, Kovalam biofouling bacteria; MNPs, marine natural products; OFAT, one factor at a time; PBS, phosphate buffer saline; PYA9, Pondicherry actinobacteria; SQA4, squid actinobacteria; YEME, yeast extract malt extract.



Primary Ovarian Insufficiency: Current Understanding and Diagnostic Approaches

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Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 17 Oct 2023	Primary ovarian insufficiency (POI) is a medical condition where ovarian function stops prematurely, typically before the age of 40. This condition leads to infertility and produces symptoms similar to those experienced during menopause. Although the origins of POIs are diverse, genetic elements substantially influence their emergence. This assessment delves into the genetic facets of POI, covering genetic triggers, detection, and genetic consultation. We scrutinize the genes linked to POI and their function in ovarian activity, as well as the genetic deviations and mutations that foster POI onset. We also examine the challenges and limitations of genetic testing and counseling for POI and suggest ways to address these challenges. This review offers a thorough examination of the existing understanding of the genetic factors linked to Primary Ovarian Insufficiency (POI) emphasizing the critical need for further investigation in this field.
CC License CC-BY-NC-SA 4.0	Keywords: Primary ovarian insufficiency, chromosome abnormalities, Genetic Factors, Mutations, Infertility, Receptors

1. Introduction

Primary ovarian insufficiency (POI), also called premature ovarian failure or early menopause, is a condition where ovarian function ceases before age 40. This leads to irregular menstrual cycles, reduced fertility, and decreased estrogen production, resulting in various symptoms and health concerns. Diagnosis involves primary or secondary amenorrhea, elevated gonadotropin levels, and low estradiol levels [1]. It affects about 1% of women under 40, with lower prevalence in younger age groups [2].

The term "primary ovarian insufficiency" encompasses related conditions like hypogonadotropic hypogonadism, premature ovarian failure, and ovarian dysgenesis, all involving disrupted ovarian function, lowered estrogen levels, irregular menstrual cycles, and reduced fertility [3]. POI was first documented by Albright and colleagues in 1942, characterized by absent or irregular menstrual cycles, insufficient sex hormone levels, and a diagnosis before age 40 [2]. Diagnosis requires two evaluations of serum FSH levels exceeding 40 IU/L at least one month apart in a woman under 40, indicating a deviation from the average age of natural menopause onset [2]. POI is significant as it can cause infertility and low sex hormone levels in premenopausal women [3]. The condition presents with

The Role of Hla Genes in Immune Response, Disease Susceptibility, and Social Behaviours: A Comprehensive Review

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Article History	Abstract
Received: 06 June 2023 Revised: 15 Sept 2023 Accepted: 01 Nov 2023	Major Histocompatibility Complexes (MHC), which assist to code for proteins that distinguish between self and non-self, are significantly influenced by the Human Leukocyte Antigen (HLA) genes. Particularly important in the suppression of immune response are the HLA genes. The bulk of the genes in the MHC region shows considerable variation. The two most important functions of HLA molecules are selection of T cell accumulation and the formation and control of immunological responses. The causes of HLA-G gene-associated illnesses and the underlying mechanisms are still up for dispute. The HLA-G gene has an impact on social behaviour as well. Numerous polymorphisms have been connected to heightened susceptibility to the beginning of autoimmune illnesses as well as heightened disease severity. The lifetime of some HLA genes is shorter. Genetic background, environmental circumstances, and certain polymorphisms have been linked to increased illness severity. certain HLA genes have shorter life spans than others, and vice versa. The major functional elements of HLA-G in both normal and autoimmune disorders are summarized in this study.
CC License CC-BY-NC-SA 4.0	Keywords: HLA-G, Polymorphism, Autoimmunity, Cancer, Immunosuppression

1. Introduction

HLA-G is one of the non-classical class I molecules that make up the Human Major Histocompatibility Complex, according to the first theory put out by Geraghty et al. in 1987. This subclass is distinguished by its restricted pattern of tissue expression and function in encoding immune-modulatory chemicals. The HLA region's class I genes were divided into three categories by Sommers (2003). These groups included the non-classical HLA-E, -L, -J, -K, -H, and -G genes as well as the classical HLA-A, -B, and -C genes. Moreau et al. (2009) noted that HLA-G has a distinct tissue-specific expression, especially in non-pathological situations, although sharing considerable similarities with classical HLA-class I genes. The crucial function of HLA-G in preserving fetal-maternal tolerance was clarified by Kovats et al. in 1990. Castelli (2014) separated HLA-G molecules (class Ib) from traditional HLA-A, HLA-B, and HLA-C molecules (class Ia) by highlighting restricted protein variability and the existence of numerous isoforms owing to alternative splicing of mRNA. HLA-G, which was originally discovered on extravillous cytotrophoblasts, is a key factor in the mother immunological tolerance for the semi-allograft fetus throughout pregnancy, according to Xu et al. (2020).

Polymorphic variations in the HLA-G gene may have an effect on the transcription level of the gene, according to Donadi et al. (2011). HLA-G genes have less polymorphism than traditional HLA

Association between Polymorphisms of Vitamin D Receptor and Lung Cancer Susceptibility: A Systematic Review and Meta-Analysis

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Abstract

The association between polymorphisms of the vitamin D receptor (VDR) gene and lung cancer susceptibility has been extensively investigated; however, the results have been inconsistent. This systematic review and meta-analysis aimed to provide a comprehensive evaluation of the relationship between VDR gene polymorphisms and the risk of lung cancer. A systematic literature search was conducted, and relevant studies published up to march 2023 were included. The studies investigating the association between VDR gene polymorphisms and lung cancer risk were selected. Data extraction, quality assessment, and meta-analysis were performed following established guidelines. Pooled odds ratios (ORs) with 95% confidence intervals (CIs) were calculated using random-effects models to estimate the overall association. A total of 10 studies were identified that met the inclusion criteria and were included in the meta-analysis. These studies consisted of 25,868 cases and 30,954 controls. The characteristics of the included studies are summarized in Table 1. The meta-analysis revealed a significant association between VDR gene polymorphisms and lung cancer susceptibility. The pooled odds ratio (OR) for the association between the FokI polymorphism and lung cancer risk was 1.19 (95% CI: 1.06-1.33, $p=0.003$), indicating a modestly increased risk of lung cancer associated with the FokI polymorphism. However, significant heterogeneity was observed among the included studies ($I^2=70\%$, $p<0.001$). Subgroup analysis by ethnicity showed that the FokI polymorphism was associated with an increased risk of lung cancer in Asians (OR=1.27, 95% CI: 1.10-1.46, $p=0.001$), but not in Caucasians (OR=1.05, 95% CI: 0.92-1.20, $p=0.44$). When stratified by smoking status, a significant association was observed between the FokI polymorphism and lung cancer risk in smokers (OR=1.22, 95% CI: 1.06-1.41, $p=0.006$), but not in non-smokers (OR=1.05, 95% CI: 0.89-1.25, $p=0.55$). This systematic review and meta-analysis suggest that the FokI and BsmI polymorphisms of the VDR gene may contribute to an increased risk of lung cancer. The findings highlight the potential role of genetic variations in the VDR gene as biomarkers for lung cancer susceptibility. Further research with larger sample sizes and diverse populations is necessary to validate these associations and explore potential gene-environment interactions. Understanding the genetic factors involved in lung cancer susceptibility can aid in risk assessment, early detection, and personalized prevention strategies.

Keywords- vitamin D receptor, VDR, polymorphisms, lung cancer, susceptibility, meta-analysis

I. INTRODUCTION

Lung cancer is a complex disease influenced by both genetic and environmental factors. The vitamin D receptor (VDR) plays a crucial role in the cellular response to vitamin D, which is involved in regulating cell growth, differentiation, and apoptosis. Given the importance of VDR in cancer-related processes, several studies have investigated the association between VDR gene polymorphisms and the risk of developing lung cancer¹.

Among the VDR gene polymorphisms studied, the FokI, BsmI, TaqI, and ApaI variants have received particular attention due to their potential impact on VDR function and subsequent signaling pathways. These polymorphisms can affect the binding affinity of VDR to its ligands, alter gene expression, and modify the cellular response to vitamin D². Consequently, variations in these polymorphisms may contribute to interindividual differences in lung cancer susceptibility.

***In silico* Docking Analysis of the FDA-Approved Drugs on Envelope Protein of SARS CoV-2 Omicron Variant**

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The current pandemic situation is created by the highly evolving SARS coronavirus 2 which is having several mutations in its structural proteins. The structural proteins of SARS CoV-2 include spike (S), Envelope (E), Membrane (M), and Nucleocapsid (N) which are primarily responsible for the infection, transmission, and pathogenesis of the virus. Envelope protein is the smallest of the four proteins containing 75 amino acids with a molecular weight of about 8 kDa. The major functions of the hydrophobic envelope protein include envelope formation, budding, replication, and release of the virion. The presence of mutation on the envelope protein results in improper formation of the pentameric structure and also hinders other functional properties. Our computational analysis majorly focuses on several FDA-approved inhibitory compounds that bind to SARS CoV-2 envelope protein that help in the inhibition of virion formation. The percentage of similarity of the envelope protein between SARS CoV and SARS CoV-2 is approximately 96 percent. The homology-modeled structure of the SARS CoV E protein was downloaded from Protein Model Database (PMDb) and the mutation which was found to be consistent among most of the SARS CoV-2 variants was selected as T9I which is present in the N-terminal region. This mutation was introduced into the SARS CoV Envelope protein and was remodeled. The ligands which were approved by FDA were selected for docking analysis to understand their binding capabilities with the envelope protein. Ligands such as Beta-D-Fucose, Mycophenolic Acid, Castanospermine, 1-Deoxynojirimycin, Nafcillin, Guaifenesin, Nabumetone, Cinametic Acid, Lauric acid were used in our study. The docking simulations revealed that Lauric acid, Nafcillin, Nabumetone, and Mycophenolic acid have high binding energy with the SARS CoV-2 wild type (Wuhan) and mutant E protein of the SARS CoV-2 (Omicron) variant. This *In silico* data gives insights to test these high binding compounds in *in vitro* studies to prove their efficacy and the protein-protein interactions of envelope protein with its other partnering proteins. These pharmaceutical compounds are a potential alternative in the future for a novel drug development to treat several emerging variants of SARS CoV-2.

Keywords: Docking; Drug Development; Envelope protein; Omicron; SARS CoV-2.

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Genotypic characterization of HPV serotypes in cervical samples from Indian women: a multi centric study

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Ganesh Kumar Sarvesan, Krupakar Parthasarathy , Chirayu Padhiar & HariBalaji V

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Abstract

Human papilloma virus (HPV infection) plays a important role in causing cervical cancer. Out of 184 different HPV genotypes, 40 diverse types only can cause anogenital infection. HPV present in >99% of cervical cancers with high risk types (16, 18) associated with cancer and Low risk types (6, 11) are associated with genital warts. Aim of the study is to determine the epidemiology of HPV infection in Indian women's population. Three hundred and thirty four liquid based cytology (LBC) samples were collected across India from the different age groups of Indian women. Pap smear, PCR and gene sequencing tests were performed for the collected LBC samples. Low risk serotype 6 (16.7%) were detected compared to other high risk serotypes. Majority of positive cases were observed in the age group between 19 and 39 groups. Northern part of India contributes high (7.1%) in HPV infection compared to other regions of India. Reports from these studies covered few regions of India showing a wide range in the prevalence of HPV infection and serotype distribution due to diversified socio economic and geo climatic conditions. This study aims to identify the epidemiology of HPV in the Indian population and concludes that early and periodic screening of women for HPV can avoid the risk of cervical cancer at the early stage of infection.

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Latent Tuberculosis: Challenges in Diagnosis and Treatment, Perspectives, and the Crucial Role of Biomarkers

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Abstract

Tuberculosis (TB) is the major cause of morbidity and mortality globally, which is caused by a single infectious agent *Mycobacterium tuberculosis*. For years, many TB control programmes are established for effective diagnosis and treatment of active TB cases, but these approaches alone are insufficient for TB eradication. This review aims to discourse on the crucial management of latent tuberculosis infection. This review will first summarize the current status, and methods for diagnosing latent tuberculosis then describes the challenges involved in the diagnosis and treatment of latent tuberculosis, and finally encounters the purpose of biomarkers as predicting tool in latent tuberculosis.

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Accelerated mutation by host protein APOBEC in Monkeypox virus

Mohamed Jameer Basha Jahankir, Vignesh Sounderrajan, Sudhanarayani S. Rao, T. Thangam, Neelagandan Kamariah, Anandi Kurumbati, Shakila Harshavardhan, Archana Ashwin, Sakthivel Jeyaraj, Krupakar Parthasarathy  

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Highlights

- Higher rate of mutation in DNA virus
- Accelerated mutation on APOBEC in MPXV
- 5 mutations (R246Q, L738S, F389L, P740L, D785N) affect proof reading mechanism.

Lung Organoids: Systematic Review of Recent Advancements and its Future Perspectives

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Abstract

Organoids are essentially an *in vitro* (lab-grown) three-dimensional tissue culture system model that meticulously replicates the structure and physiology of human organs. A few of the present applications of organoids are in the basic biological research area, molecular medicine and pharmaceutical drug testing. Organoids are crucial in connecting the gap between animal models and human clinical trials during the drug discovery process, which significantly lowers the time duration and cost associated with each stage of testing. Likewise, they can be used to understand cell-to-cell interactions, a crucial aspect of tissue biology and regeneration, and to model disease pathogenesis at various stages of the disease. Lung organoids can be utilized to explore numerous pathophysiological activities of a lung since they share similarities with its function. Researchers have been trying to recreate the complex nature of the lung by developing various “Lung organoids” models.

This article is a systematic review of various developments of lung organoids and their potential progenitors. It also covers the in-depth applications of lung organoids for the advancement of translational research. The review discusses the methodologies to establish different types of lung organoids for studying the regenerative capability of the respiratory system and comprehending various respiratory diseases.

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Designing of a novel and potent HPV66 L1 major capsid protein-epitope based therapeutic vaccine against Human Papillomavirus (HPV): A bioinformatics approach

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Abstract

Aim: Oncogenic human papillomaviruses (HPV) cause various types of cancer, including cervical cancer. The main protein of HPV is capsid, targeted in many vaccine attempts. However, these vaccines do not cover enough high-risk HPV serotypes. Therefore, a low-cost potential HPV vaccine to protect against all serovars of the α -papillomaviruses family would be promising in the future. Our study aimed to develop a therapeutic epitope vaccine for HPV using bioinformatics methods.

Methodology: Bioinformatics approach was followed to analyze and identify potential T-cell and B-cell dominant epitopes of HPV-66 L1 major capsid protein. Additionally, various aspects of this protein were examined, including its physico-chemical properties, and secondary and tertiary structures. These analyses helped us to design an effective HPV infection therapeutic vaccine.

Results: The findings revealed that the L1 major capsid protein was unstable and hydrophilic. The secondary structure of this protein composed 39% α -helices, 17.97% β sheets and 31.80% loops. Our study demonstrated 19 dominant epitopes of HPV-66 L1 major capsid protein including 5 B-cell epitopes and 14 T-cell epitopes (10 HTL epitopes, and 4 CTL epitopes) for a novel vaccine candidate.



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Analysis of L1 gene sequence variations in high-risk and low-risk Human Papillomavirus (HPV) serotypes among indian women: implications for cervical cancer research

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Bio-therapeutic intervention for dengue infection-present scenario on plant based remedies : A review article

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Abstract Dengue virus (DENV), a member of the *Flaviviridae*, is the causative organism of Dengue hemorrhagic fever and Dengue Shock Syndrome. It exists as 4 serotypes (DEN1-4), all of which can cause full spectrum of the disease. Dengue is one of the most re-emerging arboviral diseases in the world with a record of 390 million cases globally, putting 3.9 billion people at the risk of infection annually. With dengue being enlisted as a high burden disease in the tropical countries, there is a dire need to bring about efficient therapeutics that help to prevent and treat the diseased population. The present mode of treatment of Dengue is based on the management of symptoms and includes the use of Non-steroidal Anti-inflammatory Drugs (NSAIDs), antibiotics, antipyretics and corticosteroids. These provide symptomatic treatment and also bring about undesirable side effects. Presently, there are no approved vaccines or antivirals for Dengue treatment in India. It is thus important to consider the biology of the virus with special focus on identification of effective anti-viral targets for therapy. With the alleviation of symptoms alone being insufficient for the disease treatment, there is a shift in research to find alternative forms of medicine for the treatment of the disease. Herbal remedies have a long history of use in ancient medical systems and are now widely accepted as safe, natural substitutes for pharmaceutical treatments. But in order to guarantee consistency and safety, the growing demand for these items calls for strict quality control methods. In particular, leaves of *Carica papaya* and Nilavembu kudineer are potential candidates for this purpose. This review focuses on bringing light to various therapeutic interventions presently in use and emphasize the significance of quality control techniques in regulating various aspects of the

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Functionalized chitosan-G-poly caprolactone vaccine delivery system fabricated to display antigen–antibody immune complexes of *Mycobacterium tuberculosis* elicits immune response in *Ex-vivo* model

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Abstract

Background

Vaccine development against tuberculosis remains a global health imperative, necessitating robust immunogenicity and safety profiles. Nanoparticle-based delivery systems offer promising avenues to enhance vaccine efficacy while ensuring tolerability. This study explores the utilization of chitosan micelles as a delivery platform for immune complex vaccination against tuberculosis. Leveraging two key antigens of *Mycobacterium tuberculosis*, namely HspX and Mpt51, known for their relevance in latent tuberculosis and its co-infection with the human immunodeficiency virus, immune complexes were synthesized *in vitro* using antibodies raised against these antigens. The immune complexes were then conjugated onto chitosan micelles, characterized for their physicochemical properties, and evaluated for their biocompatibility and immunogenicity.

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Effects of dietary marine microalgae *Chlorella salina* on the growth, haematological, digestive enzyme, antioxidant activities and coloration of the freshwater ornamental fish Flame Red Gourami (*Tricogaster lalius*)

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Abstract

Ornamental fish trade is based on their brilliant colors and patterns, which frequently determine the fish's market worth. The aim of the study was to evaluate the effect of dietary marine microalgae *Chlorella salina* on the growth, digestibility, hematological, antioxidant enzyme activities and coloration of *Tricogaster lalius* (Flame red gourami). The experiment trial was conducted for 60 days by feeding different formulations of *C. salina* (100% algal feed (group I), algal feed mixed basal diet in the ratio of 1:1 (group II)) and a control diet with no microalgae added for comparison. From the results, the dietary inclusion of *C. salina* rich feed showed its effectiveness in enhanced growth performance, digestive enzyme, hematological and antioxidant activities in *T. lalius* when compared to the control. Color measurement lightness L* (70.19±0.12), Redness a* (4.51±0.23) and Yellowness b* (14.7±0.22) values were maximum found in *T. lalius* fed with *C. salina* rich diet after 60 days of feeding trials. It was found that increasing the amount of *C. salina* in the fish diet increases carotenoid levels in *T. lalius* skin. This study suggests that *C. salina* rich diet might be used as a necessary feed supplement as well as a coloring agent in the culture of ornamental fish *T. lalius*.

Keywords: *Tricogaster lalius*, *Chlorella salina*, skin color, CIElab, Ornamental fish.

1. Introduction

The ornamental fishes are among the most popular attractions in the world because of their beautiful colors, distinctive patterns and ability to adapt to a confined environment (Rajeswari et al. 2014). In recent years, the marine ornamental trade has increased and developed into a multibillion-dollar industry. It is estimated that over 2 million aquarium hobbyists across worldwide purchase more than 20 million marine ornamental fish from wild collections (Wabnitz 2003). The global ornamental fish trade is estimated to be worth more than US 15 billion dollars, including US 300 million dollars traded in marine ornamental fish (Satam et al. 2018; Pouil et al. 2020).

The price of ornamental fish on the global market is largely influenced by a variety of criteria, including color. Poor color performance in juveniles and adults may also result in price reduction, although this can easily be regulated by the use of a carotenoids-rich supplemental diet (Shahidi and Brown 1998). They also show their body colors for a variety of reasons, including identification, communication, camouflage, mimicry, defense, and adaptation to their environment (Saxena 1994). The fish's body colors were dependent on the special cells found in tissue known as chromatophores. There are four major categories of pigments such as melanins, carotenoids, pteridines and purines, that can be used to provide colors in these cells (Anderson 2001).

Carotenoids are important pigmenting molecules that are normally not synthesized by fish; instead, they must be obtained via eating algae, coral, or wild that have collected these pigments (Devi et al. 2016). Plants, algae, as well as bacteria and some yeast can biosynthesize the carotenoids (Pizarro and Stange 2009). The red, orange, and yellow color that can be found in fish and crustaceans are caused by carotenoids (Packer 1992). Ornamental fish traders are looking for ways to improve the color of their skin. Additionally, they are searching for natural pigments to replace synthetic pigments because synthetic pigments are more expensive (Ramamoorthy et al. 2010).

Dietary supplements of carotenoids are significantly influencing the fish color, and also essential nutrients for healthy growth and reproduction (Paripatananont et al. 1999; Gouveia et al. 2003). Several studies have investigated the usage of different algae as dietary supplements including *Porphyridium*, *Isochrysis*, *Pavlova*, *Chaetoceros*, *Gracillaria*, *Palmaria* and *Arthrospira* are effective color elicitors in cichlid fish, rainbow trout, fish larvae, bivalve molluscs and various gastropods (O'Connor and Heasman 1997; Kop and Durmaz 2008).

The consumer concern about the safety of using synthetic carotenoids in food has interested food producers to look for potential natural alternatives. As a result, the usage of natural ingredients in food and feed has expanded. Therefore, natural carotenoids generated from microalgae could serve as an alternative to synthetic carotenoids in the aquaculture industry (Pezeshk et al. 2019). The present work was undertaken to evaluate the potential of marine microalgae *C. salina* as a dietary carotenoid source for growth, haematological, digestive enzyme, antioxidant and coloring the skin of ornamental fish *T. lalius* (Flame red gourami).

2. Materials and Methods

2.1 Experimental design

Flame red gourami (*T. lalius*) (Initial weight: 4.26 ± 0.75) was purchased from a commercial aquarium fish farm and was acclimatized to the laboratory conditions. The experiments were conducted for 60 days. The fish were randomly divided into four treatments of 30 fish each and a control group. All the fish were held in transparent glass tanks (50 L, 45 (l) x

30 (w) x 45 (h) cm) with natural water supplied continuously under the experimental condition. Water quality parameters in each tank were checked and maintained at a temperature of 24-26°C, a dissolved oxygen level of 2-4 mg L⁻¹, a pH range of 7.0-7.1, and the unionized ammonia was never found in the concentrations greater than 0.01 mg L⁻¹. The experiment was carried out in a room illuminated by a fluorescent light set to a 12-hour light (1,200 lux) and 12-hour darkness cycle.

2.2 Ethics statement

Even though fish have been used in this study, no animals were stressed or sacrificed in the process, and every effort was made to minimize suffering. Principles of laboratory animal care have been followed, and experiments were carried out by the National Institutes of Health's regulations (Jenkins et al. 2014). This study was approved by the institutional ethical committee, Sathyabama Institute of Science and Technology (Ethical Clearance, Biosafety and Animal Welfare Committee).

2.3 Algal material and culture condition

The marine microalgal strain, *C. salina* was cultured in a fresh convey medium (Anderson 2001) at laboratory conditions and inoculum to fresh convey medium ratio of 1:9 under aseptic conditions. A total working volume of one litre of *C. salina* was cultivated at a temperature of 20-25°C, light intensity of about 2000 Lux, 16:8 h light–dark cycle and supplied with continuous aeration. The algal cultures were collected when they reached the early stationary phase of the first stage of cultivation (15 days) (Chong et al. 2019).

2.4 Experimental Feed Preparation

In this study, three types of diet were formulated. In Group 1, the fish were only fed an algal diet of *C. salina*. In Group II, the fish were fed with a basal diet mixed with algal feed in a ratio of 1:1. In Group III, the fish received only a basal diet. The formulation of the basal diets was prepared using the method described by (Thirumalaikumar et al. 2021), as shown in Table 1. The proximate composition of the control and experimental diets were analyzed as per the standard method (Cunniff and Washington 1997), as displayed in Table 2.

Nutrient (%)	Diet (% dry weight)
	Control
Crude protein	29.52 ± 0.13
Lipid	8.33 ± 0.28
Ash	10.36 ± 0.86
Moisture	5.28 ± 0.55

Table 1. Formulation of the experimental diet (g %).

Ingredients	(g%)
	Basal diet
Fish meal	14
Ragi flour	15
Wheat flour	14
Corn flour	17
Rice bran	10
Groundnut oil cake	18
Tapioca powder	5
Fish oil	5
Vitamin premix ^a	1
Mineral premix ^b	1

Table 2. Proximate analysis

1 g protein = 5.6 kcal; 1g lipid = 9.4 kcal.

^a Vitamin (IU or mg per kg premix): Vitamin A, 700000IU; Cobalamin, 200IU; Vitamin D3, 70000IU; Vitamin E, 250 mg; Nicotinamide, 1 g.

^b Mineral premix (per kg premix): Cobalt, 150 g; Copper, 1200 mg; Iodine, 325 mg; Iron, 1500 mg; Magnesium, 6000 mg; Manganese, 1500 mg; Potassium 100 mg; Sodium, 5.9 mg; Sulphur, 0.72%; Zinc, 9600 mg; Calcium, 25.5%; Phosphorus, 12.75%.

All dry ingredients were powdered, passed through a 650 µm sieve and mixed with water until very soft biscuit-textured dough was obtained. Then the mixture was steam sterilized (autoclaved at 120 °C for 20 min) and pelletized by hand to get pellets of 3 mm size. The pellets were dried in a hot-air oven at 55–60°C for 48 h until the final moisture content was less than 15%. After drying, lutein was mixed with experimental diets with 2.5% fish oil and dried in a hot air oven at 35°C for 30 min. All feeds were stored in opaque containers at 4°C before use.

Fishes were fed at the rate corresponding to 3% of body weight twice a day in all the experiments. Water in the experimental tanks was changed every day to provide the fish with adequate oxygen, remove the faeces matter and uneaten feed. At the end of the experiment, all fishes were starved for one day to take the final wet weight. The observations for color enhancement of the fish were measured initially and at end of the experiment.

2.5 Analysis of biological indices

The biological indices (growth performance, weight gain, specific growth rate, feed utilization and survival rate) of the *T. lalius* were evaluated by the following formulae (Gouveia et al. 2003; Kalinowski et al. 2005).

Body weight gain (BWG) = [(Final body weight (g) - Initial body weight (g))/Initial body weight].

Specific growth rate (SGR) = $[\text{In Final body weight (g)} - \text{In Initial body weight(g)} / \text{Number of days}] \times 100$.

Feed conversion ratio (FCR) = $\text{Feed intake (g)} / \text{Weight gain (g)}$.

Survival rate = $(\text{Final live fish} / \text{Initial live fish}) \times 100$.

2.6 Blood and tissue samples collection

On the 30th and 60th days of the feeding trial, each group of fish were euthanized by bath immersion in 50 μL^{-1} clove oil (Himedia, GRM340) for 5 minutes. After anaesthesia, blood samples were collected from the fish caudal vein using a 1 ml plastic syringe containing a pinch of anticoagulant (0.5% EDTA) used for haematological assessment. Additionally, organs such as the gut, and muscle were dissected from anaesthetized fishes in each experimental and control group to study digestive and antioxidant analysis (Paulpandian et al. 2023). 50 μL^{-1} clove oil (Himedia, GRM340) for 5 minutes. After anaesthesia, blood samples were collected from the fish caudal vein using a 1 ml plastic syringe containing a pinch of anticoagulant (0.5% EDTA) used for haematological assessment. Additionally, organs such as gut, and muscle were dissected from anesthetized fishes in each experimental and control group to study of digestive and antioxidant analysis (Paulpandian et al. 2023).

2.7 Haematological assessment

Haematological parameters such as total red blood cells (RBC), white blood cells (WBC), haemoglobin and haematocrit analyses from the collected blood samples with anticoagulants were analysed by using all combinations of automated haematology analyzer (Aspen/Rapid Diagnostic, India) (Levina et al. 1997).

2.8 Evaluation of digestive enzymes

After the 30th and 60th days of the feeding, digestive enzymes like protease, amylase, and lipase were evaluated in the gut tissues of both the experimental and control groups (Bernfeld 1955; Furne et al. 2005). Total amylase, protease and lipase production were determined using Nutrient agar plates supplemented with 1% (v/v) starch 1% (v/v) skimmed milk and 1% (v/v) of tributyrin, respectively. Culture Petri plates were spot inoculated and incubated at 37°C for 24 hours. After incubation, starch agar plates were flooded with iodine solution, appearance of yellow discoloration revealed amylase production. Skim milk agar plates showed a zone of clearance around the inoculum indicating proteinase production. Likewise, tributyrin nutrient agar plates showed a clear zone around the inoculum revealing lipase production.

2.9 Determination of antioxidant properties

Individual samples of the experimental and control group fish's muscle tissue were homogenized in 10% ice-cold 50mM Tris buffer (pH 7.4). The homogenized samples were centrifuged at 12,000 rpm for 15 minutes at 4°C, with the supernatant used to analyse the enzyme activity such as superoxide dismutase (SOD) and catalase (CAT) activity using a commercially available kit (Sigma-Aldrich, Chemie) according to the instruction of the manufacturer. The absorbance of SOD and CAT activities were measured at 550 nm and 405 nm, respectively. CAT activity (1 unit) is referred to as the amount of the sample required to catalyse the breakdown of 1 μmol of H_2O_2 into H_2O and O_2 per minute. Similarly, one unit of SOD activity is referred to as the sample volume required to catalyse the breakdown of one μmol of $\text{O}_2^{\cdot -}$ into H_2O_2 and O_2 per minute.

2.10 Skin Color Measurement

After 30th and 60th days of feedings, the skin color of the *T. lalius* was measured on both sides of the dorsal skin by using a CM5 spectrophotometer (Konica Minolta, Japan) with a 5 mm measuring area based on tristimulus values, L* (lightness), a* (redness) and b* (yellowness). Before taking color measurements, each fish was anaesthetized to reduce stress and placed in a petri dish to minimize handling. According to the CIE standards (C I E 1986), the color parameters were measured, L* value for the lightness factor, which ranges from 0 for black to 100 for white; a* value coordinates the red/green chromaticity; and b* value reflect the yellow and blue chromaticity. Skin color measurements were performed at the initial and the end of the experiment in both the experimental and control group.

2.11 Statistical Analysis

Statistical analysis was carried out with Graph pad Prism 8.0 software package using one-way ANOVA and Duncan's multiple range test analyses at the 5% level of probability. Data is reported as the mean $\pm$ standard deviation of triplicates (n=3) with a p-value of 0.05 considered as significant.

3 Results and Discussion

3.1 Growth and Survival

The results showed that the survival rate, specific growth rate and feed conversion ratio were not significantly affected by dietary treatments (Table 3). Fish in all experimental groups gained significant weight and length during the experimental period of 60 days. After 60 days of the feeding trial, the fish were grown normally and showed high activity. The specific growth rate of *T. lalius* fed on the experimental diet was significantly higher than that of the control diet. This finding of survival rate, specific growth rate and feed conversion ratio is consistent with the previous study, dietary supplementation of lutein has increased the specific growth rate in Lake Kurumoi rainbow fish, *Melanotaenia parva* after 56 days of observation. Additionally, *Chlorella* sp. has been used as a good source of lutein (Meilisza et al. 2017). Moreover, dietary supplementation with microalgae such as *Chlorella fusca*, *Chlorella pyrenoids*, *Chlorella sorokiniana* improve fish growth and decrease FCR in grey mullet (*Chelon labrosus*) (Garcia-Marquez et al. 2022), Common Carp (*Cyprinus carpio* L.) (Abdulrahman et al. 2019), and juvenile rainbow trout (*Oncorhynchus mykiss*) (Chen et al. 2022) respectively.

Table3. Growth performance, Survival rate and feed utilization of *T. lalius* fed with experimental diets for 30 and 60 days

Days	Diets	CD	G-I	G-II
30 days	Initial weight (g)	4.26 $\pm$ 0.44	4.4 $\pm$ 0.75	4.32 $\pm$ 0.83 ^b
	Final weight (g)	6.4 $\pm$ 0.06	7.12 $\pm$ 0.42 ^a	8.21 $\pm$ 0.09 ^b
	Weight gain	50.23 $\pm$ 0.23	61.81 $\pm$ 0.53 ^a	90.04 $\pm$ 0.87 ^b
	Specific growth rate	7.13 $\pm$ 0.34	9.06 $\pm$ 0.28 ^a	12.96 $\pm$ 0.69 ^b
	FCR	0.07 $\pm$ 0.01	0.06 $\pm$ 0.02 ^a	0.04 $\pm$ 0.01 ^b

60 days	Initial weight (g)	4.26±0.44	4.4±0.75 ^a	4.32±0.83 ^b
	Final weight (g)	7.4±0.43	8.5±0.65 ^a	9.1±0.24 ^b
	Weight gain	73.70±0.22	93.18±0.13 ^a	110.64±0.31 ^b
	Specific growth rate	5.23±0.12	6.83±0.33 ^a	7.96±0.13 ^b
	FCR	0.10±0.55	0.08±0.40 ^a	0.06±0.15 ^b
	Survival (%)	85	90	90

Values are represented as mean ± SD (n = 3), Significant difference ($p < 0.05$) vs control

3.2 Haematological parameters

The haematological parameters were significantly higher in the experimental groups (Group I & II) when compared to the control group (Table 4) after 30 and 60 days of feeding. After 60 days of feeding trials, the RBC level was significantly increased in Group II ($3.84 \times 10^6 \text{ mm}^{-3}$) when compared to other groups. In the same way, Group II had significantly higher levels of WBC ($48.80 \times 10^3 \text{ mm}^{-3}$), haematocrit (26.80%), and haemoglobin (8.46 g dl^{-1}) than the other groups. The haematological parameters were significantly increased in the experimental groups when compared to the control group after 30 and 60 days of feeding. Consistent with previous studies, dietary supplementation of β -carotene and phycocyanin extracted from *Spirulina platensis* has increased the haematological parameters in Nile tilapia, *Oreochromis niloticus* when compared with the control diet (Hassaan et al. 2021). In addition, *Trichogaster trichopterus* fingerlings fed with Iron Oxide Nanoparticle conjugated *Camellia sinensis* was shown higher levels of RBC, WBC, haematocrit and haemoglobin when compared to the control (Paulpandian et al. 2023). The observed significant improvements in haematological parameters consistently enhance their immune response and defence mechanism against pathogenic infections. The iron deficiency issue in fish would be resolved by the higher haemoglobin concentrations.

Table 4. Haematological parameters of the *T. lalius* fed with different doses of *C. salina* supplemented (Group I & II) and control diets after 30 and 60 days

Days	Haematological parameters	Different doses of <i>C. salina</i> supplemented diets		
		CD	G-I	G-II
30 days	RBC ($\times 10^6 \text{ mm}^{-3}$)	2.98 ± 0.22	3.19 ± 0.32^a	3.28 ± 0.10^b
	WBC ($\times 10^3 \text{ mm}^{-3}$)	35.23 ± 0.28	37.33 ± 0.82^a	38.20 ± 0.42^b
	Haematocrit (%)	20.12 ± 1.22	22.36 ± 0.94^a	24.80 ± 0.26^b
	Haemoglobin (g dl^{-1})	3.22 ± 0.62	5.37 ± 0.33^a	5.97 ± 0.46^b
60 days	RBC ($\times 10^6 \text{ mm}^{-3}$)	3.68 ± 0.42	3.79 ± 0.25^a	3.84 ± 0.20^b
	WBC ($\times 10^3 \text{ mm}^{-3}$)	43.22 ± 0.82	44.20 ± 1.72^a	48.80 ± 0.48^b
	Haematocrit (%)	25.82 ± 1.29	26.63 ± 1.24^a	26.80 ± 0.46^b
	Haemoglobin (g dl^{-1})	6.52 ± 0.78	7.48 ± 0.28^a	8.46 ± 0.72^b

Values are represented as mean $\pm$ SD (n = 3), Significant difference ($p < 0.05$) vs control

3.3 Digestive enzyme activity

The digestive enzymes like protease, amylase, and lipase were significantly improved in fish fed a *C. salina* supplemented diet when compared to the control diet (Fig 2a-c). The digestive enzyme activity in fish fed with different concentrations of *C. salina* supplemented diets ranged from 0.8 ± 0.26 (G-I) to 0.85 ± 0.39 (G-II) U/mg Protein for amylase, 1.3 ± 0.21 (G-I) to 1.75 ± 0.19 (G-II) U/mg protein for protease, and 0.33 ± 0.22 (G-II) to 0.42 ± 0.43 (G-I) U/mg Protein for lipase after 60 days of feeding trials. The basal diet mixed *C. salina* (1:1) fed to the fish showed the highest level of amylase and protease activity, whereas the fish fed with *C. salina* showed the maximum level of lipase activity when compared to the control diet (Fig 1a-c). These results agree with the previous study: Nile tilapia (*Oreochromis niloticus*) fingerlings fed with the green microalga *Scenedesmus quadricauda*, which improved activities of digestive enzymes such as lipase, α -amylase, and protease (Abdel-Tawwab et al. 2022a). Likewise, dietary supplementation of *Chlorella vulgaris* has enhanced the protease, amylase, and lipase enzyme levels in *O. niloticus*, when compared to the control (Abdel-Tawwab et al. 2022b). Furthermore, fish growth is influenced by the activity of their digestive enzymes, which is important for nutritional physiology. Additionally, it improves our knowledge of the potential impacts of feed on the growth and consumption of fish (Dawood et al. 2015).

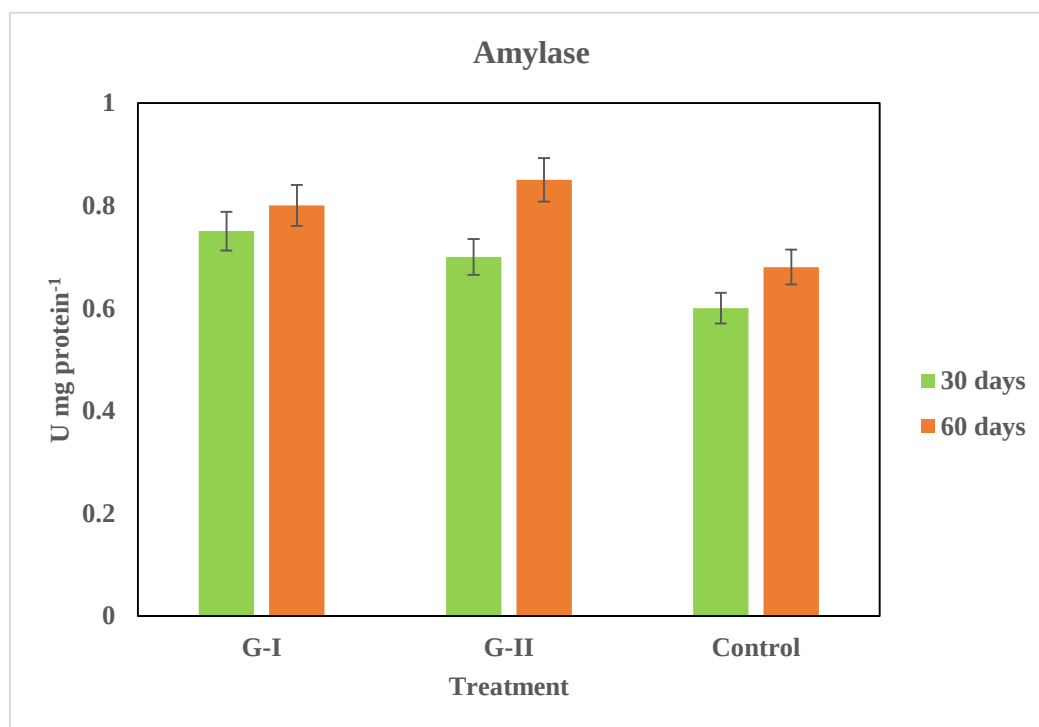


Fig 1a. Amylase enzyme activity in the gut sample of *T. lalius* fed on control and *C. salina* supplemented diets after 30 and 60 days (means $\pm$ SD, n = 3 tanks)

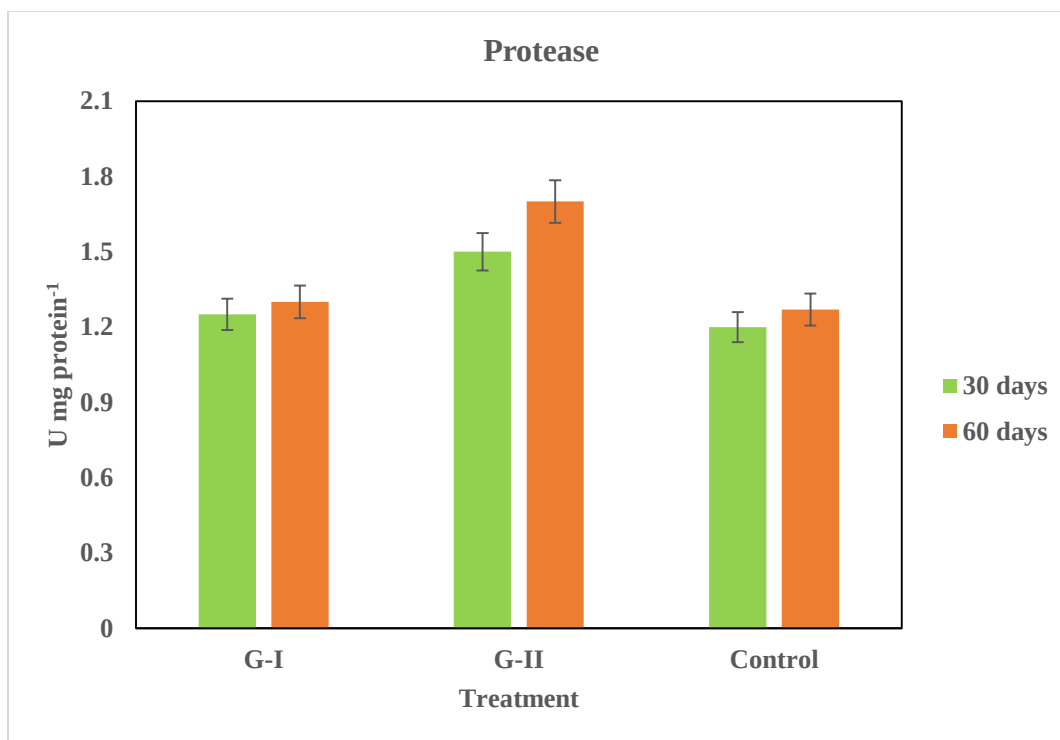


Fig 1b. Protease enzyme activity in the gut sample of *T. lalius* fed on control and *C. salina* supplemented diets after 30 and 60 days (means $\pm$ SD, n= 3 tanks)

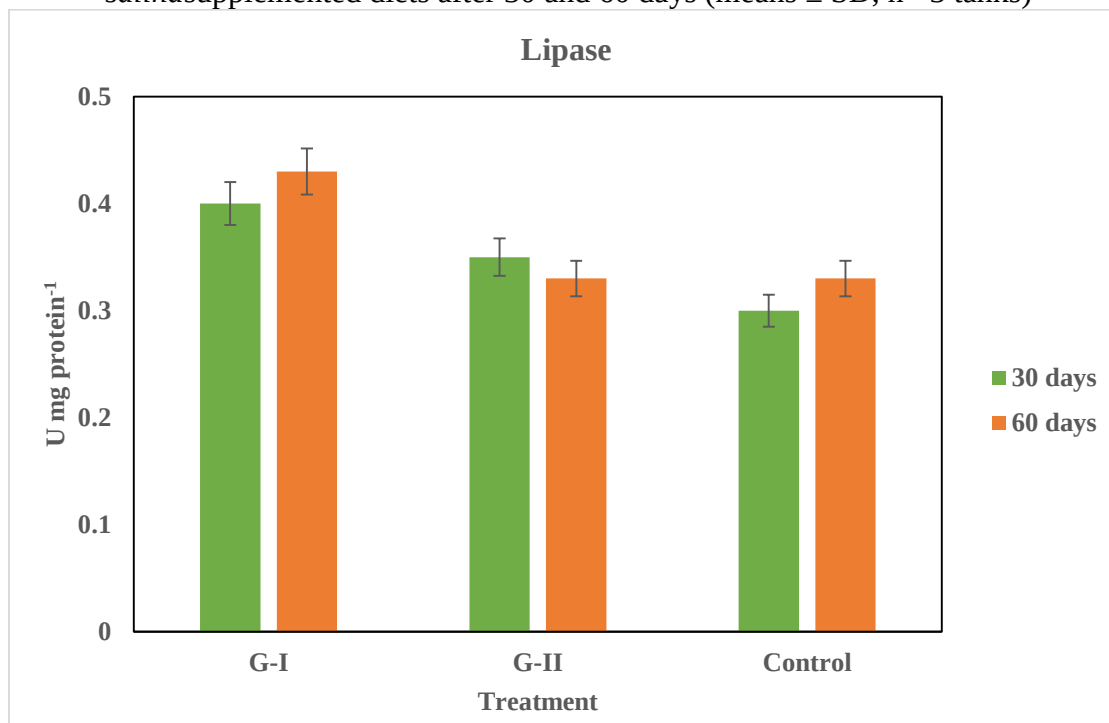


Fig 1c. Lipase enzyme activity in the gut sample of *T. lalius* fed on control and *C. salina* supplemented diets after 30 and 60 days (means $\pm$ SD, n= 3 tanks)

3.4 Antioxidant enzyme activity

The result of SOD and CAT activities were shown in Figures 2a and b, respectively. The SOD activity in the *C. salina* supplemented groups (ranged from 20 ± 0.15 (G-I) to 26 ± 0.34 (G-II) U/mg proteins) was significantly lower than the control group (33 ± 0.34 U/mg proteins) after 60 days of feeding trails. Similarly, the CAT activity was observed less in the *C. salina* supplemented groups (ranging from 14 ± 0.15 (G-I) to 15 ± 0.44 (G-II) U/mg proteins) than the control group (27 ± 0.45 U/mg proteins). In the treatment group containing basal diet mixed *C. salina* in ratio of 1:1 (G-II), the SOD and CAT activities were slightly higher than the other treatment group ($p > 0.05$). These findings were consistent with the previous study: the SOD and CAT activities of *Labidochromis caeruleus* fed the diets supplemented with extracts derived from *Sargassum boveanum*, *Gracilariapersica* and *Entromorphaintestinalis* were significantly lower than the control (Pezeshk et al. 2019). According to this study, the usage of algal extract contains antioxidant compounds, which seem to reduce the accumulation of oxidative chemicals in the kidneys and liver and, as a result prevent fish mortality (Aghajani et al. 2015). These findings are consistent with the results obtained from measuring CAT and SOD activity.

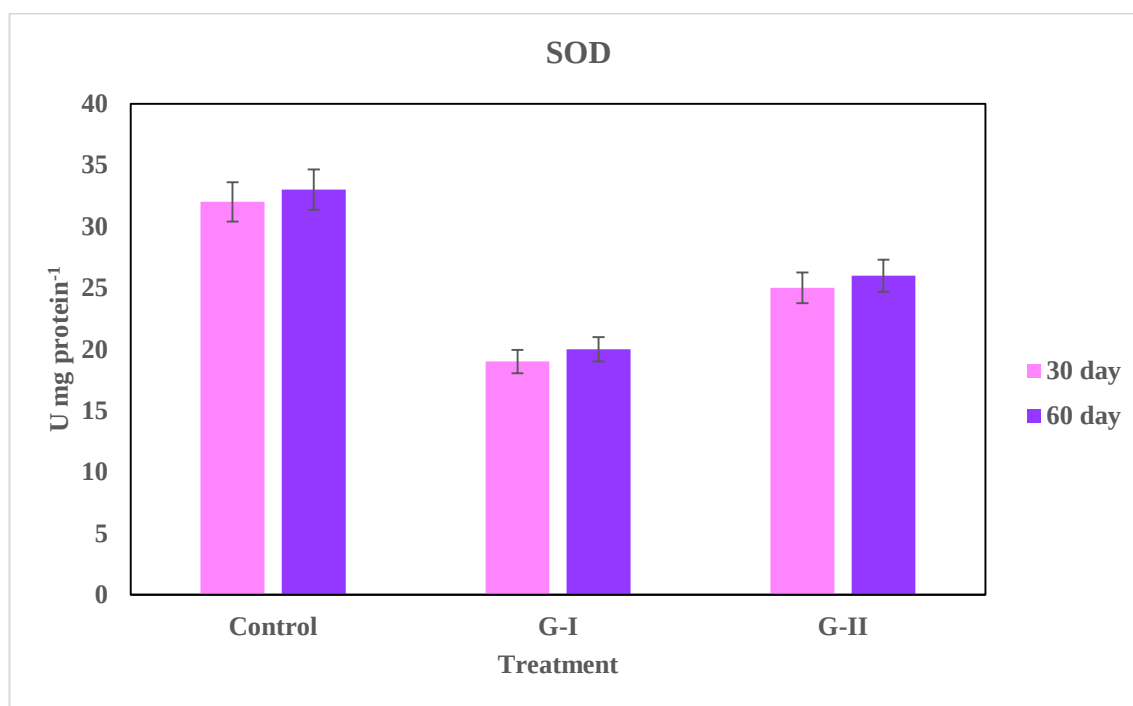


Fig 2a. SOD activity (U mg/protein) of the *T. lalius* fed with different doses of *C. salina* supplemented (Group 1 & II) and control diets after 30 and 60 days (means $\pm$ SD, n= 3 tanks).

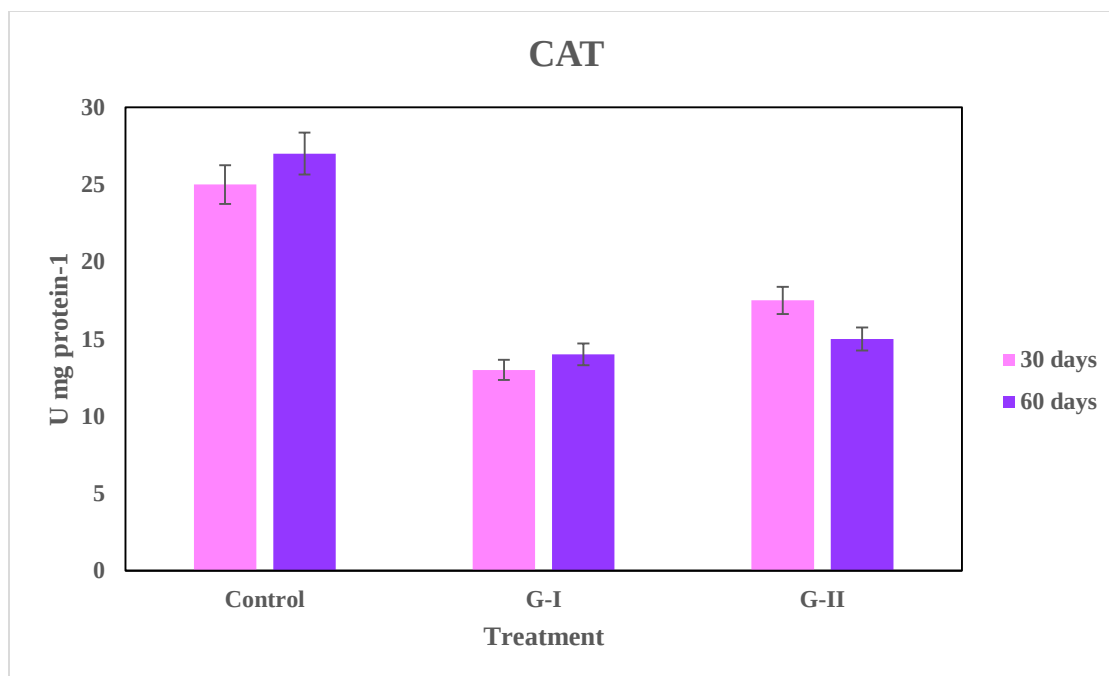


Fig 2b. CAT activity (U mg/protein) of the *T. lalius* fed with different doses of *C. salina* supplemented (Group 1 & II) and control diets after 30 and 60 days (means $\pm$ SD, n= 3 tanks).

3.5 Analysis of Skin color

The diets used in this study comprised different concentrations of *C. salina* as coloration pigment. After 15 and 30 days of feeding the fishes those diets, the results revealed that the dietary carotenoids can alter the skin color of the *T. lalius* fish. Lightness (L^*), Redness (a^*) and Yellowness (b^*) values of the dorsal skins of the *T. lalius* were displayed in Fig 3a-c. At the end of the feeding trial, the maximum L^* , a^* and b^* value was found in the treatment group fed with *C. salina* rich diet (G-I) when compared to others, it was 70.19, 4.51 and 14.7 respectively. The diet of microalgae *C. salina* rich feed showed its effectiveness in increased skin red color deposition at end of the 60th day. These results were in agreement with the previous study, in which it was indicated that dietary-supplemented carotenoids can enhance the skin coloration of fish (Pan and Chien 2009; Yasir and Qin 2010). The effect of dietary carotenoids on fish skin color change was more easily visible by direct analysis, indicating that carotenoid-supplemented diets induce skin coloration in *T. lalius*. This was consistent with the results of (Xu et al. 2006) and (Yanar et al. 2008) for goldfish. Moreover, (Pérez-Escalante et al. 2012) and (Liu et al. 2016) have reported that carotenoid supplementation may induce chromatophore production and pigment granules, resulting in increased coloration in fishes. The exposure value (L^*) and the yellow-blue color (b^*) of the fish were not affected by the experiment diets because the color of the *T. lalius* was mostly dark-orange, which did not affect the values of L^* and b^* . The characteristic change in redness (a^*) value represents the difference in orangeness or brightness of the skin color of fish among the experimental diets. According to earlier research, the skin colour of ornamental fish changes over time based on the amount of carotenoids in the experimental diet (Chatzifotis et al. 2005; Ramamoorthy et al. 2010).

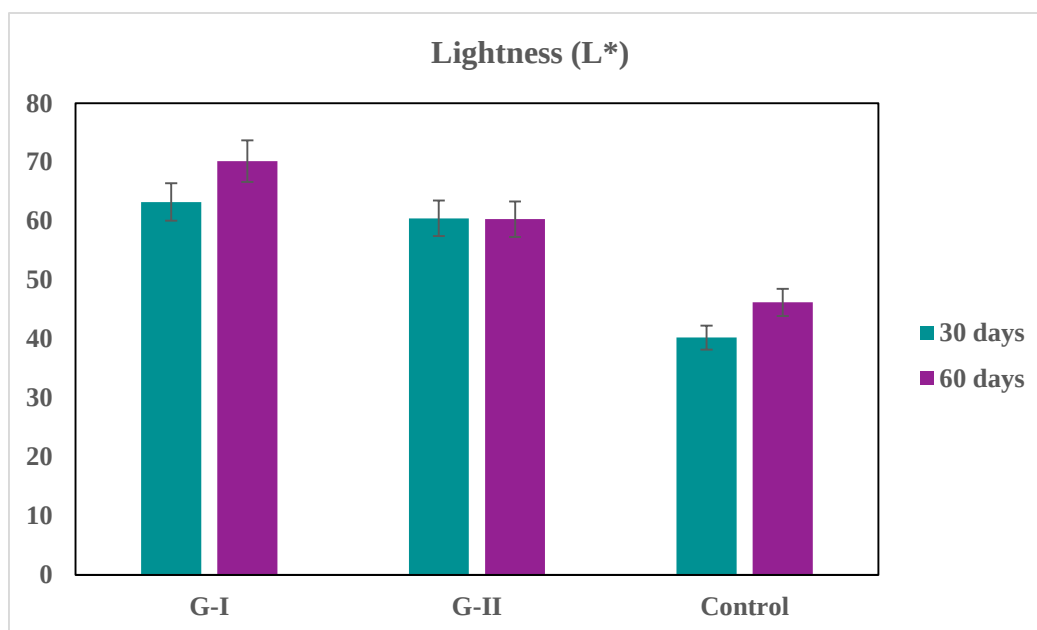


Fig 3a. Lightness (L*) values of the dorsal skin of *T. lalius* fed with different doses of *C. salina* supplemented (Group 1 & II) and control diets after 30 and 60 days (means $\pm$ SD, n= 3 tanks).

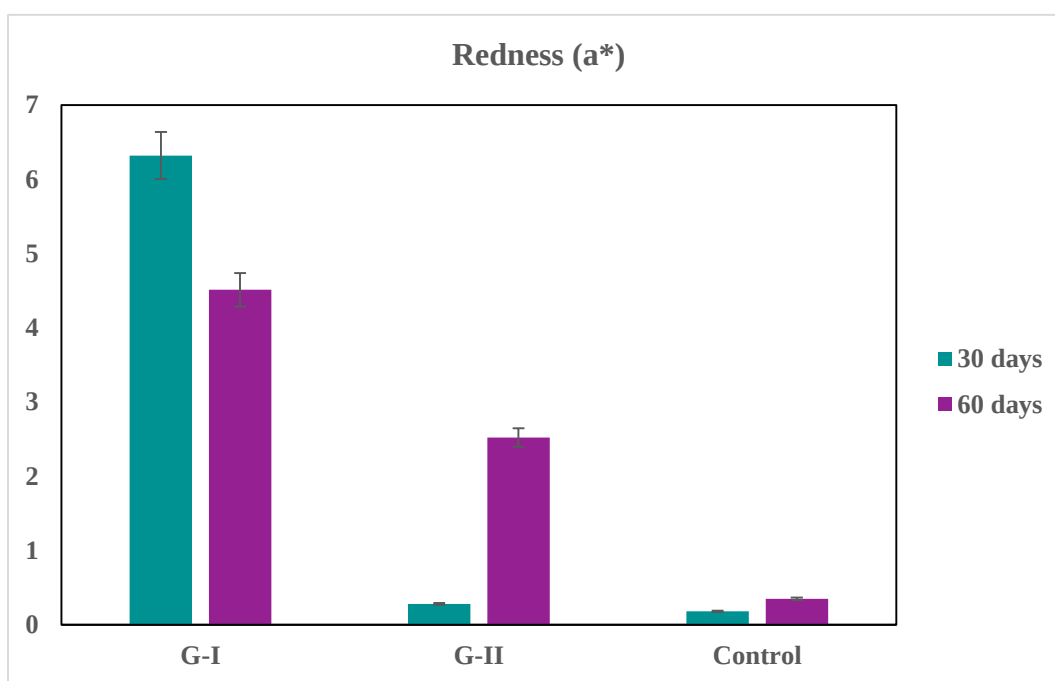


Fig 3b. Redness (a*) values of the dorsal skin of *T. lalius* fed with different doses of *C. salina* supplemented (Group 1 & II) and control diets after 30 and 60 days (means $\pm$ SD, n= 3 tanks).

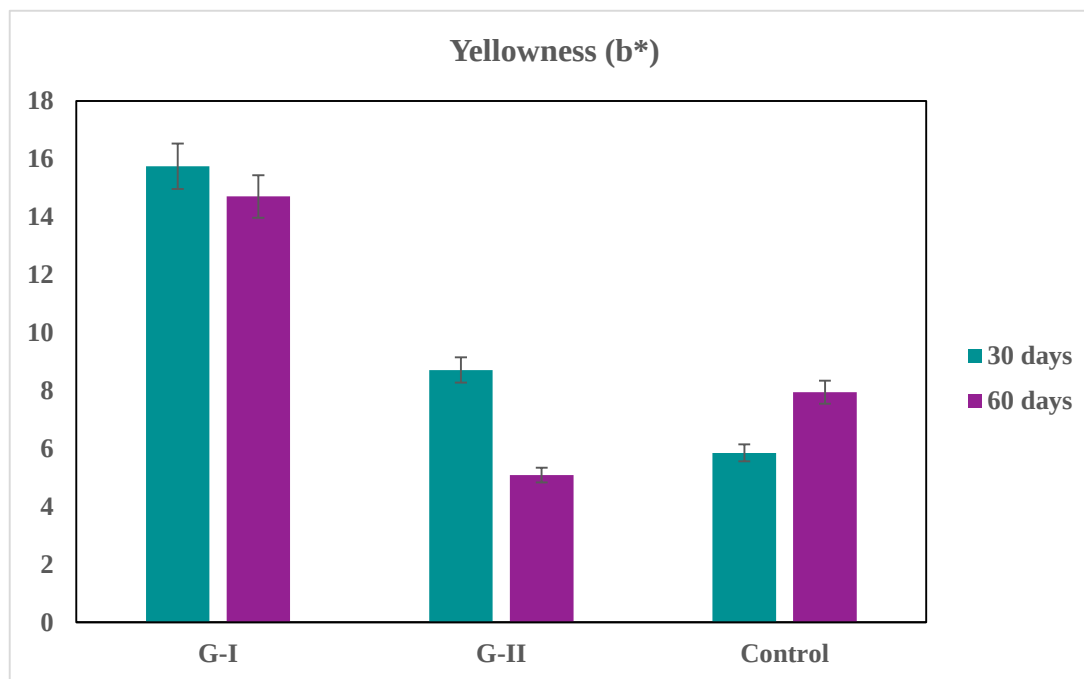


Fig 3c. Yellowness(b*) values of the dorsal skin of *T. lailus* fed with different doses of *C. salina* supplemented (Group 1 & II) and control diets after 30 and 60 days (means $\pm$ SD, n= 3 tanks).

4. Conclusions

In conclusion, feeding *T. lailus* with different concentrations of marine microalgae *C. salina* enhanced growth performance, haematological parameters, digestive, antioxidant enzyme activities and pigmentation when compared to control, suggesting that it might be used as a necessary feed supplement in the culture of ornamental fish *T. lailus*.

5. References

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Effects of dietary *Andrographis paniculata* extract on growth, haematological, immune responses, immune-related genes expression of ornamental goldfish (*Carassius auratus*) and its susceptibility to *Aeromonas hydrophila* infection

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ABSTRACT

In the present study, goldfish (*Carassius auratus*) were fed diets supplemented with different levels of methanolic crude extracts of *Andrographis paniculata* to assess their efficacy against *Aeromonas hydrophila*. The *A. paniculata* extract showed antibacterial activity against *A. hydrophila* at different concentrations and was characterised by FTIR and GC-MS analysis. The fishes (*C. auratus*) were divided into three groups, the control fishes were fed with a basal diet, the DI and DII group fishes were fed with a basal diet mixed with 0.1% and 1% of methanolic crude extracts of *A. paniculata*, respectively for 4 weeks. Then, haematological and immunological parameters were examined in each experimental and control group after the 2nd and 4th week. Finally, all group fish were challenged with *A. hydrophila* at the end of the 4th week, and their cytokine genes expression were investigated. The results showed that even at low concentrations (50 µg) of *A. paniculata* leaf extracts showed a potent antibacterial effect against *A. hydrophila*. GC-MS analysis revealed that the methanol precipitation contains Benzylamine, 2-hydroxy-N,N-DI-[2-aminoethyl]-, 18-Nonadecen-1-OL, and andrographolide. Similarly, FTIR analysis revealed aliphatic primary amine, conjugated ketone and alcohols, which are known antimicrobials. The extracts also changed the growth, haematological and immunological parameters of treated fishes by significantly increased the total RBC counts and haemoglobin level, along with the phagocytic, total immunoglobulin and myeloperoxidase activities when compared to the BD group. Moreover, fish fed with 1% of *A. paniculata* extract showed a higher survival rate (80%) against *A. hydrophila*. Furthermore, 1% of *A. paniculata* extract dietary was significantly up-regulated pro-anti-inflammatory cytokine gene expression, compared with the BD group. In conclusion, dietary *A. paniculata* extract supplementation beneficial for *C. auratus*, as it assesses their efficacy against *A. hydrophila*.

1. Introduction

Fisheries and aquaculture are not only a source of health but also of

wealth which contributes to the world's well-being and prosperity (Cooke et al., 2016). Ornamental fish also support the development of the aquaculture sector (Wei and Wee, 2014). Ornamental fisheries are a

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multibillion-dollar business and livelihood of many people, particularly in developing countries. India also contributes significantly to ornamental fisheries and holds at 26th position in the international ornamental fish trade (Narendrakumar et al., 2023). The global ornamental fish industry generates around 333 million USD, including export and import activity, and over 2 billion live ornamental fish are traded. The trade of freshwater ornamental fish generated about 250 million USD, with the remaining amount coming from the trade of marine ornamental fish in 2016 (Mulyati et al., 2023).

Although modern hatchery technology has helped in the sector's development, there have also been abnormalities in the sector's profitability because of fish infections and associated mortality. Furthermore, high densities of fish along with a lack of hygienic barriers, facilitate pathogen growth, resulting in high mortality varieties (Narendrakumar et al., 2023; Quesada et al., 2013). *Aeromonas hydrophila* is one of the Gram-native bacteria, an opportunistic pathogen that is relatively more common in freshwater aquatic zones with a high organic load and is responsible for causing ulceration, hemorrhages, ascites anemia, and abscesses (Stratev and Odeyemi, 2017). However, the fish species is susceptible to infection with *A. hydrophila*, which causes serious damage and increases the mortality rate by 80–100% within a week (Shankar et al., 2000). Dharmaratnam et al. (2018) reported that *A. hydrophila* infection caused huge mortality of *C. auratus* in India.

To overcome the disease, farmers frequently used antibiotics and synthetic drugs in aquaculture systems. Even though it gives positive outcomes and it is not advised for use in commercial aquaculture practices due to their high costs, restricted or unclear regulatory status, impractical administration routes, toxicity, biomagnification, low absorption, environmental pollution, and transmission of antibiotic-resistant bacteria to humans through handling and eating of food, resulting hazardous to human health (Citarasu, 2012; Thirumalaikumar et al., 2022). Considering the possible threat of diseases and environmental difficulties, the disease management aspects should focus on herbal components used as antibacterial and immunostimulants to control fish disease (Karina et al., 2016). Herbal compounds have better immunostimulant, antiviral, antibacterial, and antistress effects in fish and shellfish aquaculture (Anusha et al., 2014; Citarasu, 2010).

The plant species *Andrographis paniculata* is classified as part of the *Acanthaceae* family and is popularly known as kings of bitters and Nila Vembu in India and distributed in the Asian subcontinent (Mussard et al., 2019). There are various active chemical components in *A. paniculata* extract, including andrographolide, 14-deoxy andrographolide, isoandrographolide, and andrographiside have the most potential for pharmacological activity (Chao and Lin, 2010). According to previously published preclinical pharmacology studies, andrographolide has antipyretic, analgesic, and antiulcerogenic properties, 14-deoxyandrographolide has antibacterial and antifungal activity, isoandrographolide has anti-inflammatory and anticancer activity (Subramanian et al., 2012), and then andrographiside reported to have hepatoprotective activity (Kapil et al., 1993; Subash R, 2020). Recently, herbal immunostimulants have been recommended for the management of aquaculture diseases since they are safe, biodegradable, and environmentally friendly (Ortuño et al., 2002). Hence, this study aimed to investigate the immune modulatory effect of *A. paniculata* extract with different concentrations (minimum and maximum levels) on ornamental goldfish *C. auratus* against *A. hydrophila* challenge.

2. Materials and methods

2.1. Plant source and extraction

Aerial parts of healthy *Andrographis paniculata* plants were collected from the Kanyakumari surrounding area of Tamil Nadu, India. The plant parts like leaves were collected and washed thoroughly 2–3 times in tap water followed by distilled water and shade dried. The dried plants were crushed, powdered, sieved and stored in the refrigerator (−20 °C) until

used.

Methanol extract of *A. paniculata* was prepared by following the method (Cooper, 2005; Singh et al., 2007). According to the method, 100 gm of plant powder was percolated with 200 ml of methanol for two days with intermittent shaking. The extract was filtered through sterile muslin cloth and the filtrate was condensed by using a rotary vacuum evaporator (Rotavap) under pressure of 45°C to 50°C. The residue was stored at −20 °C until further experimentation.

2.2. Preliminary phytochemical screening

The crude methanolic extract of *A. paniculata* was subjected to different qualitative chemical tests to screen for phytochemical constituents (Ochi and Okwute, 2022).

- 1. Test for alkaloids:** One milliliter of 1% HCl was added to a test tube containing 3 ml of methanolic extract and then Mayer's reagent was added in a few drops along the sides of the test tubes. The appearance of a creamy white precipitate indicated the presence of alkaloids.
- 2. Test for flavonoids:** One milliliter of methanolic extract was dissolved in a few drops of dilute NaOH. A yellow solution was observed for the presence of flavonoids.
- 3. Test for anthraquinones:** 1 ml of methanolic extract was mixed with a few drops of 10% ammonia solution. The formation of pink color was observed.
- 4. Test for tannins:** The methanolic extract (0.5 ml) was mixed with 1 ml of distilled water and a few drops of ferric chloride solution were added. A brownish-green or blue-black coloration was observed.
- 5. Test for saponins:** Two milliliters of methanolic extract were shaken vigorously in a test tube for two minutes. The formation of emulsion was observed.
- 6. Test for proteins:** Two milliliters of methanolic extract were mixed with 1 ml of 40% NaOH solution and 2 drops of 1% CuSO₄ Solution. The appearance of a violet color was observed.
- 7. Test for terpenoids:** 5 ml of methanolic extract was added with 2 ml of chloroform in a test tube. The mixture was concentrated with 3 ml of H₂SO₄. A reddish brown or grey color was observed.
- 8. Test for steroids:** 1 ml of methanolic extract mixed with 1 ml of chloroform and concentrated with sulphuric acid (5 drops) sidewise. A red colour appearance at the lower chloroform layer was observed.
- 9. Test for amino acids:** 2 ml of ninhydrin reagent was added with 2 ml of methanolic extract and kept in a boiling water bath for about 20 min. The formation of purple color was observed.
- 10. Test for reducing sugars:** 2 ml of methanolic extract was mixed with a few drops of Molisch's reagent and shaken well. About 2 ml of concentrated H₂SO₄ was added dropwise along the sides of the test tube. A reddish-violet ring formed at the connection of two layers, indicating the presence of reducing sugar or carbohydrates.

2.3. Structural characterization of *A. paniculata* fraction by FT-IR analysis

The basic functional groups of the crude methanolic extract of *A. paniculata* leaf were analyzed qualitatively by Fourier Transform Infra-Red (FTIR; Shimadzu FT-IR, Japan). Two milligrams of *A. paniculata* leaf methanolic extract were mixed with 100 mg KBr and compressed in the form of a KBr pellet. The infrared (IR) spectrum was recorded in the wavelength ranging between 4000 and 400 cm^{−1} using FTIR spectrometer.

2.4. Structural characterization of *A. paniculata* fraction by GC-MS analysis

The active fraction of *A. paniculata* methanolic extract was analysed using the Gas Chromatography Mass Spectrometer (Thermo GC Trace Ultra Ver: 5.0, Thermo MS DSQ II). The oven temperature was programmed from 70° to 250°C at 10 °C/min using helium as a carrier gas at 1.0 ml/min. The column was DB 35-MS Capillary standard non-Polar column and its dimension 30 MTS, ID: 0.25 mm, Film: 0.25 µm. The diluted sample was injected, and its size was 0.1 µl prepared in methanol. The total GC-MS running time (min) was 38.02. The retention time of isolated bioactive compounds were identified and analysed by comparing the retention time of library search results (NIST-05 library).

2.5. Bacterial strains and culture condition

Fish pathogens *Aeromonas hydrophila* culture was purchased from the American Type Culture Collection Centre (ATCC 7966) in Virginia, USA. The strain was stored in 15% (v/v) glycerol stock at – 80 °C for further study and retrieved on Tryptic Soya Broth (Himedia, India) for 24 h at 37 °C.

2.6. In vitro antagonistic activity

Antagonistic screening was performed using a crude extract of *A. paniculata* against *A. hydrophila* by agar well diffusion method (Cintas et al., 1995). The *A. hydrophila* strains were grown in Nutrient broth at 37 °C for 24–48 h. After incubation, the strain was overlaid with 5 ml of sloppy agar (0.6%) layer in the Nutrient agar plate and wells were then punched (6 mm). Further, these different concentrations of 12.5, 25, 50 and 100 µg of crude extract of *A. paniculata* were added into the wells. Plates were incubated at 37 °C for 24 h and the diameter of the incubation zone was measured using a ruler and recorded in millimeters (Adlin Jenifer et al., 2019).

The minimum inhibitory concentration (MIC) was determined by the lowest concentration of *A. paniculata* extract, serially diluted with Muller Hinton broth, inoculate *A. hydrophila* bacterial strain into each tube and incubated at 37 °C for 24 h, and which completely inhibited the bacterial growth. The minimum bactericidal concentration (MBC) of the extract was calculated using the results of MIC, a loop full was taken from each test tube of the MIC test (no turbidity), streaked on sterile Nutrient agar plates and incubated at 37 °C for 24 h (Adlin Jenifer et al., 2019).

2.7. Ethical statement and experimental design

Laboratory animal care principles were followed and experimental procedures were conducted according to the guidelines established by the National Institutes of Health (Nickum et al., 2004), and every effort was made to minimize suffering. This study was approved by the institutional ethical committee of the Centre for Marine Science and Technology, Manonmanium Sundaranar University, Rajakkamangalam, Tamil Nadu, India.

Healthy goldfish (*Carassius auratus*) were obtained from the JJ Aquarium, Kanyakumari, Tamil Nadu, India. Before experimentation, the goldfish were not vaccinated or exposed to fish diseases and were considered free of pathogens by common microbiological and microscopic examinations of the skin, gills, kidney tissues and intestines of representative samples. Before plant extract administration, fish were acclimatized for 2 weeks in 150-L rectangular glass aquaria to laboratory conditions at 28 ± 2 °C.

Goldfish were divided into three groups with 20 fish in each group. In the basal diet (BD) group, the control fishes were fed with a basal diet. In the diet I (DI) group, the fish were fed with a basal diet mixed with 0.1% of crude extracts of *A. paniculata*. In the diet II (DII) group, the fish were fed with a basal diet mixed with 1% crude extracts of *A. paniculata*.

Table 1

Composition of the experimental diets (g%) fed to goldfish *Carassius auratus*.

Ingredients	Percentage of dry weight (g%)		
	Basal diet	Diet-I	Diet-II
Fishmeal	14	13.9	13
Ragi flour	15	15	15
Wheat flour	14	14	14
Corn flour	17	17	17
Rice bran	10	10	10
Groundnut oil cake	18	18	18
Tapioca flour	5	5	5
Vegetable oil	5	5	5
Vitamin premix ^a	1	1	1
Mineral premix ^b	1	1	1
Plant extract	-	0.1	1

^a Vitamin (IU or mg per kg premix): Vitamin A, 700000 IU; Cobalamin, 200 IU; Vitamin D3, 70000 IU; Vitamin E, 250 mg; Nicotinamide, 1 g.

^b Mineral premix (per kg premix): Cobalt, 150 g; Copper, 1200 mg; Iodine, 325 mg; Iron, 1500 mg; Magnesium, 6000 mg; Manganese, 1500 mg; Potassium, 100 mg; Sodium, 5.9 mg; Sulphur, 0.72%; Zinc, 9600 mg; Calcium, 25.5%; Phosphorus, 12.75%.

Each experiment was carried out in triplicate. Each group of the fish was fed with respective experimental feed for one day at the rate of 3% body weight for three times. The water quality parameters were regularly monitored and maintained at optimum levels throughout the experimental period (dissolved oxygen: 5.22 ± 0.57 mg/l; pH: 7.5 ± 0.5; nitrites: 0.013 ± 0.25 mg/l; ammonia: 0.112 ± 0.12 mg/l).

2.8. Diet formulation

Experimental diets were made following the procedure described by Kumar et al. (2013) with slight modifications. The formulation of the basal diet was shown in Table 1 and proximate composition was analyzed using the Association of Official Analytical Chemists method (Cunniff and Washington, 1997) revealing 43.2% crude protein, 52.9% carbohydrate and 24% ash, respectively. The dough was prepared by adding water to the above ingredients. The material was then steam sterilized and pelletized by hand to get pellets of 3 mm size. The pellets were dehydrated in a forced-air oven at 55–60 °C for 48 h when the final moisture content < 15%. After drying, crude extracts of *A. paniculata* was mixed with 2.5% vegetable oil onto the basal diet (BD) at two different doses 0.1% (Diet I) and 1% (Diet II) in the respective test diets (DI and DII) whereas the vegetable oil was only mixed with the BD group (Jinendiran et al., 2019).

2.9. Effects of plant extract on the growth performance of goldfish

The fish were measured and weighed at the end of the 2nd and 4th weeks to assess the growth performance of goldfish. The fish were weighed by a digital scale (electronic balance) after they had been anaesthetized. The growth parameters such as weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate were calculated at the end of the 2nd and 4th weeks of feeding trial according to the following standard formula: WG (g) (%) = [(Final weight (g) – Initial weight (g))/Initial weight (g)] × 100; SGR (%) = [(Ln Final weight (g) – Ln Initial weight (g))/Time] × 100; FCR = Feed intake (g) / Weight gain (g); Survival (%) = (final number of fish survived/initial number of fish stocked) × 100 (Gouveia et al., 2003; Kalinowski et al., 2005).

2.10. Blood and tissue sample collection

At 2nd and 4th weeks, fish samples were randomly chosen from each group and anaesthetized with 50 µl/l clover oil (Himedia; GRM340) for 5 min. Blood samples were collected from the caudal vein using a 1.0 ml plastic syringe with 24-gauge needles. A part of the blood samples was

transferred into a tube with anticoagulant (2.7% EDTA) and then stored at 4 °C for haematological analysis. The remaining blood was transferred into a tube without anticoagulant kept at room temperature for 1 h, (to collect the serum) and stored at – 20 °C for immunological analysis.

After completion of the feeding trial, all group fish were challenged with *A. hydrophila*. Tissue samples including the spleen and kidney of each group were taken for immune-related gene expression study. The collected tissue samples were stored in TRIzol reagent and kept at – 80 °C until RNA isolation.

2.11. Haematological parameters

Haematological parameters such as the total red blood cells (RBC) and haemoglobin levels were studied for both the control and experimental groups. The total red blood cells (RBC) were calculated by using an automated haematology analyzer (Cobas Micros-18 OT, France) (Levina et al., 1997). Haemoglobin levels were measured by the cyanomethemoglobin spectrophotometry method (Kumar et al., 2013).

2.12. Immunological parameters

2.12.1. Phagocytic activity

Phagocytic activity was carried out in both the experimental and control groups previously described (Park and Jeong, 1996; Thirumalaikumar et al., 2022). Equal volumes of *A. hydrophila* (1.6×10^7 CFU ml⁻¹) suspension and 100 µl of whole blood cells were placed in sterile 96-well microtitre plates and incubated at 25 °C for 30 min. Following incubation, 10 µl of blood-bacteria suspension was smeared on glass slides. The smear was dried by air, fixed in 95% ethanol for five minutes and then dried again. The air-dried smear was stained for 10 min with 7% May–Grunwald's Giemsa solution. The number of phagocytic cells were counted microscopically. Phagocytic activity was calculated by applying the formula = (phagocytic leucocytes/total leucocytes) × 100.

2.12.2. Total immunoglobulin (Ig) in plasma

Total immunoglobulin levels in blood plasma were estimated by following the method (Kumar et al., 2013; Thirumalaikumar et al., 2022). 100 µl of the blood plasma was transferred to a plastic serum vial, and an equal volume of 12% polyethylene glycol (PEG) was added and incubated at 37 °C for 2 h with constant mixing. The mixture was centrifuged at 7500 g for 10 min, and the concentration of protein in the supernatant was determined (Lowry et al., 1951). The total immunoglobulin level (mg/ml) was measured by subtracting the protein content in the supernatant (with PEG treatment) from the total protein content in the blood plasma (without PEG treatment).

2.12.3. Myeloperoxidase (MPO) activity

Myeloperoxidase (MPO) activity analysis was determined by the following method (Kumari and Sahoo, 2006; Thirumalaikumar et al., 2022). The production of endogenous peroxidases was estimated using chromogenic substrate 3', 5'-tetramethylbenzidine (TMB, Himedia), according to the method. Serum (15 µl), Hank's balanced salt solution (135 µl) (HBSS, Sigma, RNBFO710), 20 mM 3', 5'-tetramethylbenzidine, 5 mM hydrogen peroxide and 4 M sulphuric acid were diluted in a microtitre plate. The color changes were observed in a microplate reader (Bio-Rad) at 450 nm.

2.13. Challenge experiments

After 4 weeks of feeding the respective diets, thirty fishes from each group (10 fish x 3 tanks) were intraperitoneally injected with 100 ml of live *A. hydrophila* ATCC 7966 at a LC₅₀ concentration of 10^7 CFU/fish. As a negative control, 0.1 ml of 1X PBS was sham injected into a group of thirty fishes fed a basal diet during a feeding trial. Survival and pathological symptoms were examined daily for ten days. The infection status

Table 2

In vitro antagonistic activity of the methanol extract of *A. paniculata* leaves against *A. hydrophila* by agar well diffusion method.

Test organism	Zone of inhibition (mm) at different concentrations of methanol extract of <i>A. paniculata</i> (mg/ml)			
	12.5	25	50	100
<i>A. hydrophila</i>	5.3 ± 0.6	9.4 ± 0.9	12 ± 0.4	15.2 ± 0.5

Table 3

Results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract of *A. paniculata* leaves against *A. hydrophila*.

Concentration (µg)	MIC	MBC
10		+++
20		+++
30		++
40		++
50	50	+
60		
70		
80		
90		

of the fish was confirmed by re-isolating *A. hydrophila* from the deceased fish intestine using *Aeromonas* specific isolation medium (Himedia; M884). Relative percentage survival (RPS) was calculated by using the following formula: RPS (%) = (Number of surviving fish after challenge/Number of fish injected with *A. hydrophila*) × 100 (Jinendiran et al., 2019; Thirumalaikumar et al., 2021).

2.14. Gene Expression Analysis

Total RNA was extracted from the spleen and kidney tissue (50–100 mg) of each group of fish tissue using RNAiso Plus reagent (TaKaRa, Japan) following the manufacturer's protocol. The RevertAid cDNA synthesis kit (Thermo Fisher Scientific, USA) was used to synthesize cDNA from 1 mg of mRNA as we described previously (Thirumalaikumar et al., 2021).

The quantification of immune-related gene expression was determined by real-time PCR (qPCR). The qPCR reaction was performed by StepOnePlus (Applied Biosystems, CA, USA) using a 2 × SYBR Green master mix (ABI) as we described earlier (Thirumalaikumar et al., 2021). Table 8 shows the primer sequences utilized in this study. The expression of each immune-related gene was calculated by comparing it to the expression level of the housekeeping gene (β-actin).

2.15. Statistical Analysis

Graph-Pad Prism statistical software was used for all statistical analyses. Data were analyzed using one-way analysis of variance followed by Dunnett's test for between-group comparison and $p < 0.05$ was declared statistically significant. All data were expressed as a mean ± standard deviation (SD) of three experimental replicates. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicates a significant difference between the control in response to different groups.

3. Results

3.1. In vitro antibacterial screening of *A. paniculata* methanol extract

The methanol extract of *A. paniculata* leaves exhibited antibacterial properties that were able to suppress the growth of virulent *A. hydrophila* (Table 2). The MIC and MBC of the methanol extract of *A. paniculata* against *A. hydrophila* were given in Table 3. The methanol extract of *A. paniculata* helped to control *A. hydrophila* at the minimal level of 50 µg

Table 4Phytochemical screening results of the methanolic extract of *A. paniculata* leaf.

S.No	Experiments	Results
1.	Test for alkaloids	+
2.	Test for flavonoids	+
3.	Test for anthroquinones	+
4.	Test for tannins	+
5.	Test for saponins	+
6.	Test for protein	+
7.	Test for terpenoids	+
8.	Test for steroids	+
9.	Test for amino acids	+
10	Test for reducing sugar	+

Table 5Functional group for FTIR Spectra of methanol leaf extract of *A. paniculata*.

Frequency (cm ⁻¹)	Bond responsible	Types of compound	Intensity
3348.06	N-H stretching	Aliphatic Primary amine	Strong
2927.66	N-H stretching	Amine salt	Medium
2856.01	N-H stretching	Amine salt	Medium
1625.15	C=O stretching	Conjugated ketone	Strong
1395.74	O-H bending	Alcohol	Medium
1033.99	S=O stretching	Alcohol	Strong

due to the presence of antimicrobial components.

3.2. Phytochemical screening

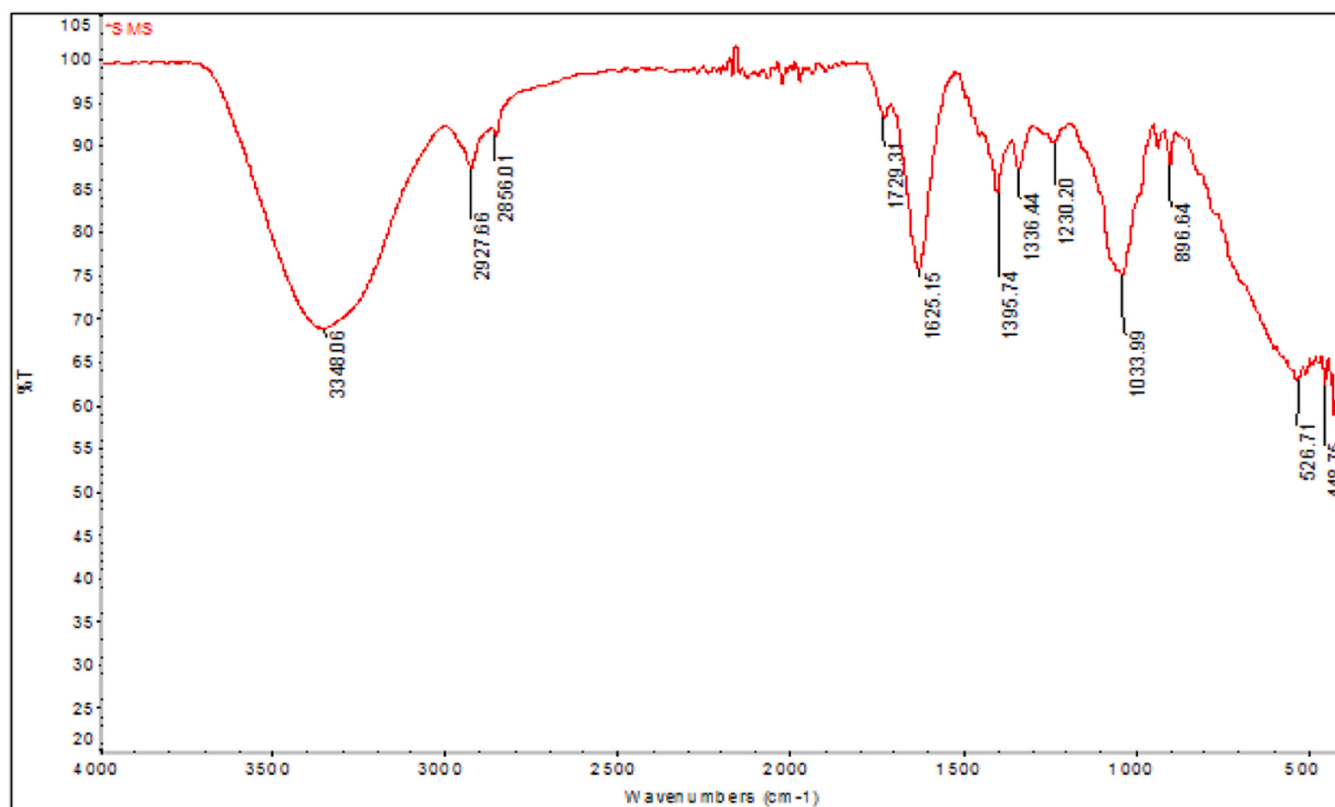
The results of the phytochemical screening of the methanolic extract of *A. paniculata* leaf are shown in Table 4.

3.3. Structural characterization of *A. paniculata* fraction by FTIR analysis

The IR spectrum of the methanol leaf extract of *A. paniculata* sample showed in Table 5 and Fig. 1. The main absorption band at around 3348.06 cm⁻¹, which is strong, broadband and attributed to N-H stretching showed the presence of aliphatic primary amine. The absorption band at 2856.01 cm⁻¹ was identified as C=O stretching and showed the presence of conjugated ketone, and the band at 1395.74 cm⁻¹ was recognised as O-H bending exhibiting the presence of alcohol.

Table 6Major components from the GC-MS analysis of the methanol extract of *A. paniculata* leaf.

Retention time (RT) (min)	Name of the compound	Molecular Formula	Molecular weight (g/mol)	Therapeutic activity
14.10	Benzylamine, 2-hydroxy-N,N-Di-[2-aminoethyl]-	C ₁₁ H ₁₉ ON ₃	209	Antibacterial (Lingfa and Ankanagari, 2023)
15.61	Heptanal	C ₇ H ₁₄ O	114	Antifungal (Li et al., 2022)
17.59	18-Nonadecen-1-OL	C ₁₉ H ₃₈ O	282	Antibacterial, Antioxidant (Biswal et al., 2020)
26.40	Alpha-farnesene	C ₁₅ H ₂₄	204	Antimicrobial (Ashari et al., 2019)
29.20	Andrographolide	C ₂₀ H ₃₀ O ₅	350	Antibacterial (Banerjee et al., 2017)
31.05	Beta-Sitosterol	C ₂₉ H ₅₀ O	414	Antimicrobial (Alawode et al., 2021)

**Fig. 1.** Structural characterization of *A. paniculata* active fraction by FTIR analysis.

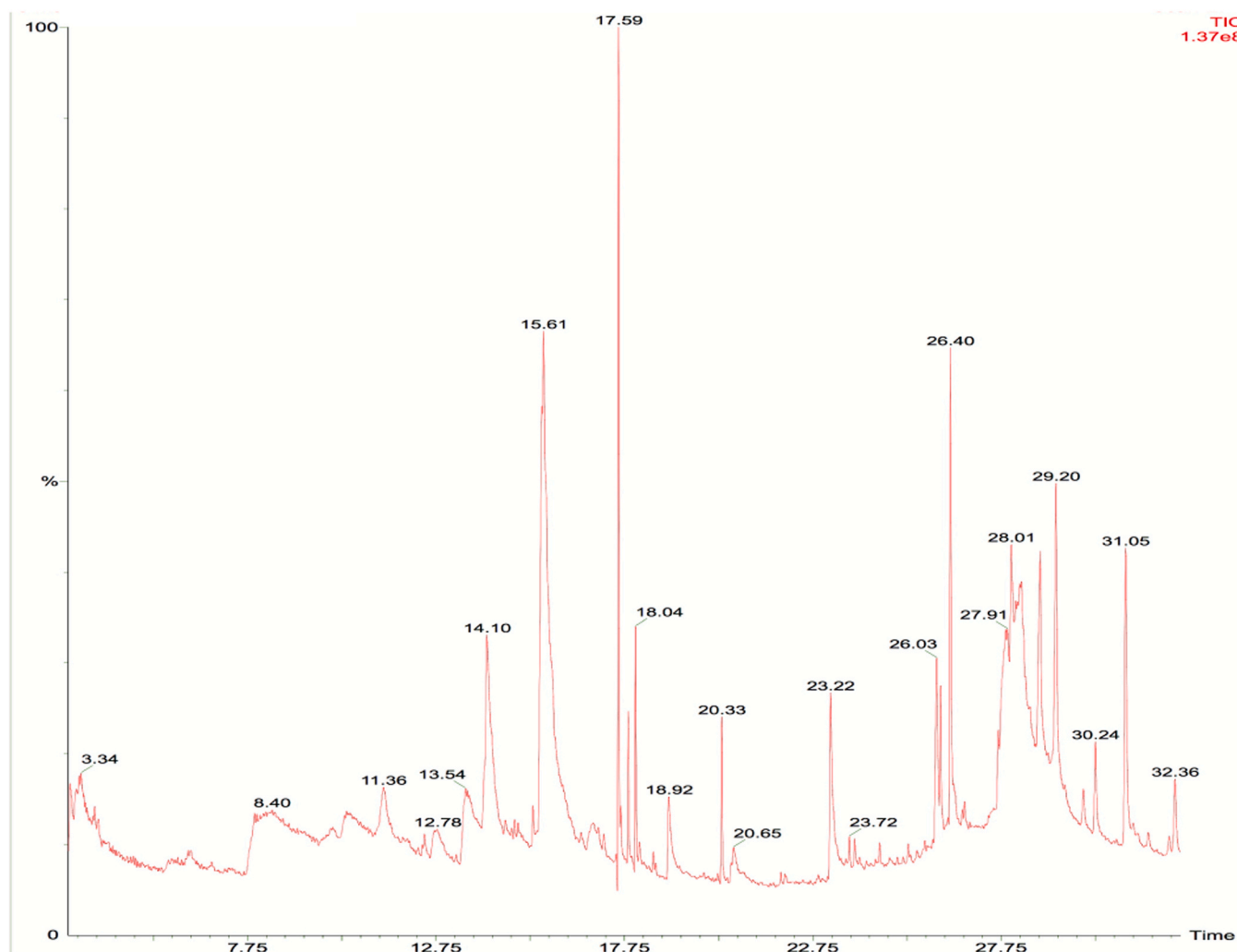


Fig. 2. Structural characterization of *A. paniculata* active fraction by GC-MS analysis.

Table 7

Growth performances of goldfish fed with different doses of *A. paniculata* extract after 2 and 4 weeks.

Sampling point	Growth parameters	Control tank (BD)	Experiment DI	Experiment DII
0–2 Weeks	Initial weight (g)	64.75 ± 0.71	64.22 ± 0.65	64.51 ± 0.88
	Final weight (g)	69.57 ± 0.44	71.55 ± 0.52 ^a	72.67 ± 0.38 ^a
	Weight gain (%)	7.44 ± 1.02	11.33 ± 0.73 ^b	11.99 ± 0.46 ^b
	SGR (% day)	34.42 ± 0.95	52 ± 0.84 ^c	55.28 ± 0.64 ^c
	FCR	3.64 ± 0.89	2.39 ± 0.94 ^b	2.26 ± 0.37 ^b
	Survival (%)	100	100	100
0–4 Weeks	Initial weight (g)	64.75 ± 1.24	64.22 ± 1.43	64.21 ± 1.32
	Final weight (g)	73.40 ± 1.35	75.92 ± 1.26 ^c	77.14 ± 1.52 ^c
	Weight gain (%)	13.35 ± 1.15	18.21 ± 1.32 ^c	19.57 ± 1.40 ^b
	SGR (% day)	30.89 ± 1.60	41.78 ± 0.98 ^b	45.1 ± 1.38 ^c
	FCR	4.06 ± 1.43	2.98 ± 1.11 ^c	2.77 ± 1.22 ^b
	Survival (%)	100	100	100

The row represented by different superscript letters was statistically significant ($p < 0.05$).

^a * $P < 0.05$ vs BD, ^b ** $P < 0.01$ vs BD, ^c *** $P < 0.001$ vs BD (SGR = specific growth rate; FCR = feed conversion ratio)

3.4. Structural characterization of *A. paniculata* fraction by GCMS analysis

The GC-MS study revealed that the presence of chemical compounds in the leaves of *A. paniculata* extract was identified with the help of the NIST library (Table 6) (Fig. 2). The peak at the retention times of 14.10, 15.61, 17.59, 26.4, 29.20, and 31.05 were confirmed as Benzaldehyde, 3-methoxy-4-[(trimethylsilyl)oxy]-, O-methyloxime, Heptanal, 18-Nonadecen-1-OL, Alpha-farnesene, Andrographolide and Beta-Sitosterol. The compounds may act as highly responsible agent for their antimicrobial and antioxidant activity.

3.5. Growth Performances

The growth parameters of goldfish fed with different doses of *A. paniculata* leaves extract as diet supplements were given in Table 7. Diet supplementation with *A. paniculata* extract had significant effects on WG and SGR compared to BD at the 2nd and 4th weeks. The percentage of WG (19.57%) and SGR (45.10%) were significantly higher in the diet DII level *A. Paniculata* extract compared to the BD and DI at 4th week. The FCR (2.77) was significantly reduced in the DII group compared to the BD group in the 4th week.

3.6. Haematological Parameters

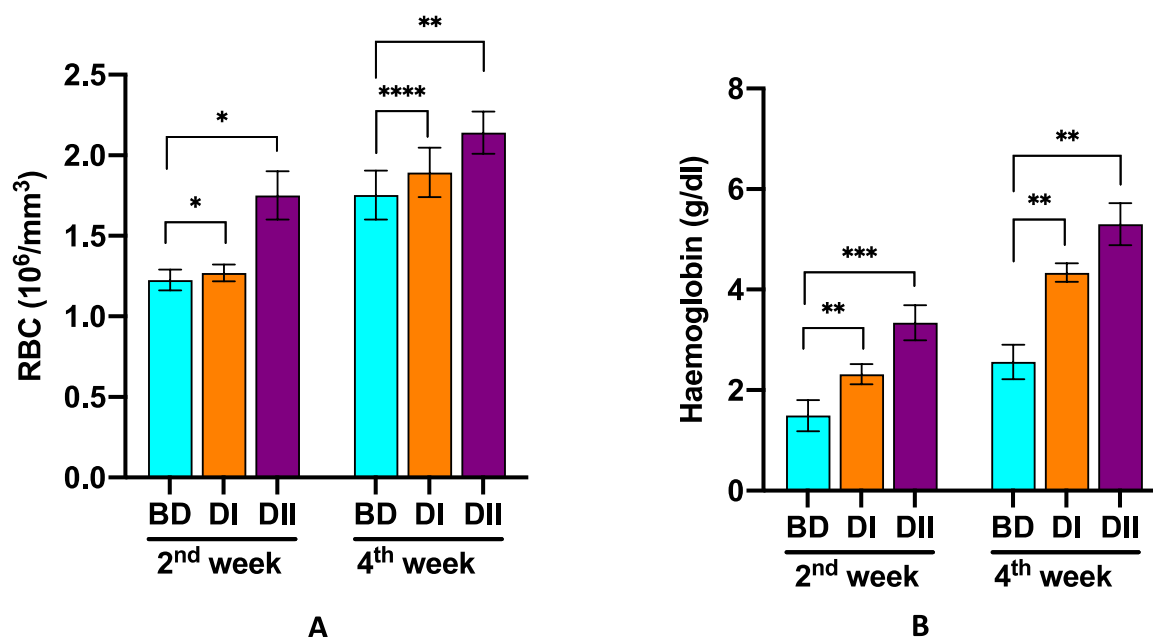
Total RBC and haemoglobin levels were significantly elevated in the

Table 8

List of primers used for real-time PCR (qPCR) amplification.

Gene	GenBank accession number	Amplicon size (bp)	Annealing temperature	Primer
IL-1 β 1	AJ249136.1	268	55 °C	FWD: 5'-TTCATTGAAGGCAGTGACG-3' REV: 5'-TAAGCTGTGCCGTCTCTTT-3'
IL-10	HQ259106.1	268	55 °C	FWD: 5'-CTTGCCAAAATCCCTTTGAG-3' REV: 5'-AGGGTGAAGTCCATTTGTGC-3'
TGF- β	EU086521.1	283	55 °C	FWD: 5'-ACCATATGCCAAAGCCTCAC-3' REV: 5'-TGATGCCTATACAGCGCAAG-3'
β actin	AB039726.2	267	55 °C	FWD: 5'-TGCTGACCGTATGCAGAAAG-3' REV: 5'-TTGAGAGGTTTGGGTTGGTC-3'

Abbreviations: bp = basepair; IL = interleukin; TGF = transforming growth factor.

**Fig. 3.** Haematological parameters – RBC (A) and haemoglobin (B) of *C. auratus* fed with *A. paniculata* extract supplementary diet after 2nd and 4th week. Data were presented as the mean $\pm$ SD ($n = 15$).

fish fed with *A. paniculata* supplementary diet compared with the BD group at the 2nd and 4th weeks (Fig. 3A). The total RBC was significantly higher in the DII group (2.14 mm³) at 4th week when compared to the BD group. Moreover, the haemoglobin level was significantly increased in the DII group (5.3 g/dL), whereas the haemoglobin level in the BD group was 2.56 g/dL in the fourth week (Fig. 3B).

3.7. Immunological parameters

After 2nd and 4th weeks, the phagocytic activity, total immunoglobulin levels and myeloperoxidase activities in the fish fed with *A. paniculata* extract supplementary diet (DI and DII) were significantly increased when compared to the BD group (Fig. 4A-C). Fish fed with 1% of *A. paniculata* supplementary diet (DII) showed the highest phagocytic activity (6.53%), total immunoglobulin levels (11.73 mg/ml) and myeloperoxidase activity (0.62) when compared to the BD group fish after 4th week.

3.8. Challenge against *A. hydrophila* infection

Dietary provision of *A. paniculata* extract enhanced the resistance of *C. auratus* against *A. hydrophila* infection. The post-challenge survival rate (80%) was significantly higher in the fish fed with the *A. paniculata* extract-supplemented DII group (Fig. 5A) whereas the survival rate (70%) was reduced in the DI group. The negative control (NC) (PBS) showed no mortality during the experiment (Fig. 5B). Pathological symptoms of haemorrhagic septicaemia, ulcers, fin rot, and tail rot, became apparent in moribund fish that were dying subsequently (Fig. 5C). We isolated colonies of *A. hydrophila* from the intestines of

dead fish.

3.9. Immune-related genes expression in *C. auratus*

The expression level of immune-related genes (IL-10, IL-1 β 1 and TGF- β) were dynamically changed when *C. auratus* fed with *A. paniculata* extract as a diet supplement (Fig. 6A-C). Following the 2nd week of feeding and then the *A. hydrophila* challenge, the IL-10 expression level was significantly up-regulated in the kidney of both groups (DI & DII), and the spleen of the DII group when compared to the BD group. Whereas the IL-10 expression was significantly down regulated in the spleen of the DII group (Fig. 6A).

Dietary *A. paniculata* extract was significantly increased the expression of IL-1 β 1 in the kidney of the DI group and spleen of both groups (DI & DII) when compared to the BD group. While the IL-1 β 1 expression was significantly down regulated in the kidney of the DII (Fig. 6B). Besides, the expression level of TGF- β was significantly up regulated in the spleen of both groups (DI & DII) when compared to the BD group. On the other hand, the TGF- β was significantly down regulated in the kidneys of the DI and DII groups (Fig. 6C).

4. Discussion

According to a recent study, the application of plant-based products for efficient and secure disease management is growing. Furthermore, the combined benefits of these natural products, such as cost-effectiveness, dependability, stability, as well as the lack of side effects, are gaining popularity. An earlier study demonstrated that methanol extracts of *A. paniculata* were particularly efficient in Indian major

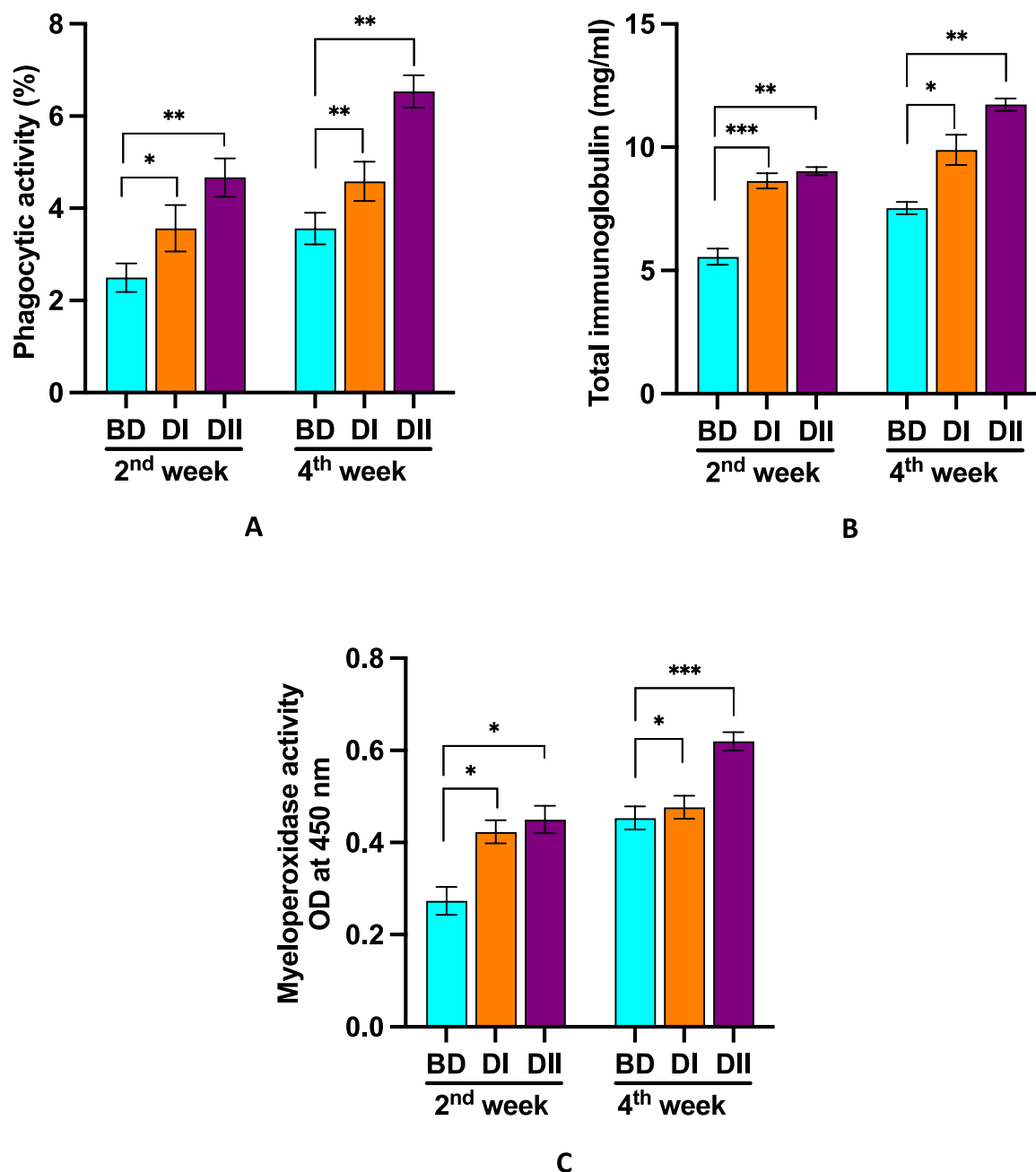


Fig. 4. Immunological assessments of *C. auratus* fed with *A. paniculata* extract supplementary diet after 2nd and 4th week. Data were presented as the mean $\pm$ SD ($n = 15$). The bar graph represents phagocytic activity (A), total immunoglobulin (B) and myeloperoxidase activity (C).

carp *Labeo rohita* against *A. hydrophila* infection. Even at low concentrations (50 μ l) (MIC) (Palanikani et al., 2020). According to Adlin Jenifer et al. (2019), MIC and MBC values revealed that the ethyl acetate extraction of *Nocardopsis* sp. AJ1 was controlled the *V. parahaemolyticus* pathogen at a low concentration due to the presence of active components. In agreement with our study, dietary *A. paniculata* extract helped to control *A. hydrophila* at the minimal level of 50 μ g due to the presence of antimicrobial components.

This study used phytochemical screening to examine the crude methanolic extract of the *A. paniculata* leaf, and the results revealed the presence of alkaloids, flavonoids, anthraquinones, tannins, saponins, protein, terpenoids, steroids, amino acids and reducing sugar (Table 4). The phytochemicals screening described in this study are known to have a wide range of pharmacological and physiological properties when used as medicines. According to several reports, tannins prevent the

replication of HIV. The primary sources of anti-inflammatory and anti-malarial chemicals are alkaloids, terpenes, and phenolic compounds. Many different terpenoids suppress the development of human cancer cells (Ochi and Okwute, 2022). Flavonoids and phenols have anti-allergic, antibacterial, and antioxidant characteristics, whereas saponins have been suggested to have anti-inflammatory, antiviral, and plant defence properties (Nahar and Sarker, 2019; Shaky, 2016).

In the present study, the methanol solvent fraction of *A. paniculata* evaluated by FT-IR analysis revealed that the leading bands in the regions between 448.75 and 3348.06 cm^{-1} belonged to aliphatic primary amine, amine salt, conjugated ketone, carboxylic acid alcohol and halo compounds. The band's intensity ranged from weak to strong with N-H stretching, C=O stretching, O-H stretching, and S=O stretching (Table 5). Similar to our study, Priya et al. (2021) reported that the FTIR study of ethyl acetate solvent fraction of *A. paniculata* indicated the

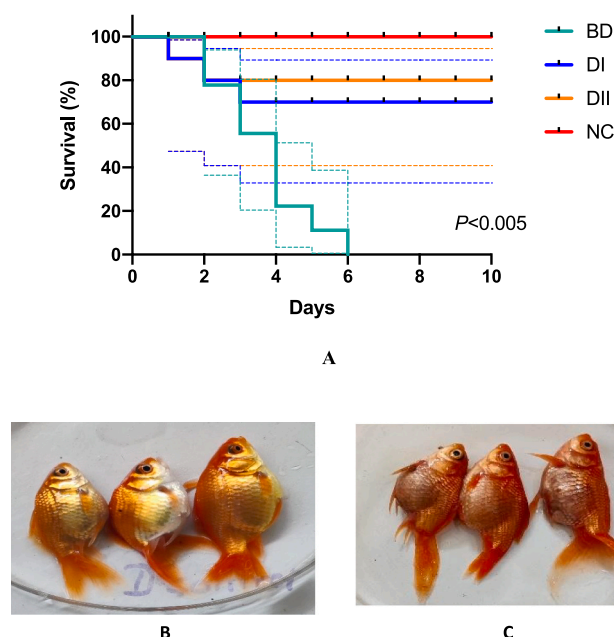


Fig. 5. Kaplan–Meier curves with log-rank (Mantel–Cox) test obtained for the survival of *C. auratus* fed with *A. paniculata* extract supplementary diet and challenged with *A. hydrophila* (A), healthy *C. auratus* injected with phosphate-buffered saline (B), and various clinical signs of *A. hydrophila* on *C. auratus* (C).

presence of aliphatic primary amine, amine salt, thiol, conjugated ketone, allene, carboxylic acid alcohol and halo compounds. Likewise, FTIR analysis of the methanol solvent fraction of *A. paniculata* revealed the presence of alkene, amine, aromatic, nitro, and alcohol (Maria et al., 2018). The presence of various functional groups suggested that the aerial parts of *A. paniculata* contain several important bioactive compounds.

Characterization of methanol extract of *A. vasica* leaf through GC-MS indicated the presence of different compounds such as Benzaldehyde, 3-methoxy-4-[(trimethylsilyl)oxy]-, O-methyl oxime, Heptanal, 18-Nona-decen-1-OL, Alpha-farnesene, Andrographolide and Beta-Sitosterol, which exhibited antibacterial, antifungal and antimicrobial activities that have been reported earlier (Alawode et al., 2022; Ashari et al., 2019; Banerjee et al., 2017; Biswal et al., 2020; Li et al., 2022; Lingfa and Ankanagari, 2023). Similar to our study, GC-MS analysis of ethanolic extracts of *A. paniculata* revealed that the presence of different compounds including, Octadecenoic acid, Oleic acid, and n-Hexadecenoic acid to possess the antibacterial, antifungal and antimicrobial activities (Kalaivani et al., 2012; Priya et al., 2021; Vasantha et al., 2013).

Dietary *A. paniculata* extract exhibited a higher percentage weight gain, specific growth rate and lowest feed conversion ratio found in the DII group compared to other groups. Consistent with previous reports, dietary supplementation with *A. paniculata* extract improves fish growth and reduces FCR in the *Pangasianodon hypophthalmus* (Bridle et al., 2011).

In this study, the effect of methanol extract of *A. paniculata* on the haematological parameters of fish were determined in terms of total red blood cells and haemoglobin levels. *A. paniculata* extracts increased the haemoglobin levels from 1.5 to 5.3 g/dL, and total RBC counts from 1.22 to 2.14×10^6 cells mm^{-3} at the end of 28 days. Consistent with previous studies, *A. paniculata* extracts have the potential to enhance the haematology status of the *Labeo rohita*, the result will be an improvement in the overall fish health (Palanikani et al., 2020). Similar to our study, haematological studies of *C. auratus* treated with *I. coccinea* following *A. hydrophila* challenge found that RBC count and haemoglobin level were significantly higher as compared to the control (Anusha et al., 2014). Sahu et al. (2007) reported that *Labeo rohita* fingerlings fed with

Mangifera indica kernels had greater WBC and RBC counts than the controls. Therefore, the fish's health condition can be determined by evaluating its haematological parameters (Cataldi et al., 1998; Sheeba, 2012).

In the present study, immunological parameters such as myeloperoxidase, phagocytic, and total immunoglobulin levels are improved in *C. auratus* fed a diet supplemented with *A. paniculata* extract. Our observation was consistent with early study, *C. auratus* fed with diets containing ethyl acetate extract of *Ixora coccinea* enhanced the immunological parameters such as phagocytic activity, serum bactericidal activity and lysozyme activity (Anusha et al., 2014). The simultaneous increase in total red blood cells corresponded to the enhanced phagocytosis activity of treated fish that maximized after 28 days. Phagocytic cells are essential parts of the non-specific immune system because they function as immune cells by engulfing and eliminating pathogens (Palanikani et al., 2020; Senthil-Nathan, 2013). Furthermore, herbal immunostimulants like *Lonicera japonica* and *Ganoderma lucidum* helped to increase the total immunoglobulin levels in *Oreochromis niloticus* against fish pathogen *A. hydrophila* (Yin et al., 2008). The methanol extract of *A. paniculata* can increase myeloperoxidase, phagocytic activities, and total immunoglobulin levels in goldfish and act as an herbal immunostimulant.

The enhanced immune systems of fish and prawns aided herbal remedies with antibacterial and immunostimulant properties in improving survival rates and lowering pathogenic load in response to pathogenic challenges (Raja Rajeswari et al., 2012). After the challenge with *A. hydrophila*, the current results found that survival of *C. auratus* supplemented with *A. paniculata* extract was improved than the fish supplemented without herbal extract. The administration of *A. paniculata* extract at 0.1% concentration to *C. auratus* improved survival by 70%, while a 1% concentration of the *A. paniculata* extract fed to *C. auratus* enhanced survival by 80%. The methanol extract of *A. paniculata* effectively improved the performance of the non-specific immunity in *C. auratus*, inhibiting the growth of *A. hydrophila*, and enhancing their survivability. There is strong evidence that the feeding of *A. paniculata* extract to *L. rohita* can alter the function of their innate immune systems and enhance the disease resistance in *A. hydrophila* (Palanikani et al., 2020). Similar to our study, Anusha et al. (2014) reported that *Ixora coccinea* extract helps to stimulate the immune system of *C. auratus* in response to the *A. hydrophila* infection.

Nowadays, characterizing cytokine genes is a remarkable progress in fish, which is regarded as useful for evaluating immunological responses (Alejo and Tafalla, 2011). The present results showed that *A. paniculata* extract significantly increases IL10, IL-1 β , and TGF β in the lymphoid organs (kidney and spleen) of the *C. auratus* when compared to the BD group. Whereas the TGF- β was significantly downregulated in the kidneys of both groups (DI and DII). The anti-inflammatory cytokine known as IL10 inhibits immune responses and regulates pro-inflammatory cytokines to prevent inflammatory damage to the living cells (Secombes et al., 2001). Jinendiran et al. (2021) revealed that IL-1 β expression in goldfish fed with *E. acetylum* S01 was balanced by the upregulation of regulatory anti-inflammatory cytokine IL-10. Defrance et al. (1992) reported that increased TGF β to stimulate IgA production leads to a rise in total immunoglobulin levels, which is related to B cell activation. Consistent with previous studies, anthocyanin extract of blackberry and *Crataegus monogyna* extract has exhibited a significant upregulation of IL-1 β and TNF- α expression in *Oreochromis niloticus* (Yilmaz, 2019) and *Trachinotus ovatus* (Tan et al., 2017), respectively. Additionally, supplementing diets with herbal additives has been shown to stimulate the pro and anti-inflammatory genes in several fish tissues (Fazelan et al., 2020). However, dietary supplementation with 1% of *A. paniculata* extract appears to be detrimental for fish since it increases the production of pro and anti-inflammatory cytokines.

Although our findings support the *A. paniculata* extract as a promising candidate for aquaculture disease management, the low bioavailability of active compounds might pose a limitation for clinical

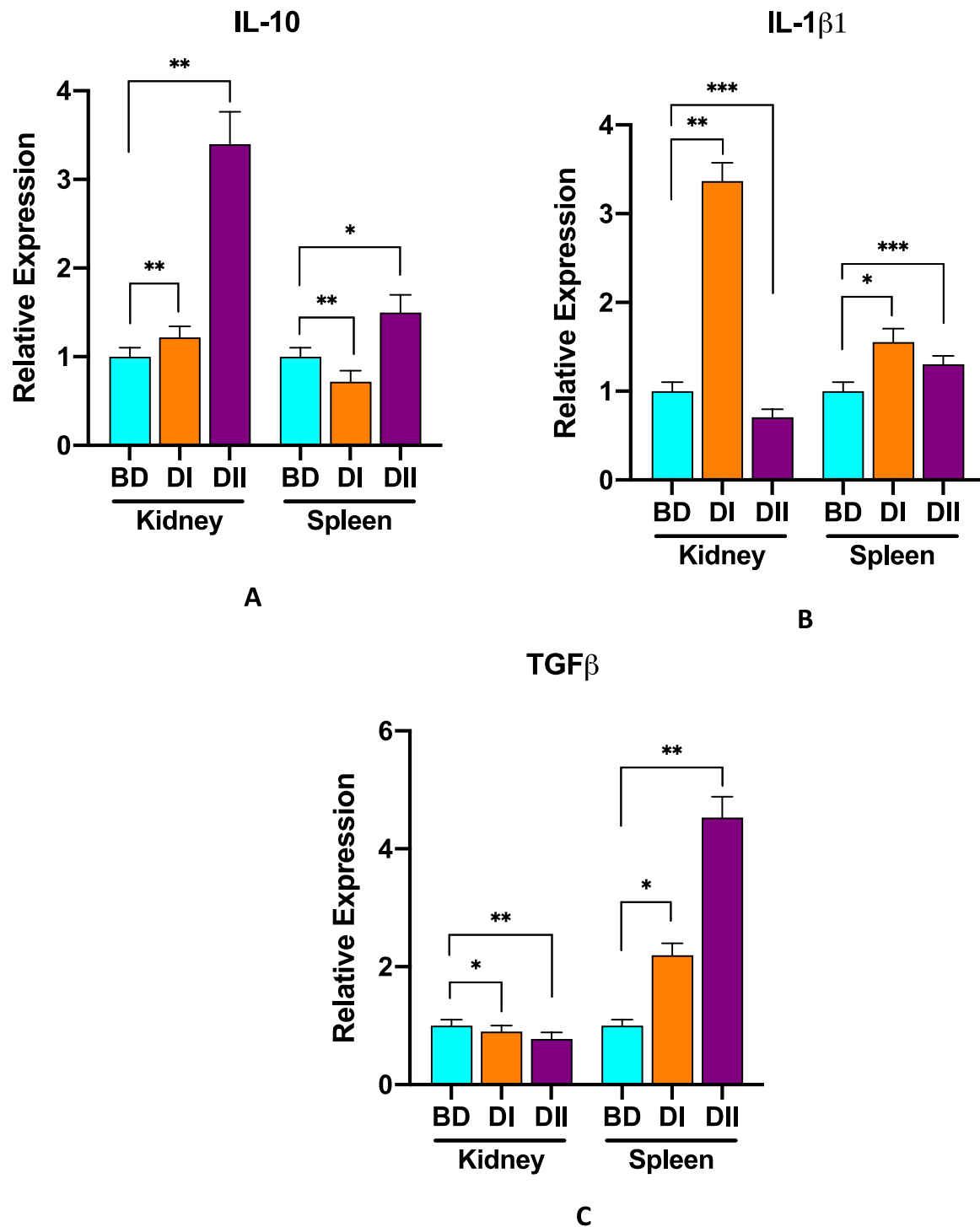


Fig. 6. Comparison of relative expressions of the genes IL-10 (A), IL-1β1 (B), and TNF-β (C) in the kidney and spleen of *C. auratus* after 4 weeks of feeding different levels of *A. paniculata* extract and challenged with *A. hydrophila*. The RT-PCR analysis was performed in triplicates, and each bar represents the mean $\pm$ SD (n = 9).

applications. Several strategies have been developed to improve active compounds by solubility, forming complexes and loading them into the nano emulsion. Furthermore, the combined benefits of these natural products, such as cost-effectiveness, reliability, and stability, as well as the lack of side effects, are gaining popularity. This study concludes that the minimum (0.1%) and maximum concentrations (0.1%) of *A. paniculata* leaf extract added to the diet of *C. auratus* would improve their growth, haematological, immunological parameters, immune-related genes expression in their lymphoid organs and resistance to *A. hydrophila* infection. It may represent as an effective

immunostimulants, potentially safe approach and an effective distribution mechanism in aquaculture system.

Authors statement

All authors agree to submit the revised MS entitled "Effects of dietary *Andrographis paniculata* extract on growth, haematological, immune responses, immune-related genes expression of ornamental gold fish (*Carassius auratus*) and its susceptibility to *Aeromonas hydrophila* infection" to your esteemed journal "Aquaculture Report". We have no

conflict of interest.

Declaration of Competing Interest

We have no conflict of interest.

Data availability

No data was used for the research described in the article.

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Personal protective equipment (PPE) pollution driven by COVID-19 pandemic in Marina Beach, the longest urban beach in Asia: Abundance, distribution, and analytical characterization

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ABSTRACT

COVID-19 pandemic has enforced the use of personal protective equipment (PPE, masks and gloves). However, the mismanagement of litter are exacerbating the increasing plastic issue worldwide. In the present study, we sampled discarded PPE in 10 sites along Marina Beach, India. We characterized the litter types by chemical analysis techniques. A total of 1154 COVID-19-associated PPE items were found on Marina beach. The highest number of items were face masks (97.9 %) and the mean PPE density in the sites studied was 4×10^{-3} PPE m⁻². The results demonstrate that poor solid waste management and lack of awareness are the main causes of pollution at Marina beach. FTIR spectroscopy revealed that face masks and gloves were principally made of polypropylene and latex, respectively. The FTIR spectra also showed signs of chemical degradation. Our results suggest that plastic pollution is increasing, possibly becoming more impactful to marine biota. Beach management measures were discussed.

1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), became a global pandemic in 2020, causing nearly 6 million deaths worldwide (WHO, 2022). During the COVID-19 pandemic, numerous disposable plastic items and disposable PPE were required as a basic precaution to prevent the spread of COVID-19 infection (Ji et al., 2021). PPE items are categorized into many types, including hazard suits, face shields, surgical masks, bouffant caps, gloves high-grade medical masks. This caused the demand for PPE to rise considerably around the world (Prata et al., 2020). It is estimated that about 65 billion gloves and 129 billion face masks are employed worldwide every month (Prata et al., 2020). In the end, adding a large charge to traditional solid waste management systems. There are several studies worldwide on the

inappropriate disposal of PPE litter (Ben Haddad et al., 2021; De-la-Torre et al., 2021; Aragaw et al., 2022; Gunasekaran et al., 2022; Mghili et al., 2022; Dioses-Salinas et al., 2022; Ribeiro et al., 2022; Sajorne et al., 2022). Benson et al. (2021) estimated that approximately 3.4 billion face masks were discarded each day. These types of litter will continue to accumulate in the future, potentially exacerbating existing plastic pollution (De-la-Torre et al., 2021). Once discarded, litter can transport from one location to another via wind, streams, and rivers to reach the marine environment (Kutralam-Muniasamy et al., 2022). It was estimated that 1.56 billion facemasks are presently disposed in the world's oceans (OceansAsia, 2020). Scientists have recorded their presence on the beaches of Peru, Brazil, Argentina, Kenya, Morocco, India, Bangladesh, Iran, Ethiopia, and the Philippines (Okuku et al., 2020a; Arduoso et al., 2021; Ben Haddad et al., 2021; De-la-Torre et al.,

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2022a, 2022b, 2022c; Gunasekaran et al., 2022; Rakib et al., 2021; Thiel et al., 2021; Aragaw et al., 2022; Hassan et al., 2022; Sajorne et al., 2022; Mohamadi et al., 2023). Nevertheless, the present data is insufficient to have a global overview of marine pollution by PPE.

Like plastic, PPE could have a significant impact on the environment and marine wildlife. PPE, particularly surgical masks, have been recognized as potential sources of microplastics in the marine environment (Morgana et al., 2021; Saliu et al., 2021). Most PPE consist of synthetic polymers, with high proportions of polypropylene, polyethylene, and polyacrylonitrile and also other polymeric materials such as polyester, polyurethane, nylon, and polystyrene (Ammendolia et al., 2021; Fadare and Okoffo, 2020; Aragaw, 2020). PPE may degrade when exposed to natural factors such as sea waves and sunlight (including UV radiation) (De-la-Torre and Aragaw, 2021; Saliu et al., 2021). The resulting pieces, smaller than 5 mm, are considered microplastics (De-la-Torre et al., 2022b). These microplastics are bioavailable to a large number of marine organisms and can produce toxic effects, the impacts of which can propagate through the food chain (De-la-Torre et al., 2022b). PPE are also able to liberate toxic chemical additives (Hajiouni et al., 2022) and serve as a vector for contaminants (Torres et al., 2021). The ingestion of face masks by marine organisms has also been documented in Brazil and Japan (Neto et al., 2021; Fukuoka et al., 2022). Recently, Hiemstra et al. (2021) and Ammendolia et al. (2022) provided an overview of the impacts of PPE interaction with various types of aquatic and terrestrial animals through entrapment and entanglement. In addition, PPE can perturb the ecosystem stability of marine habitats through the spread of invasive species (De-la-Torre and Aragaw, 2021).

Waste management is an urgent concern in India, especially with the growing population throughout the country (Banerjee et al., 2019). In India, the current COVID-19 pandemic has caused an increase in the demand for single-use plastics, adding pressure to an already out-of-control problem (Singh et al., 2022). It is estimated that the average amount of biomedical waste generated by COVID-19 in December 2021 is nearly 72.8 tons per day (CPCB, 2021). Recent reports have been published indicating that litter related to COVID-19 is not correctly discarded on Indian beaches (Gunasekaran et al., 2022). Ineffective litter management as well as the litter disposal behavior of the population are among the causes of marine litter pollution on beaches. Also, Asian rivers have discharged a huge amount of PPE into the sea and oceans (Peng et al., 2021). Only a few studies have investigated the occurrence and distribution of PPE along the beaches of Tamil Nadu (Gunasekaran et al., 2022). Data on PPE pollution on Indian beaches is still lacking.

Marina beach, located on the Southeast coast of India, is widely known for the recreational and fishing activities carried out. Recreational uses like surfing, swimming, horse riding, and picnicking, are likely drivers of plastic pollution. Preliminary marine litter surveys revealed that Marina beach is heavily polluted with marine litter (Arun kumar et al., 2016). Also, a wide range of studies have investigated the occurrence and impact of microplastics on the sediments of Marina beach and the Chennai coast (Karthik et al., 2018; Sathish et al., 2019; Sunitha et al., 2021; Venkatramanan et al., 2022). However, the current state of PPE pollution is yet to be assessed.

In this context, we have conducted wide range of surveys focusing on PPE pollution in the world's second longest beach (Marina beach), Tamil Nadu, India. In addition, a subsample of various types of PPE especially face mask and gloves was collected and analyzed by Fourier transformed infrared (FTIR), which may provide additional information on their chemical composition and degradation in the environment.

2. Materials and method

2.1. Study area

With a shoreline of >7500 km, India has been one of the main centers of intense tourism and recreational, fishing, and shipping activities along

the shore. The density of its population, the growing plastic consumption, and the presence of large rivers can constitute threatening factors for the increasing plastic pollution in the marine environment. In India, plastic consumption has increased 20-fold between 1990 (0.9 MT) and 2018 (18.45 MT; Indian Plastic Industry Report, 2019). Plastic litter mismanagement continues to be a major problem in India. The growing generation and inappropriate uses of PPE have introduced vast amounts of this type of litter into the environment and aquatic ecosystem. In India, the current average quantity of COVID-19-related biomedical waste generation during May 2022, is estimated at about 3.54 TPD (CPCB, 2022). The government of Tamil Nadu state contributed 0.338 tons per day in May 2022, while COVID-19 biomedical waste kept increasing in June (0.776 tons per day) and July (0.925 TPD) 2022. Particularly, the capital city of Tamil Nadu, Chennai, is the second highest generation of COVID-19 biomedical waste (0.216.82 TPD) during July 2022, took place. Since the outbreak of COVID-19, India and the Tamil Nadu government have proactively taken several measures for containing the disease which are in line with guidance from WHO, CDC, and other international best practices guidance and learning. Despite all these efforts, PPE litter is found on parks, beaches, roads, and sewers.

Chennai Metropolitan city is the fourth largest city in India and the capital city of Tamil Nadu state, located on the southeast coast of India (Fig. 1). According to the UN World population prospects, (2022) the Chennai population is estimated 11.5 million. Marina beach (13.05°N, 80.2824°E) is a natural urban beach in Chennai. It is the second largest urban beach in the world, situated by Fort Saint George in the north, Foreshore Estate in the south, and Bay of Bengal in the East. With a length of 6 km, it is India's longest natural city beach (Fig. 1). The mean width of the beach is 300 m with a maximum width of 437 m. It is one of the busiest beaches in the country, attracting nearly 30,000 visitors a day on weekdays and 50,000 visitors per day on weekends and public holidays. The fishing industry is the main activity for the people in the coastal areas, and the urban coastline people are working in industries and government and non-government organizations (Venkatachala-pathy et al., 2011). Furthermore, the Adyar and Cooum rivers carry the plastic litter to the shoreline and make Marina beach unhygienic (Gowri et al., 2008). However, solid waste management across the Tamil Nadu state is very poor, resulting in a significant amount of marine litter and plastic debris contaminating coastal areas (Arun kumar et al., 2016). Especially, marine litter and microplastic pollution have been evidenced in the study area (Arun kumar et al., 2016; Sathish et al., 2019; Ranjani et al., 2022; Venkatramanan et al., 2022).

2.2. PPE monitoring

A total of 10 sampling sites were selected in Marina beach (Fig. 1). The selected sites were well distributed along the beach and were representative of different coastal activities, such as recreational and fishing activities (Table 1). PPE surveys were carried out from the summer (May & June) to the beginning of the post-monsoon (July) season of 2022. During the sampling campaign, Marina beach was open to the public, thus gathering an enormous number of beachgoers and workers wearing various types of face masks. The sampling method was followed based on the previous studies conducted on the beaches of Morocco and Peru (Ben Haddad et al., 2021; De-la-Torre et al., 2021). In short, a sampling area that covered the entire extent of the beach (from the low-tide line to the upper beach limit) was determined at each location and many transects (parallel to each other) were separated by 8–10 m intervals to cover entire beach areas.

PPE sampling strategies consist of walking along each transect, visually scanning the environments, and detectable PPE litters were identified, which were categorized as face masks, gloves, face shields, and bouffant caps. Every PPE litter was photographed. Various PPE litters were carefully collected and stored in zip lock bags to be transported to the laboratory for further analysis. In each site, the sampling area was estimated using Google Earth (<https://www.google.com/earth>

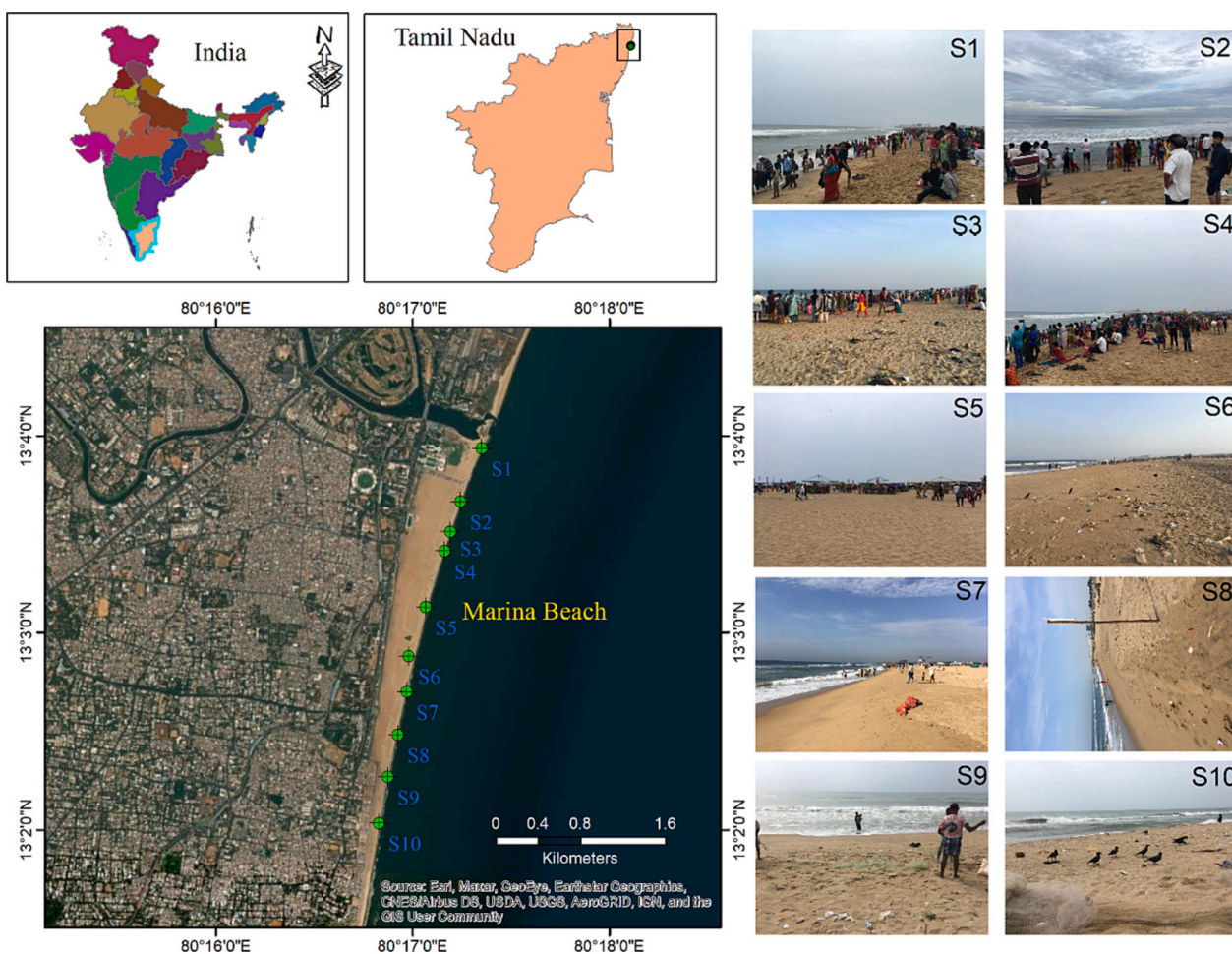


Fig. 1. Map of the sampling sites in Marina beach, Tamil Nadu, India.

Table 1

The major activity, substrate, surveyed area of each sampling site, geographical coordinates Marina beach, number and density of PPE recorded in Marina beach, Tamil Nadu, India.

Code	Site	Coordinates		Activity	Area covered (m ²)	Number of PPE	Density (m ⁻²)
		Starts	End				
S1	MGR memorial spot	13°03'58.77"N; 80°17'22.42"E	13°03'51.63"N; 80°17'18.83"E	Recreational	25,678	194	7.55×10^{-3}
S2	Marina beach 1	13°03'42.98"N; 80°17'15.26"E	13°03'33.19"N; 80°17'10.87"E	Recreational	41,476	275	6.63×10^{-3}
S3	Marina beach 2	13°03'31.69"N; 80°17'07.71"E	13°03'24.18"N; 80°17'09.15"E	Recreational	30,588	205	6.70×10^{-3}
S4	Masi Magam theerthavari spot	13°03'22.78"N; 80°17'06.16"E	13°03'14.01"N; 80°17'05.77"E	Recreational	31,798	146	4.59×10^{-3}
S5	Mobile restaurant of Tamilnadu fisheries	13°03'07.90"N; 80°17'02.47"E	13°03'00.44"N; 80°17'00.68"E	Recreational	25,623	85	3.31×10^{-3}
S6	Marina creek	13°02'56.29"N; 80°16'01.88"E	13°02'50.45"N; 80°16'57.02"E	Recreational	21,897	74	3.37×10^{-3}
S7	Gandhi beach 1	13°02'45.86"N; 80°16'58.20"E	13°02'39.27"N; 80°16'55.03"E	Recreational	21,286	76	3.57×10^{-3}
S8	Gandhi beach 2	13°02'31.79"N; 80°16'53.68"E	13°02'23.56"N; 80°16'51.12"E	Recreational	23,811	49	2.05×10^{-3}
S9	Marina beach fish market	13°02'19.87"N; 80°16'52.51"E	13°02'14.10"N; 80°16'50.67"E	Fishing	11,424	33	2.88×10^{-3}
S10	Foreshore estate beach	13°02'08.06"N; 80°16'50.41"E	13°02'01.45"N; 80°16'48.16"E	Fishing	12,687	17	1.33×10^{-3}

/) (Table 1).

2.3. FTIR analysis

The various subsamples of PPE litter ($n = 4$, 2- weathered surgical face mask, 1 glove, and 1 N-95 face mask) collected from the sampling

site of Marina beach were analyzed by Fourier- transform infrared (FTIR) spectroscopy, following De-la-Torre et al. (2022b). In order to analyze PPE litter with FTIR spectroscopy, face masks were cut open and three layer was determined separately. The readings were carried out in transmittance mode at wavelengths varied from 3500 to 500 cm^{-1} at 8 cm^{-1} resolution (De-la-Torre et al., 2022b). The adsorption bands were analyzed manually to determine the presence of various functional groups and suspected polymer types. Additionally, brand-new surgical, KN95 face masks, and gloves were analyzed by FTIR for comparison.

2.4. Statistical analysis

The PPE litter density in each sampling site was followed by Okuku et al. (2020a).

$$C = n/a$$

where C represents the density of PPE (PPE m^{-2}), n denotes the number of PPE and a is the sampled area (m^2). The mean density of PPE in each station was presented as a boxplot. Sample locations were combined by activity (recreational activities and fishing activity) to investigate its influence on PPE density. PPE density data were examined for normality and homoscedasticity. PPE densities were not normally distributed (Kolmogorov-Smirnov test and Levene test, $p < 0.05$). Therefore, nonparametric tests were employed. Significant differences in PPE density between all sites and weeks were analyzed by the Kruskal-Wallis test. The significance level was fixed at 0.05 for all statistical tests. Statistical tests were performed using SPSS software (version 20 for Windows).

3. Results and discussion

The occurrence and distribution of COVID-19-driven PPE items were



Fig. 2. Various types of surgical face masks, gloves found in different sampling sites on Marina beach.

monitored in the world's second longest beach (Marina beach), Tamil Nadu, Southeast coast of India. A total of 1154 COVID-19-associated PPE items were found on Marina beach. Fig. 2 shows examples of COVID-19-driven PPE items. The entire beach was predominantly polluted by face masks (99.81 %) and only 2 surgical gloves were found (0.17 %) (Fig. 3). Among the total face masks, 97.83 % were surgical masks, 1.21 % were cloth masks and 0.77 % were KN-95 respirators. Face shields and hazard suits were not found on the beaches. The predominance of surgical masks in the marine environment has been recorded in most studies with some exceptions (Ben Haddad et al., 2021; De-la-Torre et al., 2021; Hatami et al., 2022; Rakib et al., 2021b; Aragaw et al., 2022; Dioses-Salinas et al., 2022; Mghili et al., 2022; Ribeiro et al., 2022; Sajorne et al., 2022). This is probably due to the accessibility of surgical masks and their low cost, as well as the mandates of their use. This study showed that face masks are still abundant in the marine environment. Despite the increase in vaccination rates, the number of cases affected by the COVID-19 during the summer period is rapidly increasing. Likewise, in Tamil Nadu, the number of active cases gradually increased in May and June 2022 (10,033), whereas active cases started to reduce at end of the August 2022 (PRS, 2022). During the study period, the use of face masks is mandated by the government in public places. In addition, the WHO strongly recommends wearing masks even after vaccination to combat this virus. For this reason, visitors and tourists to Marina Beach have continued to wear masks.

A higher number of PPE items were recorded in all the stations on May 2022, followed by June 2022 and a lower number of PPE items was found on July 2022. The higher number of PPE items found in May 2022 may be due to the start of the summer holiday in India, therefore schools, colleges and the public kept visiting Marina beach. The number of tourists reached a peak during May and June which explains the large number of PPE recorded during these two months. This is consistent with the results of studies in the coastal zone, which have linked increasing numbers of beach visitors to higher PPE disposal (De-la-Torre et al., 2021; Thiel et al., 2021; Hassan et al., 2022; Sajorne et al., 2022). Following Fig. 3b, the sampling surveys with the highest densities of PPE were registered during the first four weeks. A similar temporal pattern has been recorded in Iran, Morocco, Peru, Bangladesh, Ethiopia and Brazil (Rakib et al., 2021; Aragaw et al., 2022; De-la-Torre et al., 2022a; Hatami et al., 2022; Mghili et al., 2022; Ribeiro et al., 2022). Concerning the PPE accumulation rates, we also observed a remarkable rise in the density of PPE items in weeks 2 and 3, which were marked to be Sunday and Monday. These two days of sampling coincided with the weekend. This may be attributed to the weekend effect. A total of 733 PPE litter were collected on the weekend while 421 were collected on the weekday. The number of visitors in the beach zones has increased during these weekend days, and consequently, PPE dumping increased in the coastal area. Our results were also similar to the findings of Hassan et al.

(2022), where the number of PPE litter increased during weekends in Egypt and Saudi Arabia. Sajorne et al. (2022) also observed a large amount of PPE on the weekend. There was a decrease in the number of PPE during week 6 (W6). From the beginning of W6, the number of visitors suddenly decreased, which coincided with the start of the reopening of schools. In Morocco, Ben Haddad et al. (2021) recorded a low occurrence of PPE items during closures and a suddenly raised just after the beaches were reopened to the public.

The overall mean density of PPE items was $4.00 \times 10^{-3} \text{ m}^{-2}$ and ranged from 0.00 to $2.25 \times 10^{-3} \text{ PPE m}^{-2}$ (Table 2). These findings

Table 2
Comparison of the mean density of PPE on different beaches in the world.

Country	City	PPE density (PPE m^{-2})		Reference
		Mean	Range	
Morocco	Tetouan	1.2×10^{-3a}	$0.00\text{--}3.67 \times 10^{-3}$	Mghili et al. (2022)
Morocco	Agadir	1.13×10^{-5}	$0.001.21 \times 10^{-4}$	Ben Haddad et al. (2021)
Kenya	Kwale and Kilifi	–	$0.00\text{--}5.6 \times 10^{-2}$	Okuku et al. (2020a)
Ethiopia	Bahir Dar	1.54×10^{-4}	$1.22 \times 10^{-5}\text{--}2.88 \times 10^{-4}$	Aragaw et al. (2022)
Peru	Lima	6.42×10^{-5}	$0.00\text{--}7.44 \times 10^{-4}$	De-la-Torre et al. (2021)
Peru	Multiple	6.60×10^{-4}	$0.00\text{--}5.01 \times 10^{-3}$	De-la-Torre et al. (2022a, 2022b, 2022c)
Peru	Protected areas	1.32×10^{-3}	–	Dioses-Salinas et al. (2022)
Argentina	Multiple	7.21×10^{-4}	$0.00\text{--}5.60 \times 10^{-3}$	De-la-Torre et al. (2022a, 2022b, 2022c)
Brazil	Santos	7.46×10^{-5}	$0.00\text{--}3.89 \times 10^{-4}$	Ribeiro et al. (2022)
Chile	Nationwide	6.00×10^{-3a}	–	Thiel et al. (2021)
Bangladesh	Cox's Bazar	6.29×10^{-3}	$3.16 \times 10^{-4}\text{--}2.18 \times 10^{-2}$	Rakib et al. (2021)
Iran	Bushehr	–	$7.71 \times 10^{-3}\text{--}2.70 \times 10^{-2}$	Akhbarizadeh et al. (2021)
Iran	Mazandaran	1.02×10^{-4}	$0.00\text{--}7.16 \times 10^{-4}$	Hatami et al. (2022)
Iran	Kish Island	2.34×10^{-4}	$0.00\text{--}1.18 \times 10^{-3}$	Mohamadi et al. (2023)
India	Tamil Nadu	1.08×10^{-3}	$2.80 \times 10^{-4}\text{--}2.80 \times 10^{-3}$	Gunasekaran et al. (2022)
India	Marina beach	4.00×10^{-3}	$0.00\text{--}2.25 \times 10^{-3}$	Present study

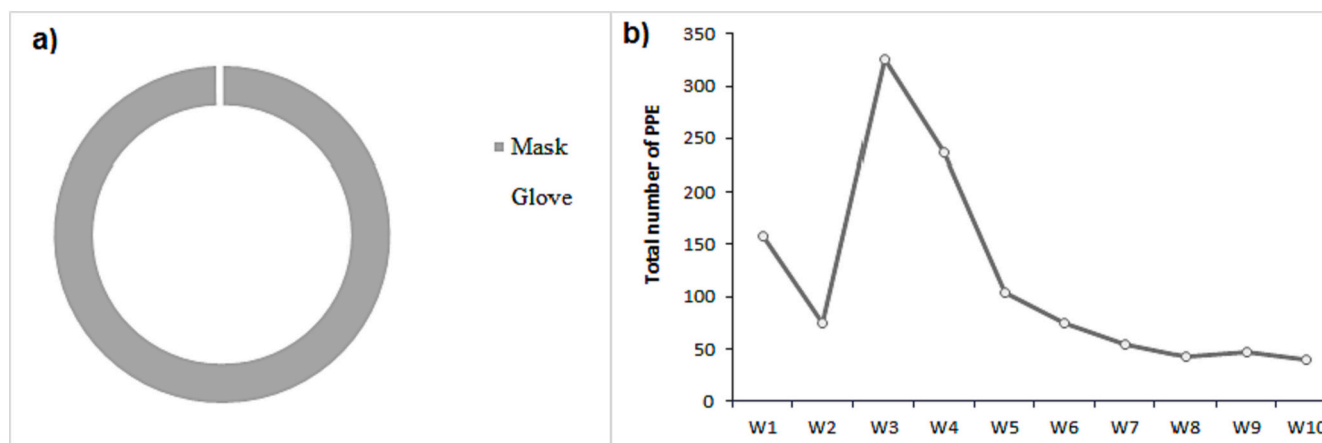


Fig. 3. a) Contribution of each type PPE items. b) Weekly evolution of the total number of PPE across sampling sites.

indicate a relatively higher abundance compared to previous studies (Table 2). The mean density of PPE in the study area was comparable to the values reported from the beaches of Tamil Nadu, India. Specifically, the abundances reported in this study are much higher than those from Morocco, Peru, Brazil, Ethiopia, Iran, and Argentina beaches. At the same time, these values are lower than the abundance recorded in Chile and Bangladesh. As the table shows, the abundance of PPE varies from one country to another. This variation may be affected by population density, COVID-19 restrictions and protocols, sampling area, weather conditions, and population density (Ben Haddad et al., 2021; Sajorne et al., 2022). The boxplot displays the mean density in each station (Fig. 4). PPE densities were grouped according to the principal activities performed in each sample area. A large number of PPE were recorded in the recreational activity ($n = 1104$; 0.0037 m^{-2}) compared to the fishing activity ($n = 50$; 0.0004 m^{-2}). PPE density differed significantly between the different activities (Kruskal Wallis test, $p < 0.05$). Previous research indicated a clear effect of the type of activity on the mean density of PPE. The present survey results observed that the highest number of PPE litter was found in more intensive touristic sites (S1 to S4) on Marina beach (Table 1). Particularly, the highest number of PPE items was found in S2 ($n = 275$), probably due to the larger number of beachgoers who occasionally gather and celebrated birthday parties, practice horse riding, and bathing. There is also a large number of commodities and snack shops located near to the S2 sampling site. Similarly, S5 to S8 are tourist sites in Marina beach where recreational and cultural activities (e.g., playing, bathing, spiritual activity) take place. Therefore, this touristic zone was also contaminated with a significant number of PPE items ($n = 284$). Kruskal-Wallis tests revealed that the PPE density differed significantly between the study sites ($p < 0.05$). Many residents and visitors were observed wearing facemasks. With a large number of visitors on recreational sites, the number of PPE litter was also reported to increase. For instance, recreational beaches in Lima, Peru (De-la-Torre et al., 2021), Cox's Bazar, Bangladesh (Rakib et al., 2021), Tetouan, Morocco (Mghili et al., 2022), and the Bushehr coast of the Persian Gulf (Akhbarizadeh et al., 2021) were considerably more polluted than the fishing beaches. Interestingly, in S9 (Marina beach fish market) and S10 (Foreshore estate beach) fishing activities primarily take place. Only 50 PPE items were found in these locations (representing 4.33 % of the total number of PPE), probably due to the reduced number of beachgoers and visitors in contrast with recreational beaches. Rakib et al. (2021) also recorded low densities in the fishing areas. Okuku et al. (2020b) indicated that beaches utilized for mixed activities had a higher density of litter than those utilized only for recreational or fishing activities.

The intertidal zone displayed a higher abundance of PPE ($n = 664$,

mean = 0.0026 items/m^2) compared to the supralittoral zone ($n = 490$, mean = 0.0019 items/m^2). In contrast, Kaviarasan et al. (2022) reported a larger amount of marine litter accumulating in the supralittoral zone compared to the intertidal zone. This suggests that the vast majority of litter found may have been brought and incorrectly discarded by beachgoers instead of washed ashore. This behavior is probably due to the lack of awareness and poor environmental education (Mghili et al., 2020). The significant abundance of PPE on the supratidal zone shows that this zone is a critical litter sink. The large amounts of PPE from the intertidal zone are not only explained by recreational activities but probably due to environmental processes. Disposed masks can be carried to surface waters where they can be transported further into marine environments. In addition, PPE is discarded in landfills without appropriate management due to the lack of resources to manage this type of litter. Drainage systems are also identified as sources of PPE on beaches (Gunasekaran et al., 2022). Wind, inundation by rainwater, and rivers are the primary pathways of face masks to marine environments. Several rivers flowing from west to east transport significant quantities of plastic litter into the study area. Neelavannan et al. (2022) previously attributed a large amount of plastic litter on Poompuhar beach to the inflow of the Cauvery River, which plays an important role in the deposition of plastic on the Tamil Nadu coast. The significant presence of PPE on the intertidal zone is likely the result of this litter washing up on the beaches. This highlights that Indian waters are widely affected by this new type of pollution, which are subject to local hydrodynamics and poor environmental awareness.

Tamil Nadu is located on the South-Eastern coast of India, with a shoreline length of 1076 km (~13 % of India's total shoreline) comprising multiple ecologically significant places, including Palk Bay (home to reefs and sea grass beds), the Gulf of Mannar Biosphere Reserve, Vedaranyam and Pichavaram (mangrove swamps), and Lake Pulicat (lagoon of ecological importance). The diversity of the environment of this region is accompanied by a great biological diversity with the presence of hundreds of endemic species of different taxa. This site is endowed with a wide range of marine biodiversity, such as fishes, bivalves, gastropods, crustaceans, corals, sea anemones, polychaete worms, echinoderms, and bryozoans (ZSI 2007; Venkatraman and Venkataraman, 2012; Tenjing et al., 2019). In addition, Marina beach supports turtle nesting sites, especially olive ridley turtles (Bhupathy et al., 2007). This region has lost its pristine and aesthetic condition due to high anthropogenic pressure, such as recreational activity, dumping untreated domestic waste, and fast-growing urbanization (Arunkumar et al., 2016; Venkatraman et al., 2022). With the increasing number of COVID-19-related litter in Indian marine environments, the threats to marine life are becoming more and more numerous. PPE will probably

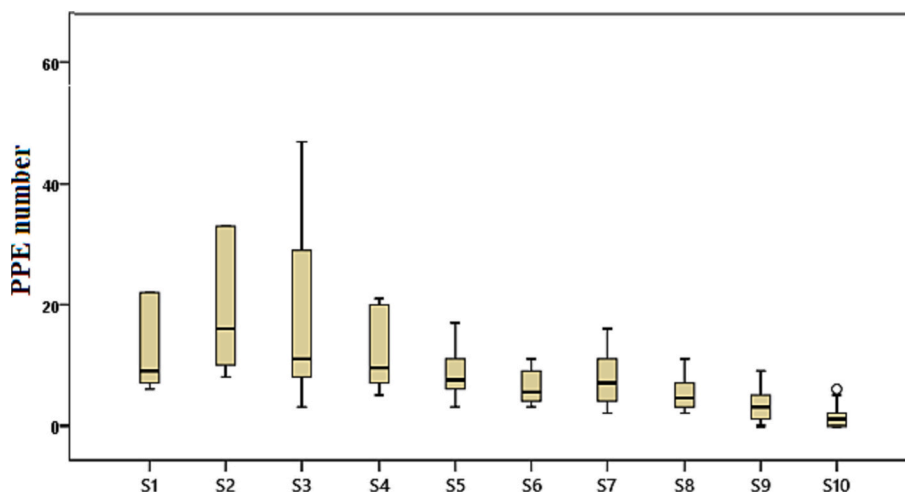


Fig. 4. Box plot diagram of the PPE number among sampling sites.

become an entanglement and ingestion threat to marine fauna in Indian waters. Entanglement is the most reported impact of PPE on wildlife. Ammendolia et al. (2022) reported 114 cases of interaction between wildlife and COVID-19-related litter, most of which were affected by entanglements. Birds, mammals, and invertebrates were most affected by entanglements. These authors have documented two entanglements of animals by PPE in India. In this study, we observed the presence of a face mask near the hole of a crab (Fig. 5b). Mohamadi et al. (2023) already recorded a crab entangled in a face mask. Marine wildlife can also ingest face masks. The first case of face mask ingestion by a Magellanic penguin (*Spheniscus magellanicus*) was documented in Brazil (Neto et al., 2021). Also, the presence of masks in the feces of a juvenile green turtle has also been documented in Japan (Fukuoka et al., 2022). In India, many studies have documented the ingestion of microplastics by marine wildlife. Bioaccumulation of microplastics in epipelagic and mesopelagic fish, commercially important fishes, Indian white shrimp, Indian edible oysters, bivalves, and other marine animals has been reported in India (Patterson et al., 2019; Daniel et al., 2020a, 2020b; Dowarah et al., 2020; James et al., 2020; Karuppasamy et al., 2020; Sathish et al., 2020). PPE can pose a hazard to many marine animals, especially those vulnerable to plastic ingestion in India. It is expected that PPE items, which are primarily composed of synthetic polymers, degrade periodically in the marine environment, leading to its fragmentation into smaller pieces (De-la-Torre et al., 2022b). PPE items may serve as a suitable substrate for the colonization of alien species. Studies have already indicated the adequacy of PPE as an artificial substrate for benthic organisms and also microorganisms, which increases the chances of biological invasion (De-la-Torre and Aragaw, 2021; De-la-Torre et al., 2021; Crisafi et al., 2022). Zhou et al. (2022) experimentally demonstrated that face masks enrich and host microbial communities, potentially acting as pathogen vectors. In addition, PPE in the marine environment acts as a carrier for the transfer of contaminants to marine organisms, which could potentially induce a range of deleterious and cytotoxic effects (Dobraradaran et al., 2018, 2021; Takdastan et al., 2021; Hajjouni et al., 2022). Recent studies have revealed that masks and wipes contain a large number of inorganic and organic pollutants used as UV stabilizers, plasticizers, and flame retardants in plastic production, including organophosphate esters, phthalates (di- and mono) and non-phthalates, bisphenols, antioxidants and plastic additives (Liu and Mabury, 2021; Sullivan et al., 2021; Wang et al., 2021a; Kutralam-Muniasamy et al., 2022). Face masks also contain metallic nanoparticles, such as Ag and Cu (Ardusso et al., 2021; De-la-Torre et al., 2022c). Based on previous work, the massive use of single-use plastics due to the COVID-19 clearly added additional stress to Indian marine ecosystems that are already threatened by numerous pressures.

3.1. FTIR results

Weathered and brand-new PPE (2 surgical face masks, 1 KN95 respirator, and 1 glove) were analyzed by FTIR to determine their polymeric composition and signs of degradation (Fig. 6). Both surgical face masks and KN95 respirators showed typical PP absorption bands

characterized by the presence of strong peaks around 2950, 2915, 2838 cm^{-1} (assigned to C—H stretching), 1455, and 1377 cm^{-1} (assigned to CH_2 and CH_3 bending, respectively), and weaker peaks around 1166, 997, 972, 840, and 808 cm^{-1} (assigned to the stretching, bending, and rocking of C—C, C—H, CH_2 , CH_3) according to Jung et al. (2018). The spectra coincide with those from the brand-new surgical face mask and KN95 respirator with slightly weaker absorption bands. The weathered samples, regardless of the mask type, showed an increase in the strength of peak at around 1700–1780 cm^{-1} , which is normally attributed to carboxyl groups (C=O). Similar observations have been reported in face masks extracted from the environment in Peru, Argentina, Ethiopia, and The Persian Gulf (Aragaw et al., 2022; De-la-Torre et al., 2022b; Mohamadi et al., 2023). These changes are due to exposure to the sun, which induced chain scission and later oxidation of the polymer chains (Gewert et al., 2018). Highly weathered plastics tend to change their structure, mostly increasing their crystallinity in the case of polyolefins, like PE and PP (Hsu et al., 2017), ultimately becoming more brittle and subject to fragmentation under a mechanical stressor (Andrady et al., 2022).

The glove was identified as Latex, characterized by the presence of a broad peak at around 3394 cm^{-1} attributed to OH stretching (Fig. 7), as well as sharper peaks at 2957, 2918, and 2851 cm^{-1} attributed to C—H stretching (Agostini et al., 2008). Weaker peaks at around 1795 cm^{-1} , and between 1650 and 1500 cm^{-1} are likely associated with vibrations of the C=O and C=C structures, respectively (Jung et al., 2018). The peak at 830 cm^{-1} was attributed to the $\text{CCH}_3\text{=CH}$ structure, typical of natural rubber (Rolere et al., 2015). Absorption bands at around 1412 and 872 cm^{-1} may be due to the presence of calcium carbonate (CO_3^{2-} stretching and out of plane deformation), which is a common additive in latex gloves (Baeta et al., 2009). The wavenumber and peak intensity of the weathered glove were similar to those from the brand-new one, except for a sharper peak at 3394 cm^{-1} (OH stretching). Wang et al. (2022) reported the occurrence of stronger peaks around 1780–1700 cm^{-1} assigned to C=O groups in weathered latex gloves, as well as a drop in the strength of O—H absorption bands ($\sim 3000\text{--}2800\text{ cm}^{-1}$). This behavior was not followed by the IR spectra of the weathered glove in the present study. However, this is likely due to the reduced/uncontrolled time exposed to the sun, resulting in photooxidation.

According to the above data, the presence of PPE in the marine environment can generate large concentrations of microplastics and continuously pollute the environment. Several studies have documented the release of micro- and nanoplastics from disposable masks and other PPE (Aragaw, 2020; Fadare and Okoffo, 2020; Ma et al., 2021; Saliu et al., 2021; Wang et al., 2021b). Some items of the face masks showed some degree of damage or physical degradation (torn layers, Fig. 5a), similar to those reported by Akhbarizadeh et al. (2021). With a large number of PPE litter entering Indian beaches, we assume that microplastic pollution may become more extensive, especially in the areas most impacted by this type of pollution.

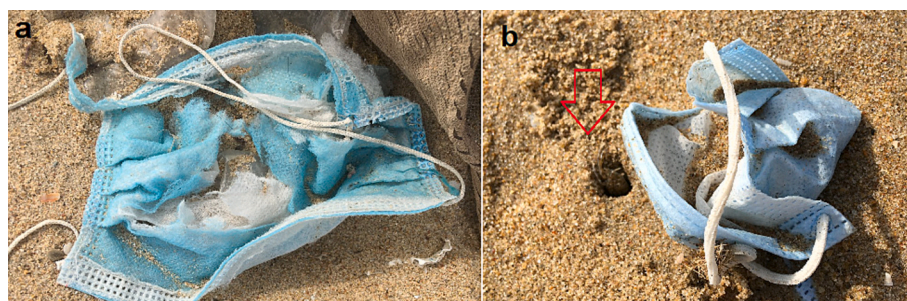
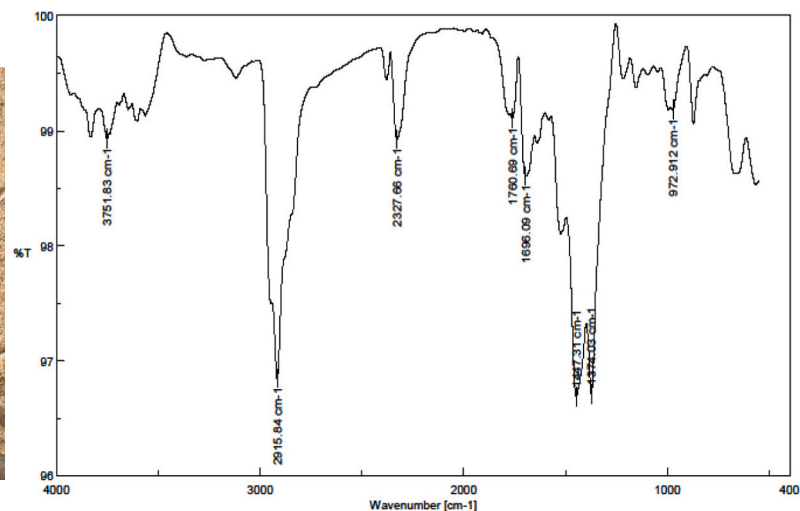
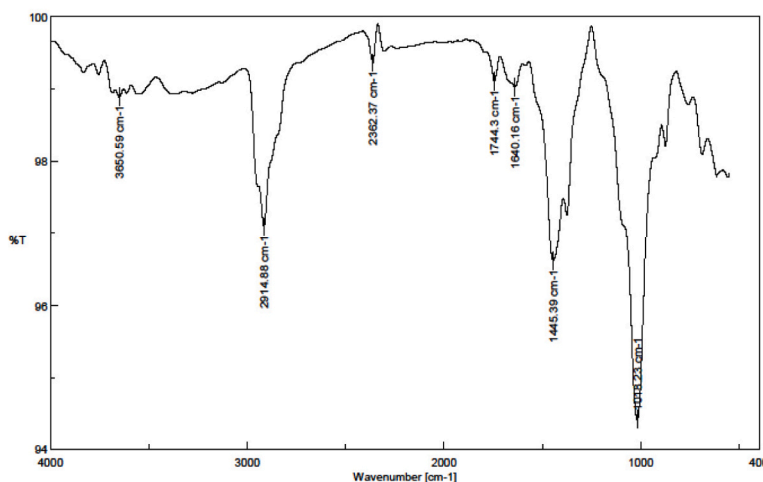


Fig. 5. a) Degraded masks found in the study area. b) a face mask near a crab hole.



[Comments]
Sample name
Comment
User
Division
Company

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Fig. 6. Photographs of a weathered surgical face mask and their corresponding FTIR spectrum.

4. Recommendations

There is an urgent need for viable management actions to save the marine resources in Marina beach from illegal littering of PPE items. As mentioned earlier, the lack of awareness is one of the major causes of PPE pollution on Indian beaches. Awareness and education may encourage more pro-environmental behaviors and decrease incorrect disposal of plastics and PPE on beaches. One of the best methods is to involve citizens and children in the cleanup campaigns, and apply citizen science to obtain litter contamination baselines while encouraging the population to get involved in educational activities (Bouzekry et al., 2022). The reappearance of the Olive Ridley turtles on the beach of Mumbai after 20 years, following the largest cleaning operation, is one of the best examples of the importance of involving people in the cleanup efforts (AFP, 2018). Extensive media coverage of the negative impact of incorrect PPE and plastic disposal is necessary to raise awareness of better practices through promotional videos and educational campaigns. During the survey, it was observed that none of the

garbage cans were placed on the beach. For this, we recommend the installation of garbage cans every 100 m on the entire beach. Periodic beach cleaning campaigns should be implemented in order to collect misplaced debris. Reusable masks should be promoted, as they can be used repeatedly and are a better choice compared to surgical masks. Recycling PPE litter provides a higher benefit to the society. Today, there are a number of initiatives to recycle PPE in India, but they are still insufficient to make up for the amount of waste generated in coastal areas. It is imperative that local and competent authorities allocate an appropriate budget to combat marine litter in India while reducing waste generation at the source by improving solid waste management systems and developing education programs.

5. Conclusions

The COVID-19 pandemic has accelerated the PPE pollution in the beach environment, negatively impacting marine biota, potentially harbour pathogens and non-native species, and release of MPs and

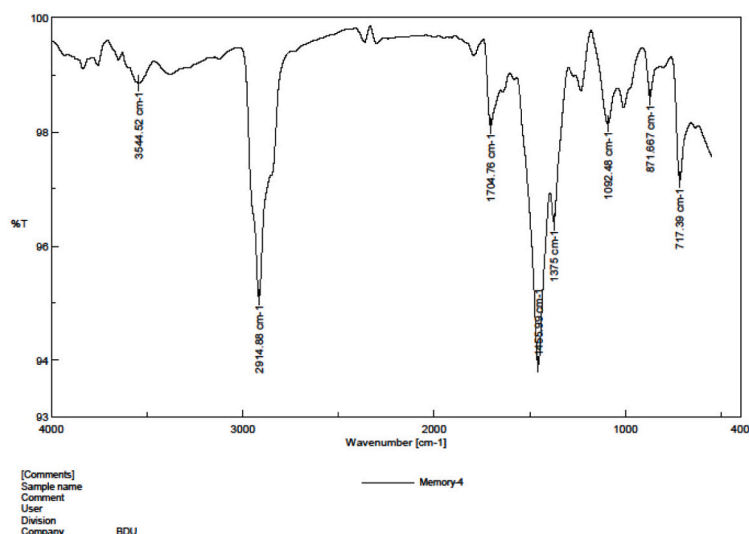
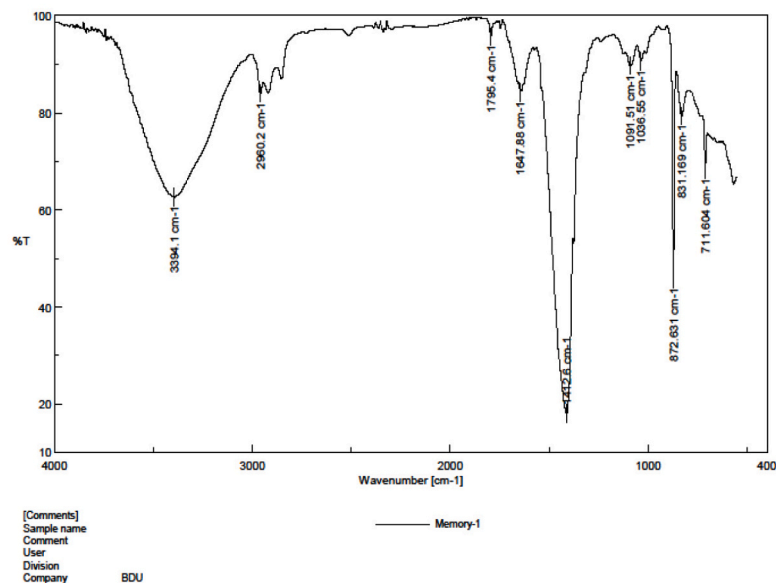


Fig. 7. Photographs of a weathered glove (top) and KN-95 face mask and their corresponding FTIR spectrum.

chemical contaminants. In the present study, COVID-19-driven PPE litter was monitored for 10 continuous weeks in 10 different sampling sites on Marina Beach, India, the longest urban beach in Asia. The observed results are comparable to those from the coastal environments around the world. The overall mean density of PPE items was 4.00×10^{-3} PPE m⁻² and ranged from 0 to 2.25×10^{-3} PPE m⁻². The entire Marina beach was predominantly polluted by face masks (99.81 %) and only 2 surgical gloves were found (0.17 %). A higher number of PPE items were recorded in all the stations on May 2022 (summer season), likely due to the summer holiday in India, where a higher number of people visited Marina beach. The results of the present surveys demonstrate that poor solid waste management and lack of environmental awareness among beachgoers on Marina beach are the main drivers of PPE pollution. The abundance of COVID-19-driven PPE items may cause entanglement, and ingestion hazards to the intertidal biota and top predators, as well as posing a potential source of microplastics and chemical contaminants. The authorities should make the alternative

mitigation routes in a durable plan with waste-to-energy recycling policies. However, further research and development are needed regarding the leaching of chemical additives and microplastic, as well as elucidating the ecotoxicological consequences in order to obtain a clear picture concerning the environmental implications of COVID-19-driven PPE pollution.

CRediT authorship contribution statement

Gunasekaran Kannan: Conceptualization, Investigation, Methodology, Writing- Original draft preparation.

Bilal Mghili: Conceptualization, Writing- Original draft preparation.

Gabriel Enrique De-la-Torre: Methodology, Writing- Reviewing and Editing.

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Mayakrishnan Machendiranathan: Writing- Reviewing and Editing,

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Ayyappan Saravanakumar: Writing- Reviewing and Editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Isolation and screening of antimicrobial biosurfactants obtained from mangrove plant root-associated bacteria

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Abstract

The unique properties of biosurfactants obtained from microbes, including their activity at extreme temperatures, make them more attractive than synthetic alternatives. Henceforth, the principle objective is to isolate and detect the antibacterial and antifungal activities of the biosurfactants produced from bacteria of the economically competitive mangrove ecosystem. Using the serial dilution method, 53 bacterial strains were recovered from the Manakudy mangrove forest in Kanyakumari, India, for the investigation. Different biosurfactant screening methods and morphological and biochemical tests were opted to select the potential biosurfactant producer. After the initial screening, two strains were discovered by 16S rRNA gene sequencing followed by extraction using chloroform: methanol (2:1) by the precipitation method. The partially purified biosurfactants were then screened for antimicrobial properties against pathogens including *Mucor* sp., *Trichoderma* sp. Morphological, biochemical, and 16S rRNA gene sequencing identified the two strains to be gram-positive, rod-shaped bacteria namely *Virgibacillus halodentrificans* CMST-ZI (GenBank Accession No.: OL336402.1) and *Pseudomonas pseudoalcaligenes* CMST-ZI (GenBank Accession No (10 K): OL308085.1). The two extracted biosurfactants viz., 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester, as well as cycloheptane efficiently inhibited human pathogens, including *Enterococcus faecalis*, and fungi, including *Mucor* sp., *Trichoderma* sp., indicated by the formation of a zone of inhibition in pharmacological screening. Thus, there is a growing interest in the prospective application of these biosurfactants isolated from marine microbes, exhibiting antimicrobial properties which can be further studied as a potential candidate in biomedical studies and eco-friendly novel drug development.

Keywords: *Pseudomonas pseudoalcaligenes* CMST-ZI; *Virgibacillus halodentrificans* CMST-ZI; Antimicrobials; Biosurfactants; Mangrove plants.

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Aruna Veerasamy; Kanimozhi Balakrishnan ✉; Godwin Antony

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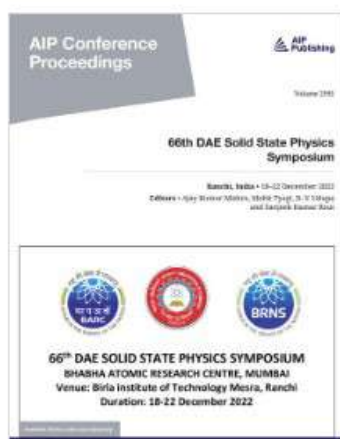
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This paper aims to improve the thermal performance of Closed loop pulsating heat pipe (CLPHP) with nanofluid fused with refrigerants. In this paper, the thermal studies were carried out by SWCNT and MWCNT nanofluids fused with R-134a. Experimentation was conducted at 10W heating power and 90° inclination angle, temperature difference from evaporator and condenser were validated. Results indicate that using nanofluid fused with R-134a, could improve the thermal performance of heat pipes. And the readings were taken every two minutes. And the obtained result was plotted to and the result shows the addition of CNT nanofluid to the refrigerant improves the Thermal performance of the closed-loop heat pipe (CLPHP). Compare to SWCNT, MWCNT with R-134a shows better heat transfer and improve the cooling capacity.



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Experimental investigation of strength characteristics of concrete reinforcement with neem powders

S. Raghuram ^a, Madhanagopal Manoharan ^{b, d}  , A. Devanandhu ^c

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Abstract

Collapsibility is the major problem faced by every structural element in buildings. Several innovations are happening every year to prevent concrete failure for better stability of these structures. This study is focused on analysing the strength aspects by using Neem powders on the concrete reinforcement and strength performance of the Neem mixed concrete at various percentages such as 0%, 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% with that of cement. Beam elements have been casted and tested using a Universal Tensile Testing

Molecular phylogeny reconstruction and biogeographic pattern of Rays (Elasmobranchii: Myliobatiformes) from Indian coastal waters

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In the present study, the phylogenetic relationship among Myliobatiformes was reconstructed from the Indian waters based on the cytochrome c oxidase subunit I (COXI) gene. Overall, 307 sequences (18 collected from Mandapam fish landing centres, Tamil Nadu, and 289 from the previous literatures) from 34 species of Myliobatiformes were clustered under two major phylogenetic clades. The families Mobulidae, Rhinopteridae, Plesiobatidae, and Gymnuridae were monophyletic, while the families Myliobatidae and Dasyatidae were polyphyletic. Further, the genera *Aetomylaeus* (Myliobatidae) and *Pateobatis* (Dasyatidae) show polyphyly by showing deep genetic divergence in the phylogenetic tree. Based on the phylogenetic tree analysis, *Himantura uarnak* appears to be *H. tulul* in the Indian waters. Similarly, *Neotrygon indica* should be consistently used instead of *N. kuhlii*, as Indian specimens form a distinct subclade within *N. kuhlii* species complex. In this study, it is also observed that several entries in the NCBI GenBank are erroneous; thus, an updation of data is recommended based on the present study. The biogeographic patterns revealed *H. tulul*, *Maculabatis gerrardi*, *Brevitrygon imbricata*, and *Gymnura poecilura* from the Indian coast form a separate haplotype compared to other geographical areas (Indo-west Pacific). In addition, *G. poecilura* and *B. imbricata* were genetically divergent between east and west coast populations of India, indicating a possibility of cryptic species.

[**Keywords:** Haplotype network, Indian coast, Marine rays, Phylogeny, Ray's taxonomy]

Introduction

Batoid rays of order Myliobatiformes form a major component of the elasmobranch fisheries in India¹. The fishing of rays in India is generally non-targeted and are mostly caught as by-catch in trawl fishing. However, in recent years, direct fishing in some areas has also been reported due to the increasing demand for international shipment owing to their biomedical values². The landing of rays in India has increased significantly during the past few decades². The east coast contributes ~70 % of rays landing in the country, while the west coast contributes the remaining ~30 % (refs. 2,3).

The order Myliobatiformes consists of ~239 valid species globally, belonging to 12 families, namely Zanobatidae, Hexatrygonidae, Dasyatidae, Urotrygoniade, Gymnuridae, Plesiobatidae, Urolophiade, Aetobatidae, Myliobatidae, Rhinopteridae, Mobulidae, and Potamotrygonidae. Only the family Potamotrygonidae consists of freshwater and marine rays, while other families have marine and estuarine species⁴. Among other families, Dasyatidae is the most

diverse, with ~97 valid species under four subfamilies, namely, Dasyatinae (35 species), Neotrygoninae (17 species), Urogymninae (39 species) and Hypolophinae (6 species)⁴. Altogether, 51 marine and estuarine species of rays in the order Myliobatiformes belonging to 8 families, namely Hexatrygonidae (1 species), Dasyatidae (30 species), Gymnuridae (3 species), Plesiobatidae (1 species), Aetobatidae (3 species), Myliobatidae (4 species), Rhinopteridae (2 species), Mobulidae (7 species) are reported from the Indian coastal waters^{1,5-15}. A list of valid species from the Indian waters is given in Table 1.

The myliobatoid rays have distinct morphological characteristics such as a flattened body, enlarged pectoral fins fused to the head, and ventrally placed gill slits. Identification up to the family level or, in some cases, up to the genus level is easier; however, the species level identity possesses problems because of similar morphological features and cryptic species complex^{1,16}. Species identity is essential to assess their natural stocks and population biology so that necessary steps can be taken for their conservation

Table 1 — Status of rays of order Myliobatiformes from Indian coastal waters (NA = Not available *COXI* sequence). Numerical values (1-5) in the superscript represents erroneous *COXI* sequences in the NCBI GenBank

Family	Common name	Genus	Total species	Species reported from Indian waters	<i>COXI</i> gene sequence available	Revised Status based on <i>COXI</i> gene phylogeny in the present study
Hexatrygonidae	Sixgill stingrays	1	1	<i>Hexatrygon bickelli</i>	No	NA
Dasyatidae	Stingray	16	31	<i>Brevitrygon imbricata</i>	Yes	Yes
				<i>Brevitrygon walga</i>	Yes	Yes
				<i>Hemitrygon bennetti</i>	Yes	Yes
				<i>Himantura leoparda</i>	Yes	Yes
				<i>Himantura uaranak</i> ¹	Yes	No
				<i>Himantura tutul</i> ¹	Yes	Yes
				<i>Himantura undulata</i>	Yes	Yes
				<i>Maculabatis arabica</i>	No	NA
				<i>Maculabatis bineshi</i>	No	NA
				<i>Maculabatis gerrardi</i>	Yes	Yes
				<i>Megatrygon microps</i>	Yes	Yes
				<i>Neotrygon indica</i> ²	Yes	Yes
				<i>Neotrygon kuhlii</i> ²	Yes	No
				<i>Pastinachus ater</i>	Yes	Yes
				<i>Pastinachus gracilicaudus</i>	Yes	Yes
				<i>Pastinachus sephen</i>	Yes	Yes
				<i>Pateobatis bleekeri</i>	No	NA
				<i>Pateobatis fai</i>	Yes	Yes
				<i>Pateobatis jenkinsii</i>	Yes	Yes
				<i>Pteroplatytrygon violacea</i>	Yes	Yes
				<i>Taeniura lymma</i>	No	NA
				<i>Taeniurops meyeri</i>	Yes	Yes
				<i>Urogymnus asperrimus</i>	Yes	Yes
				<i>Urogymnus granulatus</i>	Yes	Yes
				<i>Maculabatis pastniacoids</i>	Yes	Yes
				<i>Himantura marginata</i>	No	NA
				<i>Telatrygon crozieri</i>	No	NA
				<i>Bathytoshia lata</i> ³	Yes	No
				<i>Pateobatis uarnacoides</i>	Yes	Yes
				<i>Himantura fava</i>	No	NA
				<i>Urogymnus polylepis</i>	Yes	Yes
Gymnuridae	Butterflyrays	1	3	<i>Gymnura tentaculata</i>	No	NA
				<i>Gymnura poecilura</i>	Yes	Yes
				<i>Gymnura zonura</i>	Yes	Yes
Plesiobatidae	Deepwater stingrays	1	1	<i>Plesiobatis daviesi</i>	Yes	Yes
Aetobatidae	Pelagic eagle rays	1	3	<i>Aetobatus flagellum</i>	Yes	Yes
				<i>Aetobatus ocellatus</i>	Yes	Yes
				<i>Aetobatus narinari</i> ⁴	Yes	No
Myliobatidae	Eagle rays	2	4	<i>Aetomylaeus maculatus</i>	Yes	Yes
				<i>Aetomylaeus milvus</i>	No	NA
				<i>Aetomylaeus nichofii</i>	No	NA
				<i>Aetomylaeus vespertilio</i>	Yes	Yes
Rhinopteridae	Cownose rays	1	2	<i>Rhinoptera javanica</i> ⁵	Yes	No

(contd.)

Table 1 — Status of rays of order Myliobatiformes from Indian coastal waters (NA = Not available *COXI* sequence). Numerical values (1-5) in the superscript represents erroneous *COXI* sequences in the NCBI GenBank (*contd.*)

Family	Common name	Genus	Total species	Species reported from Indian waters	<i>COXI</i> gene sequence available	Revised Status based on <i>COXI</i> gene phylogeny in the present study
				<i>Rhinoptera jayakari</i>	Yes	Yes
Mobulidae	Devil rays	1	7	<i>Mobula alfredi</i>	No	NA
				<i>Mobula birostris</i>	Yes	Yes
				<i>Mobula kuhlii</i>	Yes	Yes
				<i>Mobula japonica</i>	Yes	Yes
				<i>Mobula tarapacana</i>	Yes	Yes
				<i>Mobula thurstoni</i>	Yes	Yes
				<i>Mobula eregoodoo</i>	No	NA

and management^{17,18}. Due to confusion and inconsistency in the species identification, several undescribed species go unnoticed, resulting in poor reporting on catch, exports, and management of rays fishery at the species level⁸.

In recent years, molecular and genetic data have been extensively used for species-level identification and to ascertain the species delimitation among morphologically similar organisms^{19,20}. In particular, DNA barcoding enables rapid and accurate species identification and differentiates cryptic lineages^{21,22}. Several genetic studies have resulted in the description of previously unreported species as well as resolving cryptic species complex, thereby increasing local diversity across the world^{16,19,23-27}. Approximately 655 nucleotide fragments of mitochondrial gene cytochrome c oxidase subunit I (*COXI*) amplified using FISH F1 and FISH R1 primers²² have been considered suitable to provide quick and reliable confirmation of ray's species²⁴. As a result, *COXI* has been used either solely or in combination with other markers to study molecular taxonomy, phylogenetic relationships among taxa, and biogeographic distribution patterns of rays, worldwide^{10,22,24,28-31}.

Description of rays from India has a long history⁸. However, most of the species described by early Ichthyologists have been either synonymized or considered invalid at present due to inability to re-examine the samples as they were not preserved properly in the national collections⁸. Exhaustive geographical sampling and molecular studies can validate additional unrecognized species³². In India, a molecular approach has been undertaken to identify ray species from the Indian coastal waters using the *COXI* gene in the last decade^{6,7,9-12,26,27,33,34}. Pavan-

Kumar *et al.*¹² described a novel species *Neotrygon indica* from the Gulf of Mannar, Tamil Nadu, which was later reported from different locations on the east coast of India¹³. The study by Bineesh *et al.*¹⁰ covered wide geographical locations for collecting rays' samples (10 fish landing centres across India) and sequenced the *COXI* gene from 161 Myliobatiformes samples. However, molecular studies of Myliobatiformes from Indian coastal waters are hitherto focused only on specific genus and lack detailed analysis such as updated taxonomic status, phylogenetic relationship among taxa, and biogeographic patterns. Hence, this study aim to fill these prevailing gaps. In the present study, the Myliobatiformes species were collected and sequenced from the Mandapam fish landing centres in Tamil Nadu and compiled almost all mitochondrial genes published so far for Myliobatiformes from the Indian coastal waters. Further, a robust phylogeny is built, updated valid species names, and constructed a phylogeographic network analysis of selected species to observe major genetic structuring among the populations.

Material and Methods

Sampling

A total of 307 sequences of rays belonging to the order Myliobatiformes reported from the Indian coastline are utilized in the present study. This includes 18 individuals of rays sampled from the fish landing centres of Mandapam (9°16'36.99" N 79°9'15.48" E), Tamil Nadu, from July 2019 to January 2020, and 289 individuals from previous published literatures^{6,7,-13,26,27}, and the NCBI GenBank nucleotide sequence database till 30th October 2020. For the specimens collected in the present study,

tissue samples from the tail region were collected and preserved in 95 % ethanol for molecular identification. The specimens' preserved tissue samples are deposited as voucher materials at the National Zoological Collections of Marine Biological Research Centre (MBRC), Zoological Survey of India, Chennai. All individuals and their gene accession used in the present study are listed in Table S1.

DNA extraction, PCR and sequencing

Total genomic DNA was extracted using OMEGA BIO-TEK E.Z.N.A. Blood & Tissue DNA Kit, USA following the manufacturer's protocol. PCR amplification was performed for the *COXI* gene using FishF1 (5'-TCAACCAACCACAAAGACA TTG GCAC-3') and Fish R1 (5'-TAG ACTTCTGG GTGGCCAAAG AATCA-30) primers²². A total of 25 µl reaction mixture containing 12.5 µL 2X PCR master mix (Ampliqon, Denmark), 2.5 µL each of the two primers (10 nM), 2.5 µL of template DNA (10-20 ng), and nuclease-free water to make a final volume of 25 µL was taken. PCR conditions were as follows: initial denaturation at 95 °C for 10 min, 35 cycles of 95 °C for 45 sec, 50 °C for 45 sec, and 72 °C for 45 min, and final extension at 72 °C for 10 min. PCR products were then visualized on 1 % agarose and products with the high-intensity band were purified using EXOSAPIT and sequenced on ABI Prism 3730 Genetic Analyzer (Thermo Fisher Scientific, USA) based on BigDye Terminator Chemistry. Sequence chromatograms were then visualized, edited, and contigs were prepared using BioEdit³⁵ for phylogenetic and biogeographic pattern analysis.

Molecular phylogeny

COXI gene sequences generated in the present study and those retrieved from the previous published literature and NCBI GenBank nucleotide sequence databases, were aligned using the ClustalW web server (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). All the species under the order Myliobatiformes were included as ingroup terminal, whereas four species belonging to three sharks (*Carcharhinus amblyrhynchos* (EF609308), *C. plumbeus* (EU398639), *Iago* sp. and one guitarfish (*Glaucostegus granulatus*) were included as outgroups terminal. Cytochrome oxidase subunit I (*COXI*) sequences for *G. granulatus* and *Iago* sp. were generated in this study from specimens collected from the fish landing centre at Mandapam, Tamil

Nadu, India and submitted in NCBI GenBank database having accession number MT317247 (*Iago* sp.) and MT317248 (*G. granulatus*).

Aligned sequences were visualized using BioEdit³⁵ for necessary modifications and were uploaded on W-IQ-TREE web server³⁶ (<http://iqtree.cibiv.univie.ac.at>) for maximum likelihood phylogenetic tree construction. The Hasegawa-Kishino-Yano (HKY) evolutionary model was found to be the best-fit substitution model on this dataset. The calculated parameters were as follows: state frequencies (empirical counts from alignment) $\pi(A) = 0.2488$, $\pi(C) = 0.269$, $\pi(G) = 0.1763$, $\pi(T) = 0.3058$, substitution rate parameter, A-C: 1.0000, A-G: 4.9512, A-T: 1.0000, C-G: 1.0000, C-T: 4.9512, G-T: 1.0000, and uniform rate of heterogeneity. The robustness of the ML tree was analyzed by reiterating the observed data using an ultrafast bootstrap approximation for 1000 generations³⁷.

Genetic divergences

The pairwise genetic divergences within species and between species of Myliobatiformes from the Indian waters were calculated using the Kimura 2-parameter distance model³⁸ as implemented in MEGA 7^(ref. 39).

Haplotype network analysis

Templeton, Crandall, and Sing (TCS) haplotype network⁴⁰ analysis was performed using PopART^{41,42} (also available online at <http://popart.otago.ac.nz>) on the nucleotide sequence matrix of the *COXI* gene. Networks were generated for four species (*Brevitrygon imbricata*, *Gymnura poecilura*, *Maculabatis gerrardi*, and *Himantura tulul*) with sequences generated in the present study as well as previously reported from different geographical locations, obtained from the NCBI GenBank. A list of individuals and their GenBank accession numbers are given in Table S2. Before TCS haplotype network analysis, the sequence matrix was trimmed to a core length. This software also provided statistics on Nucleotide diversity, number of segregating sites, number of parsimony informative sites, and AMOVA.

Results

Molecular phylogeny

A total of 18 samples sequenced in the present study belong to 4 families and 9 species of the order Myliobatiformes, namely *Aetobatus ocellatus* (3 nos.), *Rhinoptera jayakari* (1 no.), *Neotrygon indica* (4 nos.), *P. atrus* (1 no.), *Maculabatis gerrardi*

(4 nos.), *Maculabatis pastinacoides* (1 no.), *Gymnura poecilura* (1 no.), *Brevitrygon imbricata* (2 no.), and *Himantura tutul* (1 no.). These *COXI* gene sequences were submitted in NCBI GenBank with accession numbers MT308592 - MT308609. In addition, 289 *COXI* gene sequences were compiled from previous literature and public databases belonging to 39 taxa. However, based on *COXI* gene phylogeny, found the presence of only 34 species (Table 1).

The maximum likelihood phylogenetic tree based on 307 *COXI* gene sequences could divide the sequences into two major clades (Fig. 1). First major clade (Clade I) includes species from the families Mobulidae (5 species), Rhinopteridae (1 species), Plesiobatidae (1 species), Gymnuridae (2 species), and Aetobatidae (2 species). These species were clustered into a single monophyletic clade and were strongly supported by the high bootstrap values from ML analysis. In clade I, polyphyl in the family Myliobatidae was observed as two species, *Aetomylaeus maculatus* and *A. vespertilio* were clustered into separate subclades. *Aetomylaeus maculatus* formed a separate sister subclade to members of the family Aetobatidae and Gymnuridae, while *A. vespertilio* formed a sister subclade to the members of the family Dasyatidae and Plesiobatidae, suggesting the possibility of polyphyletic nature within the genus *Aetomylaeus*. Likewise, the genus *Megatrygon*, a member of the family Dasyatidae formed a separate sister subclade to the family Plesiobatidae and Myliobatidae. The other members in the clade I belong to the family Dasyatidae, such as *Taeniura*, *Hemitrygon*, *Pteroplatytrygon* and *Neotrygon*, which formed a sister subclade within the clade I supported by strong bootstrap values from ML analysis (Fig. 1a).

In the second major clade (clade II), members of the family Dasyatidae (16 species) were found with strongly supported bootstrap values from ML analysis. The genus *Brevitrygon*, *Urogymnus*, *Maculabatis*, *Himantura*, and *Pastinachus* formed a monophyletic subclade within clade II (Fig. 1b). Three species of the genus *Pateobatis* formed separate subclades based on *COXI* gene phylogeny indicating the polyphyletic nature of the genus. Species *Pateobatis jenkinsii* clustered within a sister subclade to the genus *Brevitrygon*; *P. uarnacoides* formed a sister subclade to the genus *Maculabatis*; and *P. fai* formed a separate sister subclade to other members of the family Dasyatidae. In addition, several species were observed

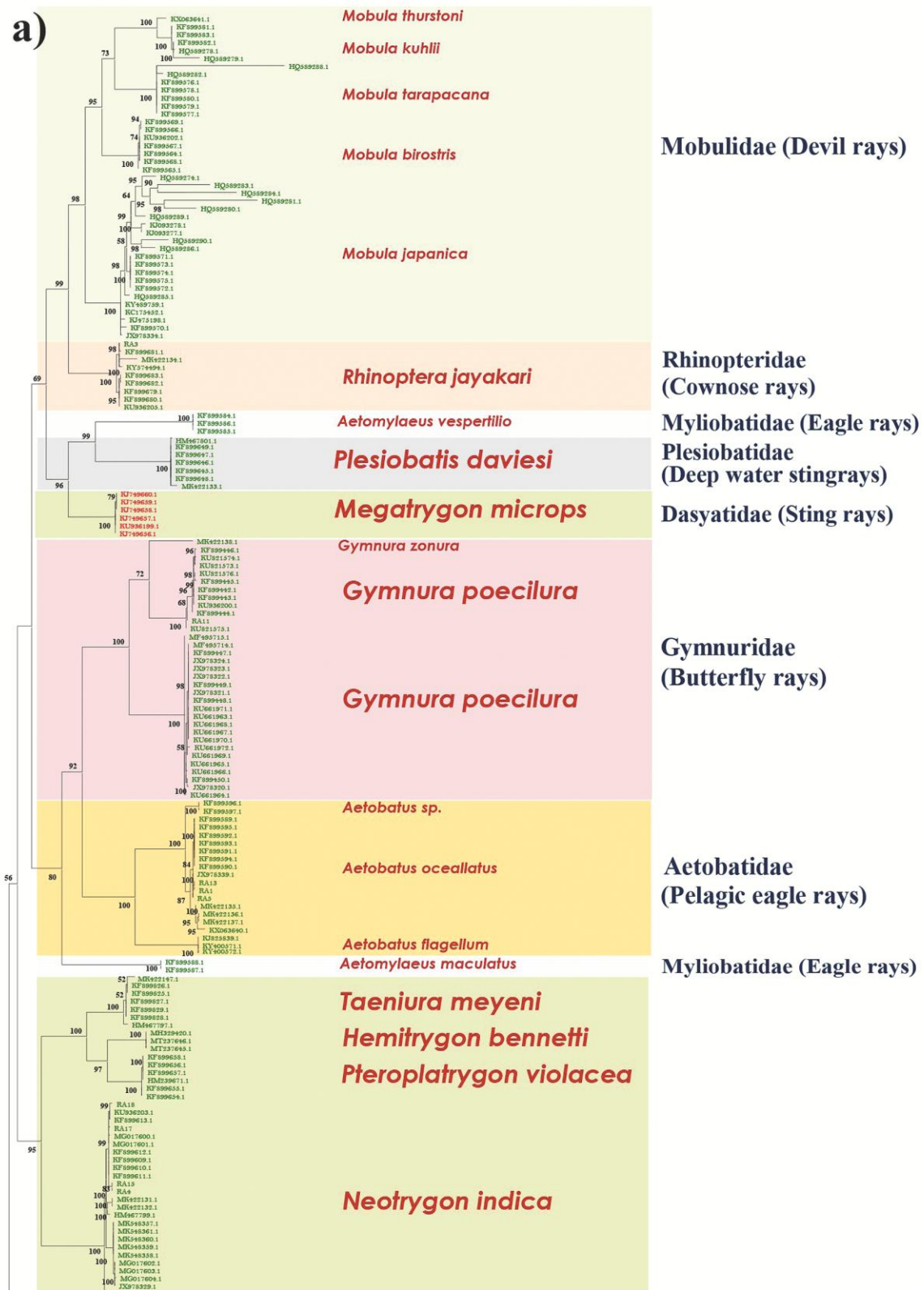
misidentified or mislabelled in the public databases. Therefore, the species list is updated for the Indian region based on the *COXI* gene phylogeny (Table 1).

Furthermore, the overall pairwise genetic divergence within species varied from 0 to 6.86 % with a minimum intra-specific genetic distance of 0.0 % in *Aetobatus flagellum*, *Aetomylaeus vespertilio*, *Aetomyleus maculatus*, *Gymnura zonura*, *Hemitrygon bennetti*, *Himantura undulata*, *Megatrygon microps*, *Mobula thurstoni*, *Pastinachus gracilicadus*, *P. sephen*, *Urogymnus asperimus*, *U. granulatus*, and *U. polylepis*; and a maximum intraspecific genetic distance of 6.86 % in *Gymnura poecilura*. The pairwise genetic divergence between the species varied from 5.87 to 26.3 %, with a minimum distance between *Pastinachus atrus* and *P. gracilicadus* and a maximum distance between *Aetobatus ocellatus* and *Aetomylaeus vespertilio* (Table S3).

Haplotype diversity

Since the results suggested more diverged clades in certain species, the objective was to describe the population genetic structure of the entities within the clades. To distinguish among the different scenarios above (phylogeny and genetic distance), the level of haplotype diversity among the populations of different geographic locations was explored assuming that they form genetically dissimilar haplotypes and was implemented through PopART.

In *Himantura tutul*, using 645 aligned sites, nine different haplotypes were found among 55 individuals belonging to different geographic locations. Out of 11 sequences from the Indian waters, three different haplotypes were found to form a separate cluster from the West Pacific (Indonesia and Malaysia) sequences. One sequence from Zanzibar (East Africa) is observed to be clustered with the Indian haplotypes (Fig. 2). In *Gymnura poecilura*, using 651 aligned sites, 26 different haplotypes were found among 62 individuals belonging to different geographical locations. The haplotype network denoted major population genetic structuring in *G. poecilura* and formed a separate cluster between India's east coast and west coast populations (Fig. 3). The east coast populations formed a network with the individuals from Bangladesh, and the west coast populations formed a network with the individuals from Qatar and Saudi Arabia. Likewise, the Indo-West-Pacific populations, such as Indonesia and Malaysia, formed a separate cluster in the haplotype network. However, the



Clade I

(contd.)

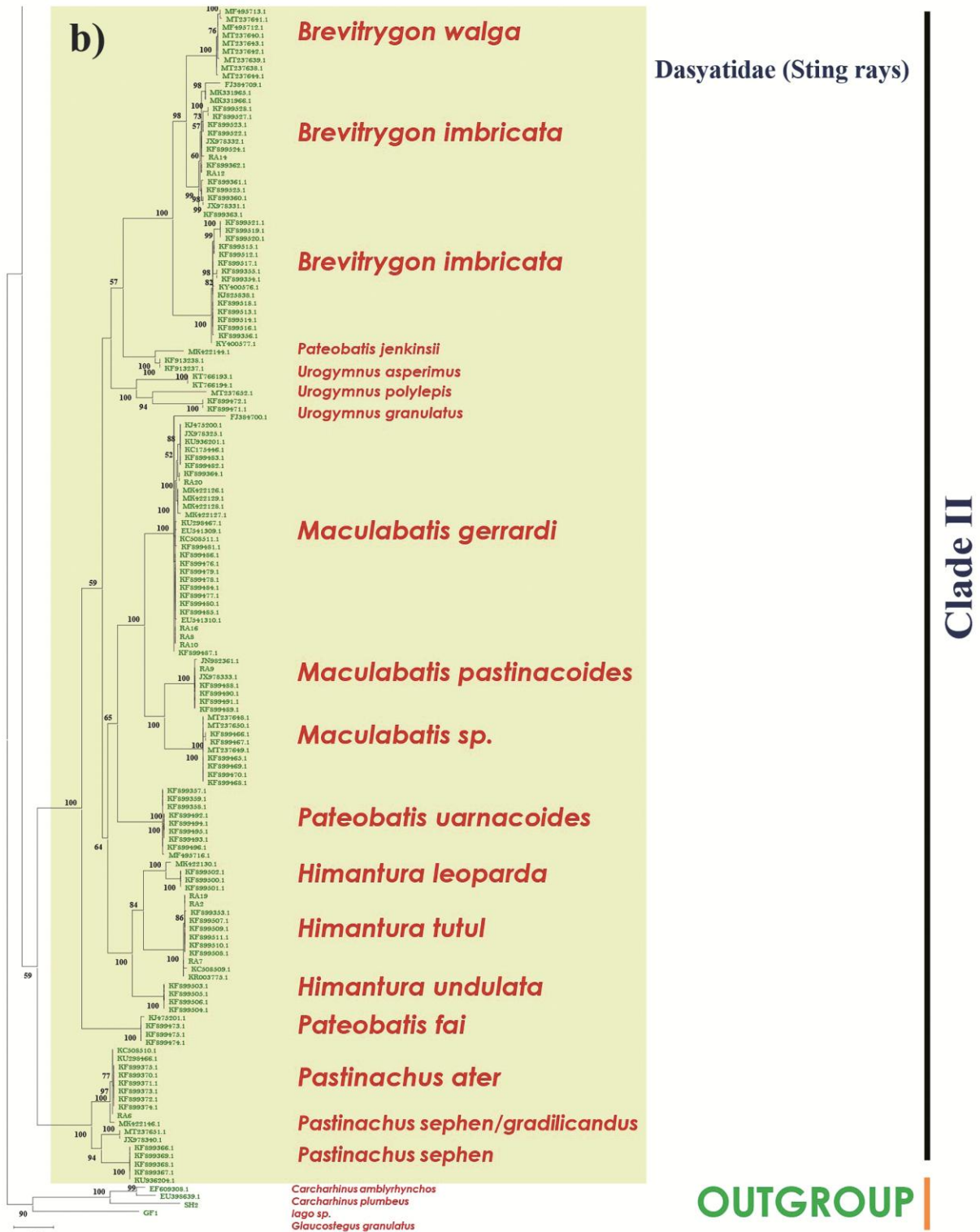


Fig. 1 — Maximum-likelihood phylogenetic tree based on partial COXI gene sequences from 307 individuals: (a) Clade I, and b) Clade II and Outgroup. The numbers above and below the branches indicate bootstrap values based on ML. GenBank accession numbers are given in the tree

number of singletons on both the east and west coast of India were 6 and 5, respectively (Fig. 3).

Among the sequences of *Brevitrygon imbricata*, 20 different haplotypes were found from 42 individuals using 617 aligned sites. Individuals of *B. imbricata* also formed a separate haplotype network between the populations of west and east coast of India. A single

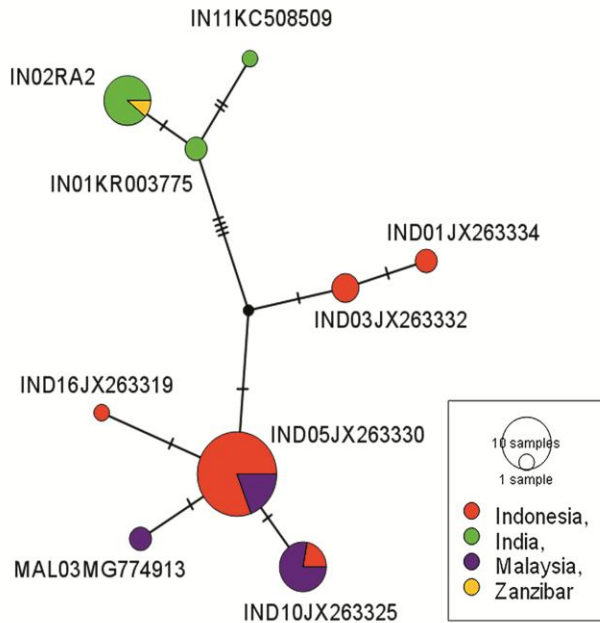


Fig. 2 — Haplotype diversity among the populations of different geographic locations of *H. tutul* using TCS network

individual from the Bangladesh has paired with populations from the east coast. In addition, a few individuals from Saudi Arabia and Kuwait formed a cluster with west coast populations. Four singletons were observed each in west and east coast populations (Fig. 4). Among *Maculobatis gerrardi*, 16 different haplotypes were observed from 46 individuals using 638 aligned sites. The Indian Ocean populations formed a separate network including Bangladesh, Myanmar, and South Africa, from the West-Pacific populations such as Taiwan, Malaysia and Indonesia (Fig. 5). Overall, 6 singleton sites were observed in the Indian Ocean populations. Lastly, the nucleotide diversity, parsimony informative sites, segregating sites and AMOVA with significance for *H. tutul*, *G. poecilura*, *B. imbricata*, and *M. gerrardi* is given in Table 2.

Discussion

The study represents a detailed molecular analysis of Myliobatoid in the Indian coastal waters using *COXI* gene sequences from 307 sequences, including 18 sequences generated in the present study and 289 sequences obtained from previously published literature and public databases such as NCBI GenBank. For the first time, an attempt has been made to resolve the current species diversity in India, nomenclature anomalies, phylogenetic relationship, and biogeographic patterns of the order Myliobatiformes using *COXI* gene.

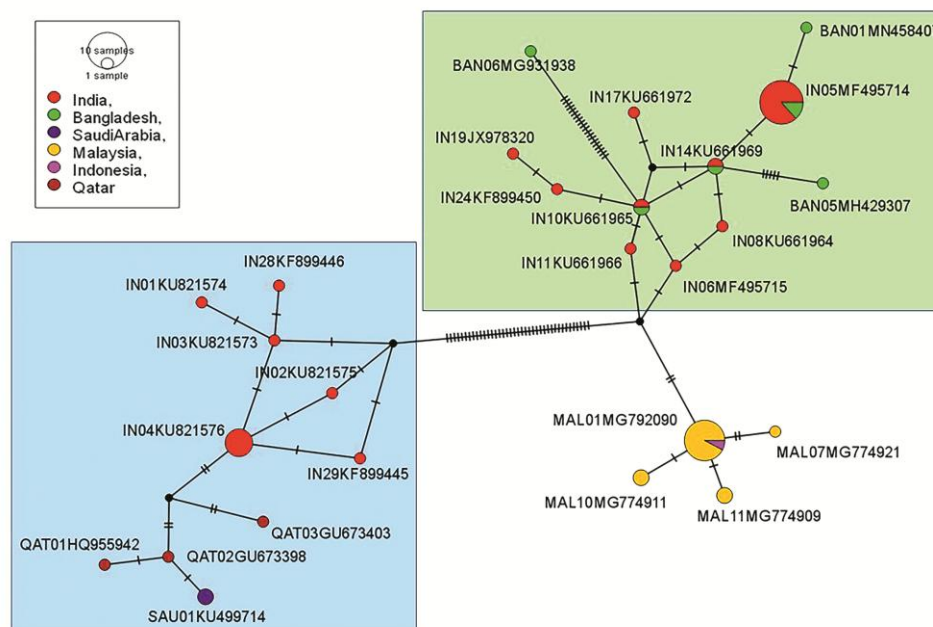


Fig. 3 — Haplotype diversity among the populations of different geographic locations of *G. poecilura* using TCS network

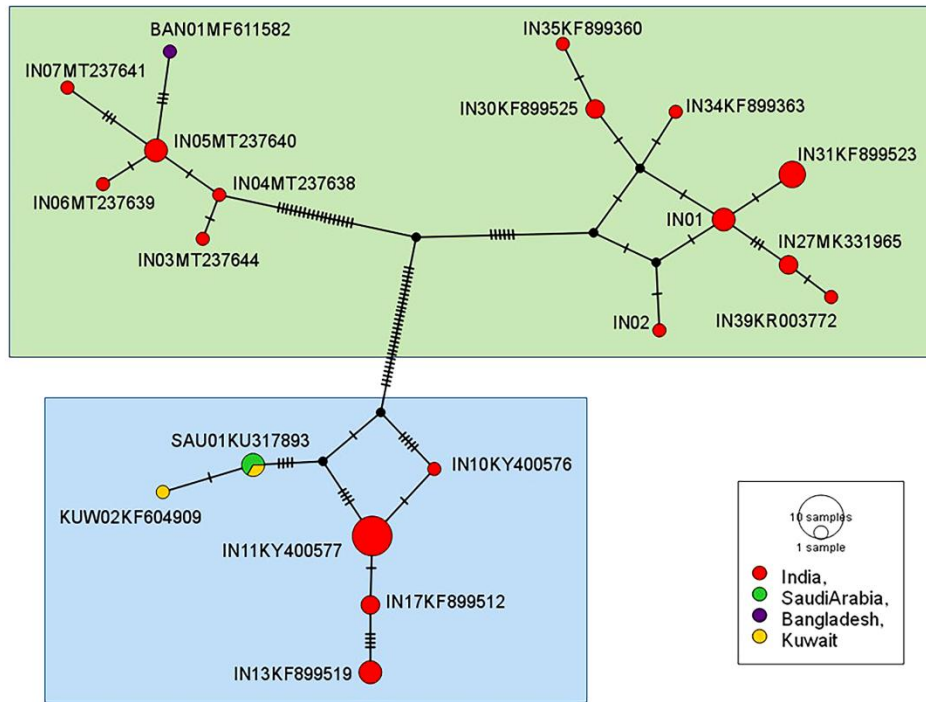


Fig. 4 — Haplotype diversity among the populations of different geographic locations of *B. imbricata* using TCS network

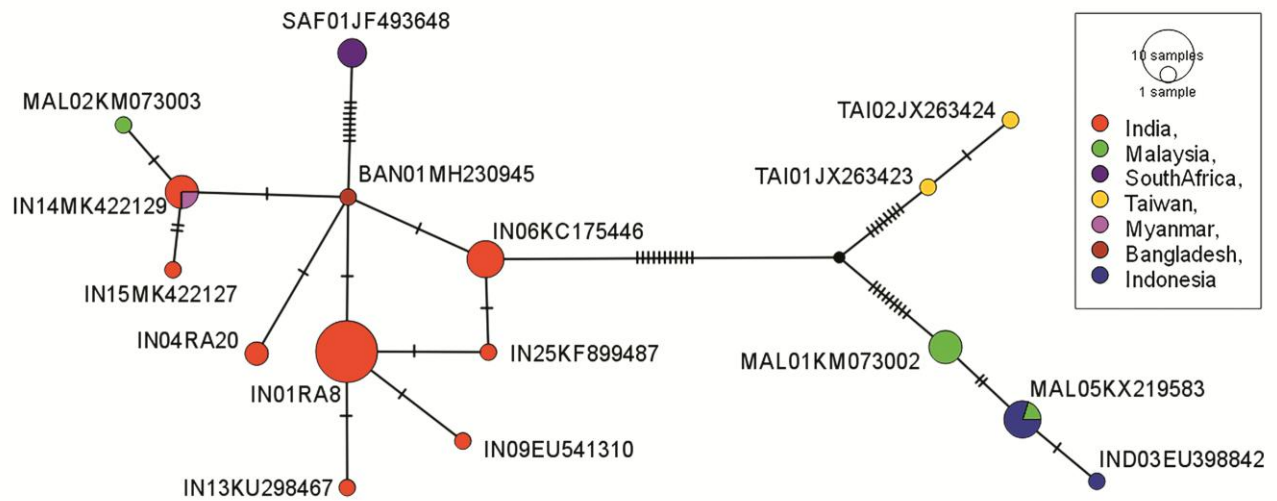


Fig. 5 — Haplotype diversity among the populations of different geographic locations of *M. gerrardi* using TCS network

Table 2 — Nucleotide diversity, parsimony informative sites, segregating sites and AMOVA with significance for *H. tutul*, *G. poecilura*, *B. imbricata*, and *M. gerrardi*

	Nucleotide diversity, PI sites, Segregating sites and AMOVA				
	Nucleotide diversity	Parsimony informative sites	Segregating sites	AMOVA (ϕ_{ST})	Significance (ϕ_{ST})
<i>H. tutul</i>	3.87	10	13	0.877	0.001
<i>G. poecilura</i>	0.036	54	81	0.375	0.001
<i>B. imbricata</i>	0.47	71	79	0.071	0.019
<i>M. gerrardi</i>	0.014	38	43	0.864	0.001

Taxonomic ambiguities

Major issues in the species delineation of the order Myliobatiformes include morphological similarities, cryptic species complex, and widespread species with intraspecific geographical variations²⁴. Mitochondrial COXI gene-based barcoding and phylogenetic approach has been effective in the ray's taxonomic identification and species delineation^{10,24,29}. However, DNA barcoding has brought another problem in the form of deposition of non-verified sequences with incorrect, inconsistent, duplicate, or outdated/unaccepted names in public databases such as NCBI GenBank. Indeed, it has been observed that several entries in the NCBI GenBank were erroneous, e.g. *Himantura uranak* (Gene accession no. FJ384700, EU541309), *Pateobatis bleekeri* (KC508511), and *Megatrygon microps* (EU541310) were clustering with *M. gerrardi* in the COXI based phylogenetic tree. Thus, it could be a case of misidentification. Similarly, sequence(s) submitted as *Urogymnus apermus* (KC508509), *Himantura leoparda* (KF899353) and *Aetobatus narinari* (KR003775) were found to be clustered with *Himantura tulul*. Three specimens of *Bathytoshia lata* (KJ825838, JX978331, JX978332) from the NCBI GenBank appeared to be *Brevitrygon* spp. Upon closer examination into the genus *Himantura*, it has been found that previously reported *Himantura uarnak* from the Indian coast is clustering with recently reported species, *H. tulul*³⁴. Hence, it is believed that the species with the name *H. uarnak* reported in the Indian waters may be *H. tulul*. Similarly, in *Neotrygon kuhlii* species complex, all the individuals clustered together in one subclade of *N. indica*¹²; thereby indicating the presence of only one single species in this species complex in the Indian waters. Further, a single sequence of *Aetobatus narinari* (JX978339) has clustered within the subclade of *A. ocellatus* and a single sequence of *Rhinoptera javanica* (KU936205) clustered within subclade of *R. jayakari*, suggesting misidentification in these two species. As the information on the voucher materials is not available (either not deposited or maintained at the museums), the species identity cannot be ascertained by re-examining the samples morphology. Overall, out of 39 species for which COXI genes were available, it is strongly argued only 34 species of Myliobatiformes are present in the Indian coastal waters (Table 1).

Phylogenetic relationships among the Indian Myliobatiformes

Utilizing COXI gene phylogeny, two major clades were observed within the Order Myliobatiformes. The major Clade I consist of monophyletic clades of the families Mobulidae, Rhinopteridae, Plesiobatidae, and Gymnuridae; and polyphyletic clades of families Myliobatidae and Dasyatidae (Fig. 1). Among the Mobulidae, 5 species namely *M. thurstoni*, *M. kuhlii*, *M. tarapacana*, *M. biostris*, *M. japonica* formed a separate subclade. However, in the subclade of *M. japonica*, deep genetic divergence among the sequences was observed, and it is suspected that the reasons could be the bad quality of sequences deposited under accession numbers HQ series (HQ589274.1, HQ589280.1, HQ589281.1, HQ589283.1, HQ589284.1, HQ589285.1, HQ589286.1, HQ589289.1, HQ589290.1). The poorly aligned positions and highly divergent regions were found in multiple sequence alignment with closely related species. Further, the location of the sample collection was not provided, besides, it was mentioned under the miscellaneous features of sequences in the NCBI GenBank labelled as “similar to cytochrome c oxidase subunit I”. However, it may also be possible that these sequences may belong to different geographical locations as *M. japonica* exhibits circum-global distribution^{30,43}.

In clade I, another interesting observation was the polyphyletic nature of the family Myliobatidae as *Aetomylaeus maculatus* did not cluster with its congeneric species *A. vespertilio*. This observation is supported by the fact that the genetic divergence between these two species was 24.7 %^(ref. 10). However, Naylor *et al.*⁴⁴ did not find the polyphyletic nature of *Aetomylaeus* spp. using additional/or another marker. Hence, to ascertain this claim, further phylogenetic studies utilizing multilocus markers or a phylogenomic approach is much warranted⁴⁵. The family Gymnuridae formed a monophyletic clade represented by two species, namely *G. zonura* and *G. poecilura*. *Gymnura poecilura* showed significant divergence among the populations forming two subclades. It indicates the possibility of geographical separation between the east and west coast of India, which was evident from recent studies¹¹. In the family Aetobatidae, subclades of *Aetobatus* sp. (2 sequences) was observed which is not yet identified and could represent a novel species¹⁰. Further, a separate subclades of *A. ocellatus*, and *A. flagellum* was also observed in this study. Based on the phylogeography

studies, it is reported that *A. ocellatus* has formed two distinct subclades belonging to the Indian population and Indo-West Pacific populations^{46,47}. In addition, the results of present study indicate that there could be two divergent populations of *A. ocellatus*, each belonging to east and west coast. However, it requires deep phylogenetic studies involving more samples and molecular markers to establish the claims.

The COXI gene phylogeny indicated the polyphyletic nature of the Dasyatidae family as few members segregated in major clade I, while others were in clade II. *Megatrygon microps* formed a separate subclade which was distantly placed from the members of the subfamily Dasyatinae (family Dasyatidae). Similar observations were made in the previous studies, suggesting the possibility of having unique characters distant from other members of the subfamily Dasyatinae. It was also hypothesized that this species may belong to a new undescribed family²⁹. Further, to strengthen the argument of polyphyly in the family Dasyatidae, several genera were observed closely, including *Taeniura*, *Hemistrygon*, *Pteroplatytrygon* and *Neotrygon* clustered within the major clade I, while other genera such as *Brevitrygon*, *Urogymnus*, *Maculabatis*, *Himantura*, *Pastinachus* and *Pateobatis* formed a separate major clade II (Fig. 1). This observation agrees to the previous reports on the phylogeny of the order Myliobatiformes based on COXI and NADH2 gene sequences^{24,29,44,48}.

The cryptic species complex of *Neotrygon* spp. was resolved based on COI phylogeny that resulted in eleven parapatrically - distributed lineages, each classified into a valid species^{12,25,28,48}. The Indian Ocean mask ray was identified recently as *N. indica*¹². Four of the sequences generated in this study (GenBank accession MT308594, MT308604, MT308606-07) clustered with *N. indica* are reported from the Indian coast^{12,13}. Also, the genetic divergence among the sequences could be linked with the geographical locations, which was also reported by Kundu *et al.*¹³. Among the *Himantura* species complex, we found only three species from the Indian coastal waters, namely *H. leoparda*, *H. undulata*, and recently reported *H. tutul*. It was observed that the sequences which were reported to be *H. uarnak* in the previous studies¹⁰ are clustered with *H. tutul* in the present (GenBank accession MT308608) and previous study³⁴. An earlier report by Arlyza *et al.*¹⁶ on *H. uarnak* species complex has clearly stated the

confusion in morphology-based taxonomy. Further, they conveyed the importance of the COI gene in separating the clades of *H. uarnak* complex. However, the possibilities of mitochondrial introgression among *H. uarnak* complex are yet to be documented¹⁶. Likewise, the recent study on the DNA barcoding of *Himantura* has confirmed *H. tutul* as a nominal species based on an integrative approach (morphology+COI phylogeny)³⁴. Additionally, *H. tutul* may have overlapping geographic ranges and shared habitats, and one can suspect other isolating mechanisms to occur as observed for its congeneric *H. uarnak* complex¹⁶. Therefore, it is sensible to include COI as one of the potential diagnostic characteristics to distinguish *Himantura* spp. complex. Recently, *H. tutul* was also reported from Sri Lankan waters, having a clear distinction from *H. uarnak* based on morphology and NADH2 sequences⁴⁹.

Haplotype and biogeography

The population genetic structure of *H. tutul* from India indicated a significant genetic differentiation ($P < 0.001$) pointing towards gene flow and migration among the individuals between the Indian Ocean (India and Zanzibar) and West Pacific (Indonesia and Malaysia). This can be supported based on the high nucleotide diversity of 3.87 with an overall ϕ_{ST} value of 0.877. However, the results presented should be seen cautiously because of the low sample sizes, *i.e.*, only 55 individuals with a limited distribution range. Therefore, it is speculated that the genetic structure of *H. tutul* may not be restricted to the Indian Ocean but will also extend to the Western Pacific, which is under the Indo-Polynesian province⁵⁰. Therefore, extensive sample sizes are necessary, along with multi-locus markers, to build strong evidence on the population structure of *H. tutul* within the restricted range of distribution.

Interestingly, in the case of *Gymnura poecilura* complex, there is a deep genetic divergence between east and west coast populations in the Indian waters. For instance, the individuals from the eastern states of India formed a separate haplotype network with Bangladesh sequences and formed a closer network with the Western Pacific populations (Malaysia+Indonesia). At the same time, the population from the Northern Arabian Sea, including Gulf waters (western states of India+Qatar+Saudi Arabia), formed a separate haplotype network. The population of *G. poecilura* between east and west

coast were separated with several mutations, indicating the possibility of a cryptic species complex. Similar, deep genetic divergence between the east and west coast populations was also observed in *G. poecilura* by Muktha *et al.*¹¹. It is possible that the geographical barriers near the Southern India and Sri Lanka such as Ram bridge, together with ancient climatic differences may act as means of isolation concerning *G. poecilura* populations between the Bay of Bengal and Arabian Sea⁵¹. Likewise, *Brevitrygon imbricata* populations in the east and west coast of India appeared to be diverged with several mutations. Lastly, the *Maculobatis gerrardi* populations also exhibit shared haplotypes within the Indian Ocean (India+Bangladesh+South Africa+Malaysia). However, some of the populations, in particular, Taiwan, East coast of Malaysia and Indonesia, have mixed haplotypes pointing towards the substantial gene flow and mixing of populations within the Indian Ocean and Western Pacific.

A recent description of *Maculabatis ambigua* from the Red Sea formed a sister clade with its closest congener *M. gerrardi* and *M. randalli*. Interestingly, the distribution range of *M. gerrardi* was further east in the Indian Ocean to Northwest Pacific; and of *M. randalli* is in the Persian and Arabian Gulf⁴⁸. This indicates the restricted gene flow within the Red Sea as a possibility of endemism as compared to the other two species, which have substantial gene flow in their distribution range, as observed in the case of coral reef fishes⁵². The biogeography of the elasmobranchs is mainly driven by migration patterns and gene flow³¹. For example, a recent study on the oceanic white tip sharks *Carcharinus longinamus* suggests a homogenous population/single stock along the Indian coast owing to their substantial migration capacity⁵³. Similarly, substantial mixing of gene pools and connectivity due to oceanic currents has been studied extensively for coral reef fishes⁵⁴. The present study also observed that the populations of rays are more homogenous if they have been collected only in Southeast and Southwest coast of India *i.e.*, Tamil Nadu (in east) and Kerala (in west). One of the possible reasons could be the collection of fish in one part of the coast and landing on the other, which has been commonly reported in the case of elasmobranch fisheries/trade¹. More samples need to be collected throughout India from the artisanal fishers as they fish in the nearby sea, directly indicating the local population of rays; thereby helping delineate

biogeographic patterns and endemism⁵⁵. Further, biogeographic information sheds light on natural habitats, which is essential to take conservation associated steps.

Eventually, it is urged to use the updated/valid species list/nomenclature for the study; as well as to submit at least a part of tissue in the national museum/collection centres as a voucher material. It is also recommended to mention the sample collection location while submitting sequences in the public database (NCBI GenBank). This would be helpful for future researches to explore the possibility of characterizing the cryptic lineages and uncovering putative new species in the Indian Ocean using the molecular taxonomy. Moreover, it is also believed that this study has proven the potential of revisiting rays' phylogeny from the Indian waters, which in turn gain insights into the species validation, the phylogenetic relationship, and biogeographic patterns in understanding the diversity. The limitation of the present study in using only one mitochondrial gene marker (*COXI*) for phylogeny and network analysis is also acknowledged. Adding more nuclear/mitochondrial markers can further improve our understanding of Myliobatiformes phylogenetic positioning. Nevertheless, the above results can be used as a framework for any effective conservation and management plans concerning ray's population in the Indian waters.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at [https://nopr.niscpr.res.in/jinfo/ijms/IJMS_52\(02\)65-78_SupplData.pdf](https://nopr.niscpr.res.in/jinfo/ijms/IJMS_52(02)65-78_SupplData.pdf)

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships

that could have appeared to influence the work reported in this paper.

Ethical Statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Specimens were collected from the bycatch of the commercial fishing harbors.

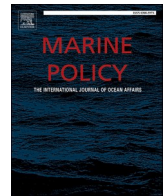
Author Contributions

AK & SP: Field sampling, lab work, data analysis, and corrections in the manuscript draft. AK: Preparation of first draft of manuscript.

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Assessing artisanal fishers' attitude and perception towards electric rays conservation along Indian coast

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ABSTRACT

In India, electric rays are not targeted by fisheries but form a significant portion of bycatch. Little information exists about their ecology and biology. Fishers' traditional ecological knowledge can fill the knowledge gap for these historically data-deprived fishes. Hence, we assessed attitudes and perceptions of artisanal fishers towards electric rays' conservation. We gathered information on catch frequency, seasonality, population trends, post-capture usages, and socio-economic values through a semi-structured questionnaire survey. We interviewed 243 fishers at 15 artisanal villages in three states: Tamil Nadu, Andhra Pradesh, and Odisha, along the east coast of India. The survey data was scrutinized, sorted into various categories: demographics, fisheries, and fishers' traditional knowledge, analysed and visualised as graphs and figures. We also presented qualitative information as general statements or quoted specific comments to better explain our findings. We found that fishers were well aware of electric rays and their electrical discharge. The data indicated that fishers knew the habitat in nearshore sandy bottoms. They get electric rays in their bottom-set gill nets. In general, December, January and February have high electric ray bycatch. Post-capture usage includes take-home consumption, discarding them dead, releasing them alive, and sell to poultry. Fishers discussed health benefits of electric rays' consumption. They perceived a downward trend in population due to anthropogenic influences: overfishing, pollution and climate change. Fishers' knowledge and scientific contributions can help develop management plans and conservation strategies. Initiating awareness campaigns within fishing communities and coastal citizens foster positive attitudes, thereby enhancing the electric rays' conservation.

1. Introduction

In the context of increasing threats to biodiversity, fisheries resources are now facing one of the greatest pressures in the world's oceans [1]. With a rapid decline in global catch rates and increasing fishing efforts, the most pressing threat to marine life is the indiscriminate capture of non-target organisms, commonly referred to as "bycatch" [2].

Electric rays (Elasmobranchii; Batoidea: Torpediniformes) form a significant portion of bycatch in the Indian fisheries [3–5]. They are a unique group of fishes that have specialized electrical organs. This allows them to produce 8–220 volts shocks, depending on the species [6]. Electric rays are bottom-dwelling fishes and play an important role as a mesopredator in the marine food web [7]. There are 68 valid species of electric rays globally [8], of which 13 were reported in the Indian waters [9]. Electric rays include the numbfishes (Narcinidae), sleeper rays (Narkidae), torpedo rays (Torpedinidae), thornback rays

(Platyrrhinidae), and coffin rays (Hypnidae). Electric rays, like other batoids, are slow growing, long-lived with prolonged gestation periods and low fecundity [3]. These life-history characteristics make them susceptible to overexploitation. They get very little time and space to recover.

In the Indian fisheries, electric rays are generally discarded because they have little or no commercial value [4,10]. Increasing mechanized fisheries for benthic organisms and high-value fish have further increased the proportion of elasmobranchs in bycatch [11]. In recent years, considerable efforts were made to document and describe elasmobranchs. However, they are largely limited to a few charismatic shark and ray species [12–15]. Less charismatic but ecologically important elasmobranchs such as electric rays are often neglected. Thus, except for a few studies on selected species of electric rays [3,5,16–18], there is a significant lack of scientific information on the ecology and biology for framing policy and implementation of conservation measures [19].

Most of the electric ray's species have been placed in the

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International Union for Conservation of Nature (IUCN) under threatened (Vulnerable, Critically Endangered, and Endangered) or Data Deficient categories. While the IUCN Red List is a valuable tool for assessing the population status, categories consulted for electric rays are generally based on total elasmobranch landings data or habitat conditions or have not been recently assessed [20]. One such critical example is the Red Sea torpedo (*Torpedo suessi*), which is not encountered for more than 30 years. Hence, it has been classified as Critically Endangered-Possibly extinct. It is alarming given the continued decline of other species of electric rays worldwide [21].

In the absence of historical data on data-sparse electric rays, fisher's traditional ecological knowledge (TEK) based on decades of their experience may prove valuable for understanding the species' distribution, and population trends, especially in a developing country like India [22–25]. In recent years, there has been increasing evidence that fishers TEK are an important source of information on elasmobranch ecology and biology [24–27]. These studies have contributed to understanding fishers' attitudes towards conservation and more importantly, decision-making in fisheries management.

In India, several regulations and policies address conservation of fishes including elasmobranchs such as closed season, boat licencing and registration, mesh size, minimum legal size, marine protected areas, and blanket ban on shark fin trade [28]. There is no specific policy and regulation for electric rays and none of the species have been included in the Wildlife Protection Act.

In the present study, we attempted to fill the knowledge gap on electric rays through semi-structured interviews with artisanal fishers on the east coast of India. We assessed the attitudes and perceptions of artisanal fishers towards electric rays' conservation and collect important information on seasonality, catch frequency, current population trends, post-capture use, and socio-economic values.

2. Methods

2.1. Study area

The study was conducted at 15 artisanal villages across the 3 states on the east coast of India. There were 6 sites in Tamil Nadu, 7 in Andhra Pradesh, and 2 in Odisha, along a total of almost 1600 km of coastline (Fig. 1). The study locations were selected based on various factors. Inclusion of artisanal fishers from rural and urban regions in each state, e.g., Rameswaram, Olaikuda, and Mandapam are rural areas while Covelong, and Neelankarai in Chennai as urban areas in Tamil Nadu. Tupilipalem and Mypadu are rural locations while Jalaripeta and Rushikonda in Vishakhapatnam as urban areas in Andhra Pradesh. Artisanal villages near ecologically relevant areas such as Pulicat Lagoon, Gulf of Mannar Biosphere Reserve and Palk Bay served as an important criterion. In addition, prior information on electric ray sightings/landings at different villages through previous field visits and on-field volunteers helped us establish the study locations (Supp. file: Appendix 1, Table S1). Each location was almost 200 km apart on the east coast, except for Tamil Nadu.

2.2. Interviews

We conducted 243 interviews with artisanal fishers between August 2022 to March 2023. Semi-structured questionnaires were developed in the English language which was later translated into Tamil, Telugu, and Odia languages. We took the help of field volunteers to communicate with fishers in their local languages in each state. There were 23 questions in the questionnaire (Supp. file: Appendix 2). Each interview lasted for about 20–25 min.

Artisanal fishers were approached randomly either in the villages or near their boats at the fish landing points. We informed them initially about the project, purpose of the survey and the intended use of collected data. We asked for their verbal consent and only upon their

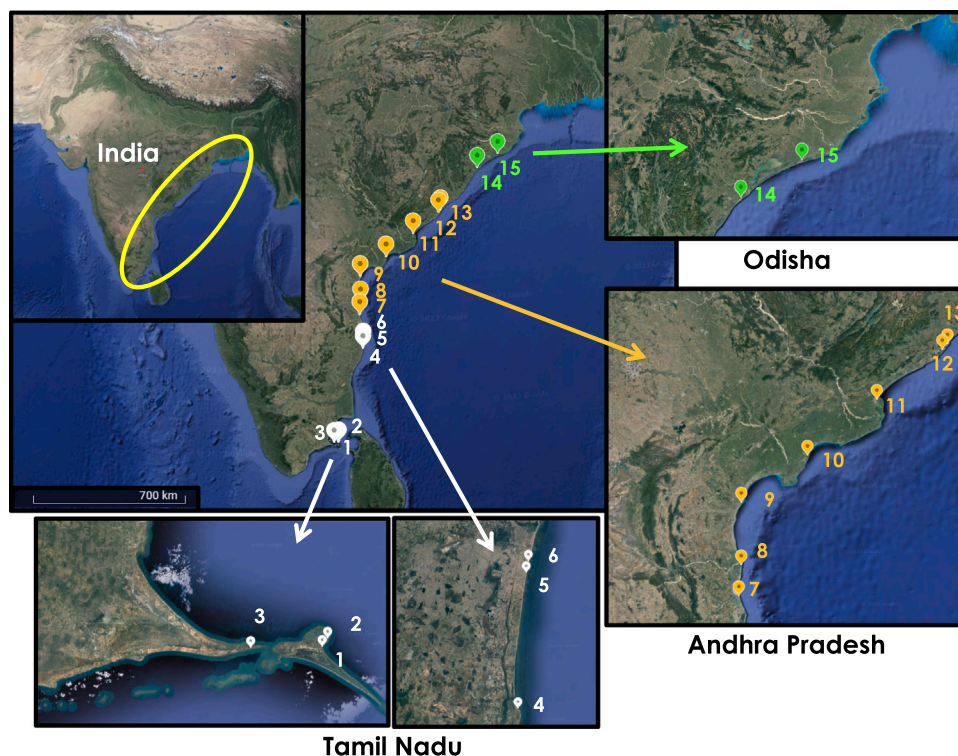


Fig. 1. Study sites. (Image is generated through Google Earth). White, yellow and green coloured pins indicate study sites in Tamil Nadu, Andhra Pradesh, and Odisha, respectively. 1. Rameswaram Jetty; 2. Olaikuda (Rameswaram); 3. Mandapam; 4. Covelong; 5. Neelankarai; 6. Thiruvannmiyur; 7. Tupilipalem; 8. Mypadu; 9. Kothapatnam; 10. Machilipatnam; 11. Kakinada; 12. Rushikonda; 13. Jalaripeta; 14. Gopalpur; 15. Puri.

agreement, we proceeded with the questions. We did not mention the name of the interviewee on the questionnaire sheets to maintain anonymity. The interview data sheets were safely stored in our research centre which will not be shared with anyone to maintain the confidentiality. The questionnaire was divided into two main sections: the first section was meant for the interviewers as it included details about the date, time, and location of the interview. Second section was meant for the fishers. In the beginning, we collected demographic details such as age, sex, primary occupation, and professional experience. We also asked if they have ever participated previously in any other marine conservation surveys. Then, we collected general information on the

fishing practices such as types of fishing gears they use, and target catch. We then, enquired specific questions on electric rays: if they know what are electric rays? have they ever caught them? are they aware of the electric shock? and other relevant information such as vernacular names for electric rays. We then further enquired about species diversity by showing them photographs of common electric rays of India. Based on their response, we continued our questions on relevant ecological information such as catch frequency, seasonality, pattern distribution, and potential habitat. We sought information on the post-capture use, precautionary measures they undertake to avoid electric shock, population status, and changing trends in the local areas. We finally enquired about

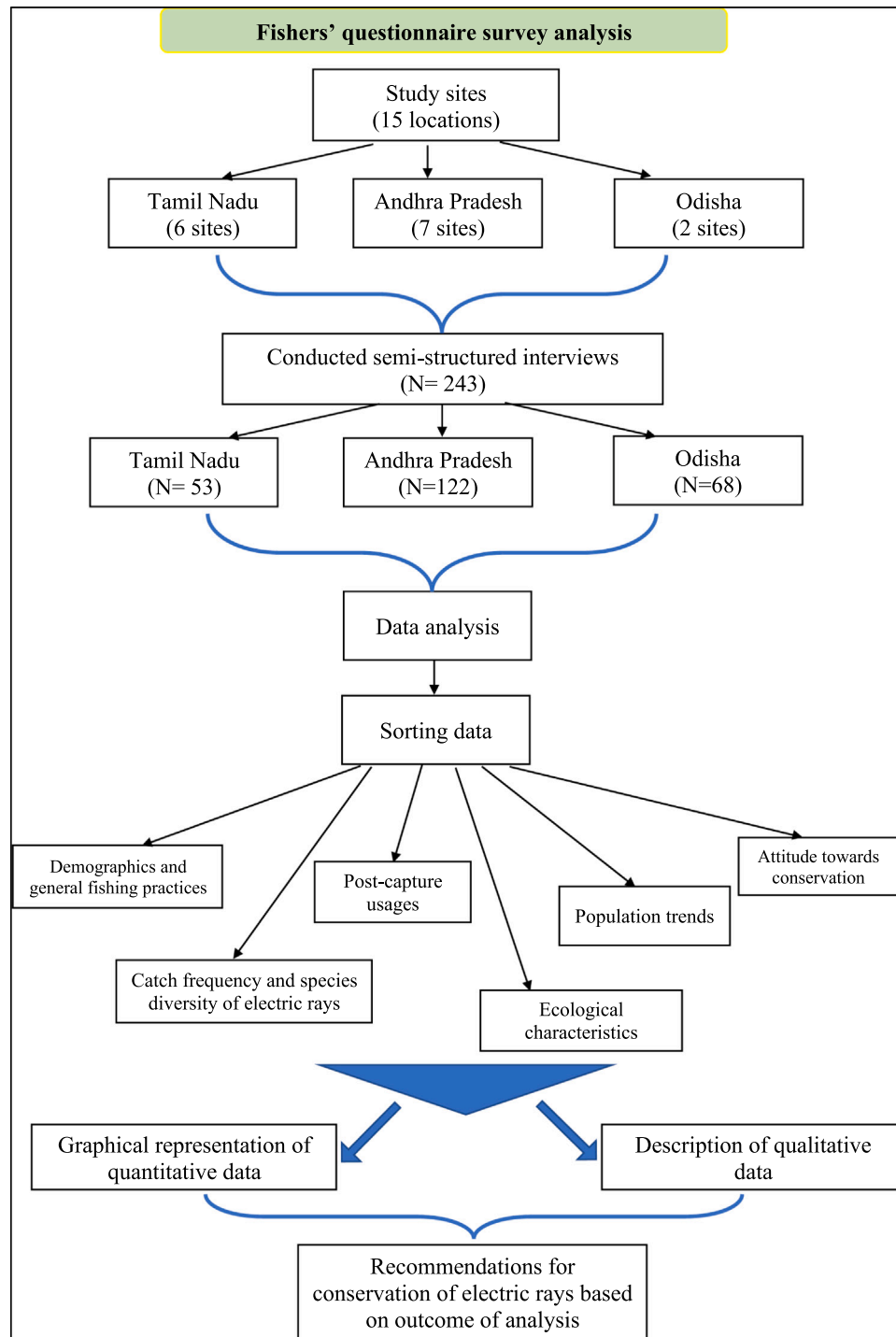


Fig. 2. PRISMA flowchart representing the steps used in the paper.

their take on the conservation status and resource management of electric rays.

We did our best to interview individual fishers. However, the interview generated curiosity among the other people in the vicinity, leading to a collective discussion, rather than an individual response. In this case, demographic details were avoided. However, we often encountered the people of same age group having fishing as their primary occupation. Since our objective was to generate fisher's TEK through interviews, this collective response would not hamper the primary objective of the study.

2.3. Data analysis

We processed the data obtained after the interview by sorting it into 6 major sections: i. demographics and general fishing practices; ii. catch frequency and species diversity of electric rays; iii. ecological characteristics including habitat, and seasonality; iv. post-capture usages; v. current population trends; vi. fisher's attitude and perception towards conservation.

The quantitative data were entered into Microsoft Excel for analysis. Charts and graphs were plotted to visualize the data. For open-ended questions, where multiple responses could be recorded, the responses were designated under broad categories: fishing practices, post-capture use, population trends, and perception towards conservation. We analysed these responses, and presented them as a general statement or quoted specific comments received from the fishers to better explain our findings. PRISMA flowchart is given in Fig. 2.

3. Results

3.1. Demographics and General Fishing Practices

In our survey, all 243 (100%) of the interviewed artisanal fishers were male. Their primary occupation was fishing and a majority of them had 30-plus years of experience. Majority of the fishers (79.8%) informed that previously they never participated in any marine conservation-related surveys. While a few from Andhra Pradesh and Odisha have contributed to fisheries (18.1%) and turtles (2.1%) related surveys. Detailed demographic information is given in the Supplementary file (Appendix 1, Fig S1-S2, Tables S2-S4). We observed that the artisanal fishers practice multi-gear and multi-species approach. They possess more than one fishing gears such as bottom set-gill net (91.4%), floating-gill net (49.8%), hook and line (49.0%), and beach seines (38.7%). Some of the fishers were also involved in other fishing practices (21.4%) such as trawl, purse-seine, trammel, and modified nets. The selection of gear was majorly dependent on the season, sea conditions, fish availability and demands, as well as their financial needs.

3.2. Fishers' Traditional Ecological Knowledge of Electric Rays

Almost all fishers (97%) were aware of electric rays and could recognize them in the photograph. The most common name for the electric rays across the study site was "Shock meen" or "Shock timli" which roughly translates to "fish that gives shock." The traditional names for electric rays at different locations are given in the supplementary file (Appendix 1).

Upon seeing the photographs, fishers could differentiate among different families but got confused with the morphologically similar species. Around 26% fishers informed that they have seen all 13 species. However, it is also possible that they could not differentiate different species due to morphological similarity. Remaining 74% fishers informed that they have encountered at least one or more species in the fishing net (Table 1, Supp. file: Appendix 1, Fig S3). According to them, *Torpedo fuscumaculata* and *Torpedo marmorata* were the most common ones.

Ninety-eight per cent of fishers were aware that electric rays

Table 1

Electric ray species identified by interviewed fishers and their IUCN categories. Values indicate number of respondents identified each species.

Species	Andhra Pradesh	Odisha	Tamil Nadu	Overall	Percent	IUCN status
<i>Torpedo panthera</i>	30	34	22	86	35.4	EN
<i>Torpedo fuscumaculata</i>	78	33	21	132	54.3	DD
<i>Torpedo sinuspersici</i>	17	6	10	33	13.6	DD
<i>Torpedo marmorata</i>	67	65	0	132	54.3	VU
<i>Torpedo zugmayeri</i>	0	0	0	0	0.0	NE
<i>Benthobatis moresbyi</i>	13	7	0	20	8.2	LC
<i>Narcine lingua</i>	22	15	0	37	15.2	VU
<i>Narcine prodorsalis</i>	25	12	0	37	15.2	EN
<i>Narcine timlei</i>	13	41	18	72	29.6	VU
<i>Narcine atzi</i>	9	15	0	24	9.9	VU
<i>Narcine maculata</i>	52	6	0	58	23.9	VU
<i>Heteronarce prabhui</i>	33	31	0	64	26.3	DD
<i>Narke dipterygia</i>	38	0	13	51	21.0	VU
ALL or could not differentiate?	42	0	23	65	26.7	-
Do not know	0	0	12	12	4.9	-

discharge shock. They learned about the shock primarily through generational knowledge transfer. Most of them believed that large-sized torpedoes would give a stronger shock than small-sized ones. While a few fishers at the Pinnivarpalem village, Andhra Pradesh claimed the opposite.

Eight percent of the fishers, all from Andhra Pradesh claimed to use rubber gloves to protect themselves from electric shock. Almost 92% of fishers either do not take any precautions or use alternative indigenous measures such as holding electric rays using nets, sand, and clothes as insulators or holding through spiracles or caudal ends. Some Tamil Nadu fishers informed that they use a combination of rubbing beach sand on their hands for insulation and then hold electric ray from the caudal end. Though the majority were not aware of the specification of the electric organs, they believed shock generates near the fish's mouth in the ventral side.

3.3. Artisanal Fisheries and Electric Rays

A hundred percent of fishers informed that electric rays are not targeted fish as it does not possess commercial value. Majority of them informed that electric rays are often bycatch in bottom set-gill net ("Every time we do crabs, shrimps, and lobsters fishing, we get these electric rays"). Eighty percent of the respondents pointed out that these fishes were generally caught in the near-shore waters up to a distance of 10 km in the sandy bottoms of < 50 m depth.

Across the study sites, 40% fishers responded that 4–10 electric rays were accidentally caught per month. Around 20% fishers get 11–20 electric rays as bycatch per month. While some fishers (17%) informed that they get more than 20 per month (Fig. 3).

3.4. Seasonality

Forty-four percent fishers responded that the majority of electric rays are caught in the month of December. 42% and 27% of respondents said maximum bycatch occurs in the months of January and February, respectively. Around 9% fishers informed that the lowest chances to get these fishes are in the month of October.

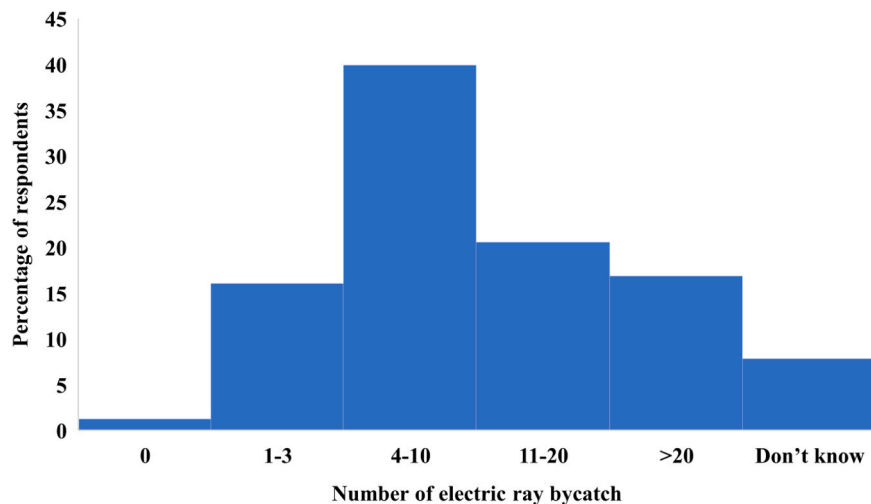


Fig. 3. Monthly frequency of electric ray's bycatch.

We also found that the seasonality of electric rays varies at different locations. Fishers in Tamil Nadu and Odisha reported the maximum ray's bycatch in the month of December, January, and February. In Andhra Pradesh, maximum bycatch occurs in the month of June and July. A few respondents from Tamil Nadu (17%) and Andhra Pradesh (28.7%) stated that they find electric rays across the year (Fig. 4). Almost a quarter of fishers from Tamil Nadu were not aware of seasonality ("We don't keep track of season for electric rays as its not targeted fishes").

3.5. Post-capture usages

Incidental catches of electric rays lead to different destinations. We found that same fishers showed different behaviours, i.e., sometime they release rays alive, or discard if dead. Other times, they bring home for consumption, or sell in market. Overall, 44% fishers responded to take-home consumption. Almost equal numbers have said they discard dead electric rays on-board. Around 35% respondents said that they release

rays alive, while 20% said that they sell them in fish markets/landing points. A small fraction of respondents (1.5%) informed that they sell it to the poultry feed industries along with other bycatches.

Post-capture usage varied among different states. In Odisha, almost half of the fishers responded in favour of take-home consumption of large-sized electric rays. They revealed the consumption of *Torpedo* spp., and *Narcine maculata* because of good taste. Around 34% respondents said they discard dead or release alive specimens. Almost a quarter testified that they sell the incidental catch of large-sized *Torpedo* spp. They disclosed that a fish of almost 1.5–2 kg can fetch around Rs.100. In Andhra Pradesh, almost half of the respondents informed that they discard dead rays as it does not have high commercial values. However, around 62% fishers have also claimed that they have released the electric rays alive in past. (Fig. 5).

The majority of the fishers in Odisha (98%), a fraction in Andhra Pradesh (4%, mostly in the northern region) and Tamil Nadu (11%) claimed that consuming electric rays have medicinal benefits, particularly in bone-related issues like arthritis, back pain, muscle pains, and

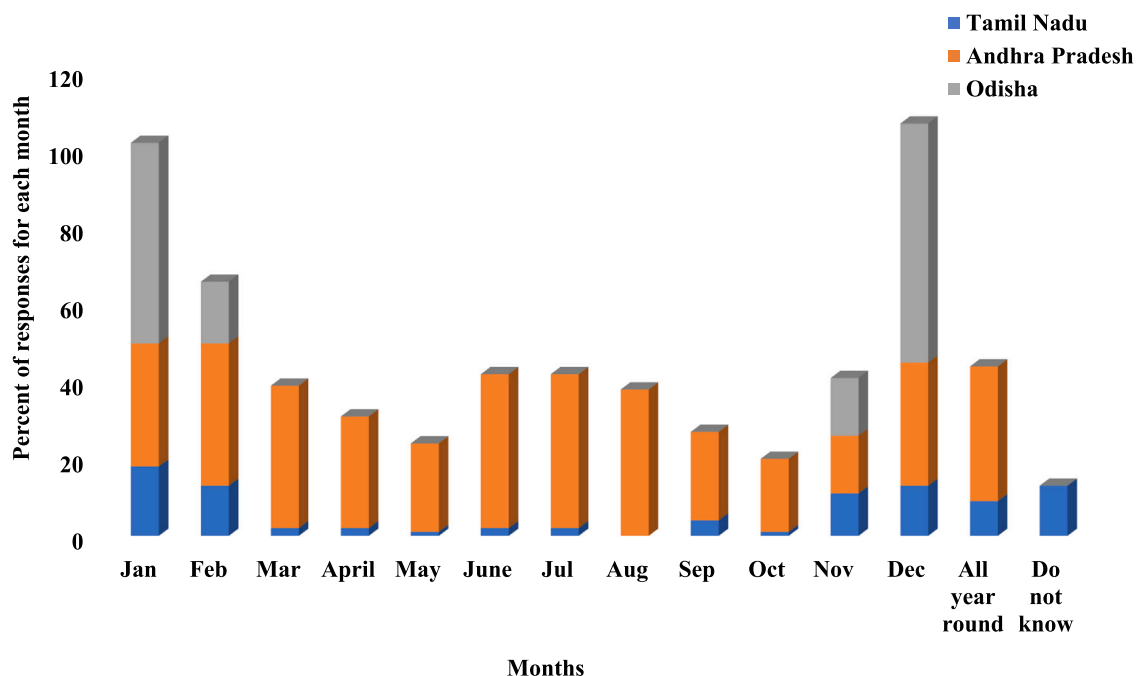


Fig. 4. Seasonality of electric rays in the eastern coastal states of India.



Fig. 5. State-wise post-capture usage of electric rays. Values indicate percent of response received for each category.

neuronal disorders, paralysis. A detailed post-capture usage is given in Table 2.

3.6. Population trends

Most respondents (43%) along the east coast of India perceived that electric ray populations have been decreasing in number. Also, they informed us that the size of the fish is also decreasing over the years ("Previously we were observing many large individuals in our bottom nets, but these days we get rarely and that too smaller ones").

Around 35% of respondents said no change in the electric ray's population in the last decade. While 21% of the fishers were unaware of the population trend as they did not bother about non-commercial fish. ("That fish is of no use to us, so we are not paying attention to their population growth or decline; sometimes we get more of them and sometimes less"). Interestingly, a fraction of fishers (1.6%) believed that the numbers of electric rays have increased in recent years as they claim to have got more in their nets (Fig. 6).

We did not find any patterns in the fisher's age and fishing experience with their perception of electric ray population change. However, we observed perception for population trend varied among the three states. The majority of fishers in Andhra Pradesh (66%) perceived a decrease in the electric ray population. While 62% of Odisha fishers said no change in electric ray catches and frequency. Around 44% of Tamil Nadu artisanal fishers were unaware of the population status of electric rays, while almost 40% perceived a decreasing trend in population (Supp. File, Appendix 1, Fig S4).

Upon further enquiring the respondents who mentioned the

declining electric ray populations about the possible reasons, each fisher gave multiple reasons. For example, 51% fishers said overfishing, 32% aggressive fishing practices ("Unsustainable fishing methods, aggressive nets, and increase in the frequency of bottom fishing are causing rapid declines of benthic fishes like rays and flatfishes"), 16% marine population, ("Factories situated on the coasts release harmful chemicals and untreated wastes in the seas due to which fishes are going away from this shore") and 12% climate change factors, were the possible reasons for decline of electric rays. A vast majority of the respondents (50%) were not aware of the possible reasons for the population decline of these rays (Fig. 6).

3.7. Fishers' attitude towards electric rays' conservation

Around 99% of the fishers were unaware of the conservation status of electric rays. Similarly, 99% of the fishers did not know about any regulatory measures on electric rays. While the remaining 1% considered that electric rays were protected by laws alongside other elasmobranchs.

We then asked if they would consider protecting electric rays, to which 38% of the respondents held positive attitudes. They suggested electric rays should be protected along with other benthic fishes as they showed concern about declining fish catches in the future. Majority of fishers (61%) had no specific opinion about electric rays' protection as they believed these fishes are not targeted. Surprisingly, a fraction of the fishers (1%) expressed a negative attitude toward electric rays' protection. They believed that the population is stable as their numbers in bycatch were constant over the years, hence they did not need protection. We did not find any trend between fisher's attitude towards electric

Table 2

Post-capture usage, perceived information with quoted sentences from fishers. Perceived information was common from fishers across state, unless it is specifically mentioned.

Use	Perceived information	Quoted comments
Take-home consumption and Medicinal use	Electric rays are enjoyed as flavoursome food	<i>It is a very delicious fish; we have e-ray curry with a native dish made of steamed rice flour and coconut and call it "Shock puttu" which is very popular among fisher households.</i>
	The skin of electric rays is very thick and tough, hence preparing, cleaning, and cooking the fish is time taking (Tamil Nadu fishers)	<i>Cleaning and cooking this fish take time as the skin does not peel off easily. It is only the men of the family who clean the fish by peeling the thick skin off the flesh and give the meat to women for cooking. The part on two sides of the fish from where we get maximum shock doesn't have much meat, so we cut that first from the fish and then go ahead with the cleaning</i>
	Fishers are aware of the electric organ located on the disc and cut it off to cook the fish	<i>After eating this fish, we feel very energetic and healthy the next day and can work better for longer hours at sea.</i>
	Fishers believe that electric rays are nutritious and provide significant energy to sustain hard-working days (Odisha fishers)	<i>We consider electric rays as a medicine to cure joint pains and muscle spasms. We also give shock-puttu to patients suffering from paralysis or other nerves related issues. The use of electric rays as a treatment for these issues has been transferred from generations and we continue to believe the same with experience.</i>
Discard dead	It is believed that the consumption of electric rays can cure bone, neurons and muscle-related issues	
	Discarding was primarily driven by anger caused due to heavy electrocution on touching electric rays	<i>I got an electric shock from this fish when I tried to handle it once. I thought it was dead and touched it. But I got a heavy shock and out of anger I threw it. Since that day I am scared so I discard these rays without even checking if it is dead or alive.</i>
Release alive	Fishers also tend to discard electric rays as it does not fetch high values. They prefer to focus on high-value species	<i>When we are busy selling fish to customers, we leave the electric rays on the ground without any ice storage as no one buys them. So, if more time passes in selling fish, these get spoiled and we leave them on the shore as such.</i>
	Since electric rays are not a targeted catch, some fishers release them alive due to low commercial values (Andhra Pradesh fishers)	<i>No one usually buy these species so we have no profit from taking them to the landing centres, so whenever we get them in our nets, we just release them back into the water.</i>
Poultry feed	When bycatch including electric rays comes in bulk amounts, it usually gets sold to poultry feed factories as fish meals (Andhra Pradesh and Tamil Nadu fishers)	<i>Usually, we collect all non-commercial and low-value species such as juvenile fish, shrimps, crabs, electric rays, etc and transport them to the poultry feed industries as raw material for fish meals. We get at least some income from</i>

Table 2 (continued)

Use	Perceived information	Quoted comments
Sale	The sale of electric rays is very rare and fetches very little economic value.	<i>these industries instead of just discarding them.</i>
		<i>There is no demand for this fish, only if any customer is curious about this fish, they will buy it for a very low price.</i>

ray conservation and fishers demographics. However, we observed difference in the fisher's attitude among the three states. For e.g., 68% of Andhra Pradesh fishers showed positive attitude towards electric ray conservation, while only 17% and 2% fishers said yes to electric ray protection in Tamil Nadu and Odisha, respectively. This observation can also be viewed in relation to the perception of the electric ray's population trends. Around 66% of the fishers from Andhra Pradesh perceived decline in the electric ray population, while only 40% and 3% fishers said decline in the electric ray population in Tamil Nadu and Odisha, respectively.

Fishers who positively favoured protection were further asked their opinion on potential mitigation approaches. However, a majority (56.4%) could not suggest any measure. A few fishers came with an array of notable suggestions including legal protection by classifying electric rays in protection acts (18%), bottom set-gill net restrictions (13%), controlling overfishing (8.2%), seasonal fishing ban (5.3%), control marine pollution (4.1%) and Government subsidies for fisheries (as a whole, but not specific to electric rays) (4.1%).

4. Discussion

This is the first-of-its-kind study for filling the knowledge gap on data-scarce electric rays through artisanal fishers' traditional ecological knowledge. This study would form the basis of vital information such as electric ray species diversity, distribution, and population trends on the east coast of India. The artisanal fisher's attitude and perception could be used for effective management and conservation measures [29]. The results are discussed under four subheadings: i. Fishers' knowledge of electric rays; ii. Anthropogenic influences as a threat for electric rays; iii. Fisher's attitude towards conservation; v. Conservation and mitigation recommendations.

4.1. Fishers' knowledge of electric rays

Electric rays form a significant portion of the bycatch in the demersal fishing gears used in India [4,9]. However, very few reports document the bycatch of this non-commercial, low-value electric rays [5,30]. Hence, undocumented biomass removal continues which may further lead to depletion of the living resources and can have damaging effects on the marine ecosystem [31]. Our study indeed revealed that electric rays are not-targeted, but are bycatch of the bottom-set gill net fishing on the east coast of India. Bottom-set gill nets were used to catch bottom dwellers such as lobsters, shrimps, crabs, and bottom-dwelling fishes. This fishing practice is particularly used if fishers do not meet their financial requirements by pelagic fishing. In some locations in Andhra Pradesh, we learnt that once or twice every week artisanal fishers undertake bottom-set fishing practices.

Fishers' electric ray knowledge was particularly remarkable as most of them were aware of electric rays and the shock it discharges. They could identify these rays up to the family level; however, had difficulty in identifying morphologically similar species. Interestingly, they identified and differentiate between *Torpedo* spp., *Narcine* spp., *Narke* spp., and *Benthobatis* sp. based on their morphological features including shape, size, and dorsal side patterns. Some of the fishers in Andhra Pradesh pinned that *Torpedo fuscomaculata* and *Torpedo marmorata* were

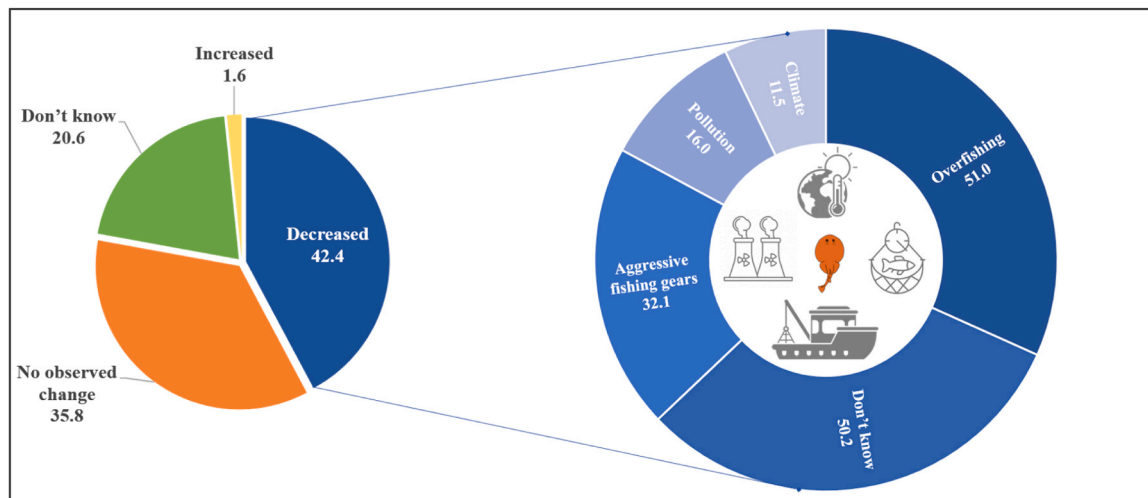


Fig. 6. Fishers' perception on the population trend for electric rays. Possible reasons for the population decline. Values indicate percent of response received for each category (sum of the percent is not 100% as many fishers indicated multiple reasons for population decline).

the most abundant catch. However, we felt that due to similar dorsal patterns, size, and colours, they were confused with species identification. Hence, we decided to restrict to report as *Torpedo* spp. as the common catch among electric rays (detailed on species-level identification by fishers is given in Table 1). The data revealed seasonality of electric rays on the east coast of India. Fishers informed that most catches of electric rays in Andhra Pradesh occur in the monsoon seasons which corroborates with the findings of the previous study in the same region [5].

Our data present new insights into the post-capture usage of electric rays. Though they are generally considered unused bycatch [4], we found take-home consumption by fishers. Due to the flavoursome meat and medicinal benefits traditionally believed by the fishers, it was a favoured option, especially in north Andhra Pradesh and Odisha. At present, these rays are low-value fishes. However, increasing global protein demand and declining target catches, it is important to consider Murawski's [32] slogan "Yesterday's bycatch may be tomorrow's target catch".

While capacity, infrastructure, and finance are limitations of developing countries like India, fishers' traditional knowledge can act as an important and cost-effective source of information at local and regional levels for data-deficient fishes [33].

4.2. Anthropogenic influences as a threat for electric rays

As informed by fishers, the decline in the electric ray population over the years was attributed to aggressive fishing practices, marine pollution, and climate change conditions. The IUCN Red List categories to order Torpediniformes state that their population is severely declining and faces extinction risks due to the increasing anthropogenic pressures [20]. There is a lack of long-term fisheries information on the population status of Torpediniformes in India. Indian Council of Agricultural Research - Central Marine Fisheries Research Institute which documents fish landing across the country gives information on total ray landing [34]. Although landing data is not a direct measure of abundance at sea [13], it can be used to infer population trends of data-scarce species.

Our data indicate that on average 15 electric rays are caught in artisanal fishing per month, which primarily depends on the type of fishing gears. Given that there are about 3461 artisanal fishing villages in the coastal states of India [35], this catch rate translates to an enormously high volume of electric rays which is alarming and requires regular monitoring. Overfishing is deemed as a primary reason for electric rays' decline which corroborates with the global scenario [36]. We also observed that artisanal fishers blame the trawling for the rapid

depletion of fisheries resources in the sea. This observation is in line with the previous report on the bycatches of India [37]. Fishers from areas near industries/ports/thermal power plant/aquaculture also blamed pollution as the reason for the fish decline in the local region. Marine pollution leads to the destruction of natural habitats and ecosystems, this forms a major stress factor [38]. Some fishers indicated that increasing ocean temperature, frequent storms, floods, and other natural calamities are also responsible for less fish catch. We have combined these reasons as potential climate change conditions. Climate change is a rapidly emerging concern for threatened elasmobranchs as it compounds effects through habitat loss and forced migration of species [36].

4.3. Fishers' Attitude towards Conservation

A few fishers were aware of the conservation status and provided suggestions for mitigation. Regarding management measures, fishers demonstrated a higher preference for species restriction through national laws. However, continuous capture of the scheduled species under the Wildlife Protection Act, 1972 does not provide much hope for electric ray conservation [24]. Suggested gear restriction through minimization of benthic nets and closed season could be considered for effective management. However, practically, it is difficult to convince fishers to gear restriction as it would result in a monetary loss [10]. Closed seasons are a commonly used management tool, but can have side effects as they can promote the temporarily concentrated and increased fishing effort in the open season [39,40]. Also, without much information on reproductive biology and ecology, it is difficult to decide the close season in this geographical area. Hence, long-term active ecological monitoring is much warranted to develop conservation and mitigation strategies.

4.4. Conservation and mitigation recommendations

8 species that were frequently caught by the interviewed fishers are currently placed in IUCN Red List's threatened categories (6 Vulnerable and 2 Endangered). Three and 2 species were in the Data deficient and Not Evaluated categories, respectively (Table 1, Supp. file: Appendix 1, Fig S5). These assessments were mainly based on the overall elasmobranch's landing data or state of their habitat or assessed long back [20]. This lack of species-specific and regional threat assessment can mask the decline of individual species and increase the risk of delaying conservation measures [41–43]. Since most electric rays have no market demand, the unused catch often goes unmanaged without any documentation. While, there are multiple pieces of evidence of decline

and proposed policies for other batoids such as rhino rays and sawfishes [25,27,44,45], almost no information exists for electric rays.

Though in recent years efforts have been taken to include elasmobranchs in the schedule list under Wildlife Protection Act, 1972, no species of Torpediniformes made it into the list (Wildlife (Protection) Amendment Bill, 2022). Our current study highlights the present declining status of electric rays on the east coast of India and hence calls for a precautionary approach, whereby management and conservation actions could be planned.

To initiate efforts toward electric rays' protection, we propose to implement awareness programs across the coastal states of India. Most fishers and coastal citizens were unaware of the threats faced by the electric rays, especially under the predicted climate change conditions. As a part of the awareness campaign, we initiated several programs including a national webinar on batoids of India, an awareness campaign at Covelong blue flag beach, Chennai, and a fishers training program at various locations. We have recently launched a website dedicated to the conservation of underappreciated electric rays of India (<https://projectrays.wordpress.com/>). Post-awareness camps, we found that fishers became more sensitive toward electric rays. For example, fishers in the Pinnivarpalem village of Kothapatnam Mandal of Prakasham district in Andhra Pradesh are readily releasing electric rays which are caught accidentally. Hence, sensitization of fishers and coastal communities can be an effective measure to mitigate population decline. We request those fishers who are interested towards the conservation of electric rays and recreational SCUBA divers to take photos and videos whenever they encounter electric rays. This will help in identification of any rare species present as well as their key habitats in this geographical location. This approach has led to the documentation of rare elasmobranch species in the Mediterranean Sea [46].

A blanket ban on fishing is not a possible option; hence we believe releasing the live catch is the best measure for electric rays. We propose that training for handling live electric rays incorporating the use of rubber gloves for the safe release of electric rays would be useful.

Domestic regulations can incorporate live-release incentives to promote the voluntary release of alive bycaught electric ray individuals. This has proved to be a successful measure through various initiatives for other endangered marine species [47–50]. While the live-release of electric rays can be a simple and cost-effective mitigation strategy, the absence of data on the at-vessel and/or post-release mortality may make management decision-making difficult.

Recent recommendations from a national consultative meeting have acknowledged the need for the conservation of non-iconic or small-sized marine fauna which is caught as bycatch [28]. They proposed that such bycaught organisms may be added to the Wildlife Protection Act, 1972 in new schedules with modification while considering no harm to the livelihoods of the fishers. Also, the promotion of Bycatch Reduction Devices and mesh-size regulations in bottom nets by the local and state government bodies is a pressing priority considering the rapid decline of endangered species [10].

Many fishers showed interest and willingness towards sustainable fishing and bycatch mitigation with growing concern towards declining catches. Their suggestions such as species restriction, gear restriction, closed season, and reduction of overexploitation were in line with various scientifically recommended fisheries management actions [10, 13,21]. Given the positive attitudes of fishers towards conservation, their suggestions could be successfully used for the development of management plans in the future as a collaborative approach of science and direct resource users to prevent excessive exploitation of elasmobranchs [51].

5. Conclusion

The present study filled the knowledge gap on the status of electric rays through a sociological approach on the east coast of India. We observed that though the electric rays were not targeted, they formed a

significant portion of the bycatch. Fishers' knowledge of the status of electric rays and their attitude and perception toward conservation can be used to develop management plans and conservation strategies. Initiating awareness campaigns within fishing communities and coastal citizens has developed positive attitudes, thereby enhancing the chance for electric ray conservation.

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CRediT authorship contribution statement

Amit Kumar: Conceptualization, Funding acquisition, Data collection and Analysis, Investigation, Methodology, Visualization, Writing – review & editing, supervision. **Shrutika Raut:** Conceptualization, Funding acquisition, Data collection and Analysis, Investigation, Methodology, Visualization, Writing-first draft. **S. Prakash:** Conceptualization, Data collection, Methodology, Writing - review & editing.

Declaration of competing interest

The authors declare no competing interest.

Data Availability

The authors do not have permission to share data.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.marpol.2023.105826.

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A New Species of the Genus *Dendronephthya* Kükenthal 1905 (Octocorallia: Alcyonacea: Nephtheidae) and Associated Crustaceans From Covelong, Chennai, India

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Abstract

In the present study, we described a new species of octocoral *Dendronephthya johnsonii* **sp. nov.** from the Covelong coast, Chennai, India. The new species is compared with the congeners *D. costatorubra* (Henderson 1909), *D. cervicornis* (Wright and Studer 1889), *D. hemprichi* (Klunzinger 1877) and *D. kluzingeri* (Studer 1888) found in the Indo-Pacific region. However, *D. johnsonii* **sp. nov.** can be distinguished based on the arrangement of polyps and structure of sclerites on the interior and exterior of basal stem, branch surface and polyp head. The *D. johnsonii* **sp. nov.** is so far known only from the type locality. Description of the holotype, paratypes, coloration and habitat were discussed in detail.

Keywords Octocorallia · Alcyonacea · *Dendronephthya* · New species · India · Associated communities

Introduction

Octocorals are distributed from warm shallow waters to the abyssal zone of the ocean including Arctic and Antarctic. Octocorals consists of 45 families that were distributed over 334 genera, and ~4000 species (Williams 2010). The subclass Octocorallia comprises three orders: Helioporacea (blue coral), Pennatulacea (sea pens), Alcyonacea (soft corals). The order Alcyonacea comprises of 360 genera under 46 families. They are found in the south-western Atlantic Ocean (Perez and Zamponi 2004), Red Sea, Indian and central-west Pacific oceans (Alderslade and Fabricius 2001). Among Alcyonacea, the family Nephtheidae have 20 genera and 481 species. It is mainly distributed in the clear water habitats, but are more diverse as their ecological requirements, and habitat preferences vary widely between genera (Alderslade and Fabricius 2001). Within the family Nephtheidae, the genus *Dendronephthya* comprises 249 species. *Dendronephthya* has a

widespread distribution in the Indo-Pacific regions, including Africa to Micronesia and French Polynesia (Alderslade and Fabricius 2001).

In Indian waters, *Dendronephthya* is found in Andaman coasts, Ganjam coasts, North Andaman Island, Palk Straits, Nicobars, Malabar Coast (Thomson and Simpson 1909), Gulf of Mannar (Gopalakrishnan et al. 2012), Bay of Bengal (Rao and Devi 2003), Gulf of Kutch (Usha et al. 2008) and Kerala coast (Mary and Sluka 2014). Earlier studies on *Dendronephthya* in Indian ocean includes Lakshadweep, Maldives and Mascarene Archipelago (Hickson 1903; Hickson and Gardiner 1905; Pratt 1903, 1905; Thomson and Henderson 1906; Thomson and Simpson 1909; Verseveldt 1974, 1971). Recent studies from India include additional reports of *Dendronephthya* from Lakshadweep (Verseveldt and Ofwegen 1991), Gulf of Mannar and Andaman and Nicobar Islands (Yogesh Kumar et al. 2016, 2019), and Tamil Nadu coast (Sivaleela and Padmanaban 2015).

The Alcyonaceans are well-known for hosting associated communities that hide among the tentacles (Haig 1981). The present specimen of *Dendronephthya johnsonii* **sp. nov.** hosts two species of crustacean decapods. Upon careful examination, they were identified as the porcelain crab *Lissoporellana quadrilobata* (Miers 1884) of the family Porcellanidae and the alpheid shrimp *Synalpheus neomeris* (de Man 1897) of the family Alpheidae. The family Porcellanidae is distinguished from other anomuran families by a crab-shaped body with a flattened abdomen. This family

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includes 30 genera and about 280 species around the world, mainly known from tropical to temperate waters (Osawa and McLaughlin 2010). Among the family Porcellanidae the genus *Lissoporcellana* comprises 10 species found in association with corals, sponges, hydrozoans, or anthozoans such as scleractinians, antipatharians, alcyonaceans, and gorgonaceans (Haig 1978), and occur from the intertidal to 128 m deep, on sandy and muddy bottoms. In India, it is also found in Gulf of Kutch and Gulf of Mannar (Prakash et al. 2013). *L. quadrilobata* has a widespread distribution in Indo-west Pacific from the Persian Gulf, east coast of Africa, and from the Indian Ocean to the Malay peninsula and Australia (Osawa and Chan 2010).

The family Alpheidae includes 50 genera and about 766 species around the world (WoRMS database 2022). Among them the genus *Synalpheus* comprises 164 species. In the present study *Synalpheus neomeris* (de Man 1897) was found associated with the soft coral *Dendronephthya johnsonii* sp. nov. This species has been commonly found in association with alcyonarians, sponges, bryozoans, pearl oysters, on reef platforms or coral heads from 3 m or less deep (Banner and Banner 1975). The general distribution of *Dendronephthya* includes Australia, Suez Canal, Red Sea, Persian Gulf, Ceylon, Maldives and Laccadive Archipelago, Singapore, Indonesia, Thailand, Shanghai, Japan and Sulu Sea (Banner and Banner 1975), Gulf of Mannar (Gopalakrishnan et al. 2012) and currently known from Covelong, Chennai, Tamil Nadu.

Materials and Methods

Covelong (also known as Kovalam) (12°47'31"N, 80°15'04"E and 12°46'42"N, 80°15'15"E) lies in north Tamil Nadu. Covelong comprises sandy and rocky intertidal zones and submerged reefs in the nearshore areas harbouring diverse life forms. It is also one of the important sites where artificial reef structures were deployed by the Government of Tamil Nadu in the coastal waters to revive marine life forms (Kumar et al. 2021). The corals are present in the reef deployment regions (12°79'05" N, 80°26'27" E) of the littoral zone (5.0 m–15.0 m), where the wave action (< 1.5 m) and bathymetry (> 5 m and < 15 m) are suitable for ideal larval settlement (Jha et al. 2022).

During the routine survey on biodiversity assessment at Covelong coast, we collected 3 soft corals caught by artisanal fishermen using a bottom-set gill net. The specimens were taken to the laboratory for taking colour photographs for identification and preserved in a deep freezer at -20 °C until further examination. Specimens were identified using morphological characteristics, such as growth form, colour, size, polyp arrangement, and sclerites. Morphological and anatomical terminologies applied to describe the specimen are taken from Sherrieffs (1922) and Bayer (1983). Sclerites

were obtained by dissolving the organic tissues with sodium hypochlorite and were then examined under a stereomicroscope (Nikon SMZ25) equipped with a digital camera. Measurements were made using NIS-D elements software attached with the microscope camera. The classifications of the taxa were based on Thomson and Simpson (1909). On further analysis, the alpheid shrimps and porcelain crabs were found attached to the stalk of the corals between the polyps. The shrimp specimens were identified based on the rostrum, telson, uropods, size of major chela, and spines on pereopods. The porcelain crab specimens were identified based on the morphological and meristic characteristics, such as rostrum, number of spines on the anterior and lateral branchial margins of the carapace, size of cheliped, carapace colouration. Examined materials were deposited in the National zoological collections of the Marine Biological Research Centre (MBRC), Zoological Survey of India (ZSI), Chennai.

Systematic Accounts

Subclass Octocorallia Haeckel 1866

Order Malacalcyonacea McFadden, van Ofwegen & Quattrini 2022

Family Nephtheidae Gray 1862

Genus *Dendronephthya* Kukenthal 1905

Dendronephthya johnsonii sp. nov. (Figs. 1, 2, 3)

Material examined. *Holotype*. ZSI/MBRC/G-27. Covelong (Kovalam), Chennai, Tamil Nadu, India, (12°47'31"N, 80°15'04"E - 12°46'42"N, 80°15'15"E), 13th January 2022, depth 10–15 m, Coll. Amit Kumar and S. Prakash.

Paratypes: ZSI/MBRC/G-27. Two individuals. Same collection data as above.

Description. The colony is distinctly divaricate (Fig. 1a, b) measuring 70 mm in height and 30 mm in breadth. Stem with distending base, measuring 11 mm, characterized by its yellowish-white appearance with linear red markings in on the stem. The polyparium is elongated, cylindrical, formed of several branches which rise at an angle of about 45° to the main stem (Fig. 1c).

The polyps are monomorphic, arranged on elongated (Fig. 2a), diverging stalks in groups of 6–8. They are oval in shape (Fig. 2b), measuring an average of 0.53 mm in length and 0.57 mm in breadth. Polyps exhibit a converging sclerite arrangement. In addition, there are 0 to 4 semi-transparent (Fig. 2c) converging spicules of the polyp, projected beyond the polyp-head, measuring 0.26 mm in length. The polyp spindles are rough, somewhat flattened, straight or slightly curved, owing to numerous small processes. The polyp wall has numerous rods and clubs (Fig. 3a).

Exterior sclerites from the basal part of stem are branched, elongated, y-shaped, and crescents. Sclerites are 2.3 mm to 5.4 mm long, 0.6 mm to 1 mm thick. Presence of

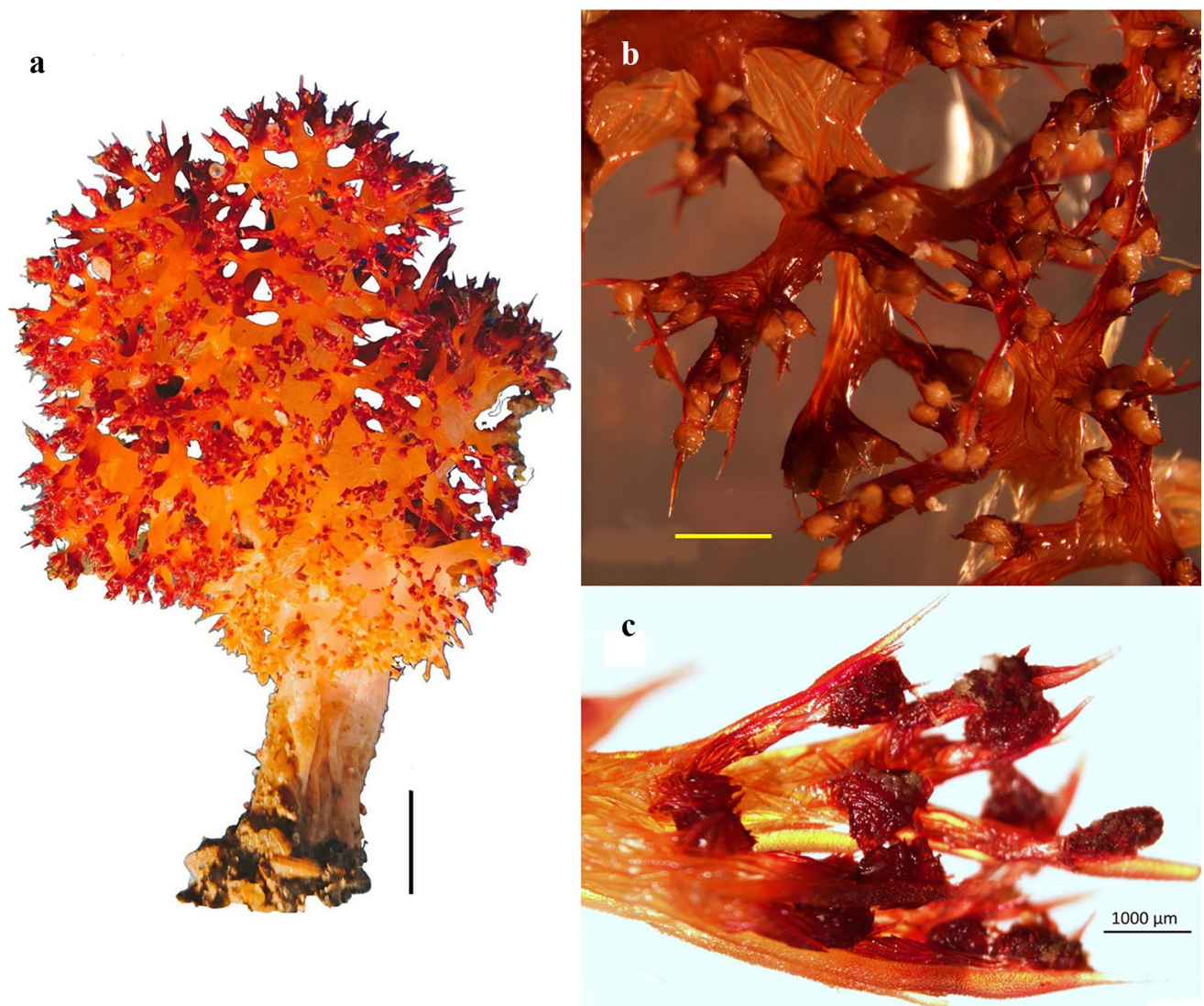


Fig. 1 a. *Dendronephthya johnsonii* sp. nov. whole specimen, dorsal view, total length 8 cm, Covelong Coast, Chennai, ZSI/MBRC/G-27; b. divaricate arrangement of branches, dorsal view; c. polyparium, lateral view (scale bar: a = 1 cm; b, c = 1000 µm)

sparse tubercular sculpture of different sizes on the spindles (Fig. 3c, f).

Interior sclerites from the basal part of stem have different shapes such as branched, bent shape, and antler-shaped spindles. Sclerites are 1.4 mm to 4.5 mm long, 0.2 mm to

0.8 mm thick. Presence of complex tubercles and blunt spines in spindles (Fig. 3d).

Sclerites from branch are elongated, straight or curved, sometimes branched. They are all furnished with minute

Fig. 2 a. Polyp arrangement on stalk; b. single polyp, dorsal view; c. converging spindles of polyp project beyond polyp head, lateral view. (Scale bar: a = 1000 µm; b, c = 500 µm)



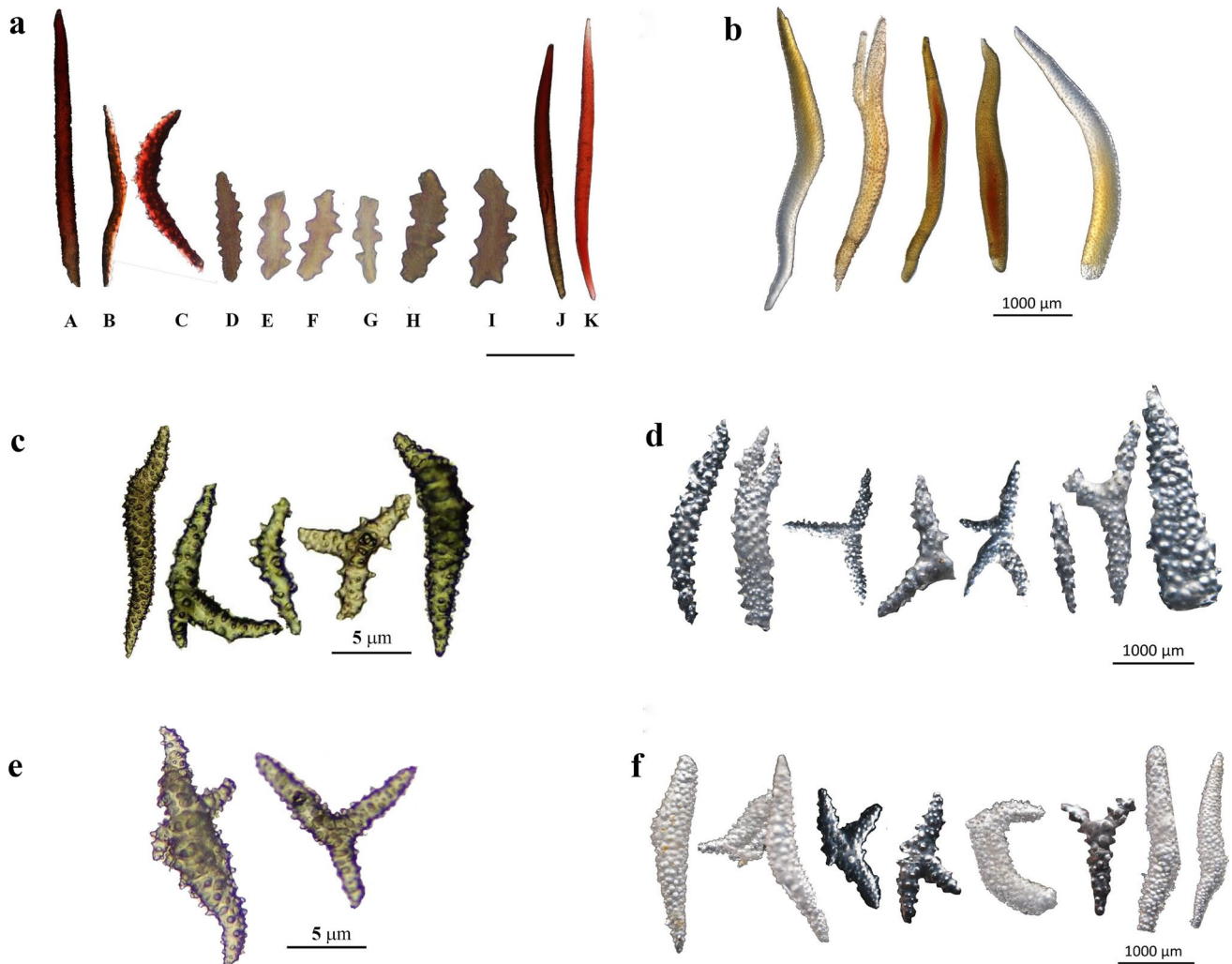


Fig. 3 Sclerites **a.** Polyp head (A, J—500 µm) (B, C, K—1000 µm) (D to I—5 µm); **b.** Branch surface; **c.** Exterior of basal stem; **d.** Interior of basal stem; **e.** Branch surface **f.** Exterior of basal stem

thorns. The spindles may reach a length of 9 mm long and a breadth of 0.8 mm (Fig. 3b, e).

Colouration. Basal stalk whitish/orangish towards the centre of the stalk, and brownish at the base. Stem and principal branches yellowish white with scattered dark red streaks appearing on the surface. Smaller branches and twigs are sharp, sometimes blunt with orange-red in color and slightly transparent toward the tips. Polyps wine red externally but appear reddish-orange under the stereo microscope (Fig. 1 and 2).

Habitat. Submerged rocks and other hard surfaces including artificial reef in a depth range from 10–15 m.

Distribution. Known only from the type locality, Covelong, Chennai, Tamil Nadu.

Etymology. The species name is in honor of Dr. Mari-azeena Johnson (Chancellor, Sathyabama Institute of Science and Technology, Chennai) and Dr. Marie Johnson

(President, Sathyabama Institute of Science and Technology, Chennai) for being an epitome of excellence in the various domains of academics and research. In addition, Dr. Mariazeena Johnson was honoured by former president of India Dr. Pranab Mukherjee as one among the 100 impactful women of India for her consistent efforts to provide world-class education and for the economic empowerment and social upliftment of underprivileged women and children. The present species name is dedicated for their valuable contributions.

Remarks. In some perspective, the colony agrees with the congeners but there are differences in terms of the branched sclerites, the distending base of stem, antler-shaped spindles of interior basal stem, long sclerites of branch with minute thorns and the sclerites in the surface layer of the basal stem. The detailed characters that are distinguishing our new species *Dendronephthya johnsonii* **sp. nov.** and other closely

Table 1 A comparison of *Dendronephthya johsonii* sp. nov. with congeners

Characters	<i>D. johsonii</i> sp. nov.	<i>D. costatorubra</i>	<i>D. cervicornis</i>	<i>D. henprichi</i>	<i>D. kluzingeri</i>
Polyp bundles	Group of 6–8 with each polyp measuring 0.53 mm in length and diameter 0.57 mm	Group of 4–5, each measuring 0.48 mm in length and 0.6 mm in diameter	1–4 mm in length and diameter is 1 mm with 3–4 polyps in a bundle	Densely packed, forming spherical groups of large globular lobes	Polyps are rarely prominent, nearly contracted into cells
Base of the main stem	Bulged and the diameter slowly diminishes to the apex	Slender and not rigid	Cylindrical in shape with presence of numerous stolons at the base	-	Thick and much branched, strengthened by slender fusiform spicules
Spicules of polyp	Rough walls with numerous small processes and rods and clubs, which are provided with cones	Flattened spindles, straight or slightly curved with simple spines.	Spicules are thickened at the base, pointed towards the outside, and covered with outwardly directed spines resembling the teeth of a saw	Cylindrical than fusiform, with blunt ends, mostly curved, with short ones blunt or pointed spines	-
Sclerites on the basal part of stem	Branched, bent shape, and antler-shaped spindles with a presence of complex tubercles and blunt spines	Flat star to spindle-shaped forms with spicules	Cross-shaped and four rayed sclerites placed at right angles or sometimes more obliquely to one another, furnished with spiny clubs and rough warts	-	-
Spindles on the stalk	Sparsely tubercular sculpture of different sizes and shapes including elongated, straight, or curved, sometimes branched also furnished with minute thorns.	Large spindles of irregular four-rayed and rough star-shaped forms.	-	-	-

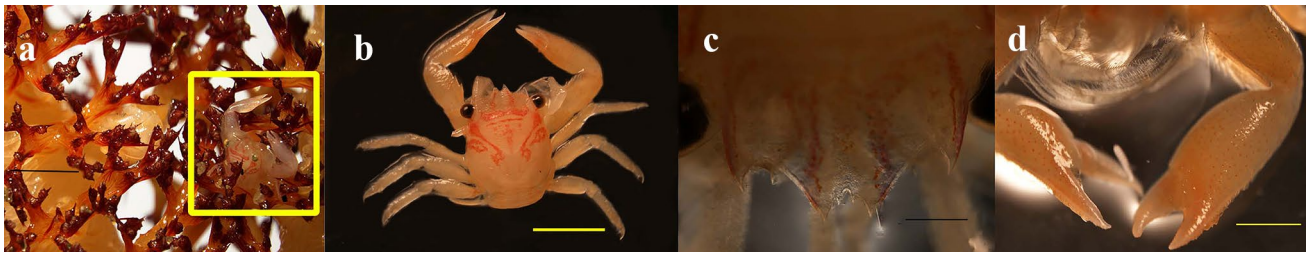


Fig. 4 a. *Dendronephthya johnsonii* sp. nov. associated organism (yellow square); b. *Lissoporcellana quadrilobata* (Miers, 1884), dorsal view; c. Trilobate rostrum, dorsal view; d. Chelipeds, dorsal view (Scale bar: a, b, d = 1000 µm, c = 100 µm)

related species such as *D. costatorubra*, *D. cervicornis*, *D. hemprichi* and *D. kluzingeri* was mentioned in the Table 1.

Associated Communities

Systematic accounts

Order Decapoda Latreille 1802

Family Porcellanidae Haworth 1825

Genus *Lissoporcellana* Haig 1978

Lissoporcellana quadrilobata Miers 1884 (Fig. 4)

Material examined. ZSI/MBRC/D1-691. 1 female, carapace length 3.76 mm, carapace width 2.9 mm, collected from Covelong (Kovalam), Chennai, Tamil Nadu, India, (12°47'31"N, 80°15'04"E -12°46'42"N, 80°15'15"E), 13th January 2022, on *Dendronephthya*. Carapace length and carapace width of other 2 specimens collected from the same location are 2.73 mm, 1.8 mm and 2.9 mm, 2.12 mm respectively.

Short description. Carapace length 1.3 times more than carapace width. Dorsal surface somewhat convex, nearly smooth. Colouration of carapace translucent, pinkish white, with pale red oblique mark on each hepatic region. Rostrum trilobate, median lobe usually with distinct median notch on frontal margin. External orbital angle terminating in small spine. Lateral branchial margin with 3 or more spines. Projection of immovable article adpressed to anterior margin of carapace. Chelipeds usually unequal in size, chelae inflated, subcylindrical, dactylus usually opening obliquely, dactylus of smaller cheliped somewhat twisted. Ambulatory legs with dactyli each usually terminating in a single claw.

Distribution. *L. quadrilobata* is widely distributed in the Indo-West Pacific: from the Persian Gulf, east coast of Africa

and throughout the Indian Ocean to the Malay Peninsula, north to the Izu Islands, and south to Queensland, Australia (Osawa and Chan 2010). The present specimen is known from Covelong, Chennai, India indicates the distribution range extension of *L. quadrilobata* which was previously reported from Gulf of Mannar and Gulf of Kutch (Prakash et al. 2013).

Habitat. Shore to 128 m deep, sandy and muddy bottoms. In the present study *L. quadrilobata* (Miers 1884) has been found associated with *Dendronephthya johnsonii* sp. nov. However, earlier reports include the association with gorgonians (*Chironophthya*, *Solenocaulon*) and soft corals.

Remarks. Description of specimen agrees well with the description of *L. quadrilobata* of Osawa and Chan 2010. It is also closely related to *Lissoporcellana nakasonei* (Kikuchi and Miyake 1978), however can be distinguished based on the relatively elongate carapace with a strongly produced rostrum and number of lateral branchial margin spines. In *L. nakasonei* the rostrum is broad with median lobe 3 times as broad as laterals, with median longitudinal, shallow groove, divided into 2 sub-lobes bearing 1- or 2-min denticles whereas in *L. quadrilobata* the rostrum is strongly produced anteriorly, median lobe with distinct notch on anterior margin. In *L. nakasonei*, lateral branchial margin of carapace possesses single spine compared to 3 or more spines in *L. quadrilobata*.

Family Alpheidae Rafinesque 1815

Genus *Synalpheus* Bate 1888

Synalpheus neomeris de Man 1897 (Fig. 5)

Material examined. ZSI/MBRC/D1-692. 1 female, carapace length 4.73 mm, total length 10.9 mm, propodus of major chela 10.8 mm and dactyl 4.1 mm, collected from Covelong (Kovalam), Chennai, Tamil Nadu, India, (12°47'31"N, 80°15'04"E -12°46'42"N, 80°15'15"E), 13th

Fig. 5 a. Coral associated organism (yellow square); b: *Synalpheus neomeris* (de Man 1897), dorsal view; c. Major cheliped, lateral view. (Scale bar: a-c = 1000 µm)



January 2022. Carapace length and total length of other specimen collected from the same location is 4.94 mm and 10.7 mm respectively.

Short description. Rostrum is long, ocular teeth arising from the margin of carapace, tip bearing several setae. Major chela 2.6 times longer than dactyl. Carpus of the second pereopod have five segments. Dactyl biunguiculate in third, fourth and fifth pereopods. Propodus of third pereopod have 8 spines in inferior margin. Telson 3.5 times as long as posterior margin is broad. Dorsal surface of telson have 2 pairs of spines. Anterior pair placed at the middle of telson and posterior pair at the distal region of telson. Posterior margin with two pairs of outer spines, inner pair more than three times as long as outer pair. Uropodal exopod have diarsis with two immovable spines laterally. 1 movable spine between two immovable spine.

Distribution. *Synalpheus neomeris* have widespread distribution including Suez Canal, Red Sea, Persian Gulf, Ceylon, Maldives and Laccadive Archipelago, Singapore, Indonesia, Australia, Thailand, Shanghai, Japan and Sulu Sea. The present specimen collected from the Covelong coast, Chennai, indicates the distribution range extension of the species.

Habitat. Shallow to sub tidal up to 250 m. In the present study *Synalpheus neomeris* (de Man 1897) has been found associated with *D. johnsonii* sp. nov., however earlier reports include association with alcyonarians, sometimes with sponges (Banner and Banner 1975).

Sampling and Field Studies

All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements, if applicable.

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Authors Contributions KK, SP and AK collected the material. KK and SP identified the soft coral and took pictures. SP identified the associated communities. KK wrote the initial draft. SP and AK reviewed, edited and approved the final draft.

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Data Availability All data generated or analyzed during this study are included in this article.

Declarations

Ethical Approval and Consent to Participate All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Human and Animal Ethics No animals were harmed during the study. Only dead animals were collected from the fish landing centre for identification purpose.

Consent for Publication The authors give consent to the publisher to publish the work upon acceptance.

Conflict of Interest The authors declare that they have no conflict of interest.

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Abstract

High altitude sickness, particularly, the effects of low pO_2 in the atmosphere above 5000 m altitude, present great difficulties for human physiology. It has long been argued that lower oxygen availability can lead to cardio-respiratory dysfunction, cognitive decline, and even lethality due to biochemical changes in the brain and lungs. While the molecular causes and biochemical background of the physiological changes are still under investigation, there is a wealth of scientific evidence pointing to a disturbed redox balance in hypoxic situations. Antioxidant therapies have been shown to have little effect while conventional and dietary oxidant supplements have been tried, but research into novel supplements to expedite the normal acclimation process is ongoing. A remarkable redox-regulating and

Research Article

Mitochondrial Genome of “Spotted Numbfish” *Narcine timlei* (Bloch & Schneider, 1801) and Phylogenetic Relationships among Order Torpediniformes

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In this study, we report, for the first time, mitochondrial genome of *Narcine timlei* (Bloch & Schneider, 1801) and its phylogenetic relationships within the order Torpediniformes. *Narcine timlei* is a medium-sized ray that occurs in nearshore waters of the Indo-Pacific and is classified as “vulnerable” category on the IUCN Red List. The mitogenome is assembled from short Illumina reads (150 bp paired end reads). It is 17,964 bp long and includes 13 protein-coding genes (PCGs), 22 tRNA genes, and 2 rRNA genes. The gene order, size, and nucleotide composition are largely consistent with mitogenomic characteristics of previously reported other *Narcine* spp. The slightly larger mitogenome length of *N. timlei* than other *Narcine* spp. may be due to the presence of a putative control region of 1,916 bp with three tandem repeats. Phylogenetic reconstruction using concatenated PCGs ($n = 13$) of 9 Torpediniformes based on maximum likelihood and Bayesian inference analysis revealed identical topologies. The tree showed two main clades: one clade containing members of the family Narcinidae and the second sister clade consisting of the families Narkidae and Torpedinidae. Our result supports the monophyletic nature of Narcinidae based on mtDNA. The information obtained in this study will contribute to a better understanding of the population genetics, phylogenetic analysis, conservation, and evolutionary biology research of *N. timlei*.

1. Introduction

With nearly 650 species in 4 orders and 23 families, the superorder Batoidea (rays) forms one of the most speciose groups of the subclass Elasmobranchii [1]. Phylogenetically, they are sister to the superorder Selachimorpha (sharks) [2, 3]. One of the 4 orders of Batoidea is Torpediniformes, which is grouped into 5 families with approximately 68 valid species (Eschmeyer’s Catalog of Fishes assessed on 15th December 2022). They are commonly referred as electric rays due to their ability to generate electrical discharges to stun prey and to defend themselves [4]. Electric rays play an important role in the benthic ecosystem as they are the predators that feed on the diverse invertebrates and small fishes; however, their contribution in benthic dynamic is least known [5, 6].

Previous studies on the phylogenetic positioning of Torpediniformes suggest that they are a sister group to other orders of batoids based on synapomorphies [7], ribosomal genes, and karyological structures [8, 9]. However, molecular phylogenetic studies using different nuclear and mitochondrial genes have shown that the Rajiformes are related to other orders of Batoids and Torpediniformes are related to the order Myliobatiformes [8]. Previous phylogenetic studies based on a few molecular markers, mostly cytochrome oxidase I (COXI) and/or NADH dehydrogenase 2 (ND2), recognized 4 subfamilies within Torpediniformes, but the relationships among them remain confusing [10, 11]. Morphological characters indicate that the families are monophyletic, i.e., separate clades for Platyrrhinidae, Narkidae, Narcinidae, Hypnidae, and Torpedinidae [7]. However,

molecular phylogenetics using ND2 markers suggests polyphyly of the genus *Narcine* [2].

In the last decade, mitochondrial DNA (mtDNA) has been widely sequenced to elucidate phylogenetic relationships among taxa because it provides finer taxonomic resolution, especially in cartilaginous fishes [11]. The number of complete mitochondrial genome sequences for elasmobranchs is increasing, mainly due to the reduced cost of sequencing and ease of bioinformatic data analysis, which improves our phylogenetic understanding of fishes [12, 13]. Based on the NCBI database (checked on 20th Jan, 2022), more than 175 complete or partial mtDNA are available for elasmobranchs. However, mtDNA is still scarcely available for fishes of the order Torpediniformes. Complete mtDNA has been reported only for 5 of the 68 valid species: *Narcine entemedor* [14], *N. bancroftii* and *N. brasiliensis* [11], *Torpedo marmorata* [2], and *Narke japonica* (GenBank accession: MZ417389.1), and 3 species have partial mtDNA, i.e., *N. tasmaniensis*, *Typhlonarke aysoni*, and *Tetronarke macneilli* [3]. Gaitán-Espitia et al. [11] established the complete phylogeny of Torpediniformes based on 6 mitogenomes, suggesting that the individual orders of Batoidea formed a separate clade, i.e., monophyletic in nature, and that Torpediniformes belong to a group that includes the orders Myliobatiformes, Pristiformes, and Rajiformes. They also showed the genus *Narcine* is monophyletic, contradicting the earlier report of polyphyly. The recent mtDNA phylogeny encompassing all elasmobranchs suggests that Torpediniformes and Rajiformes form a sister clade, albeit with low support node values [12, 13, 15]. It is worth noting that these recent mtDNA phylogeny studies had one or a few representatives of the order Torpediniformes. Therefore, it is important to generate mtDNA for more species of Torpediniformes to clarify their phylogenetic position.

In the present study, we report, for the first time, the mitochondrial genome sequence of *Narcine timlei* (Bloch & Schneider, 1801). This species belongs to the family Narcinidae, commonly known as spotted numbfishes. It is a medium-sized ray with large oval/shovel-shaped discs, stout tails, and a naked body (without dermal denticles) [16, 17]. They are known to occur in nearshore waters of the Indo-Pacific ranging from Pakistan to southern China [18]. Their IUCN conservation status was recently changed from data deficient to “vulnerable” [19], yet they are common bycatch batoids in mechanized and artisanal fisheries on the southeast coast of India [20] (authors’ per. obs.). We characterized the mitogenome organization of *N. timlei* and compared it to other available Torpediniformes to examine the evolutionary relationship within the order.

2. Materials and Methods

2.1. Specimen Collection. The specimen of spotted numbfish *N. timlei* was collected in November 2021 during our routine survey at the Covelong Fish Landing Center (12°47′31″N; 80°15′04″E) to determine the diversity of catches and bycatch. Covelong fisher folks engage in artisanal fishing, mainly using gillnets, and bottom gillnets at depths of 0–20 m within 5–7 km of shore [21]. Collected specimens

TABLE 1: Mitochondrial genome of *Narcine timlei*: arrangements and annotation.

Gene name	Gene product	Start	Stop	Strand
tRNA	tRNA-Phe	1	69	+
rRNA	12S rRNA	70	1014	+
tRNA	tRNA-Val	1015	1085	+
rRNA	16S rRNA	1086	2749	+
tRNA	tRNA-Leu	2750	2824	+
CDS	ND1	2826	3800	+
tRNA	tRNA-Ile	3801	3868	+
tRNA	tRNA-Gln	3866	3936	–
tRNA	tRNA-Met	3937	4005	+
CDS	ND2	4006	5050	+
tRNA	tRNA-Trp	5051	5119	+
tRNA	tRNA-Ala	5122	5190	–
tRNA	tRNA-Asn	5192	5264	–
tRNA	tRNA-Cys	5296	5360	–
tRNA	tRNA-Tyr	5361	5429	–
CDS	COI	5431	6987	+
tRNA	tRNA-Ser	6988	7058	–
tRNA	tRNA-Asp	7059	7127	+
CDS	COII	7130	7820	+
tRNA	tRNA-Lys	7821	7893	+
CDS	ATPase 8	7895	8062	+
CDS	ATPase 6	8041	8736	+
CDS	COIII	8737	9521	+
tRNA	tRNA-Gly	9522	9592	+
CDS	ND3	9593	9943	+
tRNA	tRNA-Arg	9944	10015	+
CDS	ND4L	10016	10312	+
CDS	ND4	10306	11686	+
tRNA	tRNA-His	11687	11755	+
tRNA	tRNA-Ser	11756	11822	+
tRNA	tRNA-Leu	11823	11894	+
CDS	ND5	11895	13721	+
CDS	ND6	13706	14221	–
tRNA	tRNA-Glu	14222	14291	–
CDS	Cyt b	14294	15436	+
tRNA	tRNA-Thr	15437	15506	+
tRNA	tRNA-Pro	15980	16048	–

were cleaned and photographed in the field before being taken to the laboratory for the detailed study of morphological and meristic characters. Specimens were identified using standard keys and descriptions [16, 17].

2.2. DNA Extraction, Library Preparation, and Sequencing.

Total genomic DNA was extracted using Omega Bio-tek E.Z.N.A. Blood and Tissue DNA Kit, as described in [22], and treated with RNase (Promega Corp, USA). The intactness of the DNA was checked by 1% agarose gel electrophoresis. Quantification was performed using the QubitTM dsDNA BR assay kit (Catalog: Q32853, Thermo Fisher Scientific), and measurements were performed in the Qubit 3.0 Fluorometer (Thermo Fisher Scientific).

After ensuring the quality of genomic DNA, whole genome sequencing libraries were prepared using the NEBNext[®] Ultra[™] II FS DNA Library Prep Kit for Illumina (Catalog: E7805S, New England Biolabs). Briefly, 500 ng of DNA was enzymatically fragmented using a fragmentation

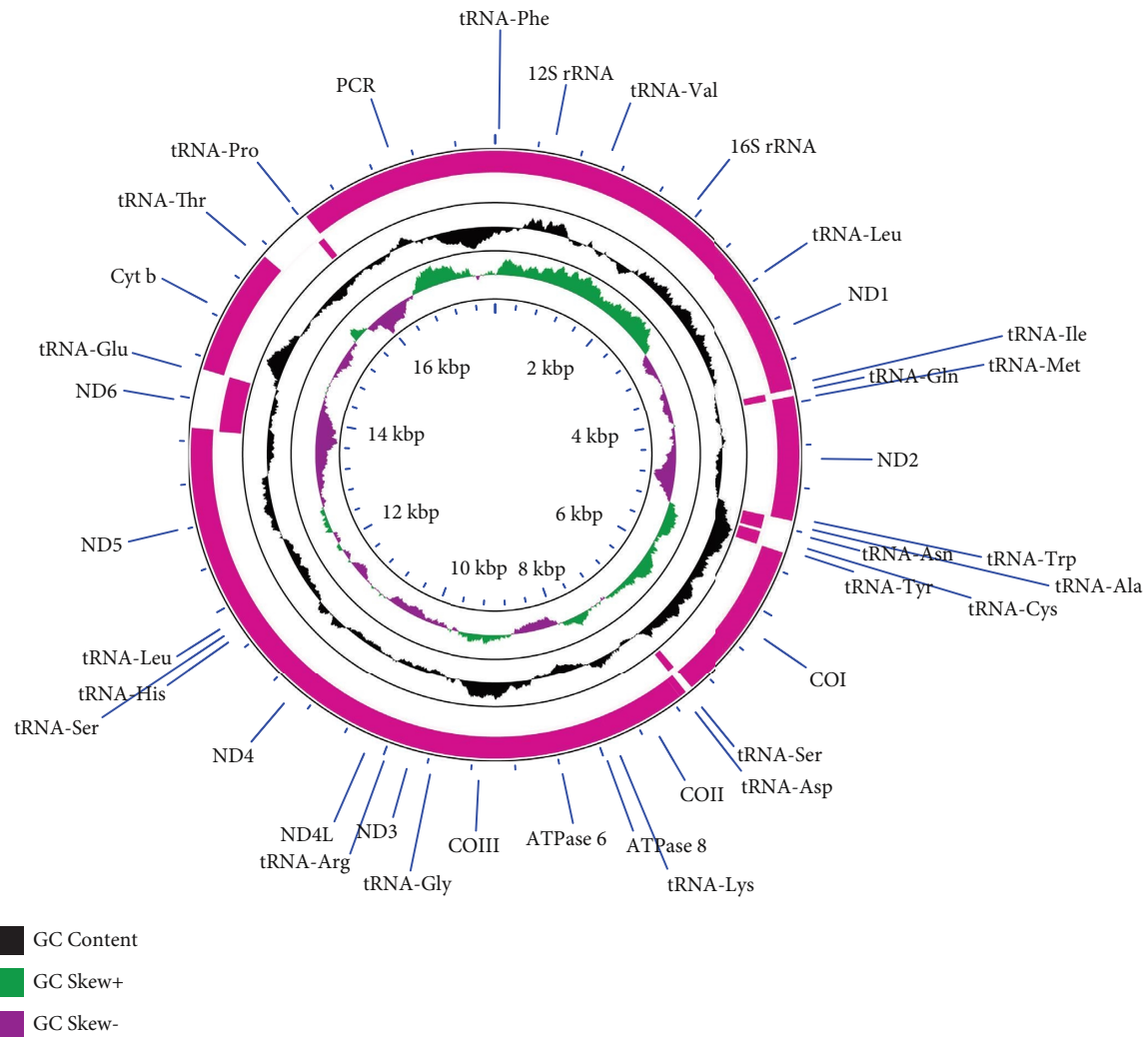


FIGURE 1: Schematic representation of the mitochondrial genome of *Narcine timlei*. The outermost circle represents a heavy strand having 12 protein-coding genes (PCGs), 14 transfer RNAs (tRNA), 2 ribosomal RNA genes, and a putative control region. The second circle represents a light strand having a PCG and 8 tRNAs. The inner circle depicts GC content along the mitogenome. COX, cytochrome oxidase; CYTB, cytochrome b; ND, nicotinamide adenine dinucleotide oxidoreductase; ATP, adenosine triphosphate synthase; PCR, putative control region.

reagent by targeting 275 bp to 475 bp in size. DNA fragments were subjected to end repair to convert them into blunt ends. The 3'-5' exonuclease activity of the end repair mixture removes the 3' overhangs and the polymerase activity fills the 5' overhangs. The fragments with blunt ends were adenylated by adding a single "A" nucleotide to the 3' ends. Loop adapters were ligated to the adenylated fragments and cleaved with the uracil-specific excision reagent (USER) enzyme. Size selection was performed according to the manufacturer's protocol with the addition of AMPure XP beads (catalog: A63881, Beckman Coulter) to achieve a final library size of 400–600 bp. In addition, DNA was amplified by 6 PCR cycles with the addition of NEBNext Ultra II Q5 Master Mix and "NEBNext® Multiplex Oligos for Illumina" to facilitate multiplexing during sequencing. The amplified products were then purified with 0.9X AMPure XP beads (Beckman Coulter), and the final DNA library was eluted in 15 µl of 0.1X TE buffer. Library concentration was

determined using Qubit 3 fluorometer, and quality was assessed using the Agilent D1000 Screen Tape System. Paired end sequencing (2 * 150 bp) was performed using Illumina NovoSeq 6000 (Illumina Inc., USA).

2.3. Mitochondrial Genome Assembly and Annotation. A total of 16,107,264 reads were generated, and the quality of the data was checked using FastQC [23] and MultiQC [24]. Low-quality reads (Phred score < 30) and adapter sequences were removed using fastp [25]. After quality filtering, the reads were assembled into contigs using Megahit v.1.1.3 [26] with kmer sizes 21, 49, 77, 105, 133, and 141. Contigs of less than 200 bp were removed from the assembly. The final assembled mitogenome of 17,964 bp was obtained and subjected to BLAST homology against the NCBI nucleotide database. In addition, annotations were performed with MitoAnnotator [27] using the genetic code of vertebrate mitochondria. Mitogenome visualization was performed

TABLE 2: Base composition and skewness of the mitochondrial genome skew of *N. timlei*.

	A (%)	G (%)	C (%)	T (%)	G + C (%)	A + T (%)	Skew_AT	Skew_GC
Overall	36.19	11.92	22.68	29.21	34.60	65.40	0.11	-0.31
PCGs	33.81	11.89	23.40	30.90	35.29	64.71	0.05	-0.33
ND1	34.26	10.15	27.28	28.31	37.44	62.56	0.10	-0.46
ND2	40.21	8.12	24.93	26.74	33.05	66.95	0.20	-0.51
COX1	30.06	15.35	23.12	31.47	38.47	61.53	-0.02	-0.20
COX2	34.91	13.59	22.03	29.47	35.62	64.38	0.08	-0.24
ATP8	43.45	6.55	20.24	29.76	26.79	73.21	0.19	-0.51
ATP6	35.67	9.21	23.39	31.73	32.60	67.40	0.06	-0.43
COX3	29.90	15.27	24.55	30.28	39.82	60.18	-0.01	-0.23
ND3	32.76	10.54	23.65	33.05	34.19	65.81	0.00	-0.38
ND4L	32.66	9.76	26.60	30.98	36.36	63.64	0.03	-0.46
ND4	35.49	10.01	23.88	30.62	33.89	66.11	0.07	-0.41
ND5	37.38	9.85	23.54	29.23	33.39	66.61	0.12	-0.41
ND6	18.22	25.78	6.98	49.03	32.75	67.25	-0.46	0.57
CYTB	32.11	11.37	25.28	31.23	36.66	63.34	0.01	-0.38
rRNA	38.83	15.78	19.93	25.44	35.71	64.28	0.20	-0.11
tRNA	31.1	15.2	35.2	18.4	50.4	49.5	0.256566	-0.39683
PCR	37	11	19.8	32.2	30.8	69.2	0.069364	-0.28571

with the CGView server [28] using the composite FASTA sequence and map file from the output of MitoAnnotator. Codon usages and relative synonymous codon usages (RSCU) for each protein-coding gene (PCGs) were predicted in the Codon Usage web server (https://www.bioinformatics.org/sms2/codon_usage.html) and MEGA X [29] using the vertebrate mitochondrial code. tRNA genes were identified using ARWEN software [30] implemented in the MITOS web server [31], and secondary structure was predicted using tRNAscan-SE v.2.0 [32]. The putative control region (POR) was analyzed for the presence of repeats using the Tandem Repeat Finder v.4.09 web server (<https://tandem.bu.edu/trf/trf.html>).

2.4. Phylogenetic Analysis. The phylogenetic position of *N. timlei* among other species of Torpediniformes was investigated. The assembled mitogenome of *N. timlei*, 8 other members of Torpediniformes, and *Gymnura poecilura* (Table S1) were used for mitophylogenetic analysis, performed using the MitoPhAST pipeline [33]. *G. poecilura*, which belongs to the order Myliobatiformes, was selected as an outgroup. The MitoPhAST pipeline extracts the nucleotide sequence for 13 PCGs from each of the 10 GenBank files of mitogenomes, aligns each gene with MAFT [34] and TranslatorX [35], trims it with Gblocks [36] to remove ambiguously aligned regions, and concatenates it into supermatrices with FASconCAT-G [37]. The best-fitting substitution models were selected for each partition using ProtTest [38]. The best model for the current dataset was mtMAM + I + G4 for ATP6, ND5, ND3, ND4L, ND4, ATP8, and ND2; mtMAM + I + G4 for COX1, COX2, COX3, ND1, and CYTB; and mtZOA + I for ND6. The rate gamma and rate invariable for ATP6, ND5, ND3, ND4L, ND4, ATP8, and ND2 were 0.823 and 0.246, respectively, and for COX1, COX2, COX3, ND1, and CYTB were 0.640 and 0.412, respectively. The rate of invariance for ND6 was 0.431.

Supermatrices along with partition information were used to perform maximum likelihood (ML) phylogenetic analysis by IQ-TREE [39]. The robustness of the ML tree was analyzed by reiterating the observed data with an ultrafast bootstrap approximation for 1000 generations [40]. In addition, gene order information was also obtained for comparative analysis. We also performed phylogenetic analysis using Bayesian inference (BI) in MrBayes [41]. The analysis was performed for 1,00,000 generations (as the standard deviation of split frequencies of <0.005 was achieved), every 100th tree was sampled from the MCMC analysis, and a consensus tree was obtained after discarding the first 25% of the sampled trees. Support for the nodes in the BI tree was obtained by the posterior probability values.

3. Results and Discussion

3.1. Mitogenome Organization. The mitogenome of *Narcine timlei* was successfully sequenced and assembled, and it was deposited in the NCBI GenBank under the accession number OM404361. The size of the assembled mitogenome was 17,964 bp, which is the expected size range for batoids [13]. However, the size is slightly longer than the previously published mitogenome of other *Narcine* spp. (Table S2), e.g., 17081 bp in *N. entemedor*, 16971 bp in *N. bancroftii*, and 16997 bp in *N. brasiliensis* [11, 14]. The mitogenome of *N. timlei* encodes typical mitochondrial DNA genes of metazoans, including 13 protein-coding genes (PCGs) (COX1, COX2, COX3, CYTB, ND1, ND2, ND3, ND4, ND4L, ND5, ND6, ATP6, and ATP8), small and large ribosomal RNAs, and a complete set of 22 tRNAs (Table 1, Figure 1). With the exception of the ND6 gene, all PCGs were transcribed from the heavy strands (H). These PCGs began with the common start codon ATG, with the exception of COX1, which began with the GTG codon. Most PCGs terminated with a complete codon (TAA/TAG/AGA), whereas incomplete termination was observed at ND4 (T).

TABLE 3: Codon usage analysis of PCGs in the mitochondrial genome of *N. timlei*.

AA	Codon	N	%	RSCU	Fraction
Ala	GCG	0	0.000	0	0
	GCA	93	2.442	1.99	0.5
	GCT	39	1.024	0.83	0.21
	GCC	55	1.444	1.18	0.29
Cys	TGT	23	0.604	0.98	0.49
	TGC	24	0.630	1.02	0.51
Asp	GAT	30	0.788	1.09	0.55
	GAC	25	0.656	0.91	0.45
Glu	GAG	6	0.158	1.85	0.07
	GAA	75	1.969	0.15	0.93
Phe	TTT	130	3.413	1.22	0.61
	TTC	83	2.179	0.78	0.39
Gly	GGG	12	0.315	0.31	0.08
	GGA	84	2.205	2.2	0.55
	GGT	16	0.420	0.42	0.1
	GGC	41	1.076	1.07	0.27
His	CAT	73	1.917	0.99	0.5
	CAC	74	1.943	1.01	0.5
Ile	ATT	220	5.776	1.31	0.66
	ATC	115	3.019	0.69	0.34
Lys	AAG	9	0.236	0.15	0.08
	AAA	110	2.888	1.85	0.92
Leu	TTG	15	0.394	0.17	0.03
	TTA	152	3.991	1.69	0.28
	CTG	16	0.420	0.18	0.03
	CTA	195	5.119	2.16	0.36
	CTT	106	2.783	1.18	0.2
	CTC	57	1.496	0.63	0.11
Met	ATG	32	0.840	0.32	0.16
	ATA	170	4.463	1.68	0.84
Asn	AAT	136	3.570	1.24	0.62
	AAC	84	2.205	0.76	0.38
Pro	CCG	10	0.263	0.18	0.04
	CCA	124	3.255	2.21	0.55
	CCT	62	1.628	1.11	0.28
	CCC	28	0.735	0.5	0.13
Gln	CAG	4	0.105	0.09	0.04
	CAA	88	2.310	1.91	0.96
Arg	CGG	7	0.184	0.37	0.09
	CGA	33	0.866	1.76	0.44
	CGT	11	0.289	0.59	0.15
	CGC	24	0.630	1.28	0.32
Ser	AGT	34	0.893	0.73	0.12
	AGC	55	1.444	1.18	0.2
	TCG	9	0.236	0.19	0.03
	TCA	106	2.783	2.27	0.38
	TCT	42	1.103	0.9	0.15
	TCC	34	0.893	0.73	0.12
Thr	ACG	6	0.158	0.08	0.02
	ACA	152	3.991	1.94	0.49
	ACT	87	2.284	1.11	0.28
	ACC	68	1.785	0.87	0.22
Val	GTG	4	0.105	0.13	0.03
	GTA	58	1.523	1.83	0.46
	GTT	42	1.103	1.32	0.33
	GTC	23	0.604	0.72	0.18
Trp	TGG	37	0.971	0.56	0.28
	TGA	95	2.494	1.44	0.72
Tyr	TAT	103	2.704	1.29	0.64
	TAC	57	1.496	0.71	0.36

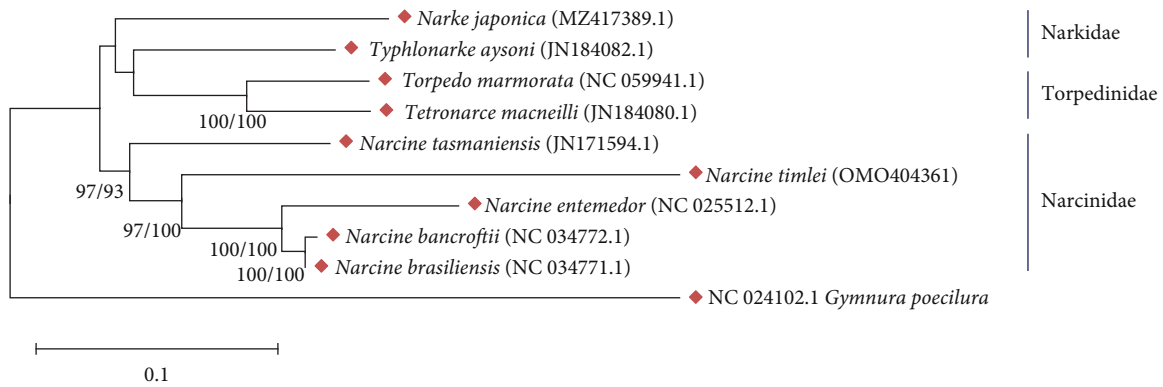


FIGURE 2: Phylogenetic tree obtained from maximum likelihood (ML) and Bayesian inference (BI) analysis based on a concatenated alignment of 13 protein-coding genes for 9 species belonging to order Torpediniformes and *Gymnura poecilura* (Myliobatiformes), which was taken as outgroup. ML bootstrap and BI posterior probability values are represented in nodes.

The incomplete termination at T could be extended to TAA through polyadenylation of the 3' end of the mRNA at the posttranscriptional level, a common phenomenon in the metazoan mitogenome [24].

The base composition of the mtDNA was in the following order: A (36.2%) > T (29.2%), C (22.7%), and G (11.9%), with a tendency towards A + T content. The A + T bias was also observed in all PCGs. The mtDNA showed a significant AC bias (skew_{AT} = 0.11 and skew_{GC} = -0.31), indicating a greater abundance of A than T and C than G (Table 2). Similar skewness was also found in the complete genomes of other *Narcine* spp. (Table S2) indicating a common pattern in this genus.

The A + T bias increases the AT-rich codons in codon usage, which appears to be a common pattern in most vertebrates [42]. The most frequently used codons were ATT_{Leu} (5.77%), CTA_{Leu} (5.11%), Met_{ATA} (4.43%), TTA_{Leu} (3.99%), and Thr_{ACA} (3.99%), followed by others (Table 3).

The two ribosomal RNAs (large, 16S rRNA, and small, 12S rRNA) were transcribed from the H-strand. 12S rRNA consisted of 944 bp and was located between tRNA-Phe and tRNA-Val. 16S rRNA consisted of 1663 bp and was located between tRNA-Val and tRNA-Leu. Both rRNA genes had a positive AT skew (~0.20) and a negative GC skew (~-0.1). Of the 22 transfer RNA genes identified, 8 were transcribed from the L-strand and the remaining from the H-strand. Their size ranges from 67 to 75 bp and exhibits a typical cloverleaf secondary structure, except for one tRNA-Ser that contained a simple loop without a D-arm (Figure S1), similar to many metazoan mitogenomes [43].

Gene order, size, and nucleotide composition were consistent with mitogenomic features of previously reported *Narcine* spp. [11, 14] (Figure S2 and Table S2).

Between the tRNA-Pro and tRNA-Phe genes, we found a putative control region (PCR) of 1916 bp, comparatively longer than in Torpediniformes, which ranges between 1060 and 1328 bp. The difference could be due to the insertion and/or tandem repeats in the control regions [13]. The base composition of the PCR was 31.1% for G, 15.2% for C, 35.2% for T, and 18.4% for T with the negative GC skew (-0.39) and

positive AT skew (0.25). We found three repeats in this region, first from 270 to 360 bp with period size 47, second from 1403 to 1427 bp with period size 10, and third from 1714 to 1758 bp with period size 22 (Table S3 and Figure S3). The larger size of the control region might be the reason for the larger mitogenome size of *N. timlei* compared to other *Narcine* spp. [13].

3.2. Phylogenetic Reconstruction. MtDNA sequences are considered to have enough phylogenetic information to reveal relationships in fishes because they show small and stable changes over a long period of time and are better than the phylogeny of a single gene or two concatenated genes [43]. We used mtDNA of 9 species representing 3 families of order Torpediniformes, which is by far the most for any mtDNA phylogenetic study on Torpediniformes. Kousteni et al. [13] took 3 species of 2 families and Amaral et al. [12] took 1 species of Torpediniformes in elasmobranch mtDNA phylogeny. The most complete mtDNA phylogeny of Torpediniformes to date was established by Gaitán-Espitia et al. [11], with 6 species from 3 families. In the present study, phylogenetic reconstruction using ML and BI analyses revealed identical topologies with similar branch lengths. We obtained two main clades: one consisted of Narcinidae, while the second consisted of Narkidae and Torpedinidae (Figure 2). Within the family Narcinidae, *N. tasmaniensis* diverged early from other species in the geological time scale. In addition, *N. timlei* branched off and formed a separate subclade containing *N. entemedor*, *N. brasiliensis*, and *N. bancroftii*. The nodes and internodes of the Narcinidae clade were supported by high bootstrap and posterior probability values. Our result supports the monophyletic hypothesis of the family Narcinidae based on the mitogenome [19], in contrast to previous studies that used the ND2 gene phylogeny and suggested polyphyly of the Narcinidae [35]. The earlier studies suggest that the Narcinidae are monophyletic only with the inclusion of the Narkidae [7]. It has also been suggested that some narkids are derived members of the Narcinidae based on comparative anatomy [15] and some genera such as *Narcine* are sister to Torpedinidae and Hypnidae, while genus *Discopyge* is sister to

Benthobatis and *Typhlonarke* [45]. The inclusion of *Narke japonica* in the phylogenetic tree suggests that *N. japonica* branched early from *Typhlonarke ayosni*, a sister genus of the Torpedinidae, although nodal support for these branches is lower (<50%). Excluding *N. japonica*, *Torpedo* has been reported to split early from other families [10, 11]. Our analysis suggests that tree topologies and interrelationships among the members of the order Torpediniformes have changed with the inclusion of additional species. Therefore, it is necessary to obtain the complete mtDNA of more species to achieve a more accurate phylogenetic resolution within the order.

Data Availability

Mitogenome generated in the present study is submitted to NCBI GenBank under the accession number OM404361.

Disclosure

A preprint has previously been published on Research Square [46], under reference no. 1804785.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Figure S1: secondary structure of tRNAs in the mitochondrial genome of *N. timlei* predicted by tRNAscan-SE v2.0. Figure S2: gene order and patterns in *N. timlei* and other Torpediniformes. Figure S3: sequence of mitochondrial putative control region highlighting tandem repeats. Table S1: species names and mitochondrial genome sequences used for phylogenetic analysis. Table S2: structural characteristics of mitochondrial genomes of species used in this study. Table S3: description of tandem repeats found in the control region of the assembled mitogenomes. (Supplementary Materials)

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UV-B Halotolerant Bacteria from Marakkanam Saltpan and Biology of UV-B Tolerant *Pontibacillus salipaludis* Based on Whole Genome Sequencing

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ABSTRACT

Salt pans in South India experience intense sunlight and ultraviolet radiation (UV). Therefore, it was hypothesized that halophilic bacteria living there could tolerate UV radiation. To test this, sediment and water samples from the Marakkanam Saltpan were collected and exposed to an artificial UV-B lamp. After irradiation, an aliquot of the sample was spread on nutrient agar (prepared in sterilized source water), and two bacterial strains were obtained, named SBO and SBY. Based on 16S rRNA gene phylogeny, these strains were identified as *Bacillus* sp. and *Pontibacillus salipaludis*, respectively. Another aliquot of the UV-B-exposed sample was inoculated into the nutrient broth (in sterilized source water), and incubated for a week in the dark. Later, it was subjected to Illumina MiSeq-based sequencing for the V3-V4 region (16S rRNA gene). Results revealed the presence of 13 bacterial phyla. However, the phylum Bacillota (48.59%) and Pseudomonadota (27.36%) accounted for more than 75% of the diversity. *Pontibacillus* was the most abundant genus, accounting for 44% of total bacterial diversity. The whole genome of *P. salipaludis* was sequenced on Illumina HiSeqX to decipher possible genes and pathways involved in UV-B tolerance. The genome size was estimated to be 4.05 MB, with a mean G + C content of 40.82%. RASTtk-based genome annotation revealed 4217 coding sequences and 78 RNAs. Related to the UV-B tolerance, sixteen genes involved in carotenoid and prodigiosin biosynthetic pathways were found. antiSMASH showed the presence of terpene-type carotenoids. More than sixty genes involved in various DNA repair pathways were found.

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Introduction

The salt pans are hypersaline environments. Halophilic organisms living in salt pans experience fluctuating salinity; hence, they have developed mechanisms to tolerate changing salinities. These include maintaining osmotic balance by accumulating osmoprotectants, maintaining internal ion concentrations, ion pumps on the cell membrane, controlling water movement in and out, and hypoosmotic conditions of intracellular space (John et al. 2019).

Besides salinity, saltpan-inhabiting microbes are often exposed to other environmental stressors, e.g., ultraviolet radiation (UV) (John et al. 2020). UV is divided into three bands: UV-C (100–290 nm), UV-B (280–320 nm), and UV-A (320–400 nm). UV-C is filtered out by the Earth's atmosphere, while 10% UV-B and 90% UV-A reach the ground surface (Zhang et al. 2022). UV-A radiation penetrates more profoundly into salt waters than other mediums (Huovinen et al. 2003). Salt pans experience stronger ionizing radiation. Further, researchers have reported that salt in the surrounding environment causes the mobilization of atmospheric chlorine, leading to more UV exposure (Stutz et al. 2002).

UV, particularly of shorter wavelengths, can damage biomolecules such as DNA, proteins, and lipids (Jones and Baxter 2017). However, members of several bacterial phyla, such as Actinomycetota, Pseudomonadota, and Bacillota, isolated from extreme environments have been shown to survive and thrive under various UV intensities (Gladka et al. 2021). Researchers have found that these UV-resistant bacteria possess several lines of defense to protect themselves against harmful radiation. It includes (a) the production of secondary metabolites such as polyphenols, and pigments (e.g., carotenoids), which act as sunscreen compounds (Borić et al. 2011; Jones and Baxter 2017; Reis-Mansur et al. 2019); (b) multiple DNA repair pathways for efficiently managing UV-induced DNA damage (John et al. 2019); and (c) the activation of antioxidant enzymes to control the oxidative damages (Jung et al. 2017). Gram-positive bacteria resist UV penetration better than gram-negative bacteria owing to the presence of peptidoglycan structure in their cell walls or because of their spore-forming capability (Fernández Zenoff et al. 2006; Kim et al. 2017). Recent studies on the UV-tolerant bacteria *Deinococcus* sp. revealed the involvement of small and non-coding RNAs in the adaptation to extreme environmental conditions (Díaz-Riaño et al. 2019).

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REPORT

Unweaving a hard taxonomic knot in coral reef dwellers: integrative systematics reveals two parallel cryptic species complexes in ‘marbled’ shrimps of the genus *Saron* Thallwitz 1891 (Caridea: Hippolytidae)

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Abstract Phenotypic dissimilarity does not always evolve in concert with genetic diversification, resulting in cryptic species complexes that represent a major challenge for documenting actual biodiversity. Resolving these complexes is of paramount importance. Herein, we tested whether *Saron marmoratus* (Olivier, 1811) and *S. neglectus* de Man, 1902, two coral reef-dwelling shrimp species distributed over contiguous biogeographic provinces in the Indo-West Pacific and crossing various biogeographic and phylogeographic breaks, are such cryptic species complexes, as indicated by their significant diversity of color

patterns. Firstly, a principal component analysis using 19 morphological traits confirmed that *S. marmoratus* and *S. neglectus* were morphologically distinctive, however, failing to detect morphologically defined groups within each of these taxa. On the other hand, molecular phylogenetic analyses (nuclear Histone 3 and mitochondrial 16S RNA markers) demonstrated a total of five well-supported clades in these two taxa, with moderate to deep genetic divergence among them. Species delimitation approaches indicated at least 10 (and a maximum of 15) putative cryptic species in the *S. marmoratus* and *S. neglectus* species complexes. Furthermore, color patterns segregated most but not all cryptic lineages. Altogether, the information above demonstrates that *S. marmoratus* and *S. neglectus* represent two cryptic species complexes, which diversified in somewhat parallel ways. Additional integrative studies, as we have shown here, to reveal the extend and magnitude of cryptic species complexes in coral reefs, are warranted given the current acute biodiversity crisis.

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Keywords Biodiversity · Caridea · Coral reef · Decapoda · Indo-Pacific · Cryptic species · Color pattern

Introduction

The scientific community is currently debating whether the planet has entered a new geological epoch, i.e., the Anthropocene, characterized by widespread human-mediated environmental impact (Lewis and Maslin 2015). Irrespective of the lexical semantics, current extensive habitat destruction and deterioration coupled with global climate change are resulting in the unparalleled extinction of species at both a local and global scale (Dirzo et al. 2014). Thus, our efforts to document the extant biodiversity and understand the

processes generating it need to be prioritized (Wilson 2016), in order to protect it. Importantly, genetic dissimilarities do not always correlate with phenotypic disparities, resulting in what are usually called cryptic species complexes (Knowlton 1986, 1993; Struck et al. 2018; Coates et al. 2018). Furthermore, phenotypic plasticity can result in remarkable anatomical disparity notwithstanding low genetic heterogeneity (West-Eberhard 2003). The above processes have deep connotations for evolution and conservation and create major taxonomic challenges for the still ongoing biodiversity inventory (Bickford et al. 2007; Coates et al. 2018).

Cryptic species are well known in both invertebrate and vertebrate taxa from terrestrial, freshwater, and marine environments (Knowlton 1986; Hebert et al. 2004; Bickford et al. 2007 and references therein). Species belonging to cryptic complexes might coexist in the same geographic area (e.g., the peppermint shrimp *Lyasmata wurdemanni* species complex—Rhyne and Lin 2006, Baeza and Prakash 2019; the snapping shrimp *Alpheus armatus* species complex—Hurt et al. 2013) or inhabit different biogeographic provinces separated by soft or hard biogeographic barriers. One of the examples of a soft biogeographic barrier is the East Pacific barrier (Cowman and Bellwood 2013), while a well-known example of a hard barrier is the Isthmus of Panama separating the eastern tropical Pacific from the western Atlantic (Lessios and Knowlton 2015). With the aid of integrative systematic approaches, previous studies have demonstrated that entities originally considered single well-defined species distributed across biogeographic barriers and/or phylogeographic breaks actually represent pairs or complexes of multiple cryptic species (e.g., Knowlton 1986; Mathews and Anker 2009; Titus et al. 2018; Dudoit et al. 2018; Baeza and Prakash 2019 and references therein). Overall, exploring genetic disparity in widely distributed species provides outstanding opportunities to explore historical and contemporary processes driving diversification and diversity patterns at multiple geographical scales (Titus et al. 2018; Dudoit et al. 2018; Struck et al. 2018; Coates et al. 2018; Baeza and Prakash 2019).

In the present study, we tested whether two closely related taxa of coral reef-dwelling caridean shrimps belonging to the genus *Saron* Thallwitz 1891 (Hippolytidae), currently defined as species (De Grave and Franssen 2011), actually represent cryptic species complexes. Each of them exhibits some morphological disparity and one of the most remarkable diversity of color patterns known in shrimps, as well as a wide geographic range encompassing most of the tropical Indo-West Pacific. Within the decapod infra order Caridea, a highly speciose clade with notable morphological, behavioral, physiological, and ecological diversity (Bauer 2004), the shrimps of the genus *Saron* are readily recognized by their relatively large size, characteristic morphology, and most easily by their vivid and complex color patterns (Chace

1997; Debelius 2001; Kuiter and Debelius 2009). All species of *Saron* inhabit coral reefs and adjacent hard and mixed bottom habitats rich in coral rubble, dwelling among coral rocks and dead or alive corals in shallow to moderately deep water (Chace 1997). Given their striking coloration (Fig. 1), these shrimps are targeted by marine ornamental trade (Calado et al. 2003; Calado 2008; Prakash et al. 2017). *Saron* is also known for its pronounced sexual dimorphism, in addition being one of the first caridean groups, in which alternative male morphotypes were reported (Coutière 1910; Kemp 1914, 1916). Whether or not the presence of dimorphic males in *Saron* (with large dominant males characterized by the extremely elongated claws, see examples in Fig. 1F and J) is a result of alternative mating strategies, as demonstrated in the non-related hinge-beak shrimp *Rhynchocinetes typus* (Rhynchocinetidae) (Baeza et al. 2014 and references therein), remains to be shown. In spite of the vivid color patterns and the presence of dimorphic males in *Saron*, remarkably little is known about the general biology and ecology of the representatives in this genus.

Saron currently comprises four species: *S. marmoratus* (Olivier 1811), *S. neglectus* de Man 1902, *S. inermis* Hayashi 1983, and *S. rectirostris* Hayashi 1984 (Hayashi 1983, 1984; Chace 1997; De Grave and Franssen 2011). The last two, viz. *S. inermis* and *S. rectirostris*, are well defined morphologically, both being characterized by a short and deeply keeled rostrum, and can also be easily recognized by their diagnostic color patterns (Chace 1997; Kuiter and Debelius 2009). On the other side, *S. marmoratus* and *S. neglectus*, both with a more elongate and dorsally curved rostrum, represent a pair of presumably geminate species with considerable disparity both in the general color (with either more greenish or more reddish individuals) and in the color patterns (Fig. 1). Both *S. marmoratus* and *S. neglectus* appear to be largely sympatric over most of their wide distributional ranges, which include almost the entire tropical Indo-West Pacific from the Red Sea and South-East Africa across the Indian Ocean and Indo-Malayan Archipelago to Japan, Australia, Hawaii, and French Polynesia (Chace 1997). In addition, *S. marmoratus* recently invaded the eastern Mediterranean Sea via the Suez Canal (Rothman et al. 2013). Their natural distribution expands over at least six ecoregions (sensu Spalding et al. 2007), divisions (sensu Green and Mous 2008) and/or biogeographic provinces (Spalding et al., 2007; Keith et al. 2013; Kulbicki et al. 2013), and also crosses at least two well-recognized major biogeographic and/or phylogeographic breaks, i.e., Wallace's Line that crosses Indonesia between Borneo and Sulawesi, and through the Lombok Strait between Bali and Lombok, and the Indian Ocean/Indo-Australian Archipelago or Indo-Pacific Barrier [IPB] that separates the Pacific and Indian Oceans (Cowman and Bellwood 2013). Recent studies have recognized major genetic discontinuities in other

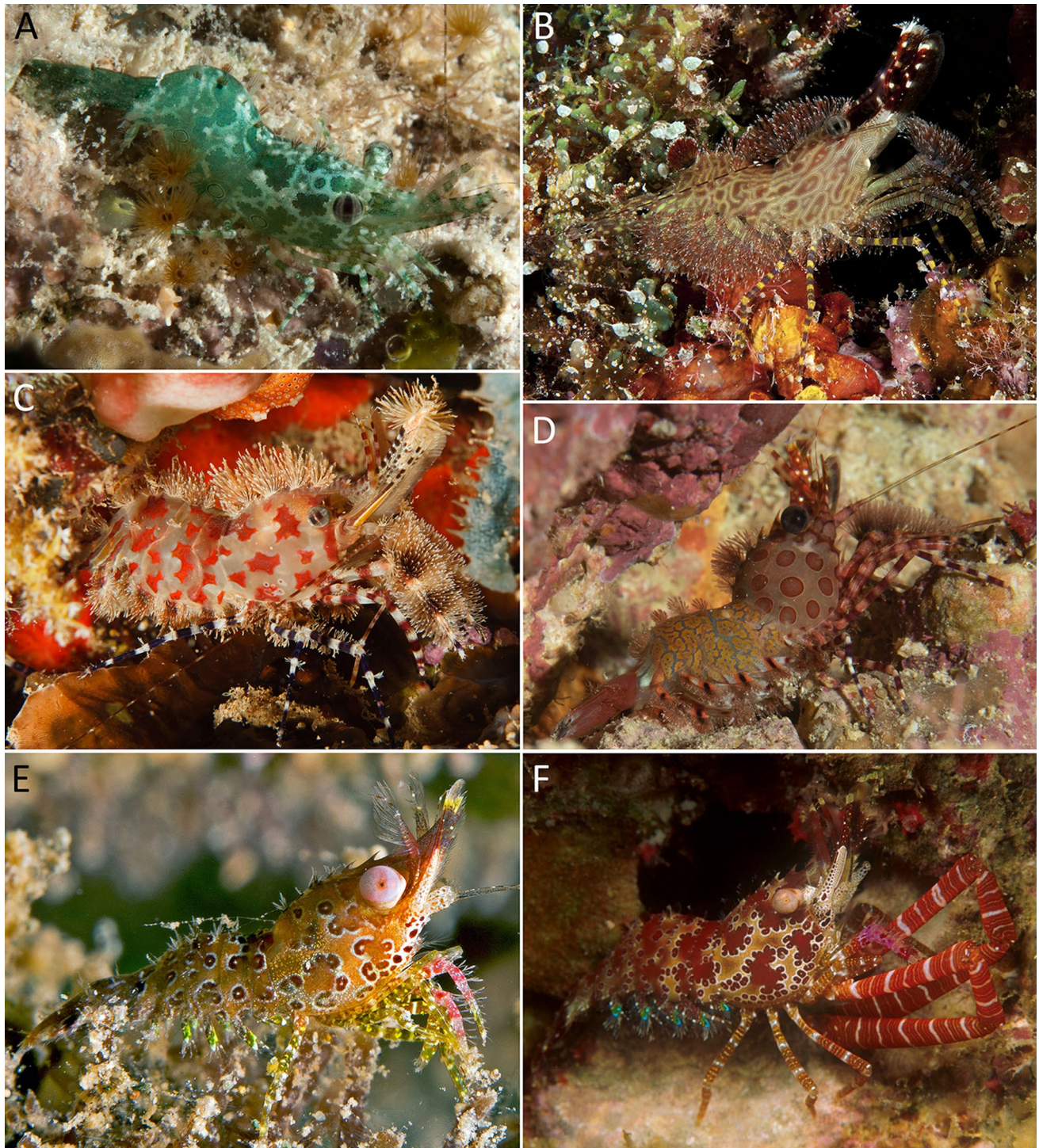


Fig. 1 Photographs in situ of six marble shrimps from the *Saron marmoratus* (A–D) and *S. neglectus* (E, F) species complexes from various Indo-West Pacific localities, illustrating the remarkable color diversity in these two taxa (specimens not collected): **A**, relatively young individual from Bali, Indonesia; **B**, adult individual, possibly male, from Raja Ampat, Indonesia; **C**, adult individual, possibly

male, from Mayotte, Mozambique Channel; **D**, adult male individual from Kimbe Bay, Papua New Guinea; **E**, young individual from Ambon, Indonesia; **F**, adult male from Mergui, Myanmar. Photographic credits: Andrey Rianskiy (A, B); Wei Tan (C); Mark Rosenstein (D); Ned Deloach (E), Mark Strickland (F)

species crossing these breaks in the Indo-West Pacific (Barber et al. 2006; DeBoer et al. 2014 and references therein). Finally, several nominal species of *Saron* described based on morphological features were later placed into synonymy with *S. marmoratus* (see De Grave and Franssen 2011 for full list). All this raises questions about the existence of a complex of cryptic species within *S. marmoratus* and perhaps also *S. neglectus*, or at least, about the existence of possible major genetic breaks among populations inhabiting different biogeographic provinces in the Indo-West Pacific.

In this study, we implement an ‘integrative systematics’ approach to investigate morphological and genetic disparity in *S. marmoratus* and *S. neglectus*. The approach aims to speed up the discovery of cryptic species and to delineate species complexes taking into account budget, personnel, and time limitations and/or constraints. We feel that the approach or similarly aimed efforts to catalog extant biodiversity need to be prioritized given the current biodiversity crisis (Wilson 2016). As the first step in this integrative systematics approach, we used morphological traits and multivariate statistical analyses to explore the possibility of morphological dissimilarity among selected specimens collected from different localities throughout the Indo-West Pacific biogeographic region and provinces. Next, we subsampled morphological disparity within each of the studied species and used fragments of one nuclear DNA marker (Histone 3, hereafter H3) and one mitochondrial DNA marker (16S RNA, hereafter 16S) to test for genetic discontinuities within each of them. Thirdly, we used the same markers to identify putative cryptic species by testing the hypothesis of genetic lineage disjointing and using the most recent species delimitation and validation methods (Yang 2015; Yang and Ranala 2014). A combination of a nuclear and a mitochondrial gene, such as H3 + 16S, is well suited for phylogenetic and species delimitation approaches (see Baeza and Fuentes 2013 for discussion of suitable genetic markers). Lastly, after a final round of subsampling, we explored the diversity of color pattern and examined if dissimilar color patterns segregated according to lineages retrieved by molecular protocols.

The present study represents the first step toward a long-awaited taxonomic revision in the *S. marmoratus* + *S. neglectus* species complexes. We aspire that the proposed approach will serve as a baseline for a planned exhaustive taxonomic revision of this genus. This approach should also aid in the exploration of cryptic lineages in putative species complexes in other groups of decapod crustaceans or marine invertebrates inhabiting coral reefs in geographic regions experiencing marked and widespread local and global climatic changes.

Materials and methods

Material of *Saron marmoratus* and *S. neglectus*

We studied a total of 214 specimens belonging to the *S. marmoratus* and *S. neglectus* species complexes deposited at the Florida Museum of Natural History, Gainesville, Florida, USA (FLMNH UF), and Muséum National d’Histoire Naturelle, Paris, France (MNHN). Most of the studied material was collected by the authors (AA, ZĎ) and colleagues across the Indo-West Pacific, often during major international expeditions or faunal surveys (see Acknowledgements). Specimens were requested on loan from FLMNH and MNHN to the Department of Biological Sciences, Clemson University, Clemson, South Carolina, USA (123 specimens), and Department of Biology and Ecology, Faculty of Science, University of Ostrava, Czech Republic (91 specimens), for both morphological and molecular studies. Many of these specimens were accompanied by photographic vouchers (see below).

Discriminating morphological entities in *Saron marmoratus* and *S. neglectus*

Specimens were identified as either *S. marmoratus* or *S. neglectus* using the dichotomous key of Chace (1997) that relies upon a few morphological characters. We then explored whether minor but consistent morphological differences existed within the material initially identified as *S. marmoratus* and *S. neglectus*, using a larger number of morphological characters and multivariate analyses. For this purpose, we measured in each specimen a total of 19 morphological characters under the dissecting stereoscope (Leica MZ FLIII or WILD MDG17, precision = 0.01 mm), as following: carapace length (CL); rostral length (RoL); propodus length (PrL); and merus length (MeL) of the 1st (P1PrL, P1MeL), 3rd (P3PrL, P3MeL), 4th (P4PrL, P4MeL), and 5th (P5PrL, P5MeL) pereopods (P); number of articles in the carpus (P2CaS) and merus (P2MeS) of the second pereopod; number of spines (= spiniform setae) on the ventrolateral margin of the merus (P3MeSp, P4MeSp, P5MeSp) of pereopods 3–5; length of the terminal article of the third maxilliped (Mxp3L); number of spines on the apex of the ultimate article of the third maxilliped (Mxp3Sp); presence of setae on the antero-dorsal region of the carapace (StC); and third maxilliped (StMxp3). The density of the setation on the mid-dorsal region of carapace and on the dorsal region of third maxilliped was divided into three categories: (1) absent, (2) present, scarce, and (3) present, dense. Specimens smaller than 4.5 mm CL were not used due to their possible juvenile status, in addition to difficulties in measuring or counting traits reliably without damaging them.

We explored whether or not the combination of above-listed morphological characters revealed cryptic morphological entities within *S. marmoratus* and *S. neglectus* using Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA). Each measured variable was normalized and log-transformed prior to PCA and DFA (Rencher 2002). Specimens with missing body parts were eliminated from the analysis, i.e., in total, 154 out of 214 specimens were used. During PCA, a new set of orthogonal variables and scores was obtained using the ‘Default Estimation Method’ (Jolliffe and Cadima 2016). Biplots were visually inspected to determine the number of principal components (PCs) to retain during the analysis. Principal component scores were analyzed using independent one-way ANOVAs to determine if collapsed sets of morphological traits differed among the two species complexes (*S. marmoratus* vs. *S. neglectus*) (Swiney et al. 2015). Lastly, we conducted DFA using the ‘Wide Linear Discriminant Method.’ DFA determines the linear combination of variables that have the greatest variance within or between specimens of *S. marmoratus* and *S. neglectus*. All the statistical tests were performed in JMP pro 12.0 software (SAS 2015, SAS Institute Inc. USA). Considering that alternative male morphotypes have been described for the genus *Saron* (Coutière 1910; Kemp 1914, 1916), we conducted the aforementioned morphological analyses three different times: once using both male and female specimens altogether, a second time using only male specimens, and a third time including only females.

PCAs and DFAs have been successfully used before to discover cryptic species in other vertebrate and invertebrate taxa from both marine and terrestrial environments (e.g., Klimov et al. 2004; Chan et al. 2007; Howlader et al. 2015), including caridean shrimps (Rhyne and Lin 2006; Baeza and Prakash 2019).

Genetic disparity between and within *Saron marmoratus* and *S. neglectus*

We used both mitochondrial (16S) and nuclear (H3) gene markers to test for genetic dissimilarity and presumed existence of cryptic species within the two studied taxa. We conducted molecular phylogenetic analyses using a total of 43 specimens (26 specimens initially identified as *S. marmoratus* and 17 as *S. neglectus*) + 6 additional terminals used as outgroups (*Nauticaris magellanica*, *Hippolyte bifidirostris*, *H. williamsi*, *Tozeuma carolinense*, *Eualus gaimardii* and *Thor amboinensis* s. lat.). In total, 85 out of 98 ingroup sequences plus two outgroup sequences (*Nauticaris magellanica* [$n=2$]) were generated by the authors. The remaining 10 outgroup sequences, generated and deposited by one of us (JAB) in previous studies, were retrieved from GenBank. Details on voucher specimens used for DNA extraction and

their respective GenBank accession numbers are provided in Supplementary Material Table S1.

DNA extraction, amplification and sequencing

We extracted total genomic DNA either from pleopods or from abdominal muscle tissue of a total of 43 shrimps using the OMEGA BIO-TEK® E.Z.N.A.® Blood and Tissue DNA Kit or the DNeasy Blood and Tissue Kit (QIAGEN, Germany), following the manufacturer’s protocol. Next, the polymerase chain reaction (PCR) was used to amplify target regions of 16S gene (~561 bp, Schubart et al. 2000) and H3 gene (~346 bp, Colgan et al. 1998) using the primers 16L2 (5’—TGC CTG TTT ATC AAA AAC AT—3’) or 16SAR (5’—CGC CTG TTT ATC AAA AAC AT—3’) and 1472 (5’—AGA TAG AAA CCA ACC TGG—3’) (Palumbi et al. 1991; Schubart et al. 2000; Baeza et al. 2009), and H3AF (5’- ATG GCT CGT ACC AAG CAG ACV GC—3’) and H3AR (5’- ATA TCC TTR GGC ATR ATR GTG AC -3’) (Colgan et al. 1998). We also attempted to sequence a fragment of the *cox1* gene, but we failed likely due to the existence of pseudogenes in the genus as reported before in other caridean shrimps (Baeza and Fuentes 2013 and references therein). Standard PCR 25 µl reactions (17.5 µl of GoTaq® Green Master Mix [Promega®], 2.5 µl each of the two primers [10 mM], and 2.5 µl DNA template) were performed on a C1000 Touch™ Thermal Cycler (BIORAD®) under the following conditions: initial denaturation at 95 °C for 5 min followed by 40 cycles of 95 °C for 1 min, 54–56 °C (depending on the specimen and primer set used) for 1 min, and 72 °C for 1 min, followed by chain extension at 72 °C for 10 min. PCR products were purified with ExoSapIT (a mixture of exonuclease and shrimp alkali phosphatase, Amersham Pharmacia) or Gel/PCR DNA Fragments Extraction Kit (GENEAID, Taiwan) and then sent for sequencing with the ABI Big Dye Terminator Mix (Applied Biosystems) to the Clemson University Genomics Institute (CUGI), Clemson University, Clemson, South Carolina, USA, or to Macrogen, Inc. (Amsterdam) which are equipped with an ABI Prism 3730xl Genetic Analyzer (Applied Biosystems automated sequencer). All sequences were confirmed by sequencing both strands, and a consensus sequence for the two strands was obtained using the software Sequencer 5.4.1 (Gene Codes Corp.).

Sequence alignment and phylogenetic analyses

Sequence alignment was conducted using Multiple Sequence Comparison by Log-Expectation in MUSCLE (Edgar 2004) as implemented in MEGA6 (Tamura et al. 2013). The alignment of the H3 gene fragment consisted of 345 bp, had no indels and was unambiguous. In contrast, the aligned sequences of the 16S gene fragment did contain several indel

‘islands.’ Therefore, positions that were highly divergent and poorly aligned in the 16S gene segment were identified using the default settings in the software GBLOCKS v0.91b (Cas- tresana 2000) and omitted for a posteriori analyses. After the highly divergent positions were pruned, the 16S dataset consisted of 516 bp.

The two aligned datasets were first analyzed with the software jModelTest 2 (Darriba et al. 2012), which compares different models of DNA substitution in a hierarchical hypothesis-testing framework to select a base substitution model that best fits the data. For the two gene fragments, the optimal models found by jModelTest 2 (selected with the corrected Akaike Information Criterion [AIC_c]) were the GTR + G. These models were implemented in MrBayes (Huelsenbeck and Ronquist 2001) for Bayesian Inference (BI) analysis and W-IQ-TREE for Maximum Likelihood (ML) analysis (Trifinopoulos et al. 2016).

The ‘total evidence’ combined analysis based on a single concatenated dataset (16S + H3) consisted of 98 sequences and 861 aligned nucleotide positions, including 6 sequences from outgroups (*N. magellanica* [sequenced during the present study], *H. bifidirostris*, *H. williamsi*, *T. carolinense*, *E. gaimardii* and *T. amboinensis*). However, the dataset was partitioned into two different segments and missing data were designated as a ‘?’ in the alignment. The GTR + G model was used for all two partitions and 1,000 bootstrap replications were conducted to find out the best-scoring Maximum Likelihood tree (Trifinopoulos et al. 2016). All the parameters used for the ML analyses were those of the default options in W-IQ-TREE.

For BI, unique random starting trees were used in the Metropolis-coupled Markov Monte Carlo Chain (MCMC) (Huelsenbeck and Ronquist 2001; Ronquist et al. 2012). The analysis was performed for 6,000,000 generations. Visual analysis of log-likelihood scores against generation time indicated that the log-likelihood values reached a stable equilibrium before the 1,000,000th generation. Thus, a burn-in of 1,000 samples was conducted, every 100th tree was sampled from the MCMC analysis obtaining a total of 60,000 trees, and a consensus tree with the 50% majority rule was calculated for the last 59,900 sampled trees. The robustness of the ML tree topology was assessed by bootstrap iterations of the observed data 2,000 times and reconstructing trees using each resampled dataset. Support for nodes in the BI tree topology was obtained by posterior probability.

We also conducted separate ML and BI phylogenetic analyses for each gene fragment to reveal possible gene-specific discordances with respect to the total evidence (combined H3 + 16S) phylogenetic hypothesis.

Species delimitation between and within *Saron marmoratus* and *S. neglectus*

Our phylogenetic analyses revealed considerable genetic distance among various well-supported clades in the two studied species (see Results below). Therefore, the next step in our approach involved a further test for cryptic species/lineages in *S. marmoratus* and *S. neglectus* using a combination of species delimitation approaches: (i) Automatic Barcode Gap Discovery (ABGD—Puillandre et al. 2012), (ii) General Mixed Yule Coalescent (GMYC—Fujisawa and Barraclough 2013), and (iii) Bayesian Poisson Tree Process (BPTP—Zhang et al. 2013, originally abbreviated as bPTB).

ABGD identifies the optimal barcoding threshold limit or ‘barcoding gap’ in a given dataset to delimit putative species boundaries (Puillandre et al. 2012). During the ABGD analysis, we employed the Kimura 2 Parameter (K2P—Kimura 1980) model of nucleotide substitution to calculate pairwise genetic distances among sequences. We applied a range of a priori intraspecific divergence estimates (P , from $P_{\min}=0.01$ to $P_{\max}=0.1$) and used a relative gap width $X=1.5$. All other parameters were set to default (steps: 10 [steps for partition]; Nb bins for distance distribution: 20), and the analysis was conducted in the server <http://www.wabi.snv.jussieu.fr/public/abgd/abgdweb.html> (Puillandre et al. 2012).

GMYC is a coalescent-based method that uses maximum likelihood to model the transition between inter- and intraspecific evolutionary branching patterns (Pons et al. 2006; Puillandre et al. 2012). Branching patterns older than an inferred threshold represent speciation events (Yule process), whereas younger branching indicates neutral coalescence within species (Blair and Bryson 2017). GMYC requires an ultrametric gene tree that we estimated within a Bayesian phylogenetic framework in the software BEAST v2.4.3 (Drummond and Rambaut 2007; Bouckaert et al. 2014; Rambaut et al. 2014). During the Bayesian phylogenetic analysis, we used the HKY + G model of molecular evolution, ‘Yule model’ (i.e., pure birth) as tree prior and a strict molecular clock. The analysis was performed for 5,000,000 MCMC generations sampling every 1,000th tree from the MCMC run. Visualization of log-likelihood scores against generation time in the software Tracer v1.7 (Rambaut et al. 2018) indicated that the log-likelihood values reached a stable equilibrium before the 100,000th generation. The effective sample size (ESS) after the 5,000,000th generation significantly greater than the ESS = 200 denotes analysis convergence (Ripley 1987). Considering all of the above, we decided to use a burn-in of 1,000 samples (total number of trees used = 4,000) to build a 50% majority rule consensus tree in the software Tree Annotator v2.4.3 (Rambaut and Drummond 2016). Finally, using this Bayesian tree, the GMYC analysis was implemented in the webserver (<http://species.h-its.org/gmyc>) (Fujisawa and Barraclough

2013). We used both single (Pons et al. 2006) and multiple (Monaghan et al. 2009) threshold limits to test the significance of branching rates in delimiting cryptic lineages.

BPTP is yet another coalescent-based method that aims to delimit species using a single-locus phylogeny (Zhang et al. 2013). This method assumes that the intraspecific substitutions require less time to accumulate than the interspecific substitutions. It also requires a rooted phylogenetic tree (not necessarily ultrametric) that we estimated using ML in the webserver W-IQ-TREE (Trifinopoulos et al. 2016). The parameters used during this ML analysis were the same as before (see phylogenetic reconstruction section for details). The robustness of the ML tree topology was assessed by bootstrap reiterations of the observed data 1,000 times. Species delimitation was assessed using one million MCMC generations. We also used the rooted tree option and other parameters, such as thinning set to 100, burn-in at 0.5, seed to default and *H. williamsi* defined as outgroup. The analysis was conducted in the server <http://species.h-its.org/ptp> (Zhang et al. 2013).

Lastly, we conducted a species validation analysis in the software Bayesian Phylogenetics and Phylogeography (BP&P v3.3a—Yang and Rannala 2014; Yang 2015, available from <http://abacus.gene.ucl.ac.uk/software.html>). BP&P uses the multi-species coalescent model, a reversible-jump Markov Chain Monte Carlo (rjMCMC) process and a single- or multi-locus phylogenetic tree to delimit species (Yang and Rannala 2014; Yang 2015). BP&P assumes that shared haplotypes (alleles) result from incomplete lineage sorting. During the analysis, we used the unguided species delimitation and validation algorithm ('A01') and thus no guide tree was provided (Yang 2015). The analysis was subjected to auto fine tune adjustments. ABGD was run twice with different combinations of ancestral population size (θ) and root age (τ) prior to determining how priors affect species assignments. The rjMCMC was run for 2,000,000 generations sampling every 5th generation. The first 10% of the trees were discarded as burn-in after the analysis.

Comparison of color pattern between and within *Saron marmoratus* and *S. neglectus* complexes

A total of 25 out of 43 sequenced specimens had photographic vouchers available from their deposition institutions (FLMNH and MNHN). We carefully examined the overall coloration and the color patterns of each of the photo-vouchered specimens, grouping them into several color pattern types. The color pattern types, or morphs were coded using capital letters (e.g., SM-A, SN-C), i.e., independently from major clades (coded with Latin numbers, e.g., SM II) and subclades or cryptic species lineages within major clades (coded with Arabic numbers, e.g., SM-4). We then determined whether the different color pattern types

segregated according to lineages recovered by our species delimitation and molecular analyses.

Results

Morphological differentiation in *Saron marmoratus* and *S. neglectus*

The first PCA (including male and female specimens from the two studied species) performed on 19 morphological traits measured in a total of 154 individuals initially identified as *S. marmoratus* ($n = 107$) and *S. neglectus* ($n = 47$) (sensu Chace 1997) resulted in two principal components representing 71.18% of the variability in our dataset (Table 1). In PC1, the highest loadings were associated with CaL, RoL, P1MeL, P3PrL, P3MeL, P4PrL, P4MeL, P5PrL, P5MeL, and Mxp3L; this PC was interpreted as representing the variability in the size of the body, pereopod and third maxilliped between the *S. marmoratus* and *S. neglectus* complexes. On the other hand, PC2 loadings were highest for P3MeS, P4MeS, P5MeS, P4PrS, P5PrS; this PC was interpreted as representing the variability of the armature

Table 1 Eigenvectors of first two axes of principal component analysis extracted from the selected variables along with the estimated eigenvalues and % variance

Variables*	PC 1	PC 2
CL	0.29	−0.06
RoL	0.30	0.02
P1PrL	0.23	−0.17
P1MeL	0.28	−0.15
P3PrL	0.30	−0.02
P3MeL	0.30	−0.04
P4PrL	0.27	0.00
P4MeL	0.30	−0.01
P5PrL	0.30	0.01
P5MeL	0.30	−0.00
P3MeSp	0.15	0.42
P4MeSp	0.14	0.42
P5MeSp	0.07	0.39
P2CaS	0.12	0.04
P2MeS	0.01	0.37
Mxp3L	0.29	−0.05
Mx3Sp	−0.09	0.17
StC	0.03	0.40
StMxp3	−0.06	0.31
Eigen value	10.17	3.35
Variance (%)	53.53	17.65
Cumulative variance (%)	53.53	71.18

*See methodology for definition of variables. Values in bold indicates higher loadings based on the principal components

on the last three pairs of pereopods (walking legs) between the *S. marmoratus* and *S. neglectus* complexes. Importantly, 2D plots between the different combinations of the first two PCs divided specimens into two moderately to well-defined clusters but with minor overlap among them, denoting morphological differences between *S. marmoratus* and *S. neglectus*. No obvious sub-clustering was observed within each of the two major clusters (Fig. 2). The second and third PCAs (including only male and only female specimens from the two studied species) also divided specimens (of each sex) into two moderately to well-defined clusters with minor overlap among them, denoting morphological differences between *S. marmoratus* and *S. neglectus*, but no obvious sub-clustering within each of the two major clusters (Fig. 2; Supplementary Materials Table S2).

The first ANOVA detected an effect of species identity (*S. marmoratus* vs. *S. neglectus*) on the derived first component scores ($F_{(1,153)} = 41.53$, $P < 0.0001$) implying the ability of a relatively large combination of traits (i.e., CaL, RoL, P1MeL, P3PrL, P3MeL, P4PrL, P4MeL, P5PrL, P5MeL, and Mxp3L) to differentiate between these two species complexes, in addition to the characters on the orbital margin, antennule, and second pereopod in Chace's (1997) key to the species of the genus *Saron*. The second independent ANOVA also detected an effect of species identity

on the derived second component scores ($F_{(1,153)} = 218.56$, $P < 0.0001$). Overall, the PCA confirmed that *S. marmoratus* and *S. neglectus* were morphologically quite distinctive; however, the same analysis failed to detect additional morphologically dissimilar entities within either *S. marmoratus* or *S. neglectus*.

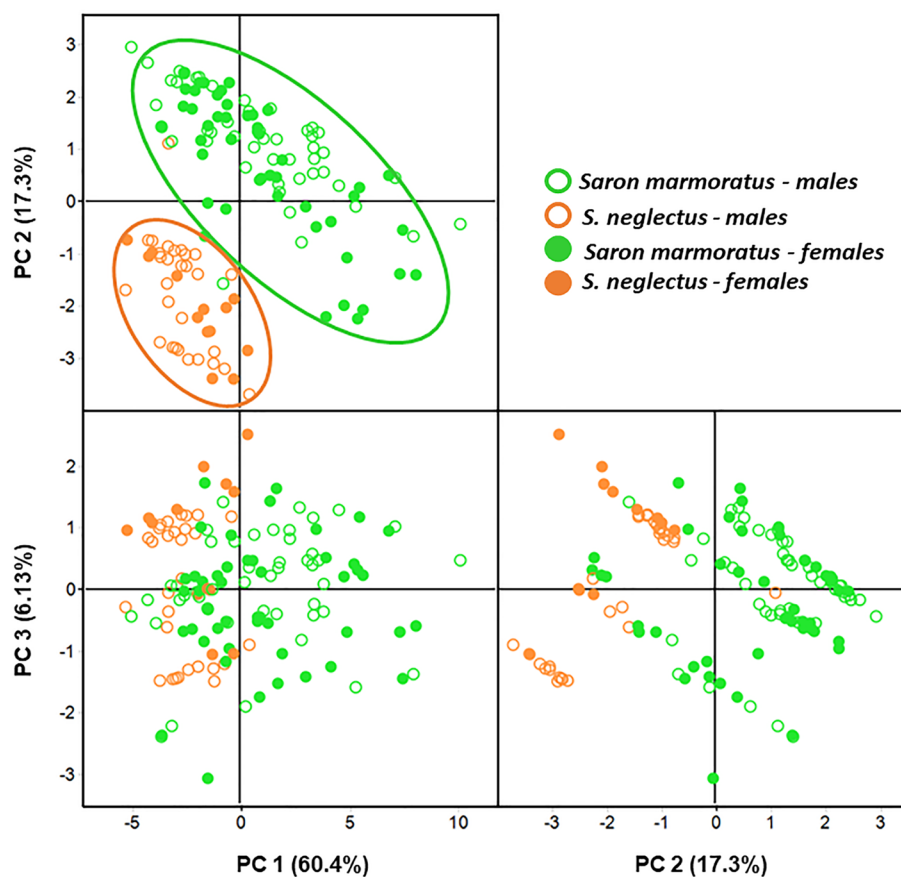
The DFA cross-validated our PCA findings. The estimated canonical scores based on a single canonical function (eigenvalue = 16.60; canonical correlation = 0.971) were statistically significant (Wilk's lambda: 0.05, $F_{(19, 138)} = 117.0605$, $P < 0.0001$). The DFA was highly successful in classifying individuals into one of the two species complexes, i.e., 98.7% of individuals were correctly assigned either to *S. marmoratus* or *S. neglectus*.

Overall, both PCA and DFA (using all specimens, only males, and only females) on morphological traits enabled to distinguish *S. marmoratus* from *S. neglectus* but did not reveal the presence of additional (morphological) entities within each of them, at least not for the measured features.

Molecular divergence in *Saron marmoratus* and *S. neglectus*

The 'total evidence' molecular data matrix comprised a total of 861 characters (16S = 516 bp; H3 = 345 bp), out

Fig. 2 Principal components analysis based on 19 morphological characters in the *Saron marmoratus* and *S. neglectus* species complexes



of which 245 sites (16S = 182; H3 = 63) were parsimony informative for a total of 43 ingroup terminals (*S. marmoratus* and *S. neglectus*) and 6 outgroup terminals. The two total evidence analyses conducted with different molecular phylogenetic inference methods (ML and BI) resulted in similar tree topologies. In the two analyses, all specimens initially identified as either *S. marmoratus* or *S. neglectus* clustered together into a single clade, strongly supported by a high posterior probability for the BI analysis and by a high bootstrap support value for the ML analysis (Fig. 3). Furthermore, the ML and BI analyses indicated reciprocal monophyly between *S. marmoratus* and *S. neglectus*. Phylogenetic trees (ML and BI) based on single gene markers, either mitochondrial or nuclear, resulted in tree topologies most similar to that of the total evidence (combined H3 and 16S) ML + BI analyses (Supplementary Material: Fig. S1). Nonetheless, as expected, the single-marker phylogenetic trees were less resolved than those produced by the combined ML + BI analyses.

Three and two clades within *S. marmoratus* and *S. neglectus*, respectively, were strongly or well supported by both ML and BI total evidence analyses. The first major clade within *S. marmoratus*, hereafter ‘SM I,’ comprised more than half of the studied specimens, i.e., 15 out of 27 (Fig. 3). Within SM I, most of the specimens were from the western Pacific (12 out of 15, Australia and Papua New Guinea), one specimen was from the western-central Pacific (French Polynesia) and two specimens from the Indian Ocean (western Australia and Reunion Island). A second major clade, hereafter ‘SM II’ comprised five specimens, four from the western-central Pacific (French Polynesia and Hawaii) and one from the western Pacific (Papua New Guinea). A third major clade, hereafter ‘SM III,’ comprised six specimens from the western Pacific (Australia and Papua New Guinea). The pairwise genetic distance between SM I and SM II was relatively large (mean $\pm$ SD = 0.1242 ± 0.0054 , range: 0.119–0.139) and comparable to the distance estimated among specimens from *S. marmoratus* and *S. neglectus* (0.1653 ± 0.0114 , range: 0.138–0.190). The pairwise genetic distance between SM I and SM III was 0.0995 ± 0.0054 (range: 0.088–0.119) and between SM II and SM III was 0.0779 ± 0.0081 (range: 0.067–0.089).

In *S. neglectus*, two major clades were strongly or well supported by bootstrap values from ML and posterior probability from BI analyses (Fig. 3). The first major clade, hereafter ‘SN I,’ comprised six specimens collected from various localities in the Indo-West Pacific, i.e., two specimens each from the western Pacific (Australia and Papua New Guinea), western-central Pacific (one each from French Polynesia and Hawaii), and Indian Ocean (one each from Western Australia and Reunion Island). The second major clade, hereafter ‘SN II,’ was sister to SN I and contained the majority of the studied specimens, i.e., 11 out of 17. In SN II, one

specimen from the western Indian Ocean (Djibouti) was sister to all other specimens forming two subclades; this result was strongly supported by bootstrap values from ML and posterior probability from BI analyses. One of the subclades comprised only specimens from the western Pacific (Australia and Papua New Guinea), while the second subclade comprised specimens from various localities in the Indo-West Pacific, i.e., one specimen from the western Pacific (Australia and Papua New Guinea) and two specimens each from the western-central Pacific (French Polynesia and Hawaii) and Indian Ocean (Western Australia and Reunion Island). The pairwise genetic distance between SN I and SN II was 0.0755 ± 0.0078 (range: 0.05–0.086).

Species delimitation in *Saron marmoratus* and *S. neglectus*

Overall, the species delimitation methods (ABGD, BPTP, GMYC -16S), as well as the species validation approach (BPP—16S + H3), recovered between five and eight cryptic lineages (= divergent mitochondrial lineages) within *S. marmoratus* and between five and seven cryptic lineages within *S. neglectus* (Fig. 4).

In the first major clade of *S. marmoratus*, i.e., SM I, the ABGD’s primary partition (hereafter ‘PP’) and BPTP methods recovered two lineages, i.e., putatively cryptic species. However, the ABGD’s recursive partition (hereafter ‘RP’) and BPP methods recovered a third cryptic species represented in our analysis by a single specimen (UF 27854, Fig. 4). In contrast, both the single [ST] and multiple [MT] threshold GMYC approaches recovered four and five divergent lineages within SM I, respectively. The two and three additional lineages that were recovered by the GMYC’s ST and MT methods seem to have a too high value, given that they contained only a single specimen (ST: UF 12847, UF 27854; MT: UF 12847, UF 27681, UF 27854) (Fig. 4).

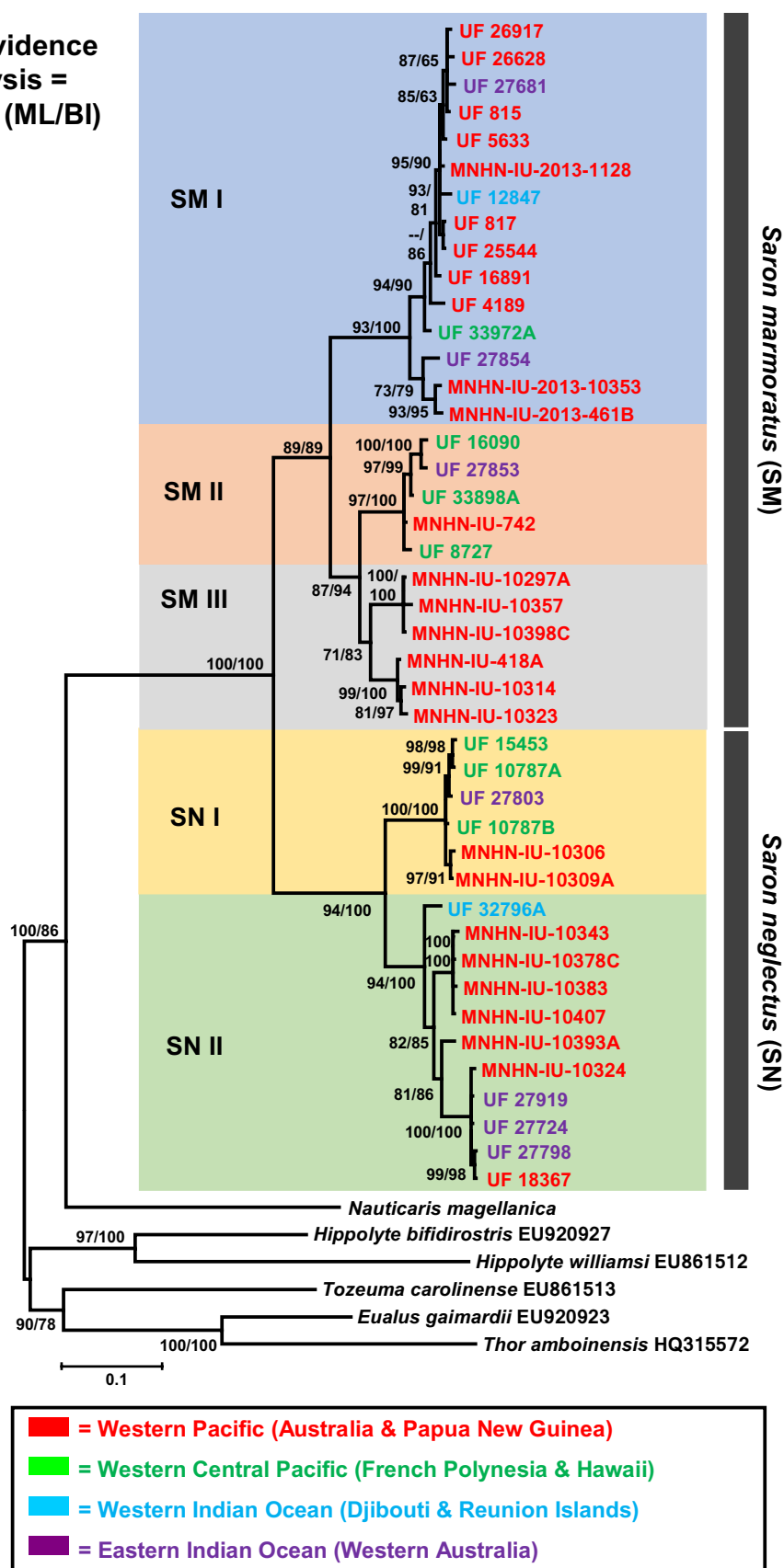
The second major clade of *S. marmoratus*, i.e., SM II, was recovered as a single distant lineage by ABGD (PP), GMYC (both ST and MT) and BPP methods. However, the ABGD’s RP and BPTP divided SM II clade into two distinct lineages containing two specimens each (MNHN-IU-742 and UF 8727). The third major clade, SM III, included two distinct lineages by all the methods each containing three specimens.

In *S. neglectus*, the first major clade, i.e., SN I, was recovered as a single distinct lineage by all species delimitation and validation methods (Fig. 4). In turn, the second major clade, i.e., SN II, was divided into four distinct lineages by all the methods but GMYC-MT. The latter method recovered six lineages, although three of them each contained a single specimen (Fig. 4).

Overall, the combination of species delimitation and validation methods indicates the presence of six and five cryptic

Fig. 3 Molecular phylogeny (ML) of the *Saron marmoratus* and *S. neglectus* species complexes based on Histone 3 (nuclear) and 16S rRNA (mitochondrial) DNA sequences. The general topology of the trees obtained from both the ML and BI analyses was similar. Different, well-supported clades within *S. marmoratus* and *S. neglectus* are indicated with Latin (major clades) and Arabic (minor subclades or cryptic lineages) numbers (see text for further details). Numbers above or below the branches represent bootstrap values obtained from ML and posterior probability values from BI analysis (ML/BI). Note: – indicates ML bootstrap support values < 50

**Total Evidence
Analysis =
16S+H3 (ML/BI)**



lineages, respectively, in *S. marmoratus* (SM-1 to SM-6) and *S. neglectus* (SN1 to SN-5) (Fig. 4, 5).

Color patterns of *Saron marmoratus* and *S. neglectus*

Color photographs (habitus, typically in lateral view) were available for a total of 19 out of 43 specimens used in the phylogenetic and species delimitations analyses. In addition, a single specimen from French Polynesia (FLMNH UF 16049, not located in the FLMNH collections, possibly misplaced) was not included in the phylogenetic and species delimitations analyses, but was still coded for its unique color pattern that strongly suggested it represents yet another cryptic lineage within the *S. neglectus* clade (see below).

A major difference between specimens initially identified as *S. marmoratus* and *S. neglectus* is the color pattern of the first to fifth pereopods. All specimens morphologically matching *S. marmoratus* exhibit broad white bands alternating with similarly broad dark purple-violet or brown-red bands, while all specimens morphologically identifiable as *S. neglectus* exhibit narrow yellow or neon-colored bands on dark brown or green background (Fig. 5).

In addition to the banding pattern of the pereopods, some details of the carapace, pleon, antennae, pleopods and tail fan permitted to distinguish six and seven distinctive color pattern types (=color morphs) within each *S. marmoratus* (SM-A to SM-F) and *S. neglectus* (SN-A to SN-G), respectively. Importantly, these color patterns segregated relatively well among molecular-based cryptic lineages in both clades, although somewhat less consistently in the *S. marmoratus* clade than in the *S. neglectus* clade. The 13 color patterns are briefly described below and illustrated in Fig. 6 (see also Fig. 1).

Color morphs in the *Saron marmoratus* clade (Fig. 6A–I)

SM-A

Carapace flanks with circular white spots, reminiscent of cells or cysts, each surrounded by orange circle among red, orange, brown or green markings with small blue spots. Rostrum red or dark green with white bands and small spots. Pleon flanks similar to carapace, especially first two pleonites; third pleonite without eye-spot; fifth pleonite with white area and small bluish spots distoventrally. Antenna with basicerite red-brown or greenish, with broad white band; scaphocerite proximally with red-brown or green areas containing small white spots. Pereopods with broad red or green transverse bands alternating with broad white bands. Pleopods red-brown or green and white, without blue or yellow markings. Uropodal exopod without eye-spot (Fig. 6A–D).

Clade SM I, cryptic lineages SM-1 (4 terminals) and SM-2 (1 terminal).

SM-B

Carapace flanks with small white circular spots (rarely doubled by orange circle) and small blue spots on larger green areas. Rostrum dark green with white bands and small spots. Pleon flanks similar to carapace; third pleonite without eye-spot; fifth pleonite with bright yellow spots or streaks and small bluish spots distoventrally. Antenna with basicerite greenish with broad white band; scaphocerite proximally with green areas containing small white spots. Pereopods with broad green transverse bands alternating with broad white bands. Pleopods green and white, with bright yellow markings. Uropodal exopod without eye-spot (Fig. 6E).

Clade SM I, cryptic lineage SM-3 (1 terminal).

SM-C

Carapace flanks with pale olive-greenish background, large red–orange amoeboid or irregular star-shaped patches, most of them containing small blue spots, and small white spots usually grouped in clusters. Rostrum dark red–purple with white spots. Pleon flanks similar to carapace; third pleonite without eye-spots; fifth and sixth pleonites with white and bright yellow spots on dark red background distoventrally. Antenna with basicerite pale greenish with purple-pink band and some white spots; scaphocerite proximally with red–purple areas containing small white spots and some bright yellow spots. Pereopods with broad red–purple or violet transverse bands alternating with broad white bands. Pleopods pale green with purple-violet bands. Uropodal exopods without eye-spots (Fig. 6F).

Clade SM II, cryptic lineage SM-4 (1 terminal).

SM-D

Carapace flanks with large white circular or oval spots reminiscent of cysts or protozoans, usually surrounded by orange circle. and small blue spots on red–orange spaces in-between. Rostrum dark red–purple and white bands, and white spots. Pleon flanks similar to carapace; third pleonite without eye-spot; fifth and sixth pleonites with white and bright yellow spots on dark red background distoventrally. Antenna with basicerite mostly white, with red–purple band more distally; scaphocerite proximally with red–purple areas containing small white spots and some bright yellow spots. Pereopods with broad red–purple transverse bands alternating with broad white bands. Pleopods white or pale yellow with purple-violet bands. Uropodal exopod without eye-spot (Fig. 6G).

Clade SM II, cryptic lineage SM-4 (1 terminal).

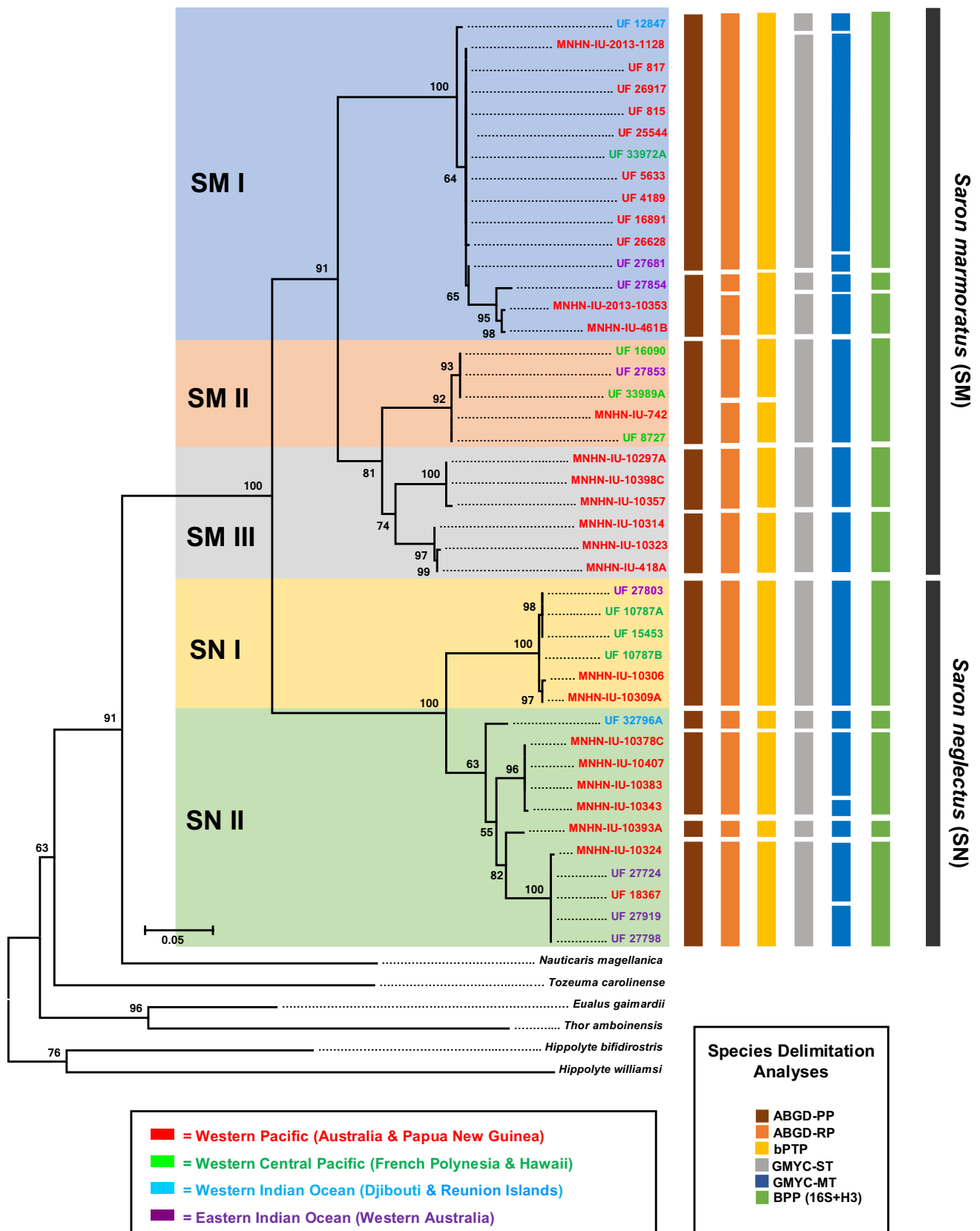


Fig. 4 Species delimitation analyses in the *Saron marmoratus* and *S. neglectus* based on 16S rRNA. Vertical bars represent dissimilar taxonomic units suggested by various species delimitation methods. ADGB-PP: Automatic Barcode Gap Discovery, Primary Partition; ABGD-RP: Automatic Barcode Gap Discovery, Recursive Partition; mPTP: Multi-rate Poisson Tree Process; bGMYC: Bayesian General Mixed Yule Coalescent Model; BPP: Bayesian Phylogenetics and Phylogeography

SM-E

Carapace flanks with very large, smooth, rounded or slightly ovoid, red or red-brown spots, each surrounded by double circle, i.e., inner red-brown or black circle and outer white circle, also containing small black spots, on pale yellow-greenish or pinkish background. Rostrum dark red–purple or red-brown, with white spots. Pleon flanks markedly different from carapace pattern, with convoluted labyrinth-like pattern of irregular orange or yellow-orange patches, each containing smaller spots; third pleonite without eye-spot; fifth pleonite with white-yellow pattern distoventrally; sixth pleonite dark red–purple. Antenna with basicerite bearing pale green-yellow or pinkish areas, surrounded by black; scaphocerite proximally with pale green-yellow or pinkish rounded patches, surrounded by black or red–purple, distally darker, with smaller spots. Pereopods with broad purple-red and black transverse bands alternating with broad white or pinkish bands. Pleopods pale yellow or greenish to pinkish, with two broad, oblique, ochre-pink bands bordering one narrow, oblique, purple-violet or almost black band. Uropodal exopod without eye-spot, entire uropod dark bluish purple (Fig. 6H; see also Fig. 1D).

Clade SM III, cryptic lineage SM-5 (2 terminals).

SM-F

Carapace flanks with large irregular patches, each containing small blue-violet spots, on pale greenish background, without circles. Rostrum pale green with brown-purple spots or bands. Pleon flanks similar to carapace; third pleonite without eye-spot; fifth pleonite with white spots on red-brown background distoventrally. Antenna with basicerite marbled pale green and red-brown; scaphocerite pale greenish with red-brown spots or short bands. Pereopods with broad olive-green or brownish transverse bands alternating with broad whitish bands. Pleopods pale greenish with broad dark purple-violet bands and yellow fringe. Uropodal exopod without eye-spot (Fig. 6I).

Clade SM III, cryptic lineage SM-6 (1 terminal).

Color morphs in the *Saron neglectus* clade (Fig. 6J–R)

SN-A

Carapace flanks marbled with paler and darker brown areas, and speckled with small to medium-sized, pale yellow spots, latter sometimes forming larger clusters but mostly not interconnected. Rostrum mostly dark green to brown, with whitish areas distally and ventrally. Pleon flanks similar to carapace; third pleonite dorsolaterally with large eye-spot (ocellus); fifth and sixth pleonites similar to first or second pleonites. Antenna with basicerite bearing conspicuous, target-like, black-and-white eye-spot (ocellus); scaphocerite with obliquely brown-white bands on proximal half, dark brown-green on distal half. Pereopods dark green with narrow whitish or pale green transverse bands. Pleopods brown-purple with narrow white and bright neon yellow-green bands and spots. Uropodal exopod with conspicuous eye-spot (ocellus) (Fig. 6J).

Clade SN I, cryptic lineage SN-1 (1 terminal).

SN-B

Carapace flanks red-brown with larger whitish spots, most interconnected and forming chains somewhat reminiscent of protein chains. Rostrum mostly brown, with broad white band. Pleon flanks similar to carapace; third pleonite with large eye-spot (ocellus) dorsolaterally; fifth and sixth pleonites similar to first or second pleonites. Antenna with both basicerite and scaphocerite bearing conspicuous, longitudinal, white and purple-red bands. Pereopods brown with narrow whitish and yellow-green transverse bands and spots. Pleopods brown with small white spots and conspicuous bright yellow spots. Uropodal exopod with large, conspicuous eye-spot (ocellus) (Fig. 6K).

Clade SN I, Subclade SN-1 (1 terminal).

SN-C

Carapace flanks with numerous small, dark red circles reminiscent of leopard spots or bacterial cultures, without outer white circle and usually not interconnected, among large irregularly shaped brown patches, on pale green background with very small yellow spots. Rostrum mostly brown, with whitish band proximally and white-yellow spots distally. Pleon flanks similar to carapace; third pleonite without eye-spot dorsolaterally, but mid-dorsal area conspicuously darker; fifth and sixth pleonites similar to first or second pleonites, much paler and more greenish. Antenna with basicerite bearing small black-and-white eye-spot (ocellus); scaphocerite pale greenish with narrow brown and white band along lateral margin proximally, dark brown with large pale yellow-green spots distally. Pereopods dark green with

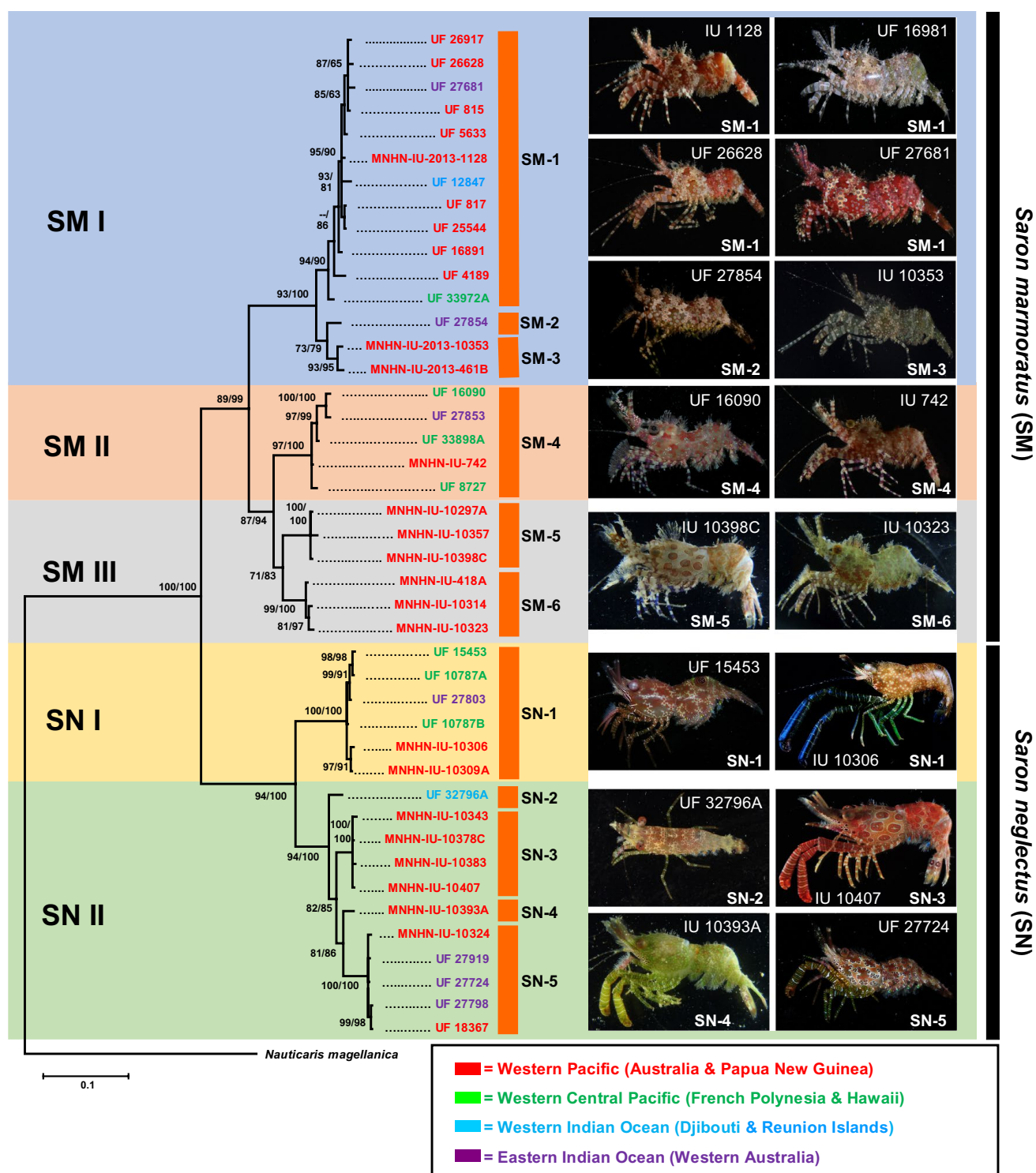


Fig. 5 Color patterns and species delimitation analyses in the *Saron marmoratus* and *S. neglectus* species complexes. Vertical bars represent dissimilar taxonomic units suggested by the combination of species delimitation methods used in this study

narrow whitish and neon green-yellow transverse bands and spots. Pleopods brown-green with narrow white and bright neon yellow-green bands, and with additional bright orange

band. Uropodal exopods with large conspicuous eye-spot (ocellus) (Fig. 6L).

Clade SN II, cryptic lineage SN-2 (1 terminal).

SN-D

Carapace flanks with background varying from pale orange to bright green, speckled with + minute dark red or black spots, with very large, rounded or oval-shaped, dark red to orange-red spots, somewhat reminiscent of agate slices, each surrounded by white circle and with smaller white spots inside. Rostrum brown to green, tip and ventral teeth whitish or yellow. Pleon flanks similar to carapace; third pleonite without eye-spot; fifth and sixth pleonites similar to first or second pleonites, with smaller spots. Antenna with basicerite with conspicuous, orange-brown or dark purple spots, each surrounded by white circle, on red–orange or pale orange background; scaphocerite white with large orange-brown or dark purple spots on proximal half, red-brown or green with whitish and yellowish spots on distal half. Pereopods dark red or green with narrow white and yellow-orange transverse bands. Pleopods dark red or green with white and yellow-orange transverse bands, with conspicuous bright blue patch distally. Uropodal exopod with large conspicuous eye-spot (ocellus) (Fig. 6M, N).

Clade SN II, cryptic lineage SN-3 (2 terminals).

SN-E

Carapace flanks with numerous small pale greenish spots on pale orange background, except for pterygostomial area, latter with dark purple spots on white. Rostrum greenish brown with pale greenish and whitish spots. Pleon flanks similar to carapace; third pleonite without eye-spot; fifth and sixth pleonites similar to first or second pleonites. Antenna with basicerite bearing purple-red spots on white background (similar to pattern on pterygostomial area of carapace); scaphocerite with purple red spots on white background on proximal half, brownish with pale green spots on distal half. Pereopods dark green with narrow pale green-bluish and neon yellow-green transverse bands. Pleopods green with whitish and brown transverse bands, with bright blue-green patch distally. Uropodal exopod with large conspicuous eye-spot (ocellus) (Fig. 6O).

Clade SN II, cryptic lineage SN-4 (1 terminal).

SN-F

Carapace flanks with numerous small, black to dark red circles, each surrounded by white circle and reminiscent of leopard spots or bacterial cultures, sometimes forming long chains, between irregular brown and orange-yellow areas. Rostrum mostly brown, marbled with whitish or yellow. Pleon flanks similar to carapace; third pleonite without eye-spot; fifth and sixth pleonites similar to first or second pleonites, with smaller spots. Antenna with basicerite with larger and smaller, conspicuous, dark red-brown or brown

spots on white background; scaphocerite with small but conspicuous, dark red-brown spots on proximal half, dark brown with small white spots on distal half. Pereopods brown-green with narrow whitish and bright neon yellow-green transverse bands and spots. Pleopods brown-purple with narrow white and bright neon yellow-green bands and spots. Uropodal exopod with large conspicuous eye-spot (ocellus) (Fig. 6P, Q).

Clade SN II, cryptic lineage SN-5 (3 terminals).

SN-G

Carapace flanks with background pale orange with large, diffuse, well-spaced, red patches and numerous, almost perfectly rounded pale violet to almost white spots, each surrounded by purple ring; anterior portion of carapace darker red, with several white, oblique or longitudinal streaks. Rostrum mostly dark red, with white bands distally and white-yellow ventral teeth. Pleon flanks similar to carapace, i.e., background pale orange with large, diffuse, well-spaced red patches and numerous, almost perfectly rounded pale violet to almost white spots, each surrounded by purple ring; third pleonite without eye-spot; fifth and sixth pleonites with dis-ventral angle deep red bordered dorsally by semi-circular white band. Antenna with ventral part of basicerite occupied by conspicuous, large, blue-and-black eye-spot or target spot (ocellus), dorsal part red with white band; scaphocerite with smaller and larger dark red to dark purple spots on largely white background, most aligned in row, distal spots larger and darker. Pereopods deep red with narrow whitish and bright yellow transverse bands. Pleopods with conspicuous bright yellow patch, bordered by white ventrally, and smaller bright blue patch distally. Uropodal exopod with large, dark-red eye-spot (ocellus). (Fig. 6R).

Possibly within Clade SN II (based on general color pattern and absence of ocelli on the third pleonite; specimen not included in the molecular and species delimitation analysis).

Discussion

In this study, we implemented an integrative systematic approach to test for the existence of cryptic species within two common and widespread shrimps of the genus *Saron*, displaying a remarkable disparity in color pattern. In fact, both *S. marmoratus* and *S. neglectus* were long suspected to contain more than just one species (Kemp 1914; Chace 1997; see also Debelius 2001; Kuitert and Debelius 2009; Minemizu 2013). Our approach joins previously proposed integrative taxonomic approaches aimed at resolving challenging taxonomic problems. For instance, Masonick and Weirauch (2020) proposed a complex strategy that integrates geometric morphometrics, molecular species delimitation

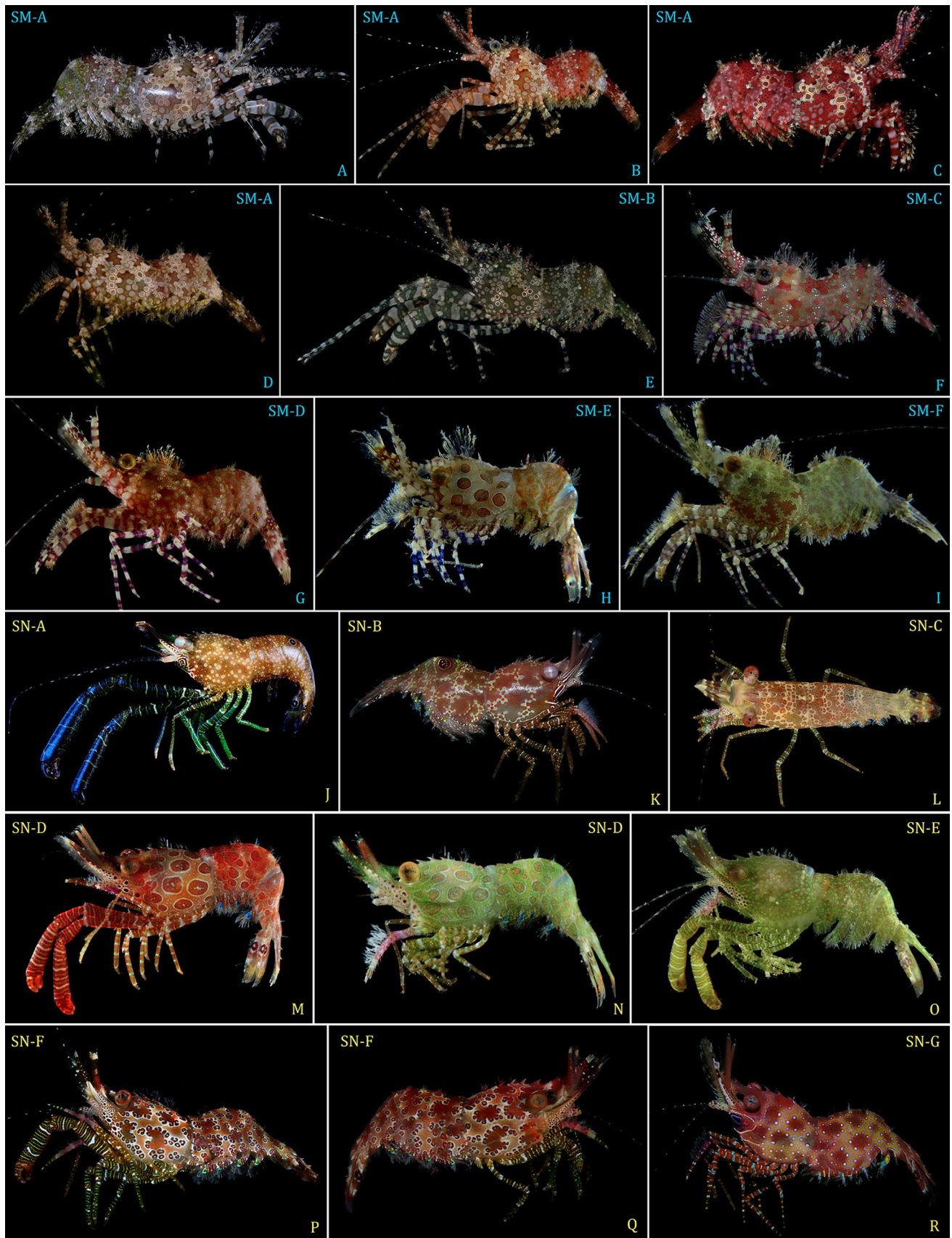


Fig. 6 Color pattern types of specimens from the *Saron marmoratus* (A–I, in light blue) and *S. neglectus* (J–R, in yellow) species complexes, analyzed in this study (except for R): **A**, specimen from Lizard Island, Australia (FLMNH UF 16981); **B**, specimen from Guam, Mariana Islands (FLMNH UF 26628); **C**, specimen from Ningaloo, Australia (FLMNH UF 27681); **D**, specimen from Lizard Island, Australia (FLMNH UF 27854); **E**, specimen from Madang, Papua New Guinea (MNHN-IU-2013–10353); **F**, specimen from Moorea, French Polynesia (FLMNH UF 16090); **G**, specimen from Kavieng, Papua New Guinea (MNHN-IU-2014–742); **H**, young specimen (damaged and *post-mortem*) from Madang, Papua New Guinea (MNHN-IU-2013–10357); **I**, specimen from Madang, Papua New Guinea (MNHN-IU-2013–10323); **J**, specimen from Madang, Papua New Guinea (MNHN-IU-2013–10306); **K**, specimen from Moorea, French Polynesia (FLMNH UF 15453); **L**, young specimen from Djibouti, Gulf of Aden (FLMNH UF 32796); **M**, specimen from Madang, Papua New Guinea (MNHN-IU-2013–10407); **N**, young specimen from Kavieng, Papua New Guinea (MNHN-IU-2014–979); **O**, specimen from Madang, Papua New Guinea (MNHN-IU-2013–10393); **P**, specimen from Lizard Island, Australia (FLMNH UF 27724); **Q**, specimen from Lizard Island, Australia (FLMNH UF 27919); **R**, specimen from Moorea, French Polynesia (FLMNH UF 16049). Color pattern types indicated in the upper right or left angle of each photograph. Photographic credits: Arthur Anker (A, B, C, D, E, F, G, K, M, P, Q, R); Zdeněk Ďuriš (H, I, N, O); Tin-Yam Chan (J); Gustav Paulay (L). A high-resolution version of this figure is available online as Supplemental Material Fig. SM-3

approaches, and ecological data to separate evolutionary lineages in Nearctic ambush bugs. Leria et al. (2020) proposed the use of morphology and molecular markers coupled with species delimitation approaches and karyological data to delineate and validate cryptic species in freshwater planarians. Importantly, these approaches are expensive both in terms of time and money, in addition to major investments in training personnel. Considering the current background of major environmental deterioration at both local and global scales, resulting in an unparalleled rate of species extinction (Dirzo et al. 2014; Wilson 2016), our approach is somewhat different (and more simple), as it aims to speed up the discovery of cryptic species taking into account monetary, personnel, and time constraints. Using this approach, we revealed two cryptic species complexes in a shrimp genus long considered a ‘taxonomic nightmare’ (Chace 1997). However, we also recognize that the following taxonomic revision of *Saron* will be both time- and money-consuming, as type material needs to be traced and eventually examined.

In this study, we clearly show that both *S. marmoratus* and *S. neglectus* together comprise at least 11 distinct evolutionarily lineages (or evolutionary significant units, ESUs), according to our molecular phylogenetic analyses and species delimitation approaches. Several lines of evidence support the hypothesis that many if not all of these lineages may represent true cryptic species for they seem to lack distinguishing morphological features within each of the *S. marmoratus* and *S. neglectus* clades, according to our preliminary multivariate analyses. On the other hand, molecular phylogenetic analyses revealed well-supported

clades within *S. marmoratus* and *S. neglectus* clades, with moderate to significant genetic distinctiveness. The absence of a relationship between genetic and phenotypic dissimilarity supports the hypothesis that these two taxa indeed represent two complexes of cryptic species, with some parallel evolution evidenced by color patterns (see below). Different species delimitation (ABGD, MPTP, BGMYC) and validation (BP&P) methods based on nuclear and mitochondrial gene fragments also suggested the existence of at least 11 cryptic lineages or ESUs in *S. marmoratus* and *S. neglectus*. We are well aware of the fact that species delimitation methods are based on over-simplistic assumptions, which, if violated, can lead to an overestimation of the actual number of cryptic species (Fontaneto et al. 2015; Sukumaran and Knowles 2017). When incongruence across results from different methods is observed, as it occurred in our study (i.e., ABGD versus GMYC), it can be interpreted as evidence that the assumptions of one or more methods employed are violated and/or that there are differences in the power of the different approaches to detect cryptic lineages (Carstens et al. 2013). Furthermore, recent studies also suggest that species delimitation approaches diagnose genetic structure, not necessarily species, and that they do not statistically distinguish genetic structure associated with population isolation versus species boundaries (Sukumaran and Knowles 2017). In an attempt to address the problems above, we employed a set of state-of-the-art species delimitation and validation analyses that collectively with our phylogenetic analyses enabled us to estimate the number of possible cryptic evolutionary lineages in *S. marmoratus* and *S. neglectus*. Different species delimitation approaches used jointly with phylogenetic inference, as we have demonstrated here, should be more reliable when dealing with challenging cryptic species complexes (Carstens et al. 2013; Fontaneto et al. 2015).

As expected, color patterns provided additional strong support to the hypothesis that both *S. marmoratus* and *S. neglectus* are two distinct cryptic species complexes. We identified a total of 13 types of color patterns / color morphs that in many (but not all) cases corresponded to genetic lineages, although one very distinctive color morph coded as SN-G was not represented by material in our analyses (see below). The general observation that can be made for both species complexes of *Saron* is an imperfect segregation of color patterns among genetically defined lineages.

In the *S. marmoratus* species complex, two closely related lineages may have either very different (e.g., the sister lineages SM-5 and SM-6) or very similar color patterns (SM-1, SM-2, SM-3) (Figs. 5, 6). In the *S. neglectus* species complex, the color patterns segregated first by larger clades, i.e., SN I (with a pair of dorsolateral eye-spots on the third pleonite) and SN II (without eye-spots on the third pleonite). However, within SN I, there was not much genetic difference between generally similar, but still fairly distinctive color

morphs SN-A and SN-B (Figs. 5, 6). Within SN II, the two very similar color morphs SN-C and SN-F were genetically widely separated in cryptic lineages SN-2 and SN-5, respectively, with the remaining, very different color morphs, i.e., SN-D and SN-E, forming cryptic lineages SN-3 and SN-4 in-between (Figs. 5, 6). The genetic identity and position of the color morph SN-G is currently unknown, but based on its general color pattern (e.g., shape of patches on the carapace and pleon) and lack of dorsolateral eye-spots on the third pleonite, this form highly likely forms an additional, e.g., fifth cryptic lineage within clade SN II (Fig. 6R). Thus, color patterns represent a valuable and useful source of easy-to-observe characters that help to differentiate among lineages and presumed cryptic species of *Saron*, despite (1) the absence of a perfect segregation between some genetically dissimilar lineages, and (2) the lack of significant genetic divergence between obviously different color morphs.

In many caridean shrimps, color patterns are important diagnostic features and therefore are a standard component of taxonomic descriptions; this is especially true for the highly diverse family Alpheidae (e.g., Knowlton and Keller 1985; Nomura and Anker 2005; Anker 2012; see also Knowlton 1986, 1993). For instance, in the large *Alpheus armillatus* species complex, all species can be separated by color patterns, with characters ranging from rather subtle (presence vs. absence of small black dots on the pleon) to very conspicuous (e.g., transverse banding on the pleon no banding, blue antennal flagella vs. yellow antennal flagella) (Anker 2012). In the smaller *Alpheus armatus* species complex, all five species are characterized by a very conspicuous color pattern dominated by red, blue and white (Knowlton and Keller 1985; Hurt et al. 2013; Anker et al. 2016). However, only four of them can be differentiated by subtle differences in the color pattern, such as the presence or absence of neon yellow spots, whereas the fifth is separated from the others mainly by the size of the uropodal spine, not by color. The peppermint shrimps of the *Lysmata wurdemanni* species complex (Lysmatidae) represent another example of an almost perfect color segregation. Each of the species originally placed in this complex has a unique and diagnostic color pattern (Rhyne and Lin 2006; Pachtelle et al. 2020). However, the recent findings of additional cryptic lineages without noticeable differences in color patterns in at least two species of the *L. wurdemanni* complex somewhat complicated the issue (Baeza and Behringer 2017; Baeza and Prakash 2019). In some other decapod groups, i.e., presumed species complexes, color patterns are homogeneous or nearly identical in genetically dissimilar lineages that have diversified over long periods of time and separated by soft and hard biogeographic barriers. Examples include the *Thor amboinensis* species complex with relatively recent speciation events (Titus et al. 2018), and the *Stenopus hispidus*

with lineages that may have diverged about 20 mya (Dudoit et al. 2018).

Our integrative taxonomic approach has demonstrated that *S. marmoratus* and *S. neglectus* each represent a complex of cryptic species comprising morphologically rather homogeneous, but genetically more or less dissimilar entities. Most genetically defined lineages can also be diagnosed by color morphs, which range from very similar to strikingly different. These results have important implications for both basic and applied research. Firstly, our study tested and supported the hypothesis that two ‘species’ with dissimilar color patterns, present in different biogeographic provinces and crossing phylogeographic + biogeographic breaks, actually represent two speciose cryptic species complexes. Our results are in line with other studies testing for cryptic entities in taxa with wide geographic ranges in the Indo-West Pacific. Genetically distinct lineages often representing cryptic or pseudocryptic species have been reported before in numerous marine invertebrate and teleost taxa across the Indo-West Pacific (e.g., Meyer et al. 2005; Barber and Boyce 2006; Barber et al. 2006; Huelsken et al. 2013; Titus et al. 2018 and references therein). Importantly, genetic discontinuities and/or cryptic species in *S. marmoratus* and *S. neglectus* did not appear to segregate according to biogeographic provinces, in disagreement to some other studies (e.g., Barber et al. 2000, 2002, 2006; Huelsken et al. 2013), although by far not all (e.g., Horne et al. 2008; Gaither et al. 2010; Dudoit et al. 2018). For instance, specimens belonging to the cryptic lineage SN-1 were collected from different provinces (Indian Ocean, western and western-central Pacific). In other cryptic lineages (e.g., SM-5 and SM-6), all specimens were collected from a single province or region in the Indo-West Pacific. However, it is presently impossible to link our data to the well-established biogeographic and phylogeographic breaks in the Indo-West Pacific due to our sample size (that we consider too small for phylogeographic inference) and a certain bias towards some better sampled areas and biogeographic provinces where most of our material was collected (e.g., Australia, Papua New Guinea, French Polynesia). Some areas of the Indo-West Pacific are generally undersampled for marine invertebrates, including *Saron*. We are also well aware of the existence of several other color morphs, especially in the *S. marmoratus* complex, for example, in the Indo-Malayan region and the Red Sea (Kuiter and Debelius 2009; A. Anker, pers. obs.); some of them are strikingly different from the ones coded by us (see Fig. 1B, C). Unfortunately, most of these color morphs are presently known only from underwater photographs, i.e., there are no specimen vouchers legally collected and deposited in natural history museums. We argue that additional sampling in populations extending throughout the different biogeographic provinces in the Indo-West Pacific is

needed to test the possible role of soft geographical barriers in driving genetic breaks and cryptic speciation in *Saron*.

Secondly, the evolutionary and ecological processes leading to genetic divergence and reproductive isolation, and consequently to speciation, in the absence or paucity of morphological differentiation in *Saron* are interesting topics that remain to be addressed. Studies attempting to estimate the divergence times between the cryptic lineages in *Saron* and allied genera, and eventually link them to biogeographic events, are underway. However, we can already state that cryptic diversification within the two major clades of *Saron* might have started anytime from the middle Pleistocene (ca. 1.2 mya) to Pliocene (5 mya), as suggested, for example, for some Indo-West Pacific mantis shrimps (Barber et al. 2006) and snappers (Gaither et al. 2010). Overall, the newly discovered cryptic species complexes in *Saron* present opportunities to study important mechanisms of speciation in the Indo-West Pacific biodiversity hotspot (Bickford et al. 2007).

Thirdly, taking into account that *Saron* spp. are an important target for the rapidly growing ornamental marine invertebrate trade (Calado et al. 2003; Calado 2008), our results imply that further studies of the genus are needed. These studies should focus on describing population- and/or individual-level parameters, as well as evaluating standing stocks of *S. marmoratus* and *S. neglectus* at several localities and for various genetically defined lineages simultaneously, if an accurate life history schedule and stock estimation of the many lineages comprising *S. marmoratus* and *S. neglectus* wants to be estimated (Baeza and Behringer 2017). It is relevant to develop a baseline of biological information for these two species complexes, as well as other ornamental shrimp species (Baeza and Behringer 2017; Baeza and Prakash 2019), so that the fisheries targeting them can be managed in a sustainable manner (Calado et al. 2003; Baeza and Behringer 2017).

Fourthly, *Saron marmoratus* is currently used as a model system to explore the role of environmental conditions in driving male dimorphism and alternative male mating tactics (J.A. Baeza, unpublished data). For the time being, the hinge-beak shrimps of the genus *Rhynchocinetes* are the only marine shrimps in which alternative mating tactics have been extensively studied (Baeza et al. 2014, and references therein). Nonetheless, it is likely that alternative mating strategies are found in other caridean shrimps where large males have well-developed weapons (i.e., chelipeds and maxillipeds), as previously reported in *Saron neglectus* and *S. marmoratus* (Kemp 1914; Baeza, unpublished data). At the same time, our results imply that field observations and laboratory experiments may not be needed for this purpose for all the cryptic lineages of *Saron marmoratus* and/or *S. neglectus*, since not all of them seem to have highly dimorphic males (A.A., pers. obs.). We consider our findings in *Saron* spp. an opportunity to develop comparative studies

using this newly discovered species complex as a model system. Future studies centered on describing the behavioral ecology of at least some of the cryptic lineages within these two species complexes will certainly aid in formally testing mating system and sexual selection theories within a comparative framework (Baeza 2013).

Lastly and importantly, the present study represents the first step toward a long overdue taxonomic revision of the genus *Saron*. Both species complexes will need more a profound taxonomic assessment. The taxonomy of *S. marmoratus* is complicated by (1) the absence of the original type material (AA and Laure Corbari, pers. comm.) in the dry specimen collection of the Muséum National d'Histoire Naturelle in Paris (MNHN), which holds most of the extant crustacean specimens described by Olivier (1811); (2) the vague type-locality data ('Nouvelle-Hollande' or New Holland), which could be anywhere in Australia (but probably southern or western Australia, see Banner and Banner 1982); and (3) the presence of five nominal taxa established during the nineteenth and early twentieth centuries, currently regarded as junior synonyms of *S. marmoratus* (De Grave and Fransen 2011). Our results suggest that future species descriptions in *Saron* will need to be based primarily on a combination of color patterns and DNA (Jörger and Schrödl 2013), although we will also try to exploit additional morphological and/or morphometric traits. Last generation pattern-recognition technologies (i.e., artificial intelligence, e.g., Cruz et al. 2013) may reveal additional taxonomic characters, such as minor but reliable color pattern variability. Furthermore, targeted experiments designed to test for reproductive isolation between/among putative cryptic species can be helpful to explore if behavioral isolating mechanisms might account for the genetic dissimilarities reported in this study. Studies focusing on the formal description of the different entities comprising the *S. marmoratus* and *S. neglectus* species complexes revealed by our study, have been initiated. However, as mentioned above, specimens of color morphs not included in the present analysis are needed for a more comprehensive taxonomic study of the genus *Saron*.

In summary, integrative taxonomic and phylogeographic studies, as exemplified in our study, are urgently needed to reveal the extent and magnitude of cryptic species complexes in the marine realm, including coral reefs. These studies must be prioritized given the current acute biodiversity crisis and the profound implications that cryptic species complexes have for conservation planning, biogeography and a great number of evolutionary questions (Bickford et al. 2007).

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Author contributions JAB and AA were responsible for conceptualization, methodology, data curation, writing, original draft preparation, visualization, reviewing and editing. JAB, PS, PF, ZD, AA performed methodology, data curation, writing, reviewing and editing.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Sexual and mating system in *Thor hainanensis* Xu & Li, 2014 (Decapoda: Natantia: Thoridae) from the Lakshadweep Archipelago, India

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ABSTRACT

We tested the hypothesis that the Hainan's squat shrimp *Thor hainanensis* Xu & Li, 2014 undergoes a sex change and displays protandric hermaphroditism, a well-known feature from other congeners (*T. dicaprio* Anker & Baeza, 2021 and *T. amboinensis* (De Man, 1888)). We used morphological traits indicative of male function to categorize shrimp as prehensile males, non-prehensile males, and females. The linear relationship among the different morphological characters and the sexual morphs revealed that *T. hainanensis* has a sexual system of partial protandric hermaphroditism, where 50% of the population stays as active males and the rest of the population changes to female. Observations on the social and mating behaviour suggest that *T. hainanensis* exhibits a 'search and defend' mating system unlike other tropical caridean shrimps featuring protandrous hermaphroditism.

KEY WORDS: allometry, Crustacea, mating behaviour, protandric hermaphrodite, sex change, *Thor* spp.

INTRODUCTION

Caridean shrimps exhibit a remarkable diversity of sexual systems. Some species exhibit typical gonochorism, where individuals grow either as a distinct female with ovaries or as a distinct male with testes (Bauer, 2004). Examples include *Processa riveroi* Manning & Chace, 1971, *P. bermudensis* (Rankin, 1900), *Ambidexter symmetricus* Manning & Chace, 1971, *Rhynchocinetes durbanensis* Gordon, 1936, and *Ancyllocaris brevicarpalis* Schenkel, 1902 (Bauer & Conner, 2012; Rasch & Bauer, 2015; Prakash *et al.*, 2017). Other species exhibit either protandrous hermaphroditism (all individuals reproduce early as a male, and later reproduce as female) or partial protandry (a portion of the adult population changes sex from male to female) (Bauer, 2000; Bergstrom, 2000). Protandrous hermaphroditism encompasses protandrous sequential hermaphroditism and protandrous simultaneous hermaphroditism. In the former, individuals reproduce early as functional males and later emerge as functional females. In the latter, individuals reproduce early as functional males, and develop as simultaneous hermaphrodites (retaining both male and female sex). For

instance, *Pandalus dispar* (Rathbun, 1902) and *Paratya curvirostris* (Heller, 1862) show strict protandrous sequential hermaphroditism (Butler, 1964; Carpenter, 1978), whereas several species of *Lyssmata* Risso, 1816 and *Exhippolysmata* Stebbing, 1915 exhibit protandrous simultaneous hermaphroditism (Kagwade, 1982; Bauer & Holt, 1998; Baeza, 2006, 2009; Bauer, 2006; Laubenheimer & Rhyne, 2008; Baeza *et al.*, 2009; Braga *et al.*, 2009).

Several variants of partial protandry have been reported in caridean shrimps. For instance, *Arete indicus* Coutière, 1903 exhibits partial protandric hermaphroditism with primary males in a population where sex changers are seen along with permanent males (Gherardi & Calloni, 1993). *Argis dentata* (Rathbun, 1902) and *Crangon crangon* (Linnaeus, 1758) also display partial protandric hermaphroditism, with primary females in which sex changers are observed along with permanent females (Frechette *et al.*, 1970 as cited in Bauer & VanHoy, 1996). Various other shrimps exhibit partial protandric hermaphroditism with populations being composed of primary males, primary females, and protandric individuals (i.e. individuals that are changing sex), as

observed for *Thor manningi* Chace, 1972 (Bauer, 1986; Bauer & VanHoy 1996).

Mating systems are quite variable in caridean shrimps, and mating happens soon after the female moults (Bauer, 1996; Correa & Thiel, 2003; Jose *et al.*, 2021). Male mating strategies depend on the potential to attract females (Wilson, 1975; Shuster & Wade, 2003). In a 'pure search' mating system, the reproductive success of males depends on their ability to find and detect receptive females (Bauer & Abdalla, 2001), whereas in the 'search and attend' mating system, adult males who live in solitary frequently search for receptive females. Once mating happens, males will be driven back to their solitary life (Nakashima, 1987).

There are currently 17 described species of *Thor* Kingsley, 1878 (WoRMS, 2023). Explicit information regarding the sexual system and mating behaviour of most of these species is scarce. The sexual systems of *T. floridanus* Kingsley, 1878 and *T. dobkini* Chace, 1972 (Bauer & VanHoy, 1996) have been confirmed as gonochoric. *Thor manningi* exhibits partial protandry (Bauer, 1986) and *T. amboinensis* (De Man, 1888) is a protandric hermaphrodite (Baeza & Piantoni, 2010). Among the mating systems, not much documentation exists except for *T. amboinensis*, as it prefers a 'pure search' mating system (Baeza & Piantoni, 2010). Another species is *T. hainanensis* Xu & Li, 2014, described from Hainan Island, China but also reported from Indian waters (Madhavan *et al.*, 2019). The species can be bred in captivity (Jose *et al.*, 2021), but information on its sexual and mating systems remained unknown until now.

We investigated the sexual system of *T. hainanensis* collected from the Lakshadweep Archipelago, India. Secondary sexual characters, such as second abdominal pleuron width (APW in mm), appendix masculina length (AML in μm), basal flange width of the second pleopod (BFW in μm), third pereopod propodus width (PW) and third pereopod propodal spines (PS) were selected to test the hypotheses that 1) whether the populations of *T. hainanensis* exhibit partial protandric species, with 50% stays as active males, and 2) whether social behaviour would result in differential mating system in *T. hainanensis* leading to brood production in females.

MATERIALS AND METHODS

Study area and collection of shrimp

The Hainan's squat shrimp *T. hainanensis* (Fig. 1) was recently reported from Agatti Island, Lakshadweep Archipelago (Madhavan *et al.*, 2019). To maintain shrimp and breed them successfully, a basic understanding of their sexual biology is required. Individuals of *T. hainanensis* were collected from the lagoon of Agatti and Bangaram islands. They are found associated with the sea anemone, *Radianthus magnifica* (Quoy & Gaimard, 1833), live corals (mainly *Pocillopora* sp.), and under rocks. Ten surveys were conducted during day hours, and 210 individuals of variable size were collected using handpicking and snorkeling 1–3 m deep. Shrimp were sampled by gently guiding individuals into plastic buckets and transferred to the Live Germplasm Resource Centre, National Bureau of Fish Genetic Resources, Agatti island, Lakshadweep. Live shrimp were acclimatized for 24 h and transferred to 50-l tanks in

the hatchery, where they were maintained with *ad-libitum* food supply (e.g. boiled clams, frozen *Artemia*, and commercial pellets). Rocks from the natural environment were provided during the acclimation. The water quality parameters were maintained at 26–27 °C, 30‰, and 4–6 mg l⁻¹ dissolved oxygen.

Sexual morphs

The morphometry of the secondary sexual characters was examined on the individuals of *T. hainanensis*. In the partially protandric *T. manningi*, Bauer (1986) identified the male-phase hermaphrodite as individuals with non-prehensile third pereopods and appendices masculinae on the second pleopods, whereas primary males are individuals with prehensile third pereopods. Bauer & VanHoy (1996) examined additional morphological characters for distinguishing male phase hermaphrodites. For instance, the maximum width of the distal half of the propodus, the length of the dactyl, the number of stout setae on the distal half of the propodus, the length of the appendix masculina, and the maximum width of a basipod flange from the second pleopod were measured. We assigned specimens to different sexual categories based on the presence (males) or absence (females) of an appendix masculina on the second pleopod, prehensibility of the third pereopod (i.e., width and number of spines on the ventral margins of the propodus), and the presence or absence of embryos as in Bauer (1986) for *T. manningi*. We recognised the following sexual categories: females (F), individuals without appendices masculinae and/or which carried embryos or, without embryos; prehensile males (PM), individuals with appendix interna and appendices masculina and prehensibility on third pereopods; non-prehensile males (NPM), individuals with appendix interna and appendix masculine and non-prehensile third pereopods.

In total, 100 individuals in variable size groups were selected and examined for their reproductive morphology: carapace length (CL in mm, i.e., distance from the posterior edge of the orbit to the mid-dorsal posterior edge of the carapace), second abdominal pleuron width (APW in mm), appendix masculina



Figure 1. The Hainan's squat shrimp *Thor hainanensis* (male) from the Agatti Island, Lakshadweep Archipelago, India.

length (AML in μm), basal flange width of the second pleopod (BFW in μm), third pereopod propodus width (PW), and third pereopod propodal spines (PS). Shrimps with a smaller number of spines (< 15) on the ventral margin of the propodus of the third pereopod were considered as non-prehensile males (NPM) and with a greater number of spines (> 15) as prehensile males (PM) (Bauer, 1986). All measurements, except CL and APW, were made under the trinocular microscope Motic B1 series (Motic Asia, Kowloon, Hong Kong), using a graduated ocular micrometer to the nearest 0.01 mm. CL and APW were measured under a stereozoom microscope GE 86-39509 (Unilab India, Bhubaneswar, India) using a graduated scale to the nearest 0.1 mm.

Social behaviour

Males and brooding females were collected from the wild and kept as outlined above. We designed experiments in a way that one pair of *T. hainanensis*, one male per three female shrimp and three males per single female, were placed separately in three tanks. The experiments were done in triplicates. Specimens were observed to describe social behaviour during six months.

Testing the sexual system

We collected 24 individuals of *T. hainanensis* for the trial. The shrimp were observed for six months, starting from June–November 2020. For testing the sex change from males to females (partial protandry; as reported in other closely related species such as *T. manningi* (Bauer, 1986) and *T. amboinensis* (Baeza & Piantoni, 2010)), the shrimp were kept in three experimental setups with six replicates each. First, to test whether the protandric hermaphrodites can simultaneously function as males, six pairs of berried females (CL 4.0–4.9 mm) were kept in separate tanks. In the second set-up, six pairs of male shrimp in the subadult stage (CL 2.8–3.4 mm) were kept in separate tanks to check whether males were able to change sex and become females and subsequently lay eggs so that protandry can be confirmed. In the third experimental setup, six mature females were kept in separate tanks to test whether the shrimp were capable of self-fertilization.

Mating behaviour

Five pairs of *T. hainanensis* were collected without causing any damage to the body parts. Observations were conducted in a non-competitive environment (i.e., 1 female: 1 male) except for one pair to avoid intra-sexual conflicts between the males. Mating events were recorded using a G1X DSLR camera (Canon, Tokyo, Japan) provided with a tripod. Although these shrimp were normally nocturnally active, mating occurred in captivity during day and night. The female shrimp were monitored continuously until they moulted and mated. Visual observations and video recordings were made on mating behaviours (touching, guarding, holding, and copulation) performed by each pair. After the successful mating and spawning of females, an egg mass could be observed attached underneath the ventral side of the abdomen as an orange mass. Initiation of spawning was marked by the bending of the abdomen in females, and spawning ended with several pleopodal beats.

Statistical analysis

The sexual morphs showed clear demarcation in the measurements of secondary sexual characters. To determine whether a linear relationship existed between the measured traits (APW, BFW, PS, PW, AML) and body size (CL), we used the allometric model $y = ax^b$ (Hartnoll, 1978, 1982). The slope b value represents positive allometry if greater than one ($b > 1$) and negative allometry if less than one ($b < 1$). One-way ANOVA was also performed to compare morphometric characters of prehensile males, non-prehensile males, and females using the SPSS statistical software package version 16.0, USA (Norusis, 2009). The data were normalized using Log10 prior to analyses.

RESULTS

Sexual morphs

We confirmed three sexual morphs: prehensile males (PM), non-prehensile males (NPM), and females (F). Males were characterized by appendices masculinae with long spines usually reaching the distal end of the endopod in the second pleopods and females by the absence of appendices masculinae in the second pleopods. Out of 100 shrimp, 58 individuals were PM, 26 individuals NPM, and 16 individuals F. PM and NPM individuals were differentiated by the number of spines on the propodus of the third pereopod. The number of spines on the propodus of the third pereopod in NPM individuals ranged 7–14 (Fig. 2B), and 15–38 individuals among PM (Fig. 2A). In the selected sample, the number of spines on the third pereopod propodus of females was observed, which ranged between 10–29 (Fig. 2C). The appendices masculinae in PM individuals exhibited numerous distal spines, longer than those of NPM individuals.

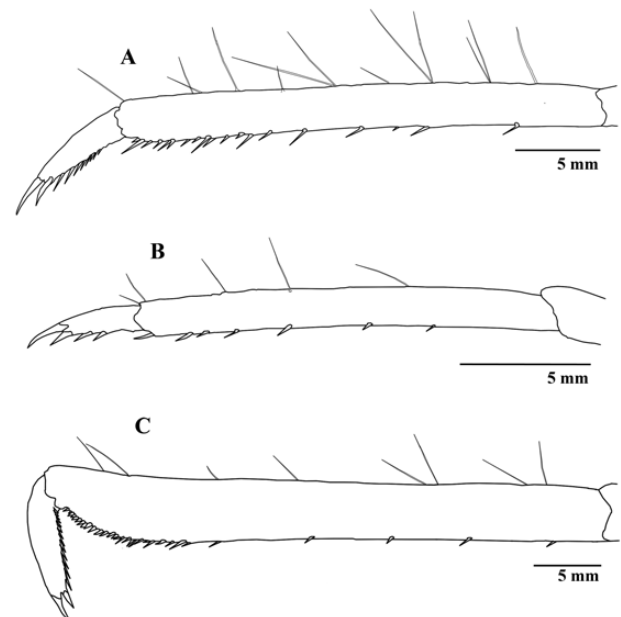


Figure 2. Third pereopods (left side, lateral view) of different sexual morphs of *Thor hainanensis* prehensile male (A); non prehensile male (B); female (C).

We analysed 58 prehensile males, 26 non-prehensile males, and 16 females for the possibility of allometric growth. The degree of allometry differed among the morphometric parts and between the sexes. The linear relationship among the secondary sexual characters such as APW, PW, PS, BFW, and AML are summarised in Table 1.

The carapace length of prehensile males, non-prehensile males and females ranged 1.15–4.65 mm (mean $\pm$ SE, 1.08 ± 0.05),

1.15–2.15 mm (2.37 ± 0.03), and 1.65–5.15 mm (2.365 ± 0.11), respectively (Fig. 3). The carapace length of the NPM individuals were significantly smaller than the PM ($F = 72.8$, $df = 2, 96$, $P < 0.0001$). The propodal spines showed a positive correlation with carapace length in all sexual morphs (Fig. 4). In PM and F individuals, the distal end of the third pereiopod is expanded and bears spinous setae. The third pereiopod propodus width (PW) shows negative allometry with respect to carapace length (CL) among all

Table 1. One-way ANOVA of morphological characters of *Thor hainanensis* (CL, carapace length; APW, width of second abdominal pleuron; AML, length of appendix masculina; BFW, basal flange width of the second pleopod; PW, width of third pereiopod propodus; PS, third pereiopod propodal spines) (PM, prehensile males; NPM, non-prehensile males; F, females)

y	x	Regression	r ²	SE	df	F	P	Allometry
PM								
APW	CL	$y = 0.09x + 1.01$	0.56	0.05	57	96.31	0.75	–
PW	CL	$y = 0.14x + 2.3$	0.03	9.16	57	78.3	0.3	–
PS	CL	$y = 6.20x + 9.74$	0.41	0.79	57	38.2	0	+
AML	CL	$y = 0.68x + 2.61$	0.54	25.41	57	45.3	0	–
BFW	CL	$y = 0.97x + 2.16$	0.67	14	57	140.12	0.74	–
NPM								
APW	CL	$y = 0.05x + 1.02$	0.14	0.03	25	3.2	0.35	–
PW	CL	$y = 0.2x + 1.94$	0.11	4.51	25	5.5	0.67	–
PS	CL	$y = 4.75x + 2.16$	0.12	0.47	25	3.27	0.6	+
AML	CL	$y = 0.85x + 2.53$	0.2	19.15	25	4.55	0.36	–
BFW	CL	$y = -0.03x + 2.30$	0	7.6	25	0.01	0.01	–
F								
APW	CL	$y = 0.06x + 1.06$	0.1	0.11	15	1.93	0.02	–
PW	CL	$y = 0.09x + 2.29$	0.01	19.6	15	8.51	0.7	–
PS	CL	$y = 5.47x - 1.85$	0.32	1.64	15	6.6	0.82	+
BFW	CL	$y = 1.81x + 2.02$	0.27	880.67	15	3.56	0.17	+

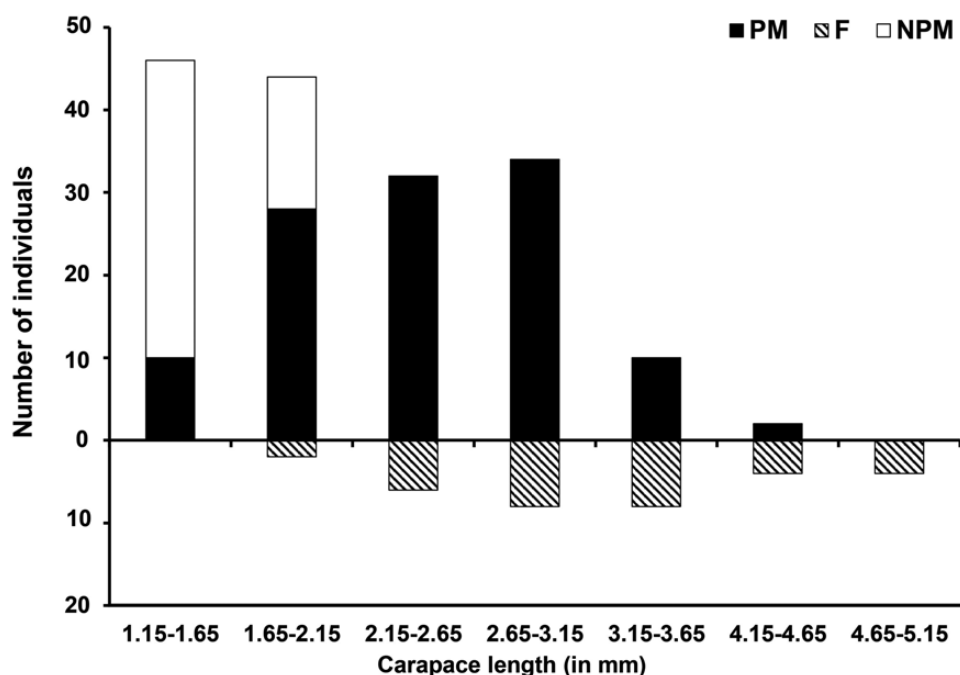


Figure 3. Size-frequency distribution (carapace length in mm) among different sexual morphs of *Thor hainanensis*: prehensile male (PM) ($N = 58$), non-prehensile male (NPM) ($N = 26$), and female (F) ($N = 16$).

sexual morphs ($P > 0.0001$), i.e., the slope depicting the relationship between PW and CL was less than unity (Fig. 5A; Table 1). Likewise, the appendix masculina length (AML) in PM and NPM individuals also showed negative allometry (Fig. 5B; Table 1). APW and BFW showed a positive correlation in mature females, compared to PM and NPM sexual morphs. APW, however, presented a negative allometry and BFW a positive allometry against CL (Fig. 5C, D; Table 1).

Social behaviour

Shrimp in both tanks, i.e., one male with three females and one female with three males took 3–4 d for initial acclimatization. The berried shrimp in the tanks hatched within 5–10 d. Frequent hatchlings followed by mating were observed in each tank. In single-paired shrimp and one female accompanied by three males, spawning was observed to happen every 10–11 d.

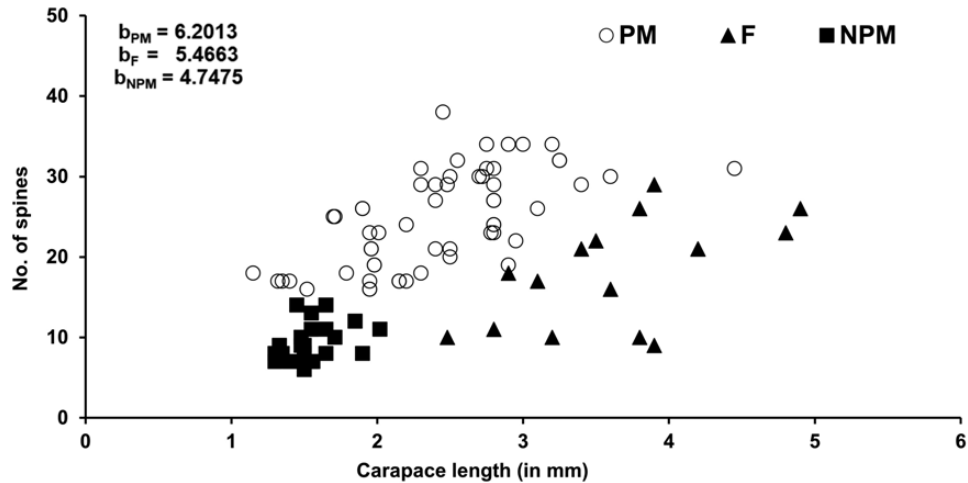


Figure 4. Number of propodal spines on the third pereiopods among different sexual morphs (PM, prehensile male; NPM, non-prehensile male, and F, female) of *Thor hainanensis*.

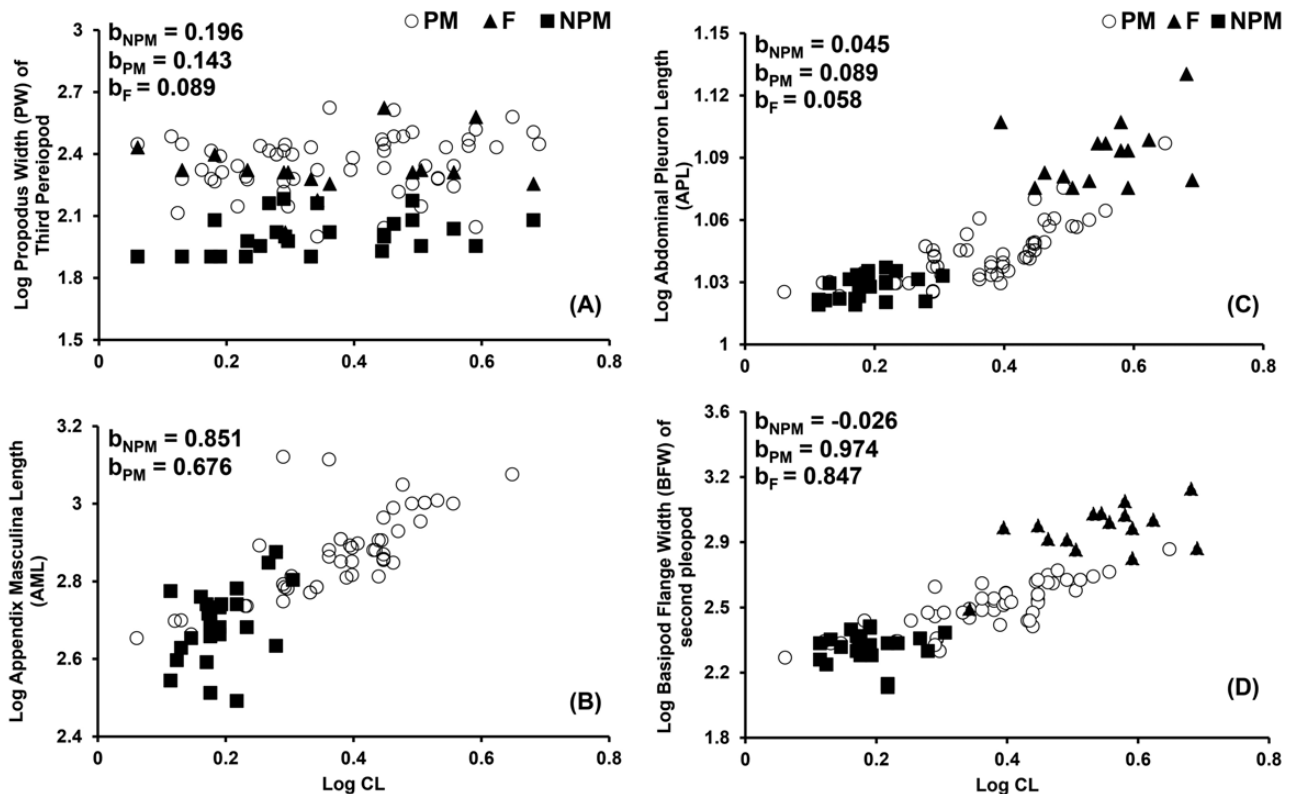


Figure 5. Linear relationship in the secondary sexual characters among different morphs (PM, prehensile male; NPM, non-prehensile male, and F, female) of *Thor hainanensis*. Width of third pereiopod propodus (A); length of appendix masculina (B); width of abdominal pleuron (C); width of basipod flange of second pleopod (D).

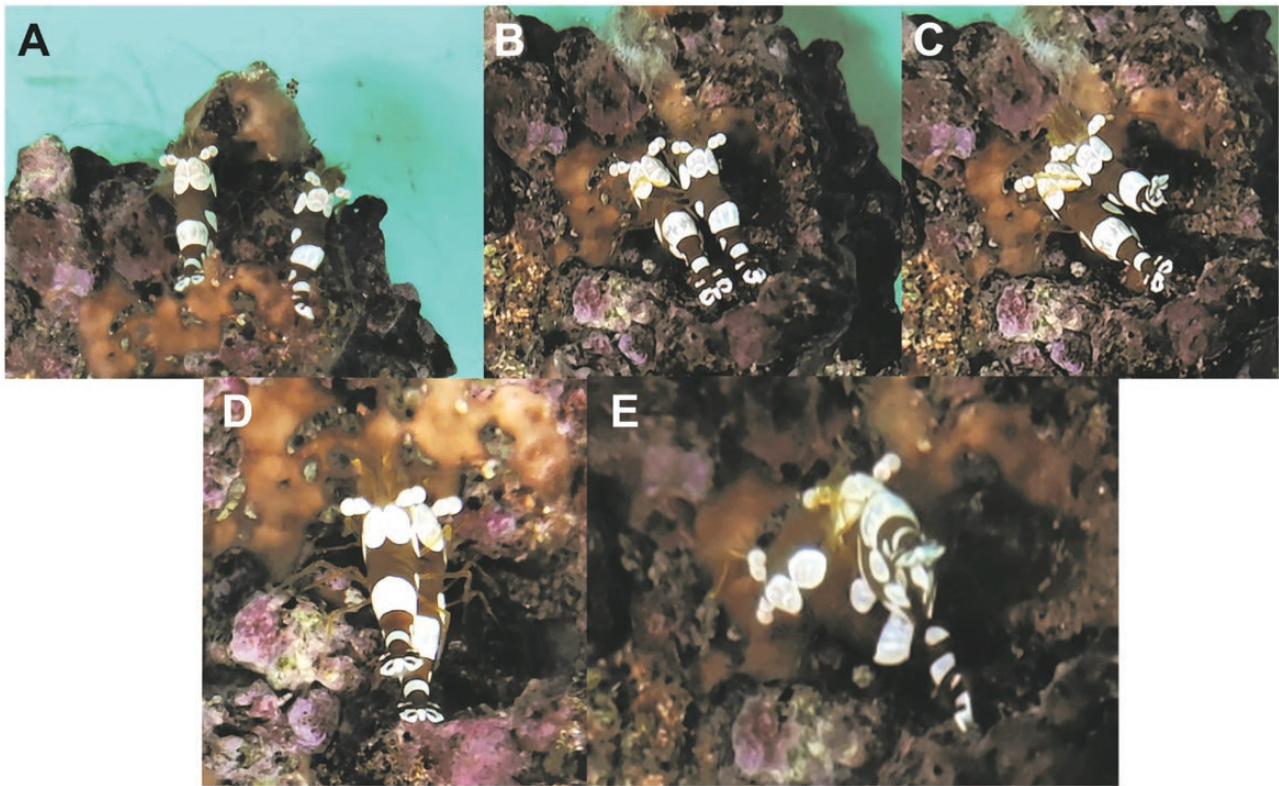


Figure 6. Mating sequence of *Thor hainanensis*. Touching with antennae (A); holding with pereopods (B); riding and overlapping (C, D) and spermatophore transfer (E).

Testing the sexual system

In the first and third setup after moulting, shrimp spawned within 24 h, but the entire egg mass was lost within 24–8 h. Moulting was observed every 10–13 d in all individuals. In the second set up, males (CL 1–1.2 mm) changed into females after two successive moults. It was observed that NPM individuals lost the appendix masculina in the second pair of pleopods and developed white spots at the second and third pleopod flange. Mating was observed after every female moult, and the females retained eggs. Moulting was recorded at least twice a month.

Mating behaviour

Five mating events were observed from initial contact, spermatophore transfer, and the final separation of the mating partners. Mating occurred during daytime and at night after the successful moult ([Supplementary material S1 Video](#)).

Touching the antenna: Males first initiated mating through bodily contact with the female by touching it with his antennae ([Fig. 6A](#)). The process took 128–232 s (mean $\pm$ SD, 181 ± 44.3).

Holding with pereopods: The male slightly perched over the female sidelong and came to the holding position ([Fig. 6B](#)). The shrimp remained in this position for 102–106 s (104 ± 1.5).

Overlapping: Males perched over the female and remained parallel to the body ([Fig. 6C, D](#)).

Spermatophore transfer: Very short sequence in which the male climbed over the female and was positioned laterally to the female's body. Males held on to the female using the pereopods, females rose the abdominal region as the male tried to twist its ventral side towards the female ventral region. With a rapid bent,

males transferred the spermatophore onto the ventral abdominal region of females ([Fig. 6E](#)). It lasted for a very short duration of 1–3 sec (2 ± 0.7). The male guards the female from other males during mating.

Post-holding: After the transfer, the male came back to the holding position and held the female within its last three pereopods; this position lasted 15–20 min. The female moved out once the pereopods were pulled off.

DISCUSSION

In the population of *T. hainanensis* studied, most of the males on average were typically smaller than the females. The NPM individuals sampled gradually converted their sex to females in a long amount of time with increased body size (after two successive moults). The information on secondary sexual characters and behavioural observations during the trials indicate that NPM individuals and females are protandric sequential hermaphrodites. Shrimp that are initially born and reproduce as non-prehensile males later changed their sex to female and stay female presumably for the rest of their lives.

The sampled population of *T. hainanensis* was composed of prehensile males ($N = 58$), which outnumbered the non-prehensile males ($N = 26$) and females ($N = 16$). Likewise, the smaller size classes of in *T. manningi* were dominated by NPM and PM individuals, which suggested protandry ([Bauer, 1986](#)), whereas the smaller size classes in *T. floridanus* and *T. dobkini* were dominated by PM individuals and juvenile females ([Bauer & VanHoy, 1996](#)). In *Crangon franciscorum* [Stimpson, 1856](#), females represented the

largest -size class, which was evident from the shorter life span of males that made an early transition to females (Gavio *et al.*, 2006). Large females and small males were seen in several species of Pandalidae, Hippolytidae and Crangonidae shrimps (Bauer, 2004). The availability of high prevalence of females during all seasons has been documented in most of free-living, as well as symbiotic, caridean shrimps (Patton *et al.*, 1985; Possenti *et al.*, 2007; Rajool *et al.*, 2012; Prakash *et al.*, 2021). The dominant availability of PM individuals in *T. hainanensis* could be due to the sampling discrepancy as the duration of the collection was restricted to a few months and did not consider potential seasonal variations. We therefore suggest additional sampling throughout the year to investigate the population dynamics of the populations of *T. hainanensis* in Lakshadweep.

Carapace and appendix masculina lengths showed a linear relationship, which agrees well with the observations by Bauer (1986) in *T. manningi*. AML, however, was observed to be greater for PM than NPM in *T. hainanensis*. The basal flange width is documented less for non-prehensile males and more for females, which agrees well with the observations of Bauer & VanHoy (1996) in *Sicyonia dorsalis* Kingsley, 1878. The length of the abdominal pleuron overlaps in NPM and PM, but it is larger in the *T. hainanensis* females.

Although *T. hainanensis* exhibits partial protandry in primary males, *T. floridanus* and *T. dobkini* were reported as gonochoric (Bauer & VanHoy, 1996). Baeza & Piantoni (2010) studied the sexual system of *T. amboinensis* under SEM and identified males, females, and a few transitional individuals in the population, suggesting simple protandry. Terossi *et al.* 2022, however, found that the *T. amboinensis* in Baeza & Piantoni (2010) was actually *T. dicaprio* Anker & Baeza, 2021.

Males have been found to identify a receptive female and, in a brief interaction, transfers the spermatophore after which both partners separate and go their own way (Bauer 1976, 1979; Boddeke *et al.*, 1991; Bauer & Holt, 1998; Bauer & Abdalla, 2001). *Thor dicaprio* exhibit a pure search mating system (Baeza & Piantoni, 2010). In another tropical caridean *Rhyncocinetes uritai* Kubo, 1942, which exhibits pure searching, males could recognize the upcoming moulting event a few minutes in advance, either by approaching or touching the female. After the moulting, females show a series of vigorous tail flips with males in pursuit (Bauer & Thiel, 2011), which is similar in *T. hainanensis*. Multiple males of *R. uritai* clung to the female and it was not possible to identify whether only one or more males transferred a spermatophore, indicating multiple copulations. By contrast, in *T. hainanensis*, where the male on top of the female did not allow any other males to come close. Females become unattractive to males after spermatophore transfer, avoiding further male contact. Males do not disengage females immediately after spermatophore transfer in *T. hainanensis* but rather continue to be in the straddle position for a long time after the transfer, which suggests a 'search and defend' mating strategy.

S1 Video. Mating sequence of *Thor hainanensis*.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

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Probing the thermo tolerant endosymbiont genus *Durusdinium* (Clade D) in the scleractinian corals of Palk Bay, Southeast coast of India

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Abstract

The world's coral reef ecosystems are built by a symbiotic relationship between reef corals and the dinoflagellates of the family Symbiodiniaceae. Climate change has already impacted the world's coral reef ecosystems. Some corals can survive in extreme environmental conditions through the acquisition of stress-tolerant endosymbionts. In the present study, the genetic diversity of endosymbionts of fourteen coral genera such as *Porites* (Five), *Favia* (Two), and *Acropora* (Seven) sampled from the reefs of Mandapam and Rameswaram, Palk Bay, Tamil Nadu, India was assessed by sequencing both large (LSU) and small subunit (SSU) gene fragments. The phylogenetic construction of LSU revealed the diversity of thermo tolerant Clade D that was monophyletic throughout various coral taxa. Comparison of thermo tolerant clade D with SST (> 32 °C) has provided valid evidence for the presence of the endosymbiont *Durusdinium* sp. (Clade D) across different coral species in the Palk Bay.

Keywords Symbiodiniaceae · India · *Durusdinium* · Thermotolerant · Coral Bleaching

Introduction

Zooxanthellae, the unicellular endosymbionts of the family Symbiodiniaceae, exist in a symbiotic relationship with varieties of marine invertebrates like Scleractinian corals, octo-corals, sea anemones, giant clams and protists (LaJeunesse et al. 2003; Meyer and Weis 2012; Shinzato et al. 2014). The biomass production of coral reef ecosystems mainly depends on the symbiont cell density in the host corals. Because ninety percent of the photosynthetic products of endosymbionts are utilized by host organisms and the inorganic waste materials released by the hosts are supplied as nutrients to symbiotic algae (Neo et al. 2015; Reich et al. 2017). This association is essential for the formation of large and important reef structures in coral reef ecosystems (Fransolet et al. 2012). It is evident, that the major risk to the coral reef ecosystem is the expulsion of symbionts and/or pigmentation (Medrano et al. 2019) from host corals due to thermal stress (increasing SST), an event known as 'bleaching of corals'. The bleaching events thus break the connectivity between them. Since the mass coral bleaching that occurred in 1998, the frequency of coral bleaching and the continuous decline of reef covers have been observed frequently till today. This could be due to the increase

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in SST coupled with El Nino events, ocean acidification, pollution and sea level rise (Hughes et al. 2018). The symbiont genetic variability between taxa may change with different environmental conditions of the reef ecosystems (LaJeunesse et al. 2010a, b; Keshavmurthy et al. 2014).

The endosymbionts are classified into nine different clades (A to I) with several sub-phylogenies (LaJeunesse et al. 2012). Normally the reef corals of Indo-Pacific regions have an association with the endosymbiont genera *Symbiodinium*, *Breviolum*, *Cladocopium*, and *Durisdinium* (formerly clades A–D) (Baker 2003) followed by *Fugacium* and *Gerakladium* (formerly clades F & G) (LaJeunesse et al. 2010a) belonging to the family Symbiodiniaceae. The genus *Durisdinium* fits into the group ‘extremophiles’ because of living in different environmental conditions such as thermal stress, high-temperature fluctuations, sedimentation, and high-latitude reef corals (LaJeunesse et al. 2010a; LaJeunesse et al. 2018; Carballo- Bolaños et al. 2019). When compared to the other symbiont genera, the establishment and abundance of *Durisdinium* symbionts of reef corals observed frequent bleaching incidences (Silverstein et al. 2015). Thus, the endosymbionts belonging to the genus *Durisdinium* have received a lot of attention from researchers because it is an important indicator for coral reef health monitoring and conservation.

Before 2018, no single study on the genetic diversity of coral endosymbionts in Indian coastal waters was published. However, Thinesh et al. (2019) just completed the genus-level identification of endosymbiont from the corals of Palk Bay. The authors indicated that two endosymbiont genera such as *Cladocopium* and *Durisdinium* were detected from the Palk Bay corals using LSU, SSU, Cyt *b* and ITS markers (Thinesh et al. 2019). The SSU rDNA allowed them to identify the family or genus level while the LSU rDNA is the most suitable marker for species-level identification (Gomez 2014). In this study, we have intended to identify single genus or multiple genera of endosymbionts harboring of reef corals of Palk Bay, India since the reef corals have a symbiotic association with six types of endosymbionts such as A to D and F and G (Baker 2003; LaJeunesse et al. 2010a). Further, we need to verify the symbiont diversity of corals of Palk Bay, India to find out the distribution of thermally adapted symbionts or any other related clades. In this study, we have sampled coral colonies from Mandapam and Rameswaram north regions of Palk Bay, India and identified the symbiont genetic diversity using LSU nr DNA and SSU nr DNA genetic markers.

Materials and methods

From 01 April 2017 to 30 August 2017, coral colonies belonging to the three major taxa such as *Acropora*, *Porites* and *Favia* were targeted for observing the bleaching. In

the present study, the coral colonies from the the genera *Acropora* ($n=7$), *Porites* ($n=5$) and *Favia* ($n=2$) were collected from the reef ecosystem of Palk Bay Coast, India (Fig. 1). The colonies (approximately 3–5 cm in size) were sampled from two sites such as Mandapam and Olaikuda, Rameswaram north regions between April 2017 and August 2017 (Fig. 1) (LaJeunesse et al. 2003) at the depth between 1.5 and 3.0 m using a hammer and chisel by SCUBA Divers. The GPS coordinates of sampling sites were recorded using Garmin GPSMAP 78sc. Before collection, the underwater photographs were taken on both the bleached as well as healthy corals using a Nikon DSLR camera (Model # D7000, Japan) including underwater housing Ikelite, USA) (Fig. 2).

Both the bleached and healthy coral colonies were collected and placed in a NASCO whirl sampling bag (HIMEDIA) containing 0.2 µm filtered seawater instead of ethanol/ice cold Zooxanthellae Isolation buffer (ZIB) (Santos et al. 2003). Upon sampling, corals were transferred to the surface and placed immediately in a Euroangel Mobile refrigerator unit (below 0 °C) and transported to the laboratory at Pudumadam in Ramnad district, Tamil Nadu, India for further analysis. The corals were identified up to species level based on their morphological characteristics of sampled corals and with the help of *in-situ* photographs based on standard literature (Veron 2000; Venkataraman et al. 2003; Venkataraman and Satyanarayana 2012; George and Sandhya 2007) (Fig. 2).

The isolation of endosymbionts was carried out (Rowan and Powers 1991a, b; Chen et al. 2003) with a modification of the protocol. Before isolation, the bags were separately wrapped and shaken vigorously for 1–3 min to disrupt the mucopolysaccharides. The centrifugation of isolated coral endosymbiont was done at 10000 rpm (REMI cooling centrifuge-Model No. CPR 27) for 5 min. The pellet was mixed with 30 mL extraction buffer containing Triton X-100 (0.01%) and the mixture was homogenized in a tissue grinder. This slurry was centrifuged at 7000 rpm in a 10 mL centrifuge tube using a REMI cooling centrifuge (CPR 27). A few drops of filtered seawater (1.2 µm) with EDTA (5 mM) were added in this pellet immediately and shaken gently for mixing. The yellowish pellet obtained after frequent washing was stored in the refrigerator at –20 °C for DNA extraction. After extraction, the coral sample was separately bleached and kept in a small museum established in the Marine Field Research Station located at Pudumadam in Ramnad District, Tamil Nadu, India and provided catalogue number.

The extraction of total genomic DNA of endosymbionts was carried out using the method described by Rowan and Powers (1991a, b) and Chen et al. (2003) and PCR amplification was done with specific LSU [28S_{zooxF}—5′—CCT CAG TAATGG CGAATGAAC-3′; 28S_{zooxR}—5′—CCT

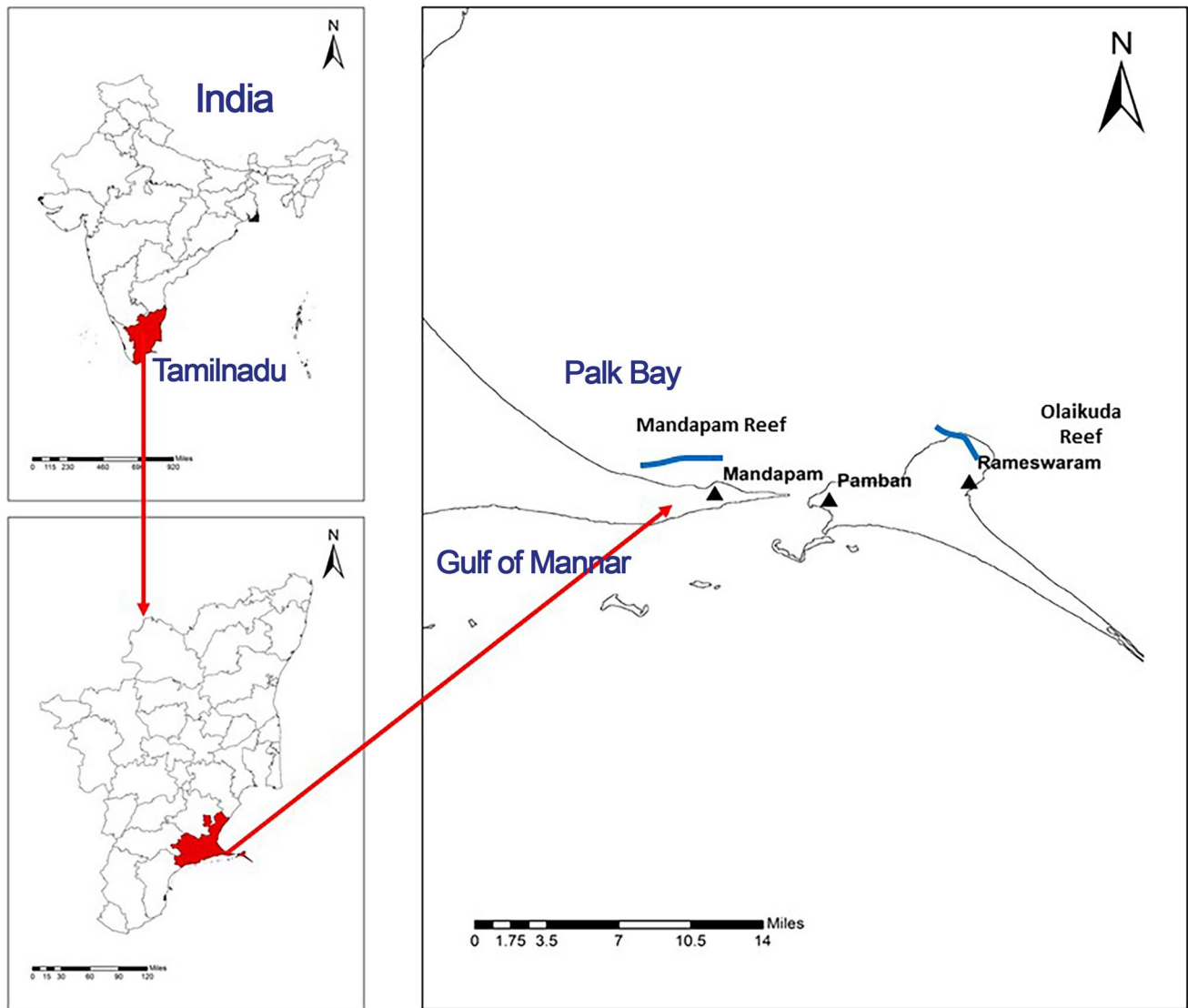


Fig. 1 Map showing the study area Palk Bay (Mandapam and Olaikuda reefs) where the coral-zooxanthellae samples were collected

TGG TCC GTG TTT CAA GA-3' and SSU [Ss5 forward-5'-GGTTGATCCTGCCAGTAGTCATATGCTTG-3'; Ss3Z reverse-5'-AGCACTGCGTCAGTCCGAATAATT CACCGG-3'-Rowan and Powers 1991a]. The PCR amplifications were done using the Thermal Cycler (Eppendorf Master Cycler) and the conditions include initial denaturation at 94 °C for 3 min, followed by denaturation at 94 °C for 1-min, annealing ranges from 48–56 °C for 1 min, elongation at 72 °C for 2 min and a final extension at 72 °C for 10 min. To check the integrity of the PCR product, we run the agarose gel electrophoresis (0.8%) and the quantification was done using Nanodrop (Thermo Scientific ND 2000). The bidirectional sequencing of PCR products was done from Macrogen Inc, Korea. The sequences were deposited in the NCBI, USA and the GenBank Accession numbers were also obtained.

Alignment of each set of sequences (LSU and SSU) was performed in MUSCLE (Edgar 2004) as implemented in MEGA 7.0 (Kumar et al. 2016). The datasets (LSU and SSU) were analyzed with the software jModelTest 2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012) to compare the different models of DNA substitution models will be selected that best fits each dataset under the hierarchical-hypothesis framework testing.

We performed a molecular phylogenetic analysis to test the phylogenetic position of the *Durusdinium* clade D based on the LSU and SSU genetic markers (Gomez 2014). Fourteen sequences each of LSU and SSU markers generated during the present study and 10 sequences: three sequences of *Durusdinium* belong to clade D, one sequence of *Cladocopium* belong to Clade C, two sequences *Symbiodinium minutum* and *S. antilloorgium*

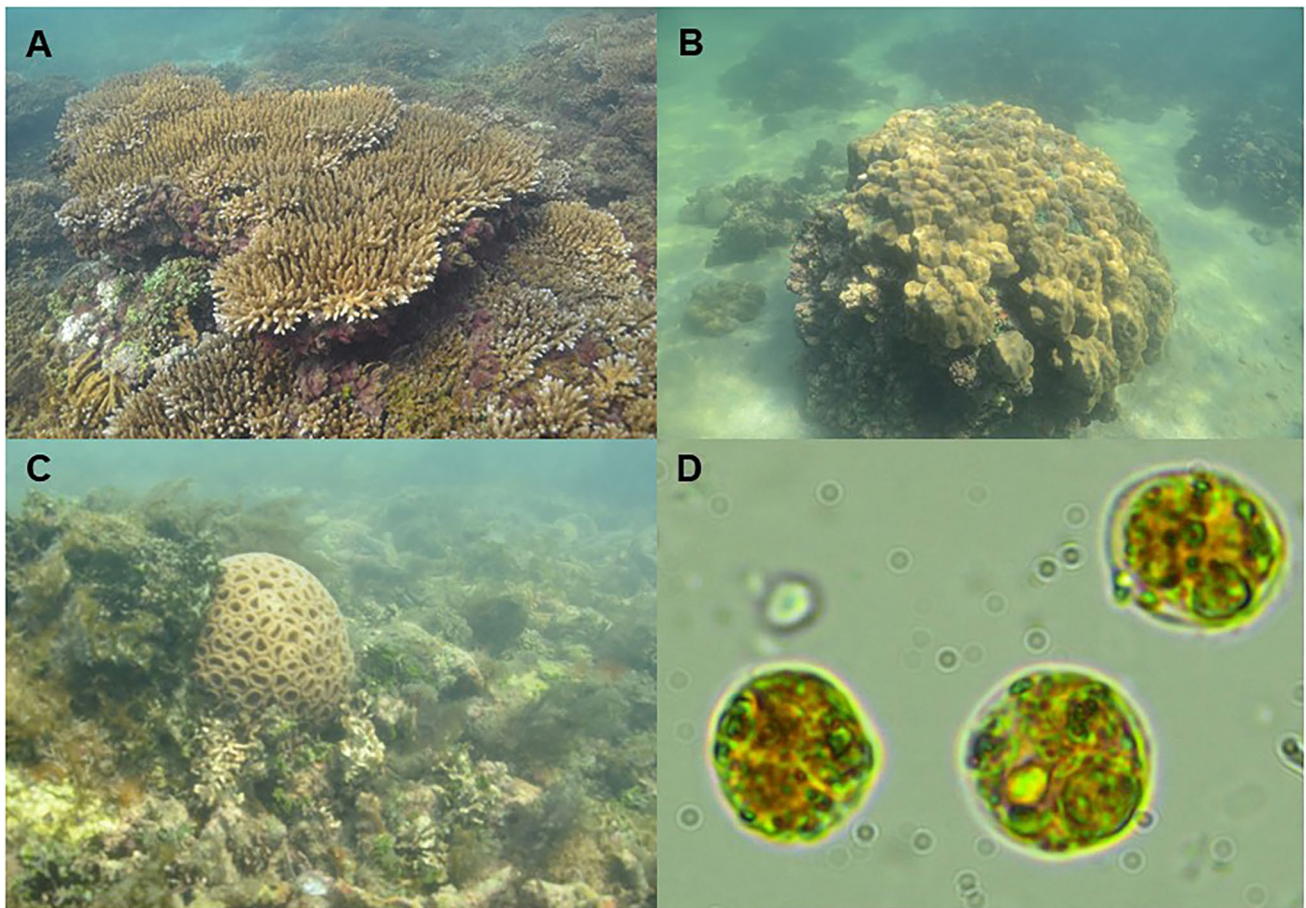


Fig. 2 Underwater photograph of *Acropora* sp. (A), *Porites* (B) and *Favia* (C). Microscopic image of *Durusdinium* sp. isolated from the corals

belong to Clade B and four sequences of *Symbiodinium* (*S. linuchae*, *S. microadriaticum*, *S. pilosum*, *S. natans*) belong to Clade A obtained from GenBank database were used as ingroup terminals and 1 sequence of *Pelagodinium beii* were used as an outgroup terminal. Since the models identified by the jModelTest were not available in the web server, we used the GTR + G + I evolutionary model that was included by the jModelTest within 95% confidence interval. To perform the phylogenetic analysis, we used the webserver W-IQ-TREE (Trifinopoulos et al. 2016; <http://iqtree.cibiv.univie.ac.at/>) for Maximum Likelihood (ML) analysis. The parameters that are used for ML analysis were those of default options that are available in the webserver W-IQ-TREE. The robustness of the ML tree.

Further, the poorly aligned positions or divergent regions of SSU were more which may misguide the tree if we perform concatenated analysis. Hence, only single gene trees were performed and the SSU did not reveal the expected outcome of delineating thermotolerant clades (Supplementary Fig. S1). Lastly, pairwise genetic distances (intra- and inter-specific) between the sequences of SSU and LSU genes were calculated in MEGA 7 (Kumar

et al. 2016) using the Kimura 2-Parameter model (Kimura 1980).

The temperature data was observed throughout the study period using Thermosensor (Hanna Instruments, USA). To confirm our ground data for temperature, we acquired additional information from National Oceanic and Atmospheric Administration (NOAA) based on real-time satellite data as suggested by Reynolds et al. (2002).

Results

The NOAA satellite data for temperature indicated the average sea surface temperature (SST) was between 28 to 32 °C (Fig. 3A-E) throughout the study period. Further, the comparison with ground truth observation of sea surface temperature at the study site (Mandapam and Olaikuda reefs) exhibits a similar range. For instance, the minimum average SST was found to be $27.42\text{ }^{\circ}\text{C} \pm 0.23$ and the maximum SST was $31.07\text{ }^{\circ}\text{C} \pm 0.26$. The minimum temperature of $26.11\text{ }^{\circ}\text{C}$ was recorded in July 2017 and the maximum temperature of 32.05 was recorded in April 2017 (Fig. 3F).

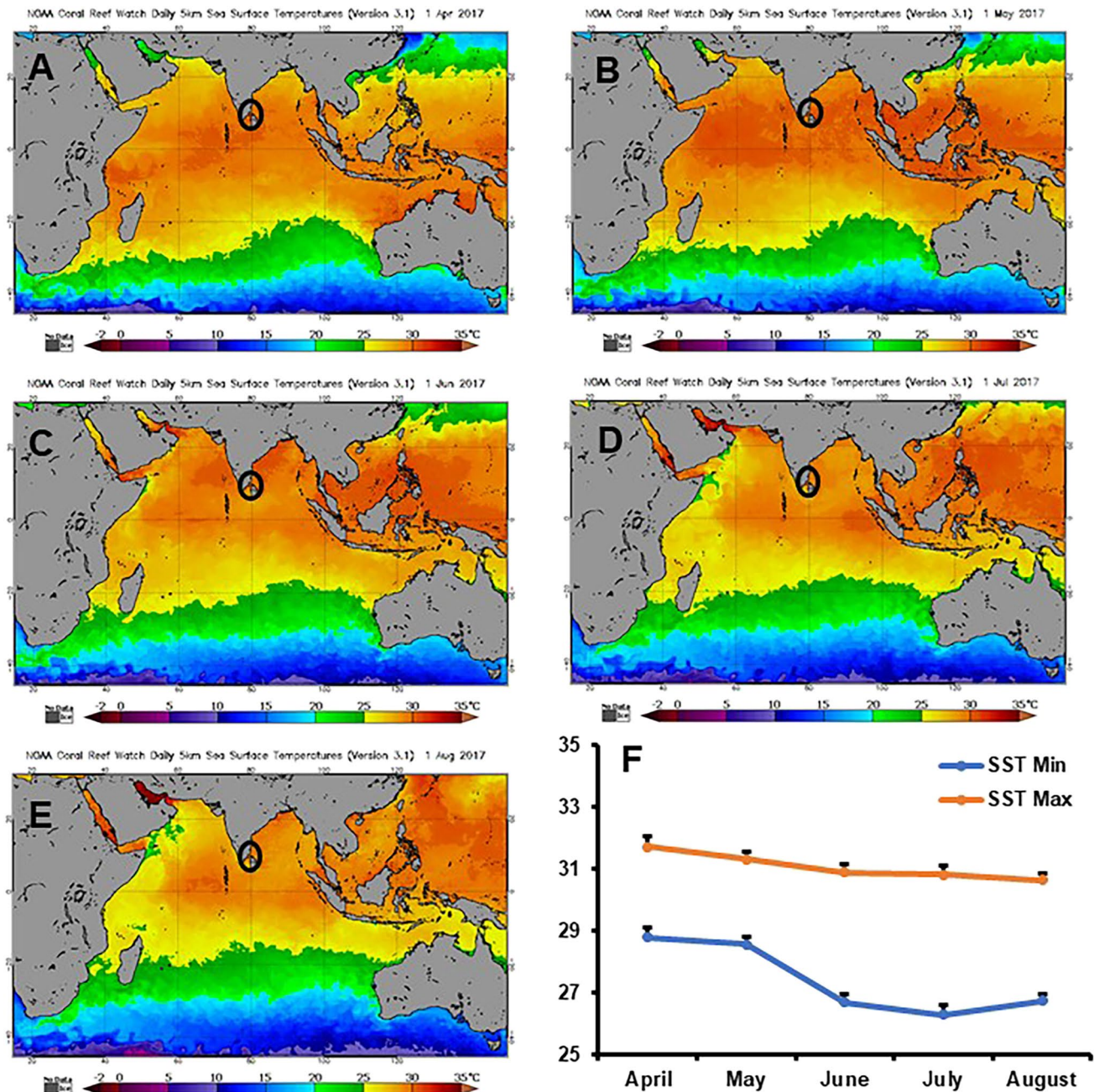


Fig. 3 Satellite imagery on Sea Surface Temperature (SST) data obtained from NOAA during the study period April to August 2017 (A–E). Sea surface temperature collected on ground during the study period (F)

Few of the coral colonies were found to be bleached during April to June when the temperature exceeds 31 °C and the slow recovery was evident in July and August 2017.

In our phylogenetic analyses, the final aligned molecular data set of LSU was 548 out of which 202 were parsimony informative sites. In SSU, the final aligned positions were 1591 out of which 200 were parsimony informative sites. The sequence analysis using the BLAST-N tool showed >99% identity with the genus *Durusdinium*. The

GenBank accession numbers obtained from NCBI were MG945916–MG945922 and MG945930–MG945936 for LSU and MG945923–MG945929 and MG945937–MG945943 for SSU regions (Table 1). For the 28 sequences we generated in this study, we found the sequences (LSU and SSU) were nested within the clade D of the thermo-tolerant genus *Durusdinium*. Based on LSU, clade D is sister to other major clades of such as Clade A, B and C. The ML analysis revealed similar tree topologies for both LSU

(Fig. 4) and SSU (supplementary file S1). These thermal tolerant clades confirmed that the Clade D *Durusdinium* was widely distributed among the coral genus *Acropora* ($N=7$), *Porites* ($N=5$), and *Favia* ($N=2$) from the Palk Bay region of South India.

In addition, the phylogenetic analysis of LSU tree was supported by the genetic divergence calculated within and between the clades. The pairwise genetic distance within the *Durusdinium* thermotolerant clade D ranges from 0 to 0.7%. Subsequently, the genetic distance between the *Durusdinium* and other clades A, B and C were ranging from 21.1 to 26.7%, 21.1 to 24.4% and 17.6 to 17.9% respectively which is well comparable to the genetic distance observed between *Durusdinium* clade D and the outgroup *Pelagodinium beii* 28.5 to 30.2%.

Discussion

Interestingly, the present study confirms the exclusive association and the dominance of thermo tolerant endosymbiont genus *Durusdinium* (formerly Clade D Type) in all 14 coral colonies in the genera *Porites*, *Favia* and *Acropora* collected from Mandapam and Olaikuda reef regions of Palk Bay based on LSU and SSU genetic markers. A similar uniform population of the genus *Durusdinium* has been reported in coral *Goniastrea aspera* sampled from Phuket, Thailand during several episodes of irradiance and thermal stress indicating that the genus *Durusdinium* has high levels of stress and thermal tolerance (Brown et al. 2002). Several studies in the past have increased our understanding of the diversity and distribution of *Symbiodinium* and their association with

coral species concerning their stress tolerance (Chen et al. 2003; LaJeunesse et al. 2010a, b; Hume et al. 2015, 2016; Thinesh et al. 2019). Intense sampling efforts and multi locus sequencing using standard markers such as ITS and mitochondrial cytochrome *b* of *Symbiodinium* clade D could reveal the hidden subtypes within the clades.

After intermittent bleaching or thermal stress, the corals has been harboring or replacing the symbionts of the genus *Durusdinium* that are considered opportunistic generalist endosymbionts, whereas other symbionts are unable to tolerate stress caused by high irradiance (Abrego et al. 2009), higher temperature (except *Symbiodinium thermophilum* see Hume et al. 2015, 2016) and sedimentation (Toller et al. 2001; Garren et al. 2006; Cooper et al. 2011). Thornhill et al. (2006) state that the corals associated with the genus *Durusdinium* is more resistant to thermal coral bleaching incidents and thus more abundant after bleaching events. The previous studies from the waters of neighboring countries such as the Persian Gulf suggests that Clade D *Symbiodinium* (= *Durusdinium*) was most abundant in the coral colonies that can withstand higher temperatures (Baker et al. 2004). Likewise, the preliminary survey on the seven species of Singapore corals revealed the prevalence of clade D *Symbiodinium* species coping against the higher temperature and sedimentation in the Singapore waters. This dominance in clade D zooxanthellae in corals indicates the importance of symbiotic relationships and the persistence of the impacted coral reefs of Singapore (Tanzil et al. 2016). Furthermore, a study carried out in the South China sea also indicates the prevalence of clade D in the common coral species and are coping with the changing environmental stress (Tong et al. 2018).

Table 1 Host coral species, sampling location, GPS coordinates, Depth, Clade type and GenBank accession numbers of Coral Symbionts of Palk Bay, Southeast cost, India

S. No	Host coral	Depth sampled (m)	GPS coordinates	LSU (28S)	SSU (18S)	Clade identified
Mandapam						
1	<i>Porites</i> sp.	3.0	9.28769°N 9.15732°E	MG945922	MG945925	<i>Durusdinium</i> (D)
2	<i>Porites</i> sp.	2.5	9.28757°N 9.15850°E	MG945918	MG945926	<i>Durusdinium</i> (D)
3	<i>Porites</i> sp.	3.0	9.28757°N 9.15850°E	MG945919	MG945927	<i>Durusdinium</i> (D)
4	<i>Porites</i> sp.	3.5	9.28757°N 9.15855°E	MG945920	MG945928	<i>Durusdinium</i> (D)
5	<i>Porites</i> sp.	2.5	9.28757°N 9.15855°E	MG945921	MG945929	<i>Durusdinium</i> (D)
6	<i>Favia</i> sp.	3.0	9.28757°N 9.15855°E	MG945916	MG945923	<i>Durusdinium</i> (D)
7	<i>Favia</i> sp.	3.0	9.28757°N 9.15855°E	MG945917	MG945924	<i>Durusdinium</i> (D)
Olaikuda, Rameswaram						
8	<i>Acropora</i> sp.	1.5	9.29996°N 79.3311°E	MG945930	MG945937	<i>Durusdinium</i> (D)
9	<i>Acropora</i> sp.	2.0	9.29996°N 79.3311°E	MG945931	MG945938	<i>Durusdinium</i> (D)
10	<i>Acropora</i> sp.	2.0	9.30433°N 79.3334°E	MG945932	MG945939	<i>Durusdinium</i> (D)
11	<i>Acropora</i> sp.	1.5	9.30433°N 79.3334°E	MG945933	MG945940	<i>Durusdinium</i> (D)
12	<i>Acropora</i> sp.	1.5	9.30692°N 79.3346°E	MG945934	MG945941	<i>Durusdinium</i> (D)
13	<i>Acropora</i> sp.	2.5	9.29996°N 79.3311°E	MG945935	MG945942	<i>Durusdinium</i> (D)
14	<i>Acropora</i> sp.	1.5	9.29996°N 79.3311°E	MG945936	MG945943	<i>Durusdinium</i> (D)

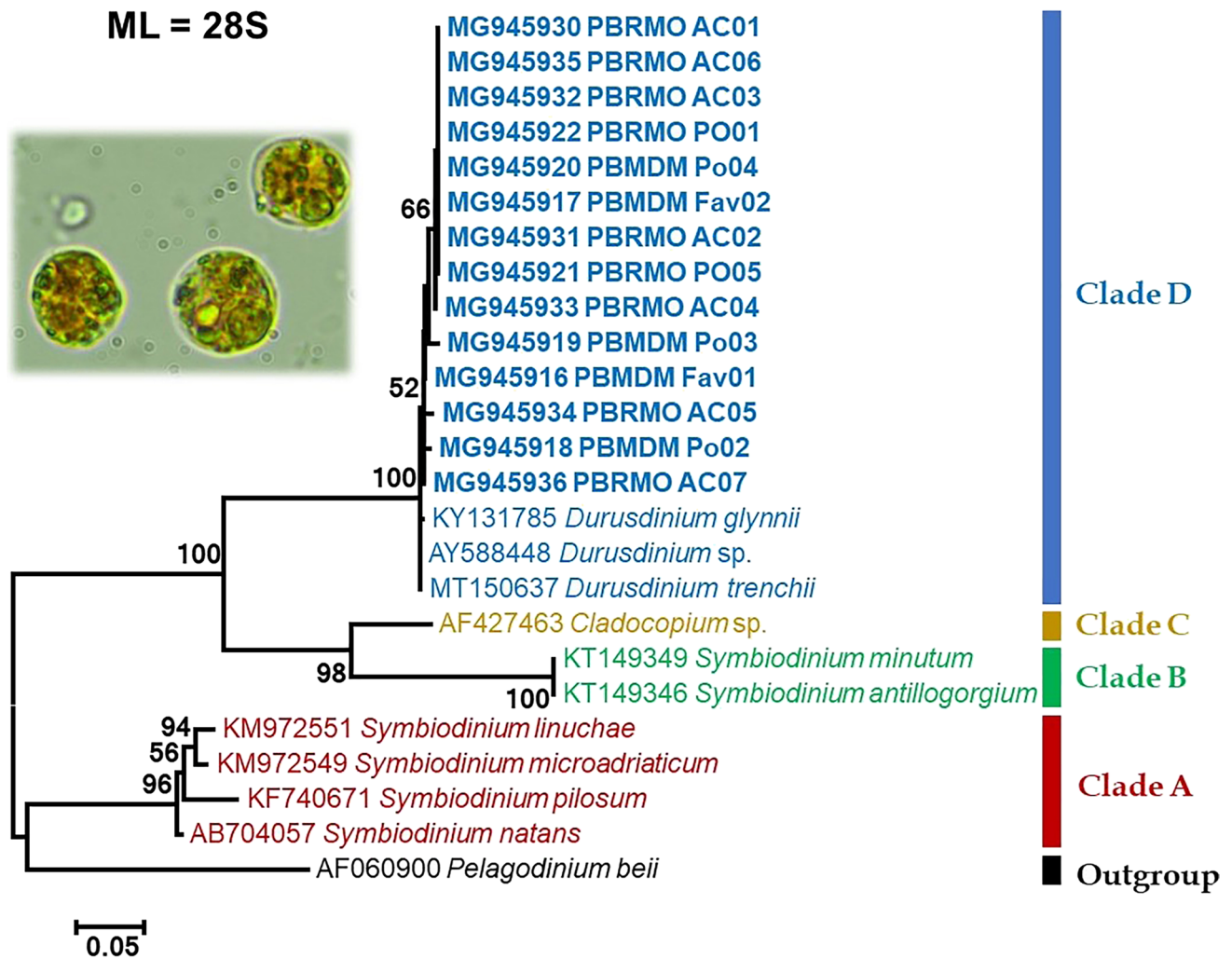


Fig. 4 A Phylogenetic tree (ML) constructed based on the 28S large subunit (LSU) gene fragments indicates clade D *Durusdinium* separated from other *Symbiodinium* group. Numbers above and below the

lines represent bootstrap values from maximum likelihood analysis. Inset picture shows the microscopic image of *Durusdinium*

Therefore, it is more authentic that the prevalence of genus *Durusdinium* clade D across the different coral colonies of *Acropora*, *Favia* and *Porites* clearly states that the Palk Bay reefs are frequently exposed to higher temperatures and bleaching events. The above stresses might indicate the ecological succession of the endosymbiont *Durusdinium* in the Palk Bay region as suggested by LaJeunesse et al. (2010a, b). Although the specific environmental conditions driving the diversity and abundance of *Durusdinium* clade D remain uncertain, there could be some underlying mechanisms that hold the secret to the persistence of coral-symbiont partnership during temperature stress in the Palk Bay.

El Niño Southern Oscillation (ENSO) driven mass coral bleaching event was evident in many parts of the world including Indian coral reefs in 1998 due to the rapid increase in sea surface temperature especially between April to July (Arthur 2000). As coral mortality has profound implications

on the ecology and economic services offered by the reef ecosystems, the mass coral bleaching event has urged researchers to continuously monitor the reefs for conservation and sustainability. Subsequently, ENSO-related bleaching events were also observed in 2010 and further as reported in several studies (Kumaraguru et al. 2003; Ravindran et al. 2012a, b; Ajith Kumar et al. 2012; Patterson et al. 2015; Manikandan et al. 2016). Most of the coral taxa that were usually susceptible to coral bleaching such as *Acropora*, *Porites* and *Favia* were relatively unaffected during the present study. We found that the coral host species were dominated by the Clade D *Durusdinium* which either showed less or no coral bleaching during the survey. However, the previous reports were mainly based on survey-based studies and did not refer much to the molecular studies to indicate the prevalence of thermo tolerant clade D. To extend this claim fully, additional sampling efforts are

required across different species of corals in the Palk Bay reefs. Because the present study includes only 3 coral genera and the endosymbiont samples only from 14 colonies.

Previous studies suggested that *Acropora* spp. associated *Symbiodinium* with both clade C and D can shift their endosymbiont following thermal stress (Baker 2003; Fabricius et al. 2004; Jones et al. 2010). This was evident from a recent study in the genus *Acropora* as both clade C and D were observed in the healthy corals following bleaching events but not in other coral genera (Thinesh et al. 2019). The absence of other *Symbiodinium* clades in this study would be due to insufficient sampling. However, our results indicate a high incidence of *Durusdinium* clade D across three coral genera sampled in the Palk Bay reefs. The shift between the endosymbiont species clade C and D is always transient and the symbionts could return to their original composition when the optimum environmental conditions are back post-bleaching (Jones et al. 2008; Coffroth et al. 2010). This flexibility in the displacements, shifting or shuffling of symbionts would provide ample opportunities to the coral host-symbiont relationship to increase the survivorship and resilience to bleaching and mortality during extreme events or other environmental stress conditions (Baker 2003; Fabricius et al. 2004).

One possible explanation for the occurrence of *Durusdinium* in our study could be associated with the above phenomenon that the coral host would have shuffled the endosymbiont species during the thermal stress when the average mean SST was more than 32 °C in Palk Bay reef areas. The present study may serve as a baseline record for the endosymbiont *Durusdinium* clade in the thermal tolerant coral host in the Palk Bay. However, the samples were collected and sequenced only for a short duration. Information on the temporal distribution pattern of *Durusdinium* and the coral-host symbiont relationships in the Palk Bay is worthy to explore. Furthermore, our study also limits the ability to detect detailed information on the *Durusdinium* clade D phylotypes as we used only a 28S large subunit marker to understand the phylogenetic relationship. More advanced methods including whole genome sequencing or identification of single nucleotide polymorphisms (SNPs) could be considered to establish a consensus nomenclature for the *Durusdinium* diversity in the Palk Bay reefs.

The second possible explanation is that the displacement or shifting of endosymbiont may protect the corals only to a certain extent. Because the presence of anti-stress defence mechanisms such as heat shock proteins, antioxidant enzymes and other proteins may also play an important role to minimize the damage to their endosymbionts during thermal stress (Lesser 2006; Brown et al. 2002; Baird et al. 2009). A recent study on the adaptive evolution of genomes in the *Symbiodinium* revealed the positive selection of genes that are involved in several functions such as photosynthesis, transmembrane ion transport, synthesis

and modification of amino acids, glycoproteins and stress response. The study also suggests that the adaptive capacity of the endosymbionts is critical in maintaining sustainable coral host-endosymbiont relationships in the face of changing environmental conditions. Hence, the presence or absence of thermo tolerant clade D may not be the only indicator of resistance in corals against climate extremes.

Lastly, the exclusive dominance of thermo tolerant endosymbiont belonging to the genus *Durusdinium* (clade D) in the Palk Bay reefs indicates that these reefs are constantly exposed to a combination of environmental stressors such as high heat, salinity and turbidity annually. Therefore, it is imperative that we must try to alleviate the stress levels on these iconic reef ecosystems. Though the global climate change including global warming, acidification and sea level rise are major threats, local or regional level damages such as fishing pressures, sedimentation and other physical disturbances to the Palk Bay reefs can be persistently reduced to ensure the sustainability and conservation of reefs.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11756-022-01235-z>.

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Author contribution **Murugesan Rajesh Kannan:** methodology, software, validation, formal analysis, investigation, data curation, writing-review and editing; **Ramkumar Balakrishnan:** methodology, formal analysis, investigation; **Muneeswaran Thillaichidambaram:** methodology, software, validation, formal analysis, writing-review and editing; **Sivakumar Natesan:** software, validation, formal analysis, writing-review and editing; **Gunsekaran Paramasamy:** Conceptualization, writing-review and editing, visualization, project administration, funding acquisition; **Sanjeevi Prakash:** software, validation, formal analysis, data curation, writing-review and editing; **Chockalinagam Muthiah Ramakritinan:** Conceptualization, methodology, validation, investigation, resources, data curation, writing-original draft preparation, writing-review and editing, visualization, visualization, supervision, project administration, funding acquisition.

Data availability All the data generated during the study were mentioned in the manuscript.

Declarations

Ethics approval For the collection of coral tissue samples from Palk Bay necessary permission have been obtained from the Tamil Nadu Forest Department and Principal Chief Conservator of Forests, Ministry of Environment, Forest and Climate Change (MoEFCC), New Delhi and Chief Wildlife Warden, Ramanathapuram via file No. WL(A)/33128/2016 prior to the study.

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Facile synthesis of Mn–Ni bimetal organic framework decorated with amine as an electrode for a high-performance supercapacitor

Original Paper | Published: 20 January 2023

Volume 27, pages 911–925, (2023) [Cite this article](#)

[Sruthi Rajasekaran](#), [B. Shalini Reghunath](#), [Sunaja Devi K. R.](#) , [B. Saravanakumar](#), [J. Johnson William](#), [Dephan Pinheiro](#) & [Madan Kumar Arumugam](#)

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Abstract

In the recent years, the whole world is looking for better energy storage devices. Supercapacitors are among the most promising high-capacity energy storage devices. Manganese-based material has gained more importance among transition metals due to its cost, easy fabrication, and wide potential applications. Here, we report the synthesis of a novel MOF using metal centers, dicarboxylate ligand, and aminoterephthalic acid as a co-ligand {Mn–Ni–NH₂(h₂fipbb)}MOF. The synthesized material has a unique hierarchical morphology consisting of manganese and nickel connected with NH₂ through h₂fipbb ligand linkage, which is highly efficient for the permeation of the electrolyte and electron transfer. The {Mn–Ni–NH₂(h₂fipbb)}MOF shows an excellent specific capacitance of 711.60 F g^{−1} using 2 M KOH at a current density of 1 A g^{−1}. The two electrode system material exhibits a high-power and energy density of 743.99 W kg^{−1} and 20.49 Wh kg^{−1}, respectively. From the above results, the synthesized {Mn–Ni–NH₂(h₂fipbb)}MOF can be



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Article

Lipidomic Analysis of Liver Lipid Droplets after Chronic Alcohol Consumption with and without Betaine Supplementation

by Madan Kumar Arumugam ^{1,2,3} , Sathish Kumar Perumal ^{1,2} , Karuna Rasineni ^{1,2} ,
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From the journal:
RSC Advances

Recent trends in macromolecule-conjugated hybrid quantum dots for cancer theranostic applications



[Lokesh Kumar Boopathy,^a](#) [Thiyagarajan Gopal,^b](#) [Anitha Roy,^c](#) [Rakhee Rathnam Kalari Kandy^d](#) and [Madan Kumar Arumugam](#)



[⊕](#) [Author affiliations](#)

Abstract

Quantum dots (QDs) are small nanoparticles with semiconductor properties ranging from 2 to 10 nanometers comprising 10–50 atoms. The single wavelength excitation character of QDs makes it more significant, as it can excite multiple particles in a confined surface simultaneously by narrow emission. QDs are more photostable than traditional organic dyes; however, when injected into tissues, whole animals, or ionic solutions, there is a significant loss of fluorescence. HQD-based probes conjugated with cancer-specific ligands, antibodies, or peptides are used in clinical diagnosis. It is more precise and reliable than standard immunohistochemistry (IHC) at minimal protein expression levels. Advanced clinical studies use photodynamic therapy (PDT) with fluorescence imaging to effectively identify and treat cancer. Recent studies revealed that a combination of unique characteristics of QDs, including their fluorescence capacity and abnormal expression of miRNA in cancer cells, were used for the detection and monitoring progression of cancer. In this review, we have highlighted the unique properties of QDs and the theranostic behavior of various macromolecule-conjugated HQDs leading to cancer treatment.

REVIEW

Potential molecular mechanisms of myrtenal against colon cancer: A systematic review

Lokesh Kumar Boopathy, Anitha Roy, Thiyagarajan Gopal, Rakhee Rathnam Kalari Kandy, Madan Kumar Arumugam✉

First published: 04 September 2023 | <https://doi.org/10.1002/jbt.23525> | Citations: 1

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Abstract

Colon cancer is a serious health problem across the globe with various dietary lifestyle modifications. It arises as an inflammation mediated crypts in the colon epithelial cells and undergoes uncontrolled cell division and proliferation. Bacterial enzymes contribute to a major outbreak in colon cancer development upon the release of toxic metabolites from the gut microflora. Pathogen associated molecular patterns and damage associated molecular patterns triggers the NLRP3 inflammasome pathways that releases pro-inflammatory cytokines to induce cancer of the colon. Contributing to this, specific chemokines and receptor complexes attribute to cellular proliferation and metastasis. Bacterial enzymes synergistically attack the colon mucosa and degenerate the cellular integrity causing lysosomal discharge. These factors further instigate the Tol like receptors (TLRs) and Nod like receptors (NLRs) to promote angiogenesis and supply nutrients for the cancer cells. Myrtenal, a monoterpene, is gaining more importance in recent times and it is being widely utilized against many diseases such as cancers, neurodegenerative diseases and diabetes. Based on the research data's, the reviews focus on the anticancer property of myrtenal by emphasizing its therapeutic properties which downregulate the inflammasome pathways and other signalling pathways.



From the journal:

New Journal of Chemistry

Synthesis, characterization, and anticancer activity of Ru(*p*-cymene) complexes bearing pyrazolyl-pyridine type ligands†



Arangasamy Mounica, ^a Arumugam Madan Kumar, ^b Nattamai S. P. Bhuvanesh^c and Mani Ganeshpandian ^{*a}

[Author affiliations](#)

Abstract

Ruthenium arene-based complexes have attracted growing interest in overcoming the lack of efficiency of platinum-based chemotherapeutic drugs. Herein, we prepared two pyrazolyl-pyridine type bidentate ligands using 2-acetyl pyridine with biphenyl-4-carbaldehyde and 1-pyrene carbaldehyde. The isolated ligands were utilized to prepare two new Ru(*p*-cymene) complexes, [Ru(η^6 -*p*-cymene)(bpy)Cl]PF₆ (**Ru-bpy(1)**) and [Ru(η^6 -*p*-cymene)(pyn)Cl]PF₆ (**Ru-pyn(2)**) and characterized well. Single-crystal X-ray diffraction analysis of **Ru-bpy(1)** revealed that the complex adopts pseudo-octahedral geometry. The MTT assay was performed on human breast carcinoma (MCF-7), cervical carcinoma (HeLa-S3) and lung adenocarcinoma (A549) cells to determine the *in vitro* cytotoxicity of the complexes. Although the complexes were found to be insensitive to cervical and lung cancer cells at concentrations of up to 100 μ M, both the complexes showed efficient cytotoxicity in breast cancer cells. Moreover, these complexes exhibited apoptosis-inducing activity and cell-free antioxidant activity.





From the journal:
Dalton Transactions

Polydiacetylene/lipid-coated red-emissive silica nanorods for the sustained release and ameliorated anticancer efficacy of a Ru(arene) complex bearing piperlongumine natural product†



Wickneswaran Ishaniya, ^a, Chezhivan Sumithaa, ^a, Muthuraman Subramani, ^b, Aswathy Karanath-Anilkumar, ^{cd}, Ganesh Munuswamy-Ramanyiam, ^{de}, Arumugam Madan Kumar, ^e, Saravanakumar Rajendran, ^b and Mani Ganeshpandian ^{*a}

[Author affiliations](#)

Abstract

A suitable drug delivery strategy for metallodrugs is as significant as the strategies adopted for an efficient metallodrug design. In this study, piperlongumine, which is isolated from long pepper, is coordinated with a Ru(II)-*p*-cymene moiety to obtain an organoruthenated complex containing the natural product (**Ru(pip)**). The isolated complex shows higher cytotoxicity in MCF-7 breast cancer cells than in THP-1 leukemia and HepG2 liver cancer cells. The IC₅₀ value of the complex in non-cancerous HEK-239 cells is also almost equal to that in MCF-7 cells. Next, with an aim to modulate the antiproliferative activity of Ru(pip) using a drug delivery strategy, the complex is loaded into mesoporous silica nanorods (**MSNRs**), which have a higher surface area than spherical silica nanoparticles. Furthermore, the outer surface of the loaded nanorods is covered with a polydiacetylene-lipid (**PL**) hybrid bilayer. Given the unique optical properties of polydiacetylene, the PL coating modifies non-fluorescent MSNRs into red-emissive particles (**PL-Ru(pip)@MSNRs**), which can be useful for diagnostic applications. The release profile studies reveal that the ene-yne conjugation in the PL coating ensures the sustained release of the complex from nanoparticles in both physiological and simulated cancer cell media. While Ru(pip) exhibits both necrotic and apoptotic modes of cell death, PL-Ru(pip)@MSNRs preferably induce the apoptotic mode of cell death in MCF-7 and THP-1 cells. Also, the nanoformulation exhibits a higher percentage of cell cycle arrest in the G0/G1 phase than Ru(pip), as measured by flow cytometry analysis. In contrast, the *in vitro* antioxidant potency of the complex is decreased after being loaded into PL-coated silica nanoparticles.

Recent advances in photoresponsive-based triazole sensors and their applications in biomaterials

Mani Rajasekar ^a  , Chiterasu Narendran ^a, Jennita Mary ^b, Meenamigai Sivakumar ^b,
Masilamani Selvam ^b

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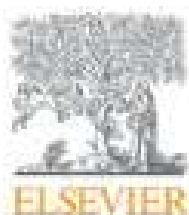
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Abstract

The photoresponsive nature of the triazole sensors introduces a dynamic element to biomaterial sensing, enabling precise control and modulation of their response. The investigation explores the innovative application of triazole sensors with photoresponsive properties in biomaterial sensing. Combining the robust binding capabilities of the triazole scaffold with the adaptability conferred by photoresponsive units, these sensors introduce a dynamic element to sensing, enabling precise control and modulation of their response. The study showcases their versatility in various applications, from cellular imaging to biomarker monitoring, within complex biological environments. Through customization based on specific biomolecules of interest, the sensors exhibit high selectivity, making them valuable tools for unraveling cellular processes and disease mechanisms. Activity testing likely involved synthesizing and characterizing these sensors and evaluating their performance through techniques such as spectroscopy, microscopy, and biochemical assays. The results underscore the



Review on biomaterial applications of photoresponsive based chromophore Hydrogels: Recent developments and future perspectives

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Review of current developments in rhodamine derivatives-based photoresponsive chemosensors for ion detection

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Revisiting diosmin for their potential biological properties and applications

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From the journal:
RSC Advances

Recent developments in sunscreens based on chromophore compounds and nanoparticles



[Mani Rajasekar](#), ^{*a}, [Jennita Mary](#), ^b, [Meenambigai Sivakumar](#) ^b and [Masilamani Selvam](#) ^b

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Abstract

Sunscreen formulations have undergone significant advancements in recent years, with a focus on improving UV radiation protection, photostability, and environmental sustainability. Chromophore compounds and nanoparticles have emerged as key components in these developments. This review highlights the latest research and innovations in chromophore compounds and nanoparticle-based sunscreens. It discusses the role of nanoparticles, such as zinc oxide and titanium dioxide, in scattering and absorbing UV radiation while remaining cosmetically acceptable. Chromophore compounds, encapsulated in nanoparticles, are explored for their potential to enhance UV protection by absorbing specific wavelengths of light. Additionally, advances in photo-stability, broad-spectrum protection, antioxidant inclusion, and biodegradability are discussed. The evolving landscape of sunscreen technology aims to provide more effective and environment-friendly solutions for safeguarding skin from the sun's harmful effects.

Recent trends in synthesis of photoluminescence based pyrene derivatives and their biomaterial applications

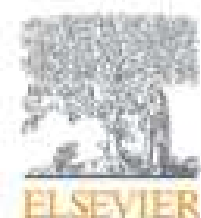
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Recent developments in photoresponse-based zinc (II) sensors and biomaterials/biomedical applications

Mani Rajasekar ^a  , Palanisamy Bhuvanesh ^b, Pramod Varada ^b, Masilamani Selvam ^b

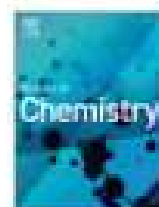
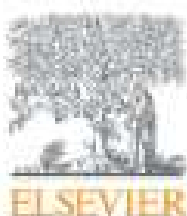
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Review on anticancer activity of flavonoid derivatives: Recent developments and future perspectives

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Research Article

Characterization of an efficient waste fat hydrolysing and detergent compactible lipase from newly isolated *Pseudomonas mosselii*

Mohana Priya Srinivasan, Dayanandan Anandan, Ajith Chandrasekar, and 1 more

This is a preprint; it has not been peer reviewed by a journal.<https://doi.org/10.21203/rs.3.rs-3972296/v1>

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Abstract

Lipase enzyme plays a major role in several industrial processes. The effective production of lipase enzyme from microorganism in a cost-effective manner is in great demand in the current scenario. This study has aimed in producing an effective and high active stable lipase enzyme from *Pseudomonas mosselii* isolated from the highly polluted cooum river bed soil, Chennai, Tamil Nadu, India. The enzyme showed the high specific activity of 157.94 U/mg. Further optimization studies which include, pH (6.5-7) 110.298 U/ml, temperature (35°C – 40°C) 112.388 U/ml, incubation time (36 hrs) 119.79 U/ml, effective substrate olive oil (1%) 118.05 U/ml and nitrogen source (Peptone 1.5% (w/v)), 150.74 U/ml enhanced the parameters to be considered for the high production of lipase enzyme. The purification process carried out in this study was ammonium sulphate precipitation, dialysis and column chromatography using Sephadex G-100 as a stationary phase. The characterization studies of partially purified lipase enzymes with parameters enhanced the stability study as follows: pH (6–8), temperature (30°C to 50°C), metal ions (Ca^{2+}) and detergent (Tween 80). The hydrolysis of the waste tallow using the produced lipase showed highest reaction ratio of 83.7% after 72 hrs at 50°C, 82.6% at 40°C and 81.2% at 30°C. The detergent compatible test confirmed that the lipase was compactible with the detergent and the stains were removed efficiently. Thus, this lipase may effectively serve as the feedstock for biodiesel production and as a detergent compactible application.



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Research Article | [Open Access](#)

Biodiesel Production from Rice-Wash Water with *Streptomyces fradiae* Fermentation: Improved Free Fatty Acid Concentration

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Issue online: June 2024

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Understanding the Multi-Functional Role of TCTP in the Regeneration Process of Earthworm, *Perionyx excavatus*

Original Article | Published: 07 November 2023

Volume 21, pages 353–366, (2024) [Cite this article](#)

[Kamarajan Rajagopalan](#), [Jackson Durairaj Selvan Christyraj](#) , [Karthikeyan Subbiahanadar Chelladurai](#),
[Puja Das](#), [Karthikeyan Mahendran](#), [Logeshwari Nagarajan](#) & [Saritha Gunalan](#)

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Abstract

Background:

Regeneration is a highly complex process that requires the coordination of numerous molecular events, and identifying the key ruler that governs is important to investigate. While it has been shown that TCTP is a multi-functional protein that regulates cell proliferation, differentiation, apoptosis, anti-apoptosis, stem cell maintenance, and immune responses, but only a few studies associated to regeneration have been reported. To investigate the multi-functional role of TCTP in regeneration, the earthworm *Perionyx excavatus* was chosen.

The molecular mechanisms underlying the regeneration process in the earthworm, *Perionyx excavatus* exhibit indications of apoptosis-induced compensatory proliferation (AICP)

Published: 19 March 2024

Volume 60, pages 222–235, (2024) [Cite this article](#)

[Kamarajan Rajagopalan](#), [Jackson Durairaj Selvan Christyraj](#) , [Karthikeyan Subbiahanadar Chelladurai](#), [Johnson Retnaraj Samuel Selvan Christyraj](#) , [Puja Das](#), [Apoorva Roy](#), [Chaughule Vrushali](#) & [Nehla Siraj M. Chemmet](#)

 224 Accesses [Explore all metrics](#) →

Abstract

Regeneration is a multifaceted biological phenomenon that necessitates the intricate orchestration of apoptosis, stem cells, and immune responses, culminating in the regulation of apoptosis-induced compensatory proliferation (AICP). The AICP context of research is observed in many animal models like in *Hydra*, *Xenopus*, newt, *Drosophila*, and mouse but so far not reported in earthworm. The earthworm *Perionyx excavatus* is used in the present study to understand the relationship between AICP-related protein expression and regeneration success in different conditions (normal regeneration and abnormal multiple bud formation). Initially, the worms are amputated into five equal portions and it is revealed that regeneration in *P. excavatus* is clitellum independent and it gives more preference for anterior regeneration (regrowth of head portion) than for posterior

[Home](#) > [Apoptosis](#) > Article

Understanding the molecular mechanism of regeneration through apoptosis-induced compensatory proliferation studies – updates and future aspects

Review | Published: 06 April 2024

Volume 29, pages 1399–1414, (2024) [Cite this article](#)

[Kamarajan Rajagopalan](#), [Jackson Durairaj Selvan Christyraj](#) , [Karthikeyan Subbiahannadar Chelladurai](#), [Kalishwaralal Kalimuthu](#), [Puja Das](#), [Meikandan Chandrasekar](#), [Nivedha Balamurugan](#) & [Karthikeyan Murugan](#)

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Abstract

AICP is a crucial process that maintaining tissue homeostasis and regeneration. In the past, cell death was perceived merely as a means to discard cells without functional consequences. However, during regeneration, effector caspases orchestrate apoptosis, releasing signals that activate stem cells, thereby compensating for tissue loss across various animal models. Despite significant progress, the activation of Wnt3a by caspase-3 remains a focal point of research gaps in AICP mechanisms, spanning from lower to higher regenerative animals. This inquiry into the molecular intricacies of caspase-3-induced Wnt3a activation contributes to a deeper understanding of the links between regeneration and cancer mechanisms. Our report provides current updates on AICP pathways, delineating research gaps and highlighting the potential for future investigations aimed at enhancing our comprehension of this intricate process.

An improved protocol for inducing the gut cleaning process in earthworm for various experiments

In: Animal Biology

Authors: Karthikeyan Kandaswamy , Kayalvizhi Vadivelu , Jackson Durairaj Selvan Christyraj , Karthikeyan Subbiahanadar Chelladurai , Kamarajan Rajagopalan , Puja Das , Meikandan Chandrasekar , Nivedha Balamurugan , Vijayalakshmi Subramanian , and Johnson Retnaraj Samuel Selvan Christyraj 

Online Publication Date: 04 Jun 2024

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Abstract

Earthworms have a complex digestive system consisting of the mouth, pharynx, esophagus, crop, gizzard, and intestine which work together to pass the soil out. The gut soil and associated microbial load in earthworms' gut hinder the outcome of many experiments like *in-vitro* regeneration, histology, immunohistochemistry, molecular expression and toxicological studies. Currently, the available gut cleaning techniques are time-consuming and costly. The tissue paper method takes 4-7 days; the agar-agar method takes around 3-5 days to clean the gut. Therefore, refined techniques are needed to reduce the gut cleaning process in a cost-effective and at the same time in a minimum number of days. Bisacodyl is a medication used to treat constipation and it acts as a laxative drug that increases peristaltic movement of the gut. It can also be used to cleanse the intestines before a bowel examination or surgery. The present study aims to enhance the gut-cleaning process in earthworm, *Eudrilus eugeniae*, using the drug bisacodyl. Briefly, the earthworm is exposed to different concentrations (1 mg, 2 mg, 4 mg, 6 mg, 8 mg and 10 mg) of bisacodyl and analyzed for its excretion rate. We observed that 4 mg is more efficient and aids in cleaning the earthworm's gut within two days. A lower bisacodyl concentration is inefficient in cleaning the gut in a shorter duration while at higher bisacodyl concentrations, worm experienced autotomy. Since

An improved protocol for inducing the gut cleaning process in earthworm for various experiments

In: Animal Biology

Authors: Karthikeyan Kandaswamy , Kayalvizhi Vadivelu , Jackson Durairaj Selvan Christyraj , Karthikeyan Subbiahanadar Chelladurai , Kamarajan Rajagopalan , Puja Das , Meikandan Chandrasekar , Nivedha Balamurugan , Vijayalakshmi Subramanian , and Johnson Retnaraj Samuel Selvan Christyraj 

Online Publication Date: 04 Jun 2024

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Abstract

Earthworms have a complex digestive system consisting of the mouth, pharynx, esophagus, crop, gizzard, and intestine which work together to pass the soil out. The gut soil and associated microbial load in earthworms' gut hinder the outcome of many experiments like *in-vitro* regeneration, histology, immunohistochemistry, molecular expression and toxicological studies. Currently, the available gut cleaning techniques are time-consuming and costly. The tissue paper method takes 4-7 days; the agar-agar method takes around 3-5 days to clean the gut. Therefore, refined techniques are needed to reduce the gut cleaning process in a cost-effective and at the same time in a minimum number of days. Bisacodyl is a medication used to treat constipation and it acts as a laxative drug that increases peristaltic movement of the gut. It can also be used to cleanse the intestines before a bowel examination or surgery. The present study aims to enhance the gut-cleaning process in earthworm, *Eudrilus eugeniae*, using the drug bisacodyl. Briefly, the earthworm is exposed to different concentrations (1 mg, 2 mg, 4 mg, 6 mg, 8 mg and 10 mg) of bisacodyl and analyzed for its excretion rate. We observed that 4 mg is more efficient and aids in cleaning the earthworm's gut within two days. A lower bisacodyl concentration is inefficient in cleaning the gut in a shorter duration while at higher bisacodyl concentrations, worm experienced autotomy. Since

Current Innovations in Intraocular Pressure Monitoring Biosensors for Diagnosis and Treatment of Glaucoma—Novel Strategies and Future Perspectives

by Rubiya Raveendran ¹ , Lokesh Prabakaran ¹ , Rethinam Senthil ² , Beryl Vedha Yesudhasan ³ , Sankari Dharmalingam ⁴ , Weslen Vedakumari Sathyaraj ^{1,*}  and Raji Atchudan ^{5,6,*}  

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Green Synthesis of Multifunctional Silver Nanoparticles Using *Plectranthus amboinicus* for Sensitive Detection of Triethylamine, with Potential *In Vitro* Antibacterial and Anticancer Activities

by Lokesh Prabakaran ¹ , Weslen Vedakumari Sathyaraj ^{1,*} , Beryl Vedha Yesudhasan ² ,
Gokulakrishnan ^{3,4,*}



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(This article belongs to the Special Issue Chemosensors for Ion Detection)

Haemostatic potency of sodium alginate/aloe vera/sericin composite scaffolds – preparation, characterisation, and evaluation

Jayavardhini Bhoopathy, Weslen Vedakumari Sathyaraj , Beryl Vedha Yesudhasan, Selvarajan Rajendran, Sankari Dharmalingam Jayashri Seetharaman, ...show all

Pages 35–45 | Received 01 Sep 2023, Accepted 20 Nov 2023, Published online: 19 Dec 2023

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Abstract

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Fabrication of haemostatic materials with excellent antimicrobial, biocompatible and biodegradable properties remains as a major challenge in the field of medicine. Haemostatic agents play vital role in protecting patients and military individuals during emergency situations. Natural polymers serve as promising materials for fabricating haemostatic compounds due to their efficacy in promoting hemostasis and wound healing. In the present work, sodium alginate/aloe vera/sericin (SA/AV/S) scaffold has been fabricated using a simple cost-effective casting method. The prepared SA/AV/S scaffolds were characterised for their physicochemical properties such as scanning electron microscope, UV-visible spectroscopy and Fourier transform infra-red spectroscopy. SA/AV/S scaffold showed good mechanical strength, swelling behaviour and antibacterial activity. In vitro experiments using erythrocytes proved the hemocompatible and biocompatible features of SA/AV/S scaffold. In vitro blood clotting assay performed using human blood demonstrated the haemostatic and blood absorption properties of SA/AV/S scaffold. Scratch wound assay was performed to study the wound healing efficacy of prepared scaffolds. Chick embryo chorioallantoic membrane assay carried out using fertilised embryos proved the angiogenic property of SA/AV/S scaffold. Thus, SA/AV/S scaffold could serve as a potential haemostatic healthcare product due to its outstanding haemostatic, antimicrobial, hemocompatible, biocompatible and angiogenic properties.

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Impact of double-strand breaks induced by uv radiation on neuroinflammation and neurodegenerative disorders

Review | Published: 08 June 2024

Volume 51, article number 725, (2024) [Cite this article](#)

[Srilakshmi Vijayakumar](#), [Beryl Vedha Yesudhason](#) , [Jenif Leo Anandharaj](#), [Weslen Vedakumari Sathyaraj](#) & [Johnson Retnaraj Samuel Selvan Christyraj](#) 

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Abstract

Exposure to UV affects the development and growth of a wide range of organisms. Nowadays, researchers are focusing on the impact of UV radiation and its underlying molecular mechanisms, as well as devising strategies to mitigate its harmful effects. Different forms of UV radiation, their typical exposure effects, the impact of UV on DNA integrity, and the deterioration of genetic material are discussed in this review; furthermore, we also review the effects of UV radiation that affect the biological functions of the organisms. Subsequently, we address the processes that aid organisms in navigating the damage in genetic material, neuroinflammation, and neurodegeneration brought on by UV-mediated double-strand breaks. To emphasize the molecular pathways, we conclude the review by going over the animal model studies that highlight the genes and proteins that are impacted by UV radiation.



Optimum performance of a novel biocompatible scaffold comprising alginate-pectin-selenium nanoparticles for cardiac tissue engineering using C2C12 cells

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Received 21 February 2023, Revised 5 August 2023, Accepted 19 August 2023, Available online 25 August 2023, Version of Record 6 September 2023.

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RESEARCH ARTICLE

Live foldscope imaging of two environmentally beneficial earthworm species and their early developmental stages

Mijithra Ganesan, Johnson Retnaraj Samuel Selvan Christyraj , Beryl Vedha Yesudhasan, Manikandan Mohan, Jackson Durairaj Selvan Christyraj 

First published: 20 April 2023 | <https://doi.org/10.1002/tqem.21997>

Mijithra Ganesan and Johnson Retnaraj Samuel Selvan Christyraj contributed equally.

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Abstract

Earthworms are extensively referred as farmer's friends, play a vital role in maintaining soil fertility and promoting agricultural sustainability. Based on the ecological distribution of numerous species in soil the epigeic earthworms were taken into consideration as they have an excellent source for vermicomposting. In addition to this, earthworms also have a tremendous regeneration ability varies depending on the species. *Eudrilus eugeniae* and *Perionyx excavatus* are the two epigeic earthworm species were taken for the study. Species identification of newly hatched and juvenile earthworms are very difficult, due to its smaller size, immature body segments, and absence of clitellum. Here, we used foldscope (a paper-microscope) as an ideal tool for species identification by live examining the morphological characteristics, internal organs, and regeneration ability of newly hatched and juvenile earthworms, precisely. Notably, clear differentiation in the arrangement of setae and numbers of hearts present in both species were observed using a foldscope microscope. Regeneration studies reveals that both the species have prodigious regeneration potential at their newly hatched and juvenile stages which is identified and documented with foldscope. However, both species are environmentally and economically important, and their precise identification is very vital for environmental restoration, research, and agriculture practices.

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Understanding the process of angiogenesis in regenerating earthworm

Published: 19 July 2023

Volume 59, pages 467–478, (2023) [Cite this article](#)

Mijithra Ganesan, [Johnson Retnaraj Samuel Selvan Christyraj](#) , [Saravanakumar Venkatachalam](#), [Beryl Vedha Yesudhasan](#), [Weslen Vedakumari Sathiyaraj](#) & [Jackson Durairaj Selvan Christyraj](#) 

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Abstract

Developing blood vessels from the existing vasculature is vital for the growth of the organism, as well as for systematic wound healing and the repair process. In this study, we investigated the role of angiogenesis during the regeneration process in the earthworm, *Eudrilus eugeniae*, animal model. Briefly, the morphological examination of blood vessels in juvenile and mature worms is documented, along with the development of new blood vessels in regenerating blastema. However, in vivo and in vitro experiments with juvenile worms revealed that geraniol retards blastemal regeneration growth with undeveloped blood vessels, as compared to the control. The results of qRT-PCR, western blotting, and immunohistochemistry confirmed a reduced expression of VEGFR2 and WNT5A in the day 3 regenerating blastema of geraniol-treated worms, as compared to the control. We conclude that geraniol acts as a potent natural inhibitor of angiogenesis, thereby retarding the regeneration process in earthworms. In addition, for studying angiogenesis and screening effective angiogenesis inhibitors as drug candidates, the earthworm is an ambient animal model system.

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ARTICLE | March 28, 2023

TLR-7/8 Agonist-Loaded Polypyrrole-Based Theranostic Nanovaccine for Second Near-Infrared Photothermal Immunotherapy of Cancer

Laxmanan Karthikeyan, Vellingiri Yasothamani, Yuvaraj Haldorai, Johnson Retnaraj Samuel Selvan Christyraj, and Raju Vivek*



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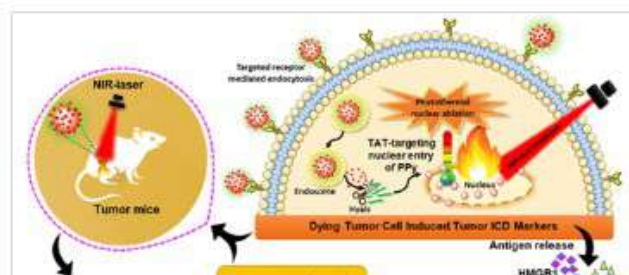
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Supporting Information (1)

Abstract

Functionalized theranostic agents have the potential to be an effective tool for targeting tumor cells with an effective response in cancer imaging and therapy. Particularly, near-infrared (NIR) light is extremely advantageous and can penetrate deeply into biological tissues with minimum attenuation and photodamage to cells. The major impediment to the translation of research into clinical applications is toxicity and long-term body retention. Designing renal clearable and theranostic qualities in a single nanoplatform remains the biggest



Article | [Open access](#) | Published: 07 March 2024

Biochemical and functional characterization of heat-inactivated coelomic fluid from earthworms as a potential alternative for fetal bovine serum in animal cell culture

[Melinda Grace Rossan Mathews](#), [Ravichandran Subramaniam](#), [Saravanakumar Venkatachalam](#), [Johnson Retnaraj Samuel Selvan Christyraj](#) , [Beryl Vedha Yesudhasan](#), [Kalishwaralal Kalimuthu](#), [Manikandan Mohan](#) & [Jackson Durairaj Selvan Christyraj](#) 

[Scientific Reports](#) **14**, Article number: 5606 (2024) | [Cite this article](#)

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Abstract

Fetal bovine serum (FBS) plays a pivotal role in animal cell culture. Due to ethical and scientific issues, searching for an alternative, comprising the three R's (Refinement, Reduction and Replacement) gained global attention. In this context, we have identified the heat inactivated coelomic fluid (HI-CF) of the earthworm, *Perionyx excavatus* as a potential alternative for FBS. Briefly, we formulated HI-CF (f-HICF) containing serum free medium which can aid the growth, attachment, and proliferation of adherent cells, similar to FBS. In this study, we investigated the biochemical characterization, sterility, stability, formulation, and functional analysis of HI-CF as a supplement in culturing animal cells. Notably, vitamins, micronutrients, proteins, lipids,

Research article

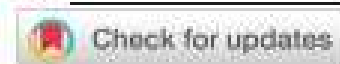
Solar light-driven photocatalysis by Co doped SnS nanoparticles towards degradation of noxious organic pollutant: Mechanism and toxicity assessment

Nachimuthu Venkatesh^a, Govindhasamy Murugadoss^b  , Govindhan Thiruppathi^c,
Palanisamy Sundararaj^c, Pachagounder Sakthivel^a  , Manavalan Rajesh Kumar^d,
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Research papers

Nano-tablets and rods-shaped Fe and Ni oxide/hydroxide encapsulated graphene for asymmetric supercapacitor and OER applications

Durai Govindarajan^a, Mohammad Etesami^a, Kasidit Nootong^a, Kamalan Kirubakaran^b,
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Synthesis of nickel–manganese sulfide decorated with reduced graphene oxide nanocomposite for ultra-fast photocatalytic degradation of organic dye molecules

Original Article | Published: 20 December 2023

Volume 34, pages 827–840, (2024) [Cite this article](#)

[Narthana Kandhasamy](#), [Govindhasamy Murugadoss](#) , [Thiruppathi Kannappan](#), [Kamalan Kirubaharan](#), [Rajesh Kumar Manavalan](#), [Sandhanasamy Devanesan](#), [Mohamad S. AlSalhi](#), [R. Mythili](#), [Surendra Shinde](#) & [Hemraj M. Yadav](#)

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Abstract

The untreated effluent dropping into the environment from various textile industries is a major issue. To solve this problem, development of an efficient catalyst for the degradation of macro dye molecules has attracted extensive attention. This work is mainly focused on the synthesis of nickel–manganese sulfide decorated with rGO nanocomposite (Ni–Mn–S/rGO) as an effective visible photocatalyst for degradation of textile toxic macro molecule dye. A simple hydrothermal method was used to synthesize Ni–Mn–S wrapped with rGO. The prepared composites were characterized using various techniques such as X-ray diffraction (XRD), high-resolution scanning electron microscopy (HR-SEM), high-resolution transmission electron microscopy (HR-TEM), Fourier transform infra-red spectrometer (FTIR), and ultra violet–visible (UV–Vis) spectroscopy. The photocatalytic performance of nickel sulfide (NiS), manganese sulfide (MnS), nickel–manganese sulfide (Ni–Mn–S), and Ni–Mn–S/rGO nanocomposite was assessed by analyzing the removal of acid yellow (AY) and rose bengal (RB) dyes under natural sun light. Among these, the Ni–Mn–S/rGO nanocomposite showed the high photocatalytic degradation efficiency of AY and RB dyes (20 ppm concentration) with efficiency at 96.1 and 93.2%, respectively, within 150-min natural sunlight irradiation. The stability of photocatalyst was confirmed by cycle test; it showed stable degradation efficiency even after five cycles. This work confirms that it is an efficient approach for the dye degradation of textile dyes using sulfide-based Ni–Mn–S/rGO nanocomposite.



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Rapid Photocatalytic Activity of Crystalline $\text{CeO}_2\text{-CuO-Cu(OH)}_2$ Ternary Nanocomposite

by Govindhasamy Murugadoss ^{1,*} , Thiruppathi Kannappan ² , Jothi Ramalingam Rajabathar ³ , Rajesh Kumar Manavalan ⁴ , Shyju Thankaraj Salammal ¹ and Nachimuthu Venkatesh ⁵

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Hydrothermal synthesis of β -NiS layer structure nanoparticles for supercapacitor applications

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Microwave assisted green synthesis of Ag doped CuO NPs anchored on GO-sheets for high performance photocatalytic and antimicrobial applications

Jarvin Mariadhas^a, Sarojini Jeeva Panchu^b, Hendrik C. Swart^b, Daniel Rani Rosaline^{a,c},
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Facile synthesis of Co-Cu metal organic framework as efficient non-noble bifunctional electrocatalysts for overall water splitting

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R.V. Mangalaraja^{b,d}  , Francisco V. Herrera Diaz^e, Saeed Farhang Sahlevani^{b,f}, S. Sasikala^a,
G. Murugadoss^h, Ilaiyaraja Perumal^a  , Moorthy Sasikumarⁱ  

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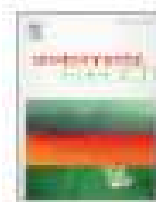
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Abstract

The rapid development of superior, highly stable, alkaline-medium-compatible, and nonprecious earth-abundant bifunctional electrocatalysts has garnered significant research interest. This interest aims to replace the costliest noble metals (Pt, Ir/IrO₂, and Ru/RuO₂) in renewable and green energy technologies for overall water splitting. However, there are still important limitations, such as lower stability and higher energy consumption. In this work, we report the synthesis of Cu-Co metal-organic frameworks (MOFs) as a bifunctional electrocatalyst using a simple chemical precipitation technique. Especially, when 11.5 mM of Co is combined with Cu MOF, it exhibits excellent bifunctional activity for overall water splitting with a lower overpotential of 0.21 V (OER) and -0.71 V (HER) at a current density of 10 mA cm⁻², which exhibits nearly several times more enhancement than that of pristine Cu and Co MOFs in a 1 M KOH electrolyte solution. The Tafel slope value of 130 mV/dec and the lower charge transfer resistance, along with relatively high stability for up to 12 h at the onset potential of OER and HER, are observed for the 11.5 mM Cu-Co MOF electrocatalyst. The present results open an alternative pathway for developing a novel design of highly efficient and scalable



Lanthanum and Indium intermetallics nanomaterial for thermal photovoltaic applications – A full potential study

D.S. Jayalakshmi^a  , G. Akshaya^b, Govindasamy Murugadoss^c, Hakim AL Garalleh^d, Albara Ibrahim Alrawashdeh^{e,f}, Mohammed Ali Alshehri^g, A Pugazhendhi^{h,i}  

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 What do these dates mean?



Silver Sulfide Anchored Anatase TiO₂ Nanoparticles for Ultrafast Degradation of Selective Textile Dyes

Govindhasamy Murugadoss ✉, Nachimuthu Venkatesh, Jothiramalingam Rajabathar, Narthana Kandhasamy, Rajesh Kumar Manavalan

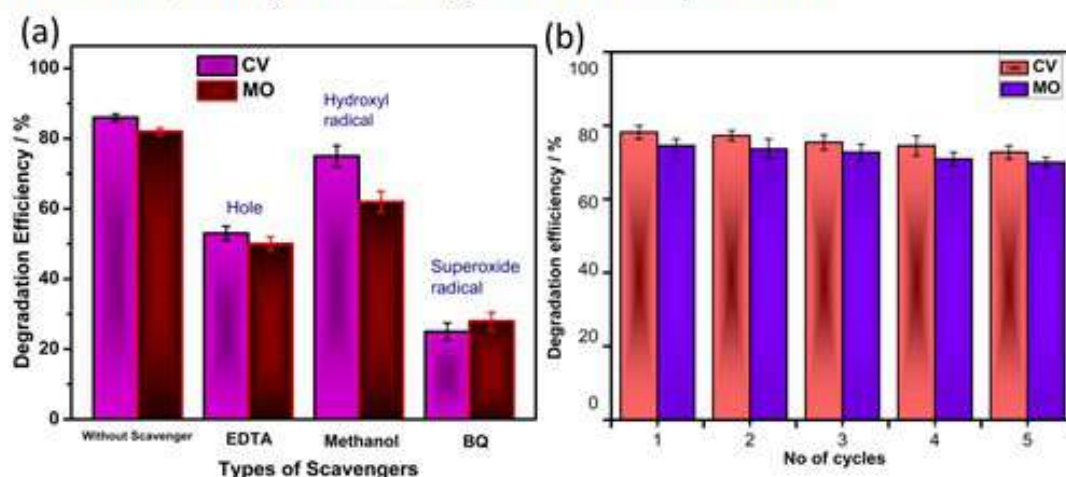
First published: 19 April 2024 | <https://doi.org/10.1002/slct.202400099>

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Graphical Abstract

Well crystalline Ag₂S decorated TiO₂ nanoparticles are synthesized by a facile chemical method. The decorated Ag₂S tiny nanoparticles on TiO₂ surface is confirmed by electron microscopic study. Crystal violet (CV) and Methyl orange (MO) dyes are degraded using the well-designed catalyst. Photocatalytic efficiency, rate constant, stability and scavenger studies also performed.



Zinc oxide nanoflakes supported copper oxide nanosheets as a bifunctional electrocatalyst for OER and HER in an alkaline medium

M. Praveen Kumar^a, Natesan Kumaresan^b, R.V. Mangalaraja^{a,c}, Irina Zaporotskova^d, A. Arulraj^e,
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Article

Hot Corrosion Behavior of Plasma-Sprayed $Gd_2Zr_2O_7$ /YSZ Functionally Graded Thermal Barrier Coatings

by Rajasekaramoorthy Manogaran ^{1,2} , Karthikeyan Alagu ^{1,*} , Anderson Arul ¹ , Anandh Jesuraj ² , Dinesh Kumar Devarajan ² , Govindhasamy Murugadoss ² and Kamalan Kirubaharan Amirtharaj Mosas ^{3,*}

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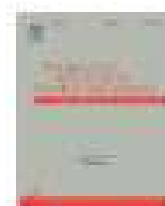
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Short communication

Improved electrochemical performance of hydrothermally synthesized Zn-Ni-S/rGO nanocomposite as an electrode for supercapacitor application

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Fabrication of Cu-doped NiO platform for the electrochemical sensing of folic acid

Published: 06 June 2024

Volume 35, article number 1084, (2024) [Cite this article](#)

[Kaveri Krishnamoorthy](#), [Govindhasamy Murugadoss](#), [Narmatha Sivaraman](#), [Velayutham Sudha](#), [Sakkarapalayam Murugesan Senthil Kumar](#) & [Rangasamy Thangamuthu](#) 

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Abstract

In this work, a wet chemical precipitation method was used to synthesize different concentrations of Cu-doped nickel oxide (NiO, Cu–NiO (5%), Cu–NiO (10%), and 20% Cu–NiO) nanoparticles. The prepared samples were characterized using various advanced characterization studies to understand the crystal structure, functional group, surface morphology, absorbance, thermal stability and oxidation state of the samples. Electrochemical sensing performance of the doped NiO (5% Cu, 10% Cu, and 20% Cu) were evaluated in 0.1 M phosphate buffer solution for the detection of folic acid (FA). In the case of 10% Cu-doped NiO modified glassy carbon electrode (GCE) showed an oxidation potential of 0.75 V with enhancing current response compared with bare GCE and other doping concentration of the samples. The Cu-doped NiO/GCE electrode achieved a wide linear range from 10–1200 μM , with a sensitivity of $40 \mu\text{A cm}^{-2} \mu\text{M}^{-1}$ and a limit of

4-Carboxyphenyl as efficient donor group in nano Zn-Porphyrin for dye sensitized solar cells

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Design and synthesis of simple quinoline-based organic molecules as dual/multifunctional chemosensors for the detection of Cu^{2+} / Fe^{3+} ions

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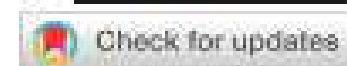
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Development of phase pure $\text{Cu}_2\text{ZnSiSe}_4$ via solid state synthesis for energy applications

Immanuel A. Sagaya Amala^a, Sivaramalingam Anandhi^b, Panneerselvam Vengatesh^c,
Salammal Shyju Thankaraj^c  

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Abstract

In recent years, quaternary diamond-like metal chalcogenide semiconductor nanocrystals have received a lot of attention in solar cell and thermoelectric applications. The optoelectronic properties of quaternary diamond-like structures are influenced by their crystal structure. In this work, a series of experiments have been performed to understand the crystal system of $\text{Cu}_2\text{ZnSiSe}_4$ materials via solid state synthesis, namely mechanosynthesis to get a single phase of quaternary chalcogenide via single step route synthesis. The materials were performed at different milling time to study the single phase compound and phase transformation of the materials. Based on the theory and existing literature, the composition of the materials has been selected as 2:1:1:4 which is the optimum composition for quaternary chalcogenides. Based on the composition, the material properties will be changed. X-ray diffraction (XRD) and Raman spectroscopy were used to analyse the structure and phase purity of $\text{Cu}_2\text{ZnSiSe}_4$ nanoparticles. Field emission scanning electron microscopy (FESEM) and Energy dispersive X-ray spectroscopy (EDS) results showed that the average size of the $\text{Cu}_2\text{ZnSiSe}_4$ particles and the composition of products were close to the stoichiometry of $\text{Cu}_2\text{ZnSiSe}_4$. Optical studies show that $\text{Cu}_2\text{ZnSiSe}_4$ nanocrystals exhibit good, strong optical absorption in the visible region of the electromagnetic spectrum and a 1.12 eV bandgap. Hall measurement shows the bulk property of the materials and it is inferred that the synthesized materials

Review on ternary chalcogenides: Potential photoabsorbers

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Abstract

The development of photovoltaic technology has brought in clean energy production. However, silicon is the most commonly used semiconductor in photovoltaics with power conversion efficiency (PCE) of about 18% to 22% under standard conditions. Recently, the chalcopyrite structure of I-III-VI₂ constitutes ternary chalcogenides and the subsequent thin films are being used in second generation thin film solar cells (TFSC). Copper-based (Cu-III-VI₂) and silver-based (Ag-III-VI₂) chalcogenides are emerged as alternatives for toxic elements & lowcost in optoelectronics. In particular, Copper Indium Diselenide (with the optical band gap of 1.04eV) cells have up to 14% efficiency with similar durability as silicon solar cells. Similarly, the optical band gap of Cu₂SnS₃ (CTS) thin films is 1.23eV and the absorption coefficient is $> 10^5 \text{ cm}^{-1}$ and hence act as potential photoabsorber in TFSC. AgGaSe₂ and AgGaTe₂ have a direct band gap of 1.42eV and 0.75eV, respectively, and strong clarity in the 500–1200nm wavelength range. Also, the optical properties of Ag-based chalcogenides (AgAlS₂) are equivalent to those of Cu-based chalcogenides (CuAlS₂). Similarly, a number of ternary semiconductors, including CuInS₂, CuSnSe₂, CuSbS₂, AgInSe₂, AgGaS₂ and AgBiS₂ with a bandgap in visible regions are created with rich crystal structures and high absorption coefficients using various deposition techniques, making them well suited for photoabsorbers. Herein, the latest progress of ternary chalcogenides is reviewed from the aspects of synthesis,

Temperature-dependent Raman and dielectric studies of Sm and Zr Co-doped BaTiO₃ ceramics

Published: 19 February 2024

Volume 35, article number 353, (2024) [Cite this article](#)

B. Vigneshwaran, P. Kuppusami , S. Ajithkumar & Madhan Kuppusamy

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Abstract

Sm and Zr co-doped BaTiO₃ ceramics were investigated for their microstructure and dielectric characteristics. (Ba_{1-x}Sm_x)(Ti_{0.75}Zr_{0.25})O_{3-δ} (BSTZO) with x = 0.02, 0.04, and 0.06 mol% of ceramic compounds were prepared by solid-state reaction route. The primary objective is to obtain higher dielectric constant with lower dielectric loss in the proposed material composition. The prepared BSTZO and sintered compounds were found to be highly crystalline with a tetragonal perovskite structure. A typical X-ray photoelectron spectroscopy spectra of BSTZO sample with Sm = 0.02 mol% calcined at 1273 K revealed the elemental composition, binding energy, and chemical state of the elements. Oxygen vacancy concentrations evaluated by in situ high-temperature Raman spectroscopy in the temperature range of 301–773 K were found to decrease with increasing Sm³⁺ concentration and the substitution of the lowest concentration of Sm³⁺ (0.02 mol%) in the A site of the BSTZO was found to be higher ($V_{\text{O}}^{\bullet\bullet} = 1.98 \times 10^{21} \text{ cm}^{-3}$, at



Short communication

High-brightness red-emitting Eu^{3+} -activated $\text{Ca}_2\text{LuZr}_2\text{Al}_3\text{O}_{12}$ garnet phosphors with excellent thermal stability for near-UV-pumped white LEDs

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Full-visible-spectrum LED lighting by using a near-UV-excitable broadband blue-emitting $\text{Ca}_2\text{LuHf}_2\text{GaAl}_2\text{O}_{12}:\text{Ce}^{3+}$ garnet phosphor

Zhe Xu ^a, Lina Cao ^a, Jingming Chan ^a, Balaji Devakumar ^b, Xiaoyong Huang ^a  

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Abstract

Highly efficient broadband blue-emitting phosphors are urgently needed for full-visible-spectrum white light-emitting diodes (WLEDs). Herein, we report a new highly luminescent garnet-type $\text{Ca}_2\text{LuHf}_2\text{GaAl}_2\text{O}_{12}:\text{Ce}^{3+}$ (abbreviated as: CLHGAO: Ce^{3+}) blue phosphor enabling full-visible-spectrum LED lighting. These CLHGAO: Ce^{3+} phosphors doped with different Ce^{3+} concentrations have been prepared by using a high-temperature solid-state reaction approach, and their phase purity, crystal structure, luminescent properties, CIE color coordinates, and quantum efficiency have been systematically studied. Importantly, CLHGAO: Ce^{3+} blue phosphors show a broadband excitation spectrum in the 300–450 nm spectral range peaking around 398 nm, matching well with the emission wavelengths of commercial near-ultraviolet LED chips (380–420 nm). Upon 398 nm excitation, the optimal CLHGAO:2% Ce^{3+} phosphor sample generates a bright broad blue emission band (emission peak: 472 nm; bandwidth: 84 nm) with high internal quantum efficiency of 79.2% and external quantum efficiency of 55.3%. Notably, the emission intensity of CLHGAO:2% Ce^{3+} phosphor is about 1.87 times higher compared to the commercial $\text{BaMgAl}_{10}\text{O}_{17}:\text{Eu}^{2+}$ blue phosphor (emission peak: 451 nm; bandwidth: 53 nm). Finally, a white LED device is fabricated with a 395 nm near-UV LED chip with CLHGAO:2% Ce^{3+} blue phosphor and commercial $(\text{Ba,Sr})_2\text{SiO}_4:\text{Eu}^{2+}$ green



From the journal:
CrystEngComm

Dazzling $\text{Ca}_2\text{LuScAl}_2\text{Si}_2\text{O}_{12}:\text{Ce}^{3+}$ green-emitting garnet-type phosphors for blue-chip-pumped white light-emitting diodes: broad emission band, high quantum efficiency and excellent thermal stability



Cheng Xian,^a Xiaoyuan Chen,^a Balaji Devakumar^b and Xiaoyong Huang^{ib} *^a

⊕ Author affiliations

Abstract

Exploring efficient, energy-saving and environmentally friendly phosphors is important for developing solid-state white light-emitting diodes (w-LEDs). Herein, we report a novel highly efficient green-emitting Ce^{3+} -activated $\text{Ca}_2\text{LuScAl}_2\text{Si}_2\text{O}_{12}$ (abbreviated as CLSAS: Ce^{3+}) garnet phosphor with high quantum efficiency and good thermal stability. These garnet phosphor samples doped with different Ce^{3+} ion concentrations were synthesized using a high-temperature solid-state method. The crystal structure and Ce^{3+} concentration-dependent luminescence properties of the phosphors were systematically studied. The as-prepared CLSAS: Ce^{3+} garnet phosphors exhibit broadband absorption in the spectral range of 250–510 nm with a maximum excitation wavelength of around 452 nm, matching well with the emission wavelengths of commercial blue LED chips. Under an excitation of 452 nm, the optimal CLSAS:4% Ce^{3+} sample exhibited a broad green emission band (emission peak: 531 nm; bandwidth: 114 nm) with CIE color coordinates of (0.3293, 0.5453) and high luminescence efficiency (internal quantum efficiency: 87.3%; external quantum efficiency: 56.6%). The CLSAS:4% Ce^{3+} sample also showed good thermal stability in the temperature range of 303–443 K. Its emission intensity at 423 K reached 79% of the emission intensity at 303 K, and it exhibited outstanding color stability with a small color chromaticity shift ($\Delta E = 1.07 \times 10^{-3}$) at 423 K. Importantly, the fabricated w-LED device exhibits excellent photoelectric properties (CCT = 4978 K; Ra = 92.4; LE = 69.59 lm W⁻¹). The obtained efficient CLSAS: Ce^{3+} green phosphors have the potential for application in high-quality w-LEDs.

High-temperature oxidation behaviours of rare earth-based pyrochlore structured $A_2B_2O_7$ /yttria-stabilized zirconia thermal barrier coatings

N. Dharuman, Gosipathala Sreedhar, Sathish Kumar Ramachandran, M. Arulmozhi - [Show less](#)

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Abstract

Addressing the challenge of cost-effectively synthesizing stable thermal barrier coating materials has been a significant hurdle. This study describes the bulk synthesis of stannate-based pyrochlore compounds, specifically erbium tin oxide ($Er_2Sn_2O_7$) and yttrium tin oxide ($Y_2Sn_2O_7$). These synthesized pyrochlore oxides and commercially available yttria-stabilized zirconia (YSZ) were coated onto nickel super alloy. The coated specimens were subjected to thermal oxidation kinetics

Enhancement of piezoelectric responses of electrospun PVDF nanofibers through mechanical stretching and annealing process

Satthiyaraju Mani ^a, Ananthakumar Kasi ^b, Thangapandian Nagamalai ^c,
Vivekananda Annamar Subramani ^d, Arul Natarajan ^e, Asiful H. Seikh ^f, Muthukumar Krishnan ^g,
Sathish Kumar Ramachandran ^h  

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Highlights

- Significant improvements in the piezoelectric properties of PVDF nanofibers.
- Mechanical stretching utilized to enhance the piezoelectric performance of the nanofibers.
- Annealing process is employed to further optimize the piezoelectric response, achieving superior performance.
- Novel and effective methodology for enhancing the piezoelectricity of PVDF nanofibers.

Membrane-based techniques for pollutants removal: An outlook on recent advancements

Sathish Kumar Ramachandran¹, Palanivel Sathishkumar²  

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Abstract

Improper disposal of emerging contaminants (ECs) such as pharmaceutical wastes, textile dyes, hazardous chemicals, toxic metals, etc., leads to severe water and environmental pollution and also poses a major threat to human health. These ECs are generated through various industrial processes like food processing, pharmaceutical manufacturing, dyeing, tanning, etc. In general, various treatment practices (biological, chemical and physical methods) have been followed in the industries to achieve effective removal of toxic ECs and also to comply with stringent environmental standards. Among the various treatment methods, membrane systems are effective over the conventional industrial effluent treatment techniques with lower operating costs and minimal energy requirements. In this work, applications of membrane systems such as microfiltration, ultrafiltration, nanofiltration, reverse osmosis, and forward osmosis processes, as well as hybrid processes for the effective removal of ECs are reviewed based on the published results of the last three years.

Nanohybrid photocatalysts in dye (Colorants) wastewater treatment: Recent trends in simultaneous dye degradation, hydrogen production, storage and transport feasibility

P. Priyadharsini^a, P. SundarRajan^b, K. Grace Pavithra^c, S. Naveen^d, S. SanjayKumar^a, D. Gnanaprakash^a, J. Arun^a, Arivalagan Pugazhendhi^{f,g}  

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Highlights

- Sustainable degradation of dye wastewater is a huge concern in industrial scale.
- Design, fabrication and synthesis of novel hybrid photocatalysts is performed.
- Photocatalysis based dye degradation provides green energy and treated water.
- Hydrogen is produced via oxidation and reduction reaction needs to be recovered.
- Overall outcome serves to SDGs 6, 7, 14 and safer environment for next generation.

Technical insights on various routes of hydrogen production from pharmaceutical, hydrothermal, sewage and textile wastewaters: Cost comparison and challenges

J. Arun ^a, V. Shriniti ^b, S. Shyam ^c, P. Priyadharsini ^a, K.P. Gopinath ^a, R. Sivaramakrishnan ^a,
Nguyen Thuy Lan Chi ^f, A. Pugazhendhi ^a  

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Highlights

- Industrial wastewater treatment-based hydrogen production is hot research area.
- Light dependent and independent are the common techniques for hydrogen production.
- Microbial electrolysis is the most cost economical way for hydrogen production.
- Future research needed in hydrogen safe storage, transport and distribution.

Review

A review on biological biohydrogen production: Outlook on genetic strain enhancements, reactor model and techno-economics analysis

Narasiman Nirmala^a, Ghodke Praveen^b, Sharma AmitKumar^c, PanneerSelvam SundarRajan^d, Athmanathan Baskaran^e, Packiyadas Priyadharsini^a, SivaPerumal SanjayKumar^a, SelvananthamShanmuganatham Dawn^a, Kirubanandam Grace Pavithra^f, Jayaseelan Arun^a, Arivalagan Pugazhendhi^{a, h}  

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Highlights

- Hydrogen is a renewable source of energy with greater energy value.
- Organic waste is a potential resource of hydrogen production.
- Molecular engineering of microbes helps in higher amount of hydrogen production.
- Integrated technologies and improved reactor design helps higher H₂ production.

Novel porous ambient temperature cured fly ash geopolymer for lead adsorption from wastewaters

Sivasakthi M  & P Priyadharsini

Pages 11604-11618 | Received 25 Jan 2023, Accepted 13 Sep 2023, Published online: 27 Sep 2023

 Cite this article  <https://doi.org/10.1080/15567036.2023.2261413> Check for updates Full Article Figures & data References Citations Metrics Reprints & Permissions

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ABSTRACT

This paper discusses the ability of the fly ash (FA) and ambient cured Alkali activated Fly ash geopolymer (FAAG) for sequestering Pb (II) ions from aqueous solution. Various physiochemical properties such as the influence of pH and contact time were investigated for achieving the conditions for maximum removal via batch mode using powdered samples. The adsorbents were characterized by employing powder X-Ray diffraction, Attenuated Total Reflection Fourier Transform Infra-Red spectroscopy, and Scanning Electron Microscopy/EDAX before and after the treatment. The adsorption capacity of Pb²⁺ ions by raw FA and FAAG were examined by the Inductively coupled plasma mass spectrometry (ICP-MS). The results represented that the maximum adsorption capacity of Pb²⁺ ions by FAAG is 75 mg/g with attained equilibrium time of 120 min whereas raw fly ash at 150 min at 21.5 mg/g capacity. The pozzolanic reactivity of fly ash in water glass hardening resulted mainly N-A-S-H gel and traces of lead silicate due to the immobilization of lead in the network. This was conformed from the binding energy lines at 139.7 eV in XPS survey lines and lead silicate at 25 and 34° 2θ as identified in XRD phase assemblages. ICP-MS test results of leachate of lead immobilized geopolymer sample are devoid of lead ions and thus sorption sites of fly ash geopolymer lead to potential safety disposal of lead. Thus, recycling of fly ash into geopolymer by less energy intensive process and subsequent use in heavy metal disposal could achieve the concept of disposal waste and recycling, which could contribute to achieve the sustainable goals.

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New insights into microbial electrolysis cells (MEC) and microbial fuel cells (MFC) for simultaneous wastewater treatment and green fuel (hydrogen) generation

Jayaseelan Arun ^a, PanneerSelvam SundarRajan ^b, Kirubanandam Grace Pavithra ^c, Packiyadoss Priyadharsini ^a, Sivaprasad Shyam ^d, Rangarajan Goutham ^e, Quynh Hoang Le ^{f,g}, Arivalagan Pugazhendhi ^{f,g}  

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Highlights

- Wastewater treatment and hydrogen production has attracted researchers globally.
- MFC and MEC are preferred for hydrogen production from wastewater.
- Catalysts, reactor design, anode and cathode materials influence the process.
- Hydrogen storage, transport mechanism needs to be identified for usage.

Futuristic advancements in phytoremediation of endocrine disruptor Bisphenol A: A step towards sustainable pollutant degradation for rehabilitated environment

Praveen Kumar Issac ¹, Gokulam Ravindiran ², Kadhirmathiyar Velumani ¹, Arun Jayaseelan ³, Babett Greff ⁴, Ravi Mani ⁵, Soon Woong Chang ⁶, Balasubramani Ravindran ⁷, Mukesh Kumar Awasthi ⁸

Affiliations [+ expand](#)

PMID: 38489980 DOI: [10.1016/j.wasman.2024.03.010](https://doi.org/10.1016/j.wasman.2024.03.010)

Abstract

Bisphenol A (BPA) accumulates in the environment at lethal concentrations because of its high production rate and utilization. BPA, originating from industrial effluent, plastic production, and consumer products, poses serious risks to both the environment and human health. The widespread aggregation of BPA leads to endocrine disruption, reactive oxygen species-mediated DNA damage, epigenetic modifications and carcinogenicity, which can disturb the normal homeostasis of the body. The living being in a population is subjected to BPA exposure via air, water and food. Globally, urinary analysis reports have shown higher BPA concentrations in all age groups, with children being particularly susceptible due to its occurrence in items such as milk bottles. The conventional methods are costly with a low removal rate. Since there is no proper eco-friendly and cost-effective degradation of BPA reported so far. The phytoremediation, green-biotechnology based method which is a cost-effective and renewable resource can be used to sequester BPA. Phytoremediation is observed in numerous plant species with different mechanisms to remove harmful contaminants. Plants normally undergo several improvements in genetic and molecular levels to withstand stress and lower levels of toxicants. But such natural adaptation requires more time and also higher concentration of contaminants may disrupt the normal growth, survival and yield of the plants. Therefore, natural or synthetic amendments and genetic modifications can improve the xenobiotics removal rate by the plants. Also, constructed wetlands technique utilizes the plant's phytoremediation mechanisms to remove industrial effluents and medical residues. In this review, we have discussed the limitations and futuristic advancement strategies for degrading BPA using phytoremediation-

Efficiency of food waste activated carbon in treating biodiesel plant effluent: Impact on process conditions and NaOH recovery

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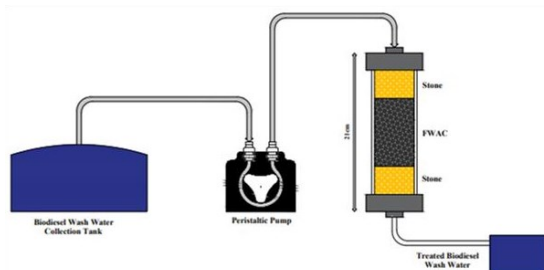
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Graphical abstract



Abstract

The technologies which provide alternate to conventional fuel has led to the production of biodiesel. The source of biodiesel wash water generation takes place during the production of biodiesel via transesterification process. Biodiesel wash water is one of the significant side streams generated during biodiesel production from waste cooking oil. Thus, the generated biodiesel wash water has high pH, turbidity, methanol, soap and glycerol. In the present study, a solution to treat and to bring down the concentration of each of the contaminants have been studied by using zinc chloride activated carbon derived from the food waste. The mixed food waste reject was carbonized and further activated with zinc chloride for biodiesel wash water treatment experiments. The food waste activated carbon (FWAC) and commercial activated carbon (CAC) were analyzed for structural and surface morphology using X-ray diffraction (XRD), Field emission scanning microscopy (FE-SEM) and IR-Raman spectroscopy. The adsorption experiments were performed at 10 to 50 minutes with an adsorbent dosage of 0.1 to 0.5 in 100 mL of biodiesel wash water. pH, turbidity and total dissolved solid (TDS) of biodiesel plant effluent before and after adsorption experiments were determined. Food waste-activated carbon used for the study reduced the effluent pH from 11.5 to 7, Turbidity from 435 NTU to 1 NTU and TDS from 1140 mg/L to 145 mg/L. The feasibility of NaOH recovery from biodiesel wash water was also studied.

Keywords: Biodiesel wash water, activated carbon, adsorption, TDS, sodium hydroxide

1. Introduction

The modernized world is attributed to various concerns like treating industrial effluents, freshwater shortage and strategies for tackling environmental pollution. The increasing demand has not only resulted in the depletion of non-renewable fossil fuels but has also contributed to higher carbon emissions. Hence, it is necessary to find renewable sources for green fuel generation with lesser environmental concerns (Aman *et al.* 2023). The development of biodiesel industries based on second-generation raw materials has been enormous in the last decade. However, higher productivity paved the way for generating a large quantum of wastewater that needs to be treated before its disposal into waterbodies (Daud *et al.* 2015) (Al-Basir *et al.* 2019). Transesterification of triglycerides catalyzed by alkali is a well-established process for biodiesel production (Atadashi *et al.* 2011; Min *et al.* 2023; Radjarejesri *et al.* 2023). Like production units, including paper and pulp, the food industry, textiles, pharmaceuticals and plastics, biodiesel production also consumes enormous amounts of water.

Washing is a significant step that defines biodiesel quality (Diaz *et al.* 2020). The number of washing cycles is determined by oil quality, the process conditions, etc. (Kilicarslan *et al.* 2023). Every batch of washing generates approximately five times the quantity of biodiesel (Bashir *et al.* 2018). The discharged biodiesel washwater is alkaline, turbid, and contaminated with unreacted esters and acids, making it unsuitable for disposal onto the land and water, affecting the metabolic activities of the organisms dwelling in that environment. These, in turn, have endangered humanity's long-term survival. Owing to these terms, finding new regimes for sustainable wastewater treatment and management is necessary. Biodiesel washing process produces huge amount of washwater effluent. An author has tried treating the washwater with the nanofiltration

A Review of Machine Learning Algorithms for Biomedical Applications

V A Binson ¹, Sania Thomas ², M Subramoniam ³, J Arun ⁴, S Naveen ⁵, S Madhu ⁶

Affiliations + expand

PMID: 38383870 DOI: 10.1007/s10439-024-03459-3

Abstract

As the amount and complexity of biomedical data continue to increase, machine learning methods are becoming a popular tool in creating prediction models for the underlying biomedical processes. Although all machine learning methods aim to fit models to data, the methodologies used can vary greatly and may seem daunting at first. A comprehensive review of various machine learning algorithms per biomedical applications is presented. The key concepts of machine learning are supervised and unsupervised learning, feature selection, and evaluation metrics. Technical insights on the major machine learning methods such as decision trees, random forests, support vector machines, and k-nearest neighbors are analyzed. Next, the dimensionality reduction methods like principal component analysis and t-distributed stochastic neighbor embedding methods, and their applications in biomedical data analysis were reviewed. Moreover, in biomedical applications predominantly feedforward neural networks, convolutional neural networks, and recurrent neural networks are utilized. In addition, the identification of emerging directions in machine learning methodology will serve as a useful reference for individuals involved in biomedical research, clinical practice, and related professions who are interested in understanding and applying machine learning algorithms in their research or practice.

Keywords: Biomedical; Convolutional neural networks; Deep learning; Dimensionality reduction methods; Machine learning; SVM.

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Review article

A review on recent advancements in extraction, removal and recovery of phenols from phenolic wastewater: Challenges and future outlook

Kirubanandam Grace Pavithra^a, Panneerselvam Sundar Rajan^b, Jayaseelan Arun^c,
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Abstract

Water pollution is the major problem seen in today's scenario and even pollutants at low concentration harms our environment. In industrial sector usage of phenol is seen even at low concentrations. The interaction of phenol in the environment provides adverse effects to living beings. This review focuses on the toxicity of phenol and its impact towards environment and human health. The treatment techniques such as distillation, extraction, wet air oxidation, membrane process, electrochemical oxidation, biological treatment and finally adsorption techniques were discussed. Among many treatment techniques so far utilized in the treatment of phenol, adsorption was considered as one of the best technique due to its advantages such as reusability, ease in operation, large availability etc., This review also highlights the adsorption technique for the cleaner removal of phenol from aqueous solution with novel as well as low-cost adsorbents in the removal of phenolic compounds. This review also discusses about the drawbacks and issues related with adsorption of phenolic compounds.

Advanced oxidation process (AOP) combined biological process for wastewater treatment: A review on advancements, feasibility and practicability of combined techniques

A. Babu Ponnusami^a, Sanyukta Sinha^a, Hridya Ashokan^a, Mathew V Paul^a,
Sai Prashant Hariharan^a, J. Arun^b, K.P. Gopinath^c, Quynh Hoang Le^{d,e},
Arivalagan Pugazhendhi^{d,e}  

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Abstract

Complexity of wastewater is the most challenging phenomenon on successful degradation of pollutant via any wastewater treatment regime. Upon availability of numerous techniques, Advanced Oxidation Processes (AOP) is the most promising technique for treating industrial wastewater. Higher operating cost is the most promising factor that possess challenge for the industrial scale usage of the AOP process. Combination of biological process with AOP helps in achieving sustainable degradation of toxic pollutant in the wastewater. AOP result in complete or partial degradation of toxic emerging pollutants with the help of free radicals like hydroxyl, superoxide, hydroperoxyl and sulphate radicals. In addition to this the presence of bio-enzymes and microorganisms helps in sustainable degradation of pollutant in an economical and environmentally friendly strategy. In this review, a detailed discussion was conducted on various AOP, focusing on catalytic ozonation, electrochemical oxidation, Sono chemical and photocatalytic processes. With the need for sustainable solutions for wastewater treatment, the use of AOP in conjunction with biological process has innumerable opportunities for not only wastewater treatment but also the production of high value by-products. Further, the effect of AOP combined biological processes needs to be analyzed in real time for the different concentration of industrial wastewater and their benefits needs to be explored in future towards achieving SDGs.

Utilization of biosilica from Bermuda grass ash on silver–grey magnesium: influence of biosilica on its mechanical and tribological properties

Original Article | Published: 24 June 2023

Volume 14, pages 23015–23030, (2024) [Cite this article](#)

[Xuezeng Jia](#), [S. Madhu](#) , [S. Naveen](#), [Suresh Vellingiri](#) & [J. Arun](#)

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Abstract

The significance of biosilica in the silver–grey magnesium (SG–Mg–10% Si) metal is to enhance its mechanical and tribological characteristics. The Bermuda grass was used to make the biosilica. This investigation employed SG–Mg–10%Si and biosilica reinforcement (0, 5, 10, 15, and 20 wt. %) as the composite matrix. Due to increased bonding between the components of the composite, the SG–Mg–10%Si/biosilica composite created by the stir casting process demonstrated remarkable mechanical and tribological qualities. Further testing was done to assess the mechanical and tribological characteristics of the composite specimens, including density, porosity, hardness, tensile strength, and wear rate. SG–Mg–10%Si was contrasted with all these outcomes. The density plummeted at a rate of 1.49 (g/cm³) when the weight percentage of biosilica particles was added. Minimum and maximum hardness values for the composite's 20% of reinforced biosilica particles are 93.4 VHN and 125 VHN, respectively, and the tensile strength is 633.18 MPa. In order to determine the wear rate of SG–Mg–10%Si/biosilica composites, a dry sliding pin–on–disc tribometer was employed. When biosilica particles were added, the rate of wear of the manufactured SG–Mg–10%Si/ biosilica composites gradually reduced. When subjected to loads of 40 N and sliding distances of 1500 m and 2500 m, respectively, the composite with

Assessment of causes and consequences of concrete deterioration and its remediation

Sudha Uthaman^a, Vinita Vishwakarma^b  

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Highlights

- Causes and consequences of concrete deterioration.
- Incorporation of supplementary cementitious materials.
- Significant impact of nanoparticles in concrete structures.
- Innovative measures to expand concrete life.

Abstract

Superiority of concrete is subject to many factors such as quality of cement, water, aggregates, its curing condition, moisture content and so on. These parameters not only abolish the concrete strength in spite of that minimize the durability of concrete prepared from ordinary Portland cement (OPC). Causes of concrete deterioration are typically concern its strength, durability and aesthetic properties. However, factors such as design, material limitations, construction practices and exposure to aggressive environmental conditions are accountable to damage and deteriorate the structural and functional properties of concrete. The defects are mainly due to exposure of concrete in different physical, chemical and biological environments which have adverse effects due to scaling

Short communication

Structural, magnetic and evanescent wave gas sensing analysis of spin-frustrated rare earth doped $\text{Bi}_2\text{Fe}_4\text{O}_9$ mullite ceramics at room temperature

Subha Krishna Rao ^a  , B. Renganathan ^b, A. Kalai Priya ^c, R. Rajesh Kumar ^d,
R. Jothi Ramalingam ^e, Manavalan Rajesh Kumar ^f, R. Kesavasamy ^g,
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<https://doi.org/10.1016/j.ceramint.2023.12.383> 

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Abstract

This study investigates the gas sensing capabilities of bulk $\text{Bi}_{2(1-x)}\text{La}_{2x}\text{Fe}_4\text{O}_9$ with $x=0$, and 0.05 systems (BFO 0, BLFO 5), using a fiber optic VOC sensor at room temperature. Structural and magnetic characterizations confirm the successful incorporation of La^{3+} into the Bi site of the BFO 0 lattice without altering the structure. BLFO 5 exhibits remarkable gas sensitivity towards benzene, with a normalized gas sensitivity of 47 counts/100ppm. This enhanced sensitivity is attributed to the presence of oxygen vacancies, which contribute to BLFO 5's weak ferromagnetism. The rapid response (43 s) and recovery (51 s) times further demonstrate the promising potential of multiferroic materials for VOC detection.

Short communication

Optimizing Gas Sensing Performance of CuO Nanoparticles via Sol-Gel Synthesis Approach for Efficient Detection of Ammonia Gas

B. Renganathan ^a  , C.K. Gopakumar ^b, A. Kalai Priya ^c, Subha Krishna Rao ^d, D. Sastikumar ^b, M. Silambarasan ^e, Nagarajan Kannapiran ^f

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Highlights

- The sensitivity of two different sensors, S1 with a length of 3 cm and S2 with a length of 1.5 cm, was calculated for their ability to detect VOCs gas at room temperature.
- CuO nanoparticles are kept at a higher temperature, the chemisorbed oxygen species remains as O^{2-} and O^- , while at low temperature (below 100°C), it exists as O_2^- .
- The refractive index of the sensing material(CuO) getting modified at minute level when there is ammonia gas interaction.

Delineating the sensing analysis of discarded pencil graphite in biofuel ethanol – Experimental and theoretical validation

B. Renganathan ^a  , Subha Krishna Rao ^b  , C. Poornimadevi ^c, Manjunath S. Kamath ^b,
E. Meher Abhinav ^d, Thrinayan Moorthy ^e, A. Kalai Priya ^f, C. Preferencial Kala ^c  

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Highlights

- Evanescent fiber optic sensing and DFT approach in graphite pencil powder introduced.
- Black graphite pencil powder exhibited better sensitivity for ethanol at room temperature.
- DFT analysis supported the experimental results with better adsorption energy and distance.

Abstract

This study investigated the ethanol-sensing properties of pencil graphite using a fiber optic gas sensor. The results were further confirmed using density functional theory (DFT). The sensor showed good sensitivity (18 counts/100ppm) and response time (70 sec). The electronic analysis of pure and ethanol-adsorbed graphite powders was also conducted using their band structures, Density of States (DOS), and projected Density of

Evodiamine release from interlinked porous polycaprolactone scaffold for cancer therapy

Lavanya S.K.^a, Manjunath Kamath S.^b, Subha Krishna Rao^b, Shantanu Patil^c,
Rajiv Janardhanan^a, Sundaresan S.^a  

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Highlights

- Evodiamine-albumin nanoparticle (EANP) in polycaprolactone (PCL)3D scaffolds.
- The characterization analysis of scaffold closely examined.
- Drug release lasted 8 days, inhibiting HeLa cancer cells and dose-dependent too.
- EANP-loaded PCL scaffold shows promise for cervical cancer therapy.

Abstract

Target drug delivery for therapeutic pharmaceuticals in a carcinogenic environment is becoming more popular because of its benefits, such as avoiding systemic adverse effects, high productivity and low-cost competence. Herein, in this study Evodiamine-albumin nanoparticles (EANPs) were incorporated into 3D porous polycaprolactone (PCL) scaffolds for sustained release, aiming to evaluate their potential as an effective and

Recent trends in phyto-mediated iron-based nanomaterials for environmental remediation and biomedical applications

Ankush Chauhan ^{a, b}, Garima Rana ^a  , Vishal Dutta ^d, Amita Kumari ^e, Subha Krishna Rao ^f, Rajasekaran Subbarayan ^g, Karthikeyan Ravi ^b, Satheesh Selvaraj ^a, Suresh Ghotekar ^b  

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Highlights

- This review highlights the various plant extract assisted methods for phyto-synthesis of iron-based nanomaterials.
- Factors affecting to the phyto-synthesis of iron-based nanomaterials are studied.
- Environmental remediation and biomedical applications of iron-based nanomaterials are also covered.
- Future perspectives and challenges are proposed in briefly.

Abstract

Advancements in nanoscience and nanotechnology have significantly impacted the widespread integration of iron-based nanomaterials across various domains of scientific investigation, research institutions, and industrial sectors. The intrinsic properties exhibited by iron oxide nanoparticles, such as their biocompatibility, low toxicity,

Annealing-induced enhancement of TiO₂-ZnO nanocomposites for high-performance room-temperature air pollutant detection in fiber optic sensors

B. Renganathan ^a  , Manjunath S. Kamath ^b, M. Silambarasan ^c, V.K. Gobinath ^d, A.R. Ganesan ^e, A. Deepak ^a, N. Kannapiran ^f, K. Guhan ^g, Narendhar Chandrasekar ^h, Subha Krishna Rao ^b 

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<https://doi.org/10.1016/j.microc.2024.110329> 

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Highlights

- Need for economic growth led to the development of fiber optic-based sensors.
- Coupling of metal oxides permits redox reactions supporting sensing property.
- TiO₂-ZnO composites (S1) and annealed 500°C (S2) & 1200°C (S3) in sensing study.
- Enhanced sensitivity and selectivity in S3 sensor for ammonia at room temperature.
- The rise and fall time of the S3 sensor were 18 sec and 15 sec respectively.

Review

A state-of-the-art review on plant-derived cellulose-based green hydrogels and their multifunctional role in advanced biomedical applications

Satheesh Selvaraj ^{a, b}, Ankush Chauhan ^{a, b}  , Vishal Dutta ^c, Ritesh Verma ^d,
Subha Krishna Rao ^e, Arunkumar Radhakrishnan ^{b, f}, Suresh Ghotekar ^g  

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<https://doi.org/10.1016/j.ijbiomac.2024.130991> 

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Highlights

- Cellulose and derivatives reviewed for tissue engineering and drug delivery.
- Exploration of cellulose with various functional groups for allowing manipulation of properties.
- Novel uses identified, emphasizing unique microstructures for diverse biomedical applications.
- Recent strides in cellulose synthesis highlighted with innovative techniques for biomedical integration.

Engineering luminescent quantum dots through laser ablation of samarium oxide (Sm_2O_3): A novel approach for enhanced fiber optic gas sensing

A. Kalai Priya ^{a,1}  , Subha Krishna Rao ^b, S. Divya ^{c,1}, Natarajan Vijay ^c, Peter Jerome ^c, Tae Hwan Oh ^c, B. Renganathan ^d, D. Sastikumar ^c  

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Highlights

- Synthesis of novel Sm_2O_3 luminescent quantum dots using a unique pulsed laser ablation technique.
- These quantum dots were employed in clad-modified fiber optic gas sensor at room temperature.
- Ethanol displayed remarkable sensitivity and selectivity, with a response of 104×10^3 Counts/kPa at 25 °C for 500 ppm.
- These results establish Sm_2O_3 quantum dots as a promising and highly effective material for ethanol sensing.

ARTICLE | July 28, 2023

Laser-Ablated Transmutation of Bulk TiO_2 into Nanospheres onto a Clad-Polished Optical Fiber for Room-Temperature Gas Sensing

Kalai Priya Anbalagan, Subha Krishna Rao, Devendiran Subbaiyan, and Dillibabu Sastikumar*



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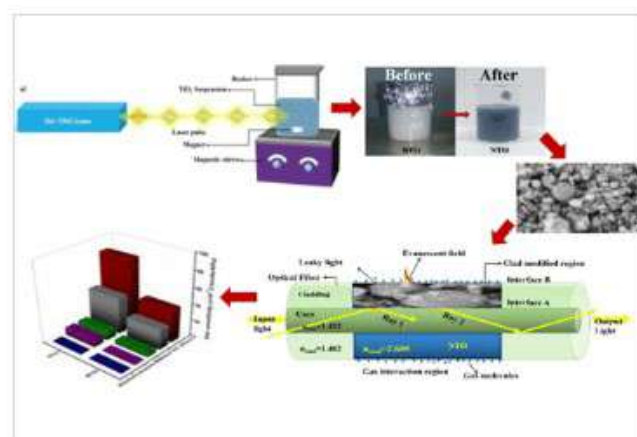
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Abstract

The impact of environmental pollution on climate change has necessitated the development of highly efficient gas sensors. Among the various sensing materials, metal oxides have shown great potential for gas sensing applications. However, the quest for highly efficient room-temperature gas sensors is ongoing. In this study, we present an approach for the fabrication of nanospheres from bulk TiO_2 using the template-free pulsed laser ablation technique in liquids. The obtained nanoparticles were comprehensively characterized using X-ray diffraction, Raman analysis, scanning electron microscopy (SEM), high-resolution transmission electron microscopy (HRTEM), ultraviolet–visible (UV–vis) spectroscopy, and photoluminescence (PL) studies to evaluate their structural, morphological, and optical properties. The room-temperature gas sensing performance of the fabricated TiO_2 nanospheres (NTO) was evaluated using clad-modified fiber optic gas sensors for various gas concentrations. Remarkably, the NTO gas sensor exhibited interesting results, where the performance of NTO was twice that of its bulk counterpart, indicating its suitability for highly efficient gas sensing applications. Additionally, a theoretical calculation of light modulation through optical fibers was presented, providing insights into the sensing mechanism.



Cellular temperature probing using optically trapped single upconversion luminescence

K. Suresh ^a, K. Monisha ^a, Aseefhali Bankapur ^a, Subha Krishna Rao ^b, Srinivas Mutalik ^c,
Sajan D. George ^{a,d}  

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Highlights

- Design and development of optical tweezers setup coupled with luminescence measurement.
- Optical trapping of single upconversion nanoparticle.
- Power and time dependent emission studies of single upconversion particle.
- Optical trapping of single upconversion particle inside the biological cell.
- Use of single upconversion particle for intercellular temperature probing.

Sustained release of resveratrol from fused deposition modelling guided 3D porous scaffold for bone tissue engineering

K. Harini Sri ^a, Dhanraj Ganapathy ^a, Deepak Nallasamy ^a, Suresh Venugopalan ^a,
Vinay Sivaswamy ^a, S. Manjunath Kamath ^{b, c}  

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Highlights

- 3D printed embedment scaffold fabricated with resveratrol (Res) release.
- Res albumin nanoparticle (RSV-Np) concentration influenced rate of release.
- RSV-Np concentration imparted hydrophilicity in the PCL embedment.
- Res was amorphized during encapsulation – beneficial for drug dissolution.
- *In vitro* analysis – not cytotoxic, enhanced alkaline phosphatase & mineralization.

New Ways to Protect the Host from SARS-CoV-2? Lung Microbiome Metabolites Inhibit STAT3 and Modulate the Immunological Network

Jency Roshni ¹, Mahema Sivakumar ¹, Faris Ahmed Bahammam ², Shilpa Bhandi ^{3 4}, Shankargouda Patil ^{3 5}, Manjunath Kamath ⁶, Ali Abusharha ⁷, Shiek S S J Ahmed ¹

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PMID: 37140561 DOI: [10.1089/omi.2023.0002](https://doi.org/10.1089/omi.2023.0002)

Abstract

COVID-19 caused by the SARS-CoV-2 infection is a systemic disease that affects multiple organs, biological pathways, and cell types. A systems biology approach would benefit the study of COVID-19 in the pandemic as well as the endemic state. Notably, patients with COVID-19 have dysbiosis of lung microbiota whose functional relevance to the host is largely unknown. We carried out a systems biology investigation of the impact of lung microbiome-derived metabolites on host immune system during COVID-19. RNAseq was performed to identify the host-specific pro- and anti-inflammatory differentially expressed genes (DEGs) in bronchial epithelium and alveolar cells during SARS-CoV-2 infection. The overlapping DEGs were harnessed to construct an immune network while their key transcriptional regulator was deciphered. We identified 68 overlapping genes from both cell types to construct the immune network, and Signal Transducer and Activator of Transcription 3 (STAT3) was found to regulate the majority of the network proteins. Furthermore, thymidine diphosphate produced from the lung microbiome had the highest affinity with STAT3 (-6.349 kcal/mol) than the known STAT3 inhibitors ($n = 410$), with an affinity ranging from -5.39 to 1.31 kcal/mol. In addition, the molecular dynamic studies showed distinguishable changes in the behavior of the STAT3 complex when compared with free STAT3. Overall, our results provide new observations on the importance of lung microbiome metabolites that regulate the host immune system in patients with COVID-19, and may open up new avenues for preventive medicine and therapeutics innovation.

Keywords: COVID-19; biomarkers; drug discovery; immune networks; lung microbiome; planetary health.

Controlled release of kaempferol from porous scaffolds augments *in-vitro* osteogenesis in human osteoblasts

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Shiek SSJ. Ahmed ^b , V. Gopinath ^c , Ganesh Munuswamy-Ramanujam ^d,
Subha Krishna Rao ^e , , Manjunath Srinivas Kamath ^{f,g} , 

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Abstract

Bone tissue engineering (BTE) is a multidisciplinary approach intending to treat bone abnormalities by using a porous polymer matrix that has the capacity to host cells, promote proliferation and continuously release drugs/growth factors to enhance bone regeneration. This study fabricated a 3D porous scaffold releasing kaempferol with varying concentrations of kaempferol-entrapped albumin nanoparticles (KaeNP) and polycaprolactone (PCL). The morphology of scaffolds was studied to ensure porous structures and the results revealed that the diameter of pores ranged between 50µm–300µm which is essential for cell penetration and angiogenesis. Further, X-ray diffraction analysis revealed amorphized form of kaempferol in scaffolds which is beneficial for delivering drugs, as it enhances dissolution. It was observed that kaempferol was controlled released for a period of 20 days. Moreover, the release of kaempferol from scaffolds implies that the concentration of KaeNP alters drug release. *In vitro* analysis with Human osteoblasts revealed the scaffolds were not toxic and had significant osteogenic potential by virtue of enhancing alkaline phosphatase activity and mineralization. In summary, these scaffolds possessing high osteogenic potential by virtue of controlled kaempferol release will be highly desired for bone tissue engineering.

Design development and optimisation of multifunctional Doxorubicin-loaded Indocyanine Green proniosomal gel derived niosomes for tumour management

[Jaison Darson](#), [Radha Thirunellai Seshadri](#), [Kajal Katariya](#), [Mothilal Mohan](#), [Manjunath Srinivas Kamath](#), [Meher Abhinav Etyala](#) & [Gopalakrishnan Chandrasekaran](#) 

[Scientific Reports](#) **13**, Article number: 1697 (2023) | [Cite this article](#)

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Abstract

This study presents the design, development, and optimization of multifunctional Doxorubicin (Dox)-loaded Indocyanine Green (ICG) proniosomal gel-derived niosomes, using Design of Experiments (2^3 factorial model). Herein, the multifunctional proniosomal gel was prepared using the coacervation phase separation technique, which on hydration forms niosomes. The effect of formulation variables on various responses including Zeta potential, Vesicle size, entrapment efficiency of Dox, entrapment efficiency of ICG, Invitro drug release at 72nd hour, and NIR hyperthermia temperature were studied using statistical models. On the basis of the high desirability factor, optimized formulation variables were identified and validated with the experimental results. Further, the chemical nature, vesicle morphology, surface charge, and vesicle size of optimized proniosomal gel-derived niosomes were evaluated. In addition, the effect of free ICG and bound ICG on NIR hyperthermia efficiency has been investigated to demonstrate the heating rate and stability of ICG in the aqueous environment and increased temperature conditions. The drug release and kinetic studies revealed a controlled biphasic release profile with complex mechanisms of drug transport for optimized proniosomal gel-

Sensing performance optimization by refining the temperature and humidity of clad engraved optical fiber sensor in glucose solution concentration

B. Renganathan^{a,d}  , Subha Krishna Rao^b, Manjunath S. Kamath^c, A. Deepak^a,
A.R. Ganesan^d

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Highlights

- To ensure the accuracy and reliability of the detection results, the ambient temperature and humidity must be calibrated during the glucose concentration measurement.
- Clad removed multimode plastic fiber optic analyte for studying the power response of glucose solution at low wavelength.
- Different temperatures (20°C, 30°C, 40°C). Normal temperature (25°C) was found to have a better reaction than the other values.
- The maximum intensity recorded at various concentrations as a function of the refractive index of the medium.
- The principle of evanescent wave absorption.

Performance evaluation of Ce doped ZnO clad modified fiber optic non-enzymatic glucose sensor at varying ambient temperatures for blood sugar detection applications

B. Renganathan ^{a, d}  , Subha Krishna Rao ^b  , Manjunath S. Kamath ^c, K. Ajitha ^b,
A.R. Ganesan ^d, A. Deepak ^a

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<https://doi.org/10.1016/j.microc.2022.107890> 

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Highlights

- Clad removed and clad modified fiber as a function of time at different temperatures (20 °C, 30 °C).
- This optical fiber based glucose sensor works on the principle of evanescent wave absorption phenomena.
- A clad modified rare earth (Ce) doped metal oxide (ZnO) fiber optic glucose sensors.

Sustained delivery of andrographolide from 3D porous scaffolds imparting anticancer activity

S.K. Lavanya ^a, Manjunath Kamath S ^b, Subha Krishna Rao ^c, P. Rajapriya ^a, Shantanu Patil ^d,
S. Sundaresan ^a  

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Highlights

- Andrographolide albumin nanoparticles loaded porous scaffold fabricated.
- Concurrent sustained releases of 12% Scaf-A-1, A-2 & A-3 were observed for 8 days.
- Andrographolide amorphized during preparation of scaffolds by leaching method.
- 12% Scaf A-1, A-2 & A-3 were inhibited the HeLa cancer cell growth using MTT assay.
- DAPI stain showed morphological changes in 12% Scaf A1, A2 & A3-treated HeLa cells.

Unraveling the potential of Gd doping on mullite $\text{Bi}_2\text{Fe}_4\text{O}_9$ for fiber optic ethanol gas detection at room temperature

Subha Krishna Rao ^a, A. Kalai priya ^b, S. Manjunath Kamath ^c, E. Meher Abhinav ^d,
B. Renganathan ^e, K. Jeyadheepan ^f, C. Gopalakrishnan ^g  

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Highlights

- Gadolinium doped $\text{Bi}_2\text{Fe}_4\text{O}_9$ (BGFO3) synthesized – ball milling method.
- Improved conduction in BGFO3- confirmed by Narrow band gap.
- Clad modified BGFO3 – Fiber optic gas sensor.
- Increased Sensitivity (%) for Ethanol-rapid response (38s) and recovery time (67s).
- BGFO3, a potential multiferroic oxide for ethanol sensing in industrial applications.

Annealing-induced enhancement of TiO₂-ZnO nanocomposites for high-performance room-temperature air pollutant detection in fiber optic sensors

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Highlights

- Need for economic growth led to the development of fiber optic-based sensors.
- Coupling of metal oxides permits redox reactions supporting sensing property.
- TiO₂-ZnO composites (S1) and annealed 500°C (S2) & 1200°C (S3) in sensing study.
- Enhanced sensitivity and selectivity in S3 sensor for ammonia at room temperature.
- The rise and fall time of the S3 sensor were 18 sec and 15 sec respectively.

Current Advances on Biomedical Applications and Toxicity of MWCNTs: A Review

Published: 29 April 2023

Volume 13, pages 860–878, (2023) [Cite this article](#)

[Sandhya Selvakumar](#), [Thyagarajan Rajendiran](#) & [Kunal Biswas](#) 

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Abstract

Recently, carbon nanotubes (CNTs) have become a possible new choice in variable disciplines such as gene therapy, bioengineering, cancer treatment and nanotechnology applications in devices, and optics. This is because multi-walled carbon nanotubes (MWCNTs) are more durable and possess high electrical and thermal conductivity along with some of the significant unique physico-chemical characteristics. As the manufacturing and usage of CNTs rises, the likelihood of exposure to CNTs, whether directly or indirectly, is higher in the general population. As a result, it becomes crucial to examine the problems related to the toxicity of CNTs and identify the most effective solutions to address such concerns. Available data demonstrates unequivocally that nanotubes may, in some circumstances, pass membrane barriers and enter the organ which may lead to adverse consequences such as inflammatory and fibrotic responses. So, the first step in assessing the biosafety and biocompatibility of CNTs in living organisms is to quantify their abundance. This review article discusses the toxicity mechanisms of

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Exploring the Potentials of Magnetic Nanoscale Material for Different Biomedical Applications: a Review

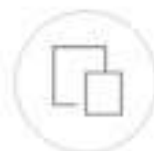
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Rough edges of reduced graphene oxide (rGO) sheets elicit anticancerous activities: An *in vitro* study

Kunal Biswas^{a,1}, Gopalarethinam Janani^{b,1}, Saranya Udayakumar^b, Balasubramanian Deepika^b, Koyeli Girigoswami^b  

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Abstract

In this study, reduced graphene oxide nanosheets (rGO) were synthesized using a chemical bottom-up technique of modified Hummer's method. The as-synthesized graphene sheets were characterized optically by Fourier Transform Infra Red spectroscopy (FTIR) and Raman spectroscopy. The morphological characterizations were performed using Field-Emission Scanning Electron Microscopy (FE-SEM), High-Resolution Transmission Electron Microscopy (HR-TEM), Atomic Force Microscopy (AFM) and Confocal Microscopy, respectively. It could be evident from the images that wrinkles and surface corrugation are clearly visible from the morphological analysis. The biomedical applications of rGO nanosheets have been studied in the cancer cell lines of A375 and A549 cells at the *in-vitro* conditions. MTT assay and live dead assay results clearly indicated that rGO exhibited robust anticancerous activities against the chosen cell lines. Although, it could not elicit any antimicrobial activity at the selected doses. Understanding the primary mechanism and the underlying phenomenon involving the interaction of the rGO nanosheets with the cell systems reveals the potentialities of the nanosheet in designing future-based anticancerous therapeutics at the nanoscale level.

Nanostructure-assisted wound dressing materials: a literature review

Review | Published: 24 June 2024

(2024) [Cite this article](#)

P. S. Sharon Sofini, [Kunal Biswas](#), [Devadass Jessy Mercy](#), [Agnishwar Girigoswami](#) & [Koyeli Girigoswami](#)



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Abstract

When wounds are caused by burns, traumatic injuries, chronic ulcers, or diabetic injuries, bacterial infection can be a major problem. It can even be life-threatening. The wound-healing process is slowed by multidrug-resistant microorganisms, so antimicrobial wound dressings are generally used. Nanostructures comprise nanoparticles, nanorods, nanospheres, nanoshells, nanoflowers, hybrid polymers, etc., which can be used for wound healing or can be used to deliver the drug at the site of the wound to improve the drug's efficacy and stability. In this review, we shall discuss the different therapeutic strategies of nanoparticle-based wound dressing materials with or without the incorporation of natural products. The recent research on nanostructure-based wound dressing materials will also be discussed.

Unveiling the properties of layered 2D-based nano-material flexible electronics in biomedical applications: a review

Review | Published: 03 June 2024

Volume 59, pages 11218–11245, (2024) [Cite this article](#)

[S. Preethi](#) , [Sony Varghese](#), [Kunal Biswas](#) & [N. Vijayalakshmi](#)

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Abstract

Nanomaterials are the key to the success of cutting-edge monitoring and diagnostics systems enabled by flexible electronics that are revolutionizing healthcare systems. Among them, layered 2D-based materials with exceptional qualities including high electrical conductivity, mechanical flexibility, thermal stability, and optical adaptability stand out. These include graphene and transition metal dichalcogenides. This comprehensive examination covers the fabrication and characterisation of these nanomaterials, as well as their immense potential for usage in flexible electronics for biomedical applications. The layered 2D-based nanomaterials have changed the face of healthcare technology by providing examples ranging from wearable health monitoring devices to implantable sensors. This has improved both device performance and user experience. There are many difficulties including biocompatibility and long-term stability while simultaneously addressing moral and legal issues, highlighting the significance of responsible technology development in this game-changing area. The extraordinary characteristics of layered 2D-

Potentialities of Bio-functionalized Carbon Nanotubes for Different Anti-cancerous Activities

Review | Published: 06 March 2024

Volume 34, pages 2325–2350, (2024) [Cite this article](#)

[Granap Blessy Ramesh](#), [Prabhakar Singh](#) & [Kunal Biswas](#) 

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Abstract

The utilization of carbon nanotubes (CNTs) in the field of cancer therapeutics has emerged as a promising avenue of research in recent years. This inquiry delves into the multifaceted potential of bio-functionalized carbon nanotubes for diverse anti-cancer activities. Carbon nanotubes, with their distinct structural and physicochemical properties, offer a versatile platform for innovative approaches in cancer diagnosis, treatment, and comprehension of the underlying molecular mechanisms. The process of bio-functionalizing CNTs plays a pivotal role in enhancing their compatibility with living organisms and specificity, thereby facilitating precise targeting of cancer cells. This scholarly article elucidates the strategies

Drug-loaded polymer-coated silver nanoparticles for lung cancer theranostics

Ranjita Misra ¹, Subhenjit Hazra ², Suraiya Saleem ³, Sushmitha Nehru ²

Affiliations [+ expand](#)

PMID: 38687401 DOI: [10.1007/s12032-024-02372-y](https://doi.org/10.1007/s12032-024-02372-y)

Abstract

Lung cancer is the leading cause of death in cancer across the globe. To minimize these deaths, the replacement of traditional chemotherapy with novel strategies is significant. We have developed a nanotheranostic approach using silver nanoparticles for imaging and treatment. Silver nanoparticles (AgNPs) are fabricated by chemical reduction method. The formulation of AgNPs was confirmed by different characterization techniques like stability test, UV-Visible spectroscopy, Confocal Raman Spectroscopy, and Energy-Dispersive X-ray analysis. Further, AgNPs are coated with poly lactic-co-glycolic acid (PLGA) and then loaded with paclitaxel (Pac). Then the drug-loaded PLGA-coated AgNPs were characterized for size and zeta potential measurement by zetasizer, surface morphology study by atomic force microscopy, Fourier transform infrared spectroscopy, and release kinetics study. The imaging and anticancer properties of these nanoformulations are investigated using lung cancer cell lines. The results proved that the particles are in the nanometer range with smooth surface morphology. Moreover, the drug-loaded NPs showed a sustained release of the drug for a longer period of time. Further the formulations showed imaging property with greater anticancer efficacy. Thus, the results suggest the effective use of these nanoformulation in both lung cancer imaging and treatment using a simple and efficient approach.

Keywords: Lung cancer; Nanotheranostics; PLGA; Paclitaxel; Silver nanoparticles.

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Multi-functional bioactive silver- and copper-doped diamond-like carbon coatings for medical implants

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Mihalis I. Panayiotidis^e, Thomas J. Joyce^f, Murtaza M. Tambuwala^g, Ángel Serrano-Aroca^h

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Abstract

Diamond-like carbon (DLC) coatings doped with bioactive elements of silver (Ag) and copper (Cu) have been receiving increasing attention in the last decade, particularly in the last 5 years, due to their potential to offer a combination of enhanced antimicrobial and mechanical performance. These multi-functional bioactive DLC coatings offer great potential to impart the next generation of load-bearing medical implants with improved wear resistance and strong potency against microbial infections. This review begins with an overview of the status and issues with current total joint implant materials and the state-of-the art in DLC coatings and their application to medical implants. A detailed discussion of recent advances in wear resistant bioactive DLC coatings is then presented with a focus on doping the DLC matrix with controlled quantities of Ag and Cu elements. It is shown that both Ag and Cu doping can impart strong antimicrobial potency against a range of Gram-positive and Gram-negative bacteria, but this is always accompanied so far by a reduction in mechanical performance of the DLC coating matrix. The article concludes with discussion of potential synthesis methods to accurately control bioactive element doping without jeopardising mechanical properties and gives an outlook to the potential long-term impact of developing a superior multifunctional bioactive DLC coating on implant device performance and patient health and wellbeing.

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Dielectric relaxation of ice in a partially crystallized poly(*N*-isopropylacrylamide)microgel suspension compared to other partially crystallized polymer–water mixtures[†]

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Polyhydroxybutyrate (PHB)-Based sustainable bioplastic derived from *Bacillus* sp. KE4 isolated from kitchen waste effluent

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Monti Gogoi ^c, Swapnali Hazarika ^c, Pranab Jyoti Koch ^d, Nipu Dutta ^e,
Gobichettipalayam Balasubramaniam Maadurshni ^f, Jeganathan Manivannan ^f, Sony Kumari ^d,
Bendangtula Walling ^a, Bhagyudoy Gogoi ^a, Alemtoshi ^a, Viphrezolie Sorhie ^a,
Vinita Vishawkarma ^g

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Highlights

- *Bacillus* sp. KE4 strain was able to accumulate 70.4 % of PHB.
- Physico-chemical characterization confirmed biopolymer's excellent thermostability.
- Studied biopolymer exhibited biocompatibility to HepG2 cells.
- Approximately 60% of the PHB film was found to degrade within 28 days.
- Obtained biopolymer confirmed its sustainability and eco-benign nature.

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Production of bacterial nanocellulose as green adsorbent matrix using distillery wastes for dye removal: a combined approach for waste management and pollution mitigation

Original Article | Published: 02 April 2024

(2024) [Cite this article](#)

[Bendangtula Walling](#), [Alimpia Borah](#), [Swapnali Hazarika](#), [Pranjal Bharali](#) , [D. Ramachandran](#), [Viswanathan Kanagasabai](#), [Nipu Dutta](#), [Gobichettipalayam Balasubramaniam Maadurshni](#), [Jeganathan Manivannan](#), [Pronab Mudoi](#), [Pranjal Kumar Kaman](#), [Viphrezolie Sorhie](#), [Bhagyudoy Gogoi](#), [Alemtoshi](#), [Shiva Aley Acharjee](#), [Vinita Vishwakarma](#) & [Palash Deb Nath](#)

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Abstract

The possibility of using stillage of rice beer as a source of carbon for the manufacture of bacterial nanocellulose (BNC) was investigated in this work. 16.434 g L⁻¹ of BNC was produced by *Komagataeibacter saccharivorans* NUWB1 using the rice beer stillage along with the other media components of Hestrin–Schramm (HS) medium. Findings showed that obtained BNC possessed dense nano-fibrous network with a signature of cellulose I type, crystallinity index of 86.23%, and higher thermostability up to 380 °C could withstand tensile loads of 68.044 MPa, and exhibited a water holding capacity of 97.53 g g⁻¹. N₂ adsorption–desorption isotherm of the BNC was found to be of type IV, suggesting mesoporous type structure with a total pore volume and surface area of 3.294e-03cc g⁻¹

In-situ biofabrication of bacterial nanocellulose (BNC)/graphene oxide (GO) nano-biocomposite and study of its cationic dyes adsorption properties

Bendangtula Walling^a, Pranjal Bharali^a  , D. Ramachandran^b, K. Viswanathan^b, Swapnali Hazarika^c, Nipu Dutta^d, Pronab Mudoi^e, Jeganathan Manivannan^f, S. Manjunath Kamath^b, Sony Kumari^g, Vinita Vishwakarma^h, Viphrezolie Sorhie^a, Bhagyudoy Gogoi^a, Shiva Aley Acharjee^a, Alemtoshi^a

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Highlights

- A possible *in situ* amalgamation of Graphene oxide on BNC is presented.
- GO did not exhibit any antibacterial activity against the bacteria *Ksaccharivorans*.
- GO nanoparticles were uniformly dispersed in the BNC matrix.
- Efficient selective adsorption of BNC might show a promising utility in effluent treatment.
- BNC-GO composites showed no cytotoxicity.



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Article

Hot Corrosion Behavior of Plasma-Sprayed $\text{Gd}_2\text{Zr}_2\text{O}_7/\text{YSZ}$ Functionally Graded Thermal Barrier Coatings

by Rajasekaramoorthy Manogaran ^{1,2} , Karthikeyan Alagu ^{1,*} , Anderson Arul ¹ , Anandh Jesuraj ² , Dinesh Kumar Devarajan ² , Govindhasamy Murugadoss ² and Kamalan Kirubaharan Amirtharaj Mosas ^{3,*}

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Bacterial valorization of agricultural-waste into a nano-sized cellulosic matrix for mitigating emerging pharmaceutical pollutants: An eco-benign approach

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<https://doi.org/10.1016/j.ijbiomac.2024.133684> 

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Highlights

- Residual maize stalk syrup was found to be an efficient and novel carbon source.
- Obtained BNC exhibited impressive physicochemical properties.
- Produced BNC confirmed its cellular compatibility and eco-benign nature.
- Synthesized BNC is efficient in the sequestration of emerging pharmaceutical pollutants.
- Confirms valorization of agro-waste for the production of BNC.

Article

Multifunctional biomedical applications of MXene-based hydrogels: A review

April 2024 · [Inorganic Chemistry Communications](#)

DOI: [10.1016/j.inoche.2024.112457](#)

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