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## DECLARATION

I declare that I conducted the research presented in this thesis at the Department of Biotechnology, University of North Bengal, Raja Rammohunpur-734014, West Bengal, India, under the guidance of Dr. Ranadhir Chakraborty, Professor in the Department of Biotechnology at the University of North Bengal. I further confirm that this work is entirely original and has not been previously submitted, either in whole or in part, for any academic degree, diploma, or other awards at this university or any other educational institution.

Chandana Basak  
02/02/2024  
(Chandana Basak)

Department of Biotechnology  
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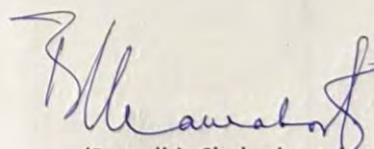


**UNIVERSITY OF NORTH BENGAL**  
**DEPARTMENT OF BIOTECHNOLOGY**  
**RAJA RAMMOHUNPUR-734013, WEST BENGAL, INDIA**

**CERTIFICATE**

The research work presented in this thesis entitled "Exploring gut microbiome of a unique **intestinal air-breathing fish *Lepidocephalichthys guntea***" has been carried out under my direct supervision by **Chandana Basak**. This work is original and has not been submitted for any degree or diploma to this or any other University or Institution.

Date: 05-02-2024



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Ranadhir Chakraborty, Ph.D  
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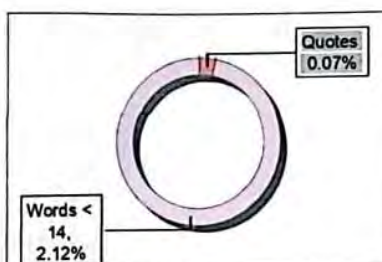
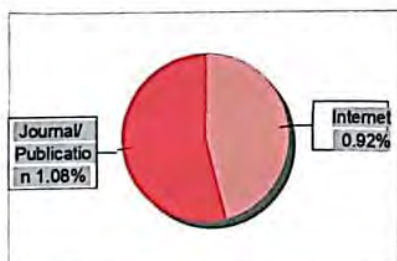
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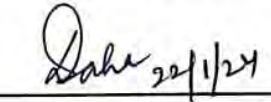
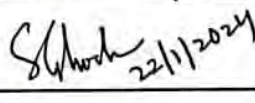
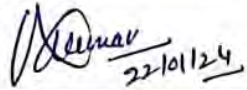
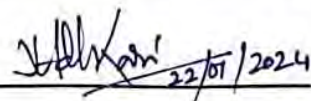
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## DRC CERTIFICATE

It is certified that the work contained in the thesis titled “Exploring gut microbiome of a unique intestinal air-breathing fish *Lepidocephalichthys guntea*” by “Chandana Basak,” has been carried out following University guideline and has been approved by the Departmental research Committee for processing by the Board of Research Studies.

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## PREFACE

The intricate interplay between fish physiology, microbial communities, and the surrounding environment has been a subject of scientific curiosity for centuries. As we delve into this fascinating realm, the pioneering work of Metchnikoff, dating back 110 years, serves as a cornerstone. His astute observation on the correlation between the activity of the intestinal microbiota and host longevity ignited a continuous exploration into the microbial ecosystems inhabiting the gastrointestinal tracts of animals.

Fish, the aquatic, gill bearing, chordate animals are evolutionary common ancestor of amphibians, reptiles, mammals and birds. The first air breathing fish evolved during Silurian period, prior to the conquest of terrestrial environment. This air breathing capacity potentially increases the chance of living in challenging environments. Several different accessory respiratory organs were evolved in fish that includes air bladder, buccal cavity, alimentary canal, skin etc. Fish with accessory respiratory systems have evolved these adaptations to enhance their ability to obtain oxygen, especially in environments with lower oxygen levels. This preface introduces a comprehensive journey into the dynamic world of fish microbiota, focusing on the gastrointestinal tract of loaches, with a special spotlight on *Lepidocephalichthys guntea* that is a gill-intestinal breather, which means that during hypoxia these fish could breathe from air through its posterior intestine. From the early misconceptions about the simplicity of fish microbiota to the revelation of their intricate role in fish physiology, the narrative unfolds. The fish gut, dense with microbial communities, emerges as a vital ecological niche influencing host development, nutrition, and immune responses. The delicate balance of symbiosis within the gut microbiota is crucial for maintaining the health of fish hosts, preventing dysbiosis, and averting potential lethal infections.

The research progress on loaches, with a focus on *L. guntea*, takes center stage in this narrative. A panoramic review traverses diverse disciplines such as taxonomy, conservation, ecology, genetics, reproductive biology, microbiology, and more. The precarious conservation status of loach species,

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their ecological preferences, feeding behaviours, and unique adaptations come under scrutiny. Molecular and genetic studies unravel the evolutionary history of loaches, shedding light on their genetic diversity and population structures. The spotlight then shifts to the specific research progress on *L. guntea*, emphasizing its morphological and anatomical features. The unique gastro-respiratory traits of this species and its adaptation to variable environmental conditions provide a rich backdrop for scientific inquiry. The limited research on *L. guntea*, spanning toxicity studies, life history traits, reproductive biology, and bacterial profiles, prompts an urgent need for deeper investigations. As we embark on this scientific voyage, the quest to bridge the knowledge gap on the gut bacterial population of *L. guntea* takes center stage. The impending research endeavors promise a nuanced understanding of the physiological, biochemical, and genomic attributes of the gut microbiota. Furthermore, a comparative analysis with gill-breathing fish adds a layer of complexity to the exploration, offering insights into the modulation of microbial communities in different respiratory environments.

In essence, this preface sets the stage for a meticulous exploration into the gut bacterial intricacies of *L. guntea*, shedding light on its composition, responses to environmental stressors, hypoxic stress, and immune reactions to pathogenic challenges. The use of various molecular techniques and genomic analyses contributes to a comprehensive understanding of the fish's gut ecology and host-microbe interactions.

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## **ABBREVIATIONS**

<b>Abbreviation</b>	<b>Description</b>
ANI	Average nucleotide identity
AR	Antibiotic resistance
AMR	Antimicrobial drug resistance
ARG	Antibiotics-resistance genes
ATM	Aztreonam
BLAST	Basic Local Alignment Search Tool
°C	Degree Celsius
CAZ	Ceftazidime
cDNA	Cyclic deoxyribo nucleic acid
CDS	Coding DNA sequence
CFU	Colony-forming unit
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
COG	Cluster of orthologous group
CPCSEA	Committee for the purpose of Control and Supervision of Experiment
DO	Dissolved oxygen
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ESBL	Extended-spectrum $\beta$ -lactamase
g	Gram
gDNA	Genomic DNA
GGDC	Genome genome distance calculator
GO	Gene ontology
h	Hour(s)
HGT	Horizontal gene transfer
IMP	Imipenem

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Kb	Kilo base pair
KEGG	Kyoto Encyclopedia of Genus and Genomes
LD <sub>50</sub>	Lethal dose for 50% of treated individuals
LEV	Levofloxacin
M	Molar
m	Minute
MDR	Multi-drug resistant
mg	Miligrams
MIC	Minimum inhibitory concentration
ml	Mililiters
mM	Mili molar
µg	Micro gram
µl	Micro liter
µM	Micromolar
NCBI	<i>National Center for Biotechnology Information</i>
NGS	Next-generation sequencing
OD	Optical density
PBP	Penicillin-binding protein
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
RND	Resistance nodulation-cell division
RPM	Rotation per minute
TE	Tetracycline
UV	Ultraviolet
WGS	Whole-genome sequencing

