MINISTRY OF EDUCATION
AND TRAINING

VIETNAM ACADEMY OF
SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

Nguyen Thi Hoang Ha

DIVERSITY OF MONOGENOIDEA IN MARINE FISH FROM CAT BA ISLAND, HAI PHONG

SUMMARY OF DISSERTATION ON PARASIOLOGY

Major: Parasitology
Code: 9 42 01 05

Hà Nội – 2025

The dissertation is completed at: Graduate University of Science and Technology, Vietnam Academy Science Technology.
Department of Parasitology, Faculty of Ecology and Biological Resources, Graduate University of Science and Technology, Vietnam Academy Science and Technology.

Supervisors:
1.Nguyen Van Ha, Assoc. Prof., PhD.
Institute of Biology, Vietnam Academy of Science and Technology
2.Nguyen Manh Hung, Assoc. Prof., PhD.
Institute of Biology, Vietnam Academy of Science and Technology

Referee 1: Assoc. Prof., PhD. Thai Thanh Binh
Fisheries and Technical Economic College (FTEC) – Ministry of Agriculture and Environment
Referee 2: Assoc. Prof., PhD. Tran Duc Hau
Hanoi National University of Education – Ministry of Education and Training
Referee 3: Assoc. Prof., PhD. Nguyen Khac Luc
Vietnam Society of Parasitology – Vietnam Biologycal Association

The dissertation is examined by Examination Board of Granduate University of Science and Technology, Vietnam Academy of Science and Technology at ……………………………………..

The dissertation can be found at:
1. Graduate University of Science and Technology Library
2. National Library of Vietnam

INTRODUCTION

The dissertation necessary

The marine fish fauna of Cat Ba Island is highly diverse, with 361 species distributed across various ecosystems, including coral reefs, mangrove forests, seagrass beds, lagoons, coastal areas, and swamps. The diversity of host species and their habitat significantly contributes to the richness of the monogenean fauna. It is estimated that the monogenean fauna in Cat Ba Island includes approximately 1000 species, but only 24 species have been recorded to date. Understanding the diversity of monogenean fauna is critically important, as it enhances our knownleadge of marine biodiversity, serves as an indicator of ecosystem health and stability, and provides a scientific basis for the management of natural ecosystems. Moreover, it enables proactive responses to disease outbreaks caused by monogenean, thereby supporting the sustainability of marine aquaculture.

Therefore, the research topic: “Diversity of Monogenoidea in marine fish from Cat Ba Island, Hai Phong” is not only of scientific significance but also of great practical importance, contributing to disease prevention in marine fish species.

Objectives

To determine the species composition and infection status of monogeneans in marine fish from Cat Ba Island area, Hai Phong.

Research contents

- Study the prevalence and intensity of monogenean infections in marine fish species from Cat Ba Island area, Hai Phong.
- Identification and description of monogeneans parasitizing marine fish in Cat Ba Island area, Hai Phong.

Scientific and practical significance of the dissertation

1. Provides new and reliable data on the species composition of monogeneans and their distrubution in certain marine fish species, including the identification of new host species.
2. Contributes to expanding the knowledge of monogenean fauna parasitizing marine fish in Vietnam, while enhancing the understanding of marine ecological diversity in the Cat Ba Island, Hai Phong.

Dissertation structure

The dissertation consists of 117 pages and includes the following sections: Introduction 3 pages. Literature review 16 pages; Materials and Research methods 7 pages; Results and Discussion 78 pages; Conclusion and Recommendation 1 page, Publications related to the dissertation 1 page. References 11 pages and Appendix 17 pages. The dissertation also contains of 6 tables, and 64 figures, illustrations and diagrams.

Chapter 1. LITERATURE REVIEW

1.1. Brief history of research and classification of monogeneans

Monogenoidea van Beneden, 1858, is one of the most species-rich classes within the phylum Platyhelminthes. Initially, monogeneans were classified as an order within the Trematoda. However, van Beneden separated Monogenoidea as a distinct taxon, dividing Trematoda divided into two groups, *monogénèses* và *digénèses.* Price considered van Beneden's French term *monogénèses* to be vernacular and attributed the term Monogenea to Carus, who first used it in its Latinized form. Price's interpretation was accepted by contemporary scholars. Nevertheless, the transition from *monogénèses* to monogenea was simply a modification of the original French into Latini, in accordance with standard nomenclature practices. Consequently, this terminological change does not affect authorship, and the term monogenea is still attributed to van Beneden.

Bychowsky elevated the taxonomic rank of monogenea from order to class and renamed the group Monogenoidea, while still crediting van Beneden as the author. The term Monogenoidea became widely used in the former Soviet Union and some Eastern European countries, although most Western scientists continued to use the Monogenea. At the 4th International Congress of Parasitology (ICOPA IV) in 1978 in Warsaw, Poland, scientists from 11 countries convened to resolve nomenclature, classification, and terminology discrepancies. The participants agreed to use the term Monogenea as the class name instead of Monogenoidea, following the International Code of Nomenclature, which reserves the suffix -oidea for the superfamilies. However, this decision was deemed invalid because Article 1b of the International Code of Nomenclature stipulates that such aconference has no authority over taxa above the family level. Lebedev

(1988) opposed the adoption of Monogenea and retained Monogenoidea, attributing its authorship to van Beneden based on Bychowsky's amendment. Boeger & Kritsky (1993) supported Lebedev's opinion, further legitimizing the use of Monodenoidea.

Llewellyn (1970) divided monogeneans into two suborders (later elevated to subclasses) Monopisthocotylea and Polyopisthocotylea, based on the morphological and structural characteristics of the posterior attachment organs. In contrast, Boeger & Kritsky (1993), using 47 morphological characteristics, proposed dividing monogeneans into three subclasses: Polyonchoinea, Polystomatoinea and Oligonchoinea. These two classification systems differ only at higher taxonomic levels, while classifications from the family level downward are largely consistent.

This dissertation adopts Boeger & Kritsky's stance, using the name Monogenoidea to refer to the class of monogeneans. However, for practical reasons, the classification system proposed by Llewellyn (1970) has been used in this disseertation. This decision aligns with the World Register of Marine Species (marinespecies.org), the largest global database for monogeneans, which also follows Llewellyn's system for data updates.

1.2. Summary of research on monogeneans in the East Sea region

The first study on monogenean fauna in Southeast Asia was conducted in 1930. However, data on monogenean fauna in the East Sea region remains limited compared to other regions. Lim (1998) reported 146 species of monogeneans parasitizing various hosts, including 113 species found on 69 species of marine fish. The author estimated that the known monogenean species represent nearly 8% of the total possible species.

In 2000, the Fauna of China (Volume 22) was published, briefly describing 584 species of monogenean belonging to 37 genera and 8 families. These species were parasitic on freshwater fish, some migratory fish species moving between sea and inland waters, and estuarine fish. Later, Zhang et al. (2003) listed 337 species of monogeneans infecting marine fish, with 278 species recorded in the South China Sea. In comparison, other seas, such as the Yellow Sea, Bohai Sea, and East China Sea, had fewer recorded species. This difference in the number of monogenean species across regions can be attributed to two primary factors: (1) The South China Sea, located in tropical and subtropical zones, naturally supports greater species diversity, while the temperate East China Sea hosts fewer species but with larger population sizes; (2) he East Sea has been more extensively studied, especially by former Soviet scientists, whose research has been widely cited, including in Zhang et al.'s work. Between 2003 to 2020, Chinese scientists discovered one new genus and 22 new species of monogeneans in the South China Sea (Gulf of Tonkin), significantly contributing to the knowledge of monogenean diversity in the region.

From 1998 to April 2021, data compiled from sources such as Google Scholar, Web of Sciences, PubMed, ScienceDirect, AGRICOLA, Aquatic Science & Fisheries, CABI, Scopus, and Zoological Record revealed 36 research studies on monogeneans parasitizing marine fish in Southeast Asia's border regions. These studies described 46 new species of monogeneans.

Research on monogenean infections has primarily focused on fish farms and aquaculture facilities (Park, 2009). For example, in Korea, parasitic diseases, including monogenean infections, have significantly impacted sea bream (Sparidae) farming (Kim, 2000). Woo et al. (2023) updated data on monogenean and copepod infections in fish farms in the Gyeongsangnam-do region, reporting an average infection rate of 98,4% and an infection intensity of 7 worms/fish. In India, Jithendran et al. (2005) studied monogenean infections in grouper (*Epinephelus tauvina*) farms near Chennai. The study revealed that *Benedenia* sp. infected 27% of the sampled fish. Ogawa (2014) provided data on the infection status of flatworm classes (Platyhelminthes) in marine fish cultivated in Japan, concluding that monogeneans had the highest infection rates and caused the most harm. Ogawa's research also included wild fish populations. Similarly, Liu et al. (2022) reported significant damage caused by *Neobenedenia girellae* to *Trachinotus ovatus* farms in Zhanjiang, China. Infected fish were notably smaller than uninfected individuals. The infection rate and intensity of *N. girellae* remained high from January to April but decreased in the subsequent months.

In other Southeast Asian counties, studies have also documented monogenean infections in fish. Lestari et al. (2018) investigated monogenean infections in grouper (*Epinephelus fuscoguttalus lanceolatus*) farms in Lamong Bay, Surabaya, Indonesia. The fish were infected with *Benedenia* *epinepheli*, *Neobenedenia girellae* and *Pseudorhabdosynochus epinepheli*, with infection rates of 4%, 3%, and 26%, respectively. Athur & Lumalan-Mayo (1997) summarized the monogenean fauna parasitizing fish in the Philippines, citing numerous publications, including a study by Mayes & Brooks (1977) on monogenean infections in catfish. Lim (1998) provided an extensive overview of monogenean diversity in marine fish from Malaysia, Thailand, and the Southeast Asian. Her studies noted that, on average, each fish species hosted more than one monogenean species (ranging from 1 to 14 species) with infection intensities ranging from a few individuals to thousands per host.

Despite the increasing threat of parasites, especially monogeneans, to aquculture, information on these species and the diseases they cause remains limited, even in developed countries such as Korea (Maran et al. 2014)

In monogenean taxonomy, condensed methods of describing and comparing morphological characteristics among species and groups have been the standard. However, molecular techniques are increasingly being used, particularly for species-rich genera, such as *Dactylogyrus*, *Gyrodactylus*, *Haliotrema*, and *Ligophorus*. Morphological characteristics often fail to clearly distinguish species in these genera, making molecular tools essential for accurate identification and classification.

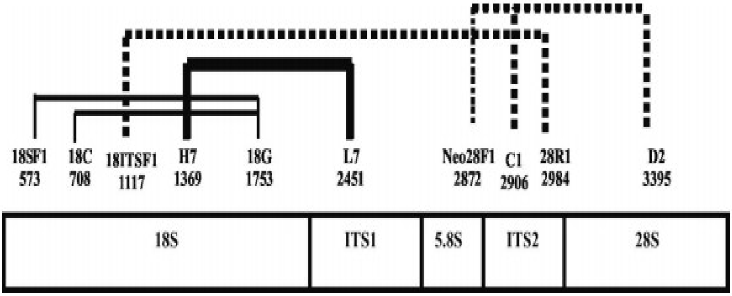
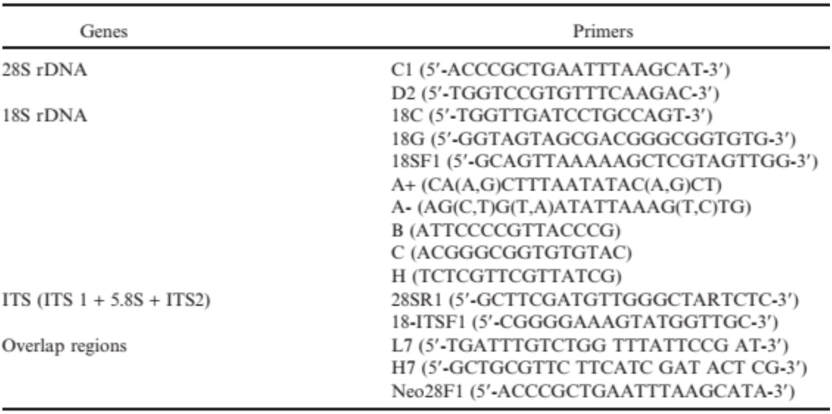


Figure 1.4. Location of gene segments and corresponding primers used for cloning

Studies frequently utilize nuclear genes to construct phylogenetic trees. Commonly analyzed gene segments include the *28S* rDNA (large subunit, LSU rDNA), the combined *ITS1-5.8S-ITS2* and *28S* segments, or the *18S* rDNA segment (small subunit, SSU rDNA). Dang et al. (2013) synthesized primers for amplifying these nuclear gene segments. Table 1.2. summarizes the primers used and the corresponding gene segment locations in *Haliotrem* spp.

Table 1.2. Primers used for rDNA in monogenoidea



Genes	Primers
28S rDNA	C1 (5′-ACCCGCTGAATTTAAGCAT-3′)
	D2 (5′-TGGTCCGTGTTTCAAGAC-3′)
18S rDNA	18C (5′-TGGTTGATCCTGCCAGT-3′)
	18G (5′-GGTAGTAGCGACGGGCGGTGTG-3′)
	18SF1 (5′-GCAGTTAAAAAGCTCGTAGTTGG-3′)
	A+ (CA(A,G)CTTTAATATAC(A,G)CT)
	A- (AG(C,T)G(T,A)ATATTAAAG(T,C)TG)
	B (ATTCCCCGTTACCCG)
	C (ACGGGCGGTGTGTAC)
	H (TCTCGTTCGTTATCG)
ITS (ITS 1 + 5.8S + ITS2)	28SR1 (5′-GCTTCGATGTTGGGCTARTCTC-3′)
	18-ITSF1 (5′-CGGGGAAAGTATGGTTGC-3′)
Overlap regions	L7 (5′-TGATTTGTCTGG TTTATTCCG AT-3′)
	H7 (5′-GCTGCGTTC TTCATC GAT ACT CG-3′)
	Neo28F1 (5′-ACCCGCTGAATTTAAGCATA-3′)

For mitochodrial genes, Yang et al. (2020) sequenced the complete genome of *Capsala pricei*. Phylogenetic analysis revealed that this species is closely to the genus *Neobenedenia*.

In addition to studies on species diversity and systematics, research has also addressed various aspects of monogenean biology, including developmental cycles, spatial population dynamics within the host (e.g., distribution on the gill arches), species distribution across geographical locations, the identification of disease-causing species in caged fish, and the testing of measures to prevent infections.

In summary, although the number of known monogenean species in the East Sea area has increased in recent years, it remains only a small fraction of the estimated number. To further explore the diversity of monogenean species and achieve a more cpmprehensive understanding of the fauna, greater collaboration among parasitologists is needed.

1.3. Summary of research on monogeneans in Vietnam

Research on monogeneans in Vietnamese marine fish has primarily focuses on understanding species diversity, fauna composition, and specfic host-parasites relationships (Nguyen et al. 2020).

The earliest studies on monogeneans in Vietnamese marine fish date back to the 1950s. These investigations, often conducted by Vietnamese and Soviet parasitologists, provided foundational insights into parasitic worms in marine fish (Bychowsky & Nagibina 1954, 1959). Subsequent publications contributed to the identification of numerous new monogenean species and included data on the prevalence and intensity of infection (Athur & Bui, 2006). Tu et al. (2017) published findings on parasitic infections in cage-cultured cobia (*Rachycentron canadum*) in Kien Giang Province, documenting the prevalence and intensity of infection by *Pseudorhabdosynochus* sp., *Neobenedenia* sp. Vo et al. (2010) authored a book on parasites of grouper and sea bream in Vietnam, offering detailed information on the rate and intensity of infection for various monogenean species on their hosts.

From 1961 to 1989, investigations into monogenean fauna in Vietnamese marine fish resulted in over 30 scientific journal articles in and monographs. These works described numerous new genera and species, significantly expanding the list of monogeneans in Vietnam. By 2006, Arthur and Bui had listed a total of 55 species of Monogenoidea in Vietnamese marine fish. However, their review overlooked several publications by foreign scientists.

In 2020, Nguyen et al. updated the list of Monogenoidea to include 220 species parasitizing 152 species of Vietnam marine fish. Their study detailed the distribution and host specificity of each parasite species, cited relevant source, and discussed changes in the taxonomic classification of various taxa.

By the end of 2020, five additional monogenean species had been described, along with records of new hosts for several previously known species. As of March 2021, the total number of Monogenoidea species recorded from Vietnamese marine fish stands as 225.

1.4. Research status of monogeneans from marine fish in the Cat Ba Island area, Hai Phong

In the Cat Ba Island area and its surrounding, over 10 studies have been conducted on the diversity and infection status of monogeneans in marine fish, including both wild and cultured species. Garasev et al. (2011a,b) and Dmitrieva et al. (2018) reported a total of 15 monogenean species, primarily belong to the genus *Ligophorus*, parasitizing mugilid hosts (mullets). Their studies provided data on infection intensity and prevalence for each species. Krisky et al. (2016) reviewed and revised the taxonomy of the genus *Metahaliotrema*, including specimens collected from *Scatophagus argus* in Ha Long, Cat Ba areas. Additional contributions by Nguyen (2012), Nguyen

& Nguyen (2017), Nguyen et al. (2020a, b) have further expanded knowledge base on monogenean infections in wild marine fish from the region.

For cultured marine fish, studies by Truong et al. (2017) and Truong (2018) updated the infection status and evaluated the pathological impact of monogeneans on farmed fish. Their research focused on groupers (*Epinephelus* spp.) and snubnose pompano (*Trachinotus blochii*) raised in offshore zones of Hai Phong Province.

Research on monogenea fauna in fish in Cat Ba Island and adjacen waters reveals that the recorded number of monogenean species rangnes from 2,43% to 11,22% of the total known species in the Gulf of Tonkin. At the same time, the number of host species examined is very small relative to the number of fish species present. Additionally, the number of fish host species examined remains low compared to the overall fish diversity in the region. Although Cat Ba has yielded a higher number of monogenean species than other surveyed locations, this figure likely underrepresents the true biodiversity of the area due to the limited scope and number of studies, which compromises statistical reliability.

Table 1.3. The number of Monogenoidea species recorded from 1930-2020 in Cat Ba island waters and surrounding areas

Location	**No. monogenean species**	**No. host species**
Cat Ba	23	8
Do Son	8	2
Ha Long	13	14
Mong Cai	5	1
Tien Yen	12	10
The Guft of Tonkin	205	140

Consequently, one of the critical objectives for parasitologists is to continue investigating the diversity of monogenean fauna in Vietnamese marine fish, particularly in the Cat Ba Island region. Expanding the application of molecular techniques will be essential to resolving taxonomic ambiguities within species complexes and identifying new species..

Chapter 2. RESEARCH OBJECTS AND METHODOLOGY

2.1. Research objects

2.1.1. Research objects and scopes

Research object: The research focused on adult monogeneans parasitizing wild fish species in the Cat Ba Island area, Hai Phong, Vietnam.

Research scope: Specimens analyzed include both archived samples since 2014 and newly collected samples gathered throughout the thesis implementation period.

2.1.2 Research contents

- Study the prevalence and intensity of monogenean infections in marine fish species from Cat Ba Island area, Hai Phong.
- Identification and description of monogeneans parasitizing marine fish in Cat Ba Island area, Hai Phong.

2.1.3. Studying site and period:

Time: May 2021- May 2024

Location: monogeneans were analyzed and identified in the laboratory of the Parasitology department, Institute of Biology, Vietnam Academy of Science and Technology.

2.2. Methodology

Given the small size and complex morphology of monogeneans, many of which possess chitinized structures, an integrative approach combining morphological and molecular techniques was employed.

2.3.1. Collection, preservation and identification of fish

Wild fish were either obtained directly from local fishermen or purchased from local markets in areas around Cat Ba Island. The fish were stored in an ice box (at 4^0C) and transported fresh to the laboratory, where they were identified to species using available taxonomic keys and verified with data from FishBase (https://www.fishbase.se/).

Following identification, fish specimens were stored at 4°C until dissection. Additionally, muscle tissue from selected fish was preserved for potential use in internal PCR assays to confirm host species identification when necessary.

2.3.2. Isolation and preserved monogeneans

Fish specimens were examined under a stereomicroscope for monogeneans in the skin, fins, gills, oral cavity, and gastrointestinal tract.

Parasites were carefully removed from infected sites using fine forceps and dissection needles.

Preservation for molecular analysis: Monogeneans intended for genetic studies were preserved in absolute ethanol.

2.3.3. Mounting and specimen preparation

- *the gelatin-glycerin technique:* For members of the subclass Monopisthocotylea, specimens were mounted on microscope slides for morphological characterization, including spines, hooks, anchors, and copulatory structures. The glycerin-gelatin mounting method, based on Malmberg (1970), was employed, and involved the following steps: (i) Arrange 1–5 monogenean specimens parallel to each other on a clean glass slide; (ii) Allow the slide to air-dry for 2–5 minutes; (iii) Add one drop of host glycerin-gelatin over the specimens and gently place a coverslip; (iv) Label the slide and allow it to set for 2–3 hours before final storage.
- *staining techniques:* Samples for staining were preserved in 4% formalin solution or 70% ethanol. After a few days, the specimens could be stained to study internal organs and perform morphological measurements.

Large worm specimens can be using the carmine alumi staining method (according to Bullard et al., 2004): leave the worm specimen in the staining solution for 10-30 minutes; then decolorize the dye with 70% ethanol + HCl solution for a few minutes; dehydrate through 70%, 80%, 95% and 100% ethanol solution; clarify with clove oild and mount the specimen with Canadian balsam; label the specimen and let it air dry for a few days.

Another staining solution can be used is the Gomori trichrome (according to Kritsky et al., 1978). Monogenea samples taken from the preservation solution were soakesd in the staining solution for 1-2 minutes; 70% ethanol was used to dilute the dry solution to stop staining immediately, avoiding the sample from being too darkly stained; water was removed through 70%, 80%, 95% and 100% ethanol solution; the specimens were labeled and air-dried for several days.

2.3.4. Morphological analysis and identification of mongenoidea

The mongenoidea specimens were measured, drawn and described in terms of morphology and structure under Olympus CH40 and BX53 microscopes; then compared and contrasted with the descriptions of the corresponding taxa in the appropriate classification systems. The mongenoidea species were described in the order prescribed in the book Zoology: Scientific name, distribution, host, parasitic site, description, drawings or photos,…

2.3.5. Molecular biology techniques

Samples for PCR are preserved in 95% alcohol and wsahed with distilled water for DNA extraction. DNA is extracted using Qiagen kit, USA. Total DNA is stored in a refrigerator at a deep cold temperature (–30ºC).

Polymerase chain reaction (PCR) was used to amplify *28S* ribosome DNA (rDNA) with primers DIG12 (5'-AAG CAT ATC ACT AAG CGG-3′) (Littlewood & Olson, 2001) and 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′) as described by Tkach et al., 2003.

PCR products were sepenced directly using the ABI Big Dry Cycle Sequencing Kit v.3.1 (Applied Biosystems, USA), following the manufacturer's recommendations, with the sequencing primers described by Littlewood & Olson (2001) and Tkach et al. (2003) for *28S* rDNA. PCR products were analyzed using a Mastercycler nexus gene analyzer (Eppendoft). Sequences were submitted to GenBank (NCBI).

rDNA sequences were assembled using SeqScape software version 2.6. Alignment and estimation of the number of variable sites and sequence differences were performed using MEGA 7.0 (Kumar et al., 2016).

Phylogenetic analysis of nucleotide sequences was performed using the Bayesian algorithum with Mr Bayes software v.3.1.2 (Huelsenbeck et al, 2001). The *28S* rDNA sequences of several parasite species from GenBank were used as outgroups.

2.3.6. Statistical analysis

Data were entered into an Excel worksheet (Microsoft Corporation, Redmond, Washington) and analyzed using STATA/IC 12 (Stata Corp LP, College Station, TX). Parasites count data from fish samples were transformed to binomial data. Fish with infection of any parasite species was coded to "1" and uninfected fish was coded to "0". Parasite infections of fishes were analyzed using logistic regressions, with fish species, fish family, fish order, and season as predictors after adjusting for groups of fish collected from a single fish haul and collection times. Diferences with P-values below 0.05 were considered signifcant

Chapter 3. RESULTS

3.1. Assessment of the status of monogenea infection in studied fish species

The total of 1.066 marine fish individuals belonging to 82 species, 36 families, 18 orders were collected for research during the period 2014-2024 (Table 3.1.). The Perciformes order was the most diverse, with 17 species sampled, followed by the Mugiliformes order with 13 species. The remaining fish order had only 1-8 species dissected. The Mugilidae and Carangidae were the most diverse, with 13 and 10 species, respectively. The remaining fish families had species numbers ranging from 1-6 species.

Five fish species including three mullet species *Osteomugil engeli* (Bleeker), *Planiliza melinoptera* (Valenciennes), *Crenimugil seheli* (Fabricius), and one rabbitfish species *Siganus fuscescens* (Houttuyn) and one sculpin *Rhynchorhamphus georgii* (Valenciennes) were the most studied, accounting for approximately one-third of the total fish samples examined.

Research on the prevalence and intensity of Mongenoidea infection in marine fish in the Cat Ba Island area showed that more than half of the studied fish species (45 out of 82 species) were infected with mongeneans, accounting for 55.6% (Table 3.2.). The order Mugiliformes had the highest number of species infected (10/13 species), followed by Beloniformes (7/8 species) and Perciformes (7/17 species).

Among the 45 species found to be infected, 28 species harbored monopisthocotylean monogeneans, while 17 species were infected with polyopisthocotylean monogeneans. Two species of croaker *Nibea albiflora* (croaker) and *Siganus canaliculatus* (rabbifish) were found to host both types of monogeneans.

Of the 22 fish species with a sample size of more than 10 individuals, those with a high infection rate (over 70%) included *Siganus fuscescens* fish (82,8%), *Argyrosomus japonicus* (91,7%), *Scomberomorus commerson* (91,7%), and *Sillago sihama* (92,9%).

The total of 3,260 mongenean specimens were collected, including 2,792 monopisthocotyleans and 468 polypisthocotyleans. The average infection intensity was 11.2 flukes per fish, with a range from 1 to 240 flukes perfish. The average intensity of monopisthocotylean infection (13.47 fluke/fish) was significantly higher than that of polypisthocotylean infection (5,68 flukes/fish) (p<0,01)

No statistically significant differences were observed in the prevalence or intensity of monogenean infections between the rainy and dry seasons.

3.2. Classfication system of Monogenoidea parasitizing marine fish in the Cat Ba Island area

Based on the analysis of 3,260 monogenoidean specimens, including 2,792 monopisthocotylean and 468 polyopisthocotylean specimens, a total of 45 monogenoidean species were identified. These species belong to 29 genera, 13 families, and 2 orders. The list of species is presented below, organized according to their taxonomic classification.

CLASS MONOGENOIDEA VAN BENEDEN, 1858/
SUBSTITUTE MONOPISTHOCOTYLEA ODHNER, 1912
ORDOE DACTYLOGYRIDEA Bychowsky, 1937/

Family Ancyrocephalidae Bychowsky, 1937

Genus *Glyphidohaptor* Kritsky, Galli & Yang, 2007

1. *G. sigani* Kritsky, Galli & Tingbao, 2007
2. *Glyphidohaptor* sp.

Genus *Tetrancistrum* Goto & Kikuchi, 1917

3. *T. indicum* (Paperna, 1972) Kritsky, Galli & Tingbao, 2007
4. *T. sigani* Goto & Kikuchi, 1917
5. *Tetrancistrum* sp.

Family Dactylogyridae Bychowsky, 1933

Genus *Haliotrema* Johnston & Tiegs, 1922

6. *Haliotrema epinepheli* Young, 1968
7. *Haliotrema holocentri* Young, 1968
8. *Haliotrema johsntoni* Bychowsky & Nagibina, 1970
9. *Haliotrema platycephali* Yin & Sproston, 1948
10. *Haliotrema tiegsi* Bychowsky & Nagibina, 1970

Genus *Hemirhamphiculus* Bychowsky & Nagibina, 1969

11. *Hemirhamphiculus armatus* Bychowsky & Nagibina, 1969
12. *Hemirhamphiculus similis* Bychowsky & Nagibina, 1969

Genus *Ligophorus* Euzet & Suriano, 1977

13. *Ligophorus fenestrum* Soo & Lim, 2012
14. *Ligophorus hamulosus* Pan et Zhang, 1999

15. *Ligophorus leporinus* (Zhang & Ji, 1981) Gussev, 1985
16. *Ligophorus macrocolpos* Euzet & Suriano, 1977

Genus *Lobotrema* Tripathi, 1959

17. *L. sciaenae* (Bychowsky & Nagibina, 1977) Oliver, 1987
18. *L. argyrosomi* (Bychowsky & Nagibina, 1977) Oliver, 1987

Genus *Paradiplectanotrema* Gerasev, Gayevskaya & Kovaleva, 1987

19. *P. trachuri* (Kovaljova, 1970) Gerasev, Gayevskaya & Kovaleva, 1987

Genus *Parahemirhamphiculus* Bychowsky & Nagibina, 1969

20. *P. pinguis* Bychowsky & Nagibina, 1969

Genus *Protogyrodactylus* Johnston & Tiegs, 1922

21. *P. alienus* Bychowsky & Nagibina, 1974
22. *P. gussevi* Bychowsky & Nagibina, 1974
23. *P. perforatus* Bychowsky & Nagibina, 1974

Family Diplectanidae Monticelli, 1903

Genus *Calydiscoides* Young, 1969

24. *C. flexuosus* (Yamaguti, 1953) Young, 1969

Genus *Murraytrema* Price, 1937

25. *M. pricei* Bychowsky & Nagibina, 1977

Genus *Paradiplectanum* Domingues & Boeger, 2008

26. *P. blairense* (Gupta & Khanna, 1974) Domingues & Boeger, 2008

CLASS POLYOPISTHOCOTYLEA ODHNER, 1912

ORDER MAZOCRAEIDEA Bychowsky, 1937

Family Allodiscocotylidae Tripathi, 1959

Genus *Allodiscocotyla* Yamaguti, 1953

27. *A. chorinemi* Yamaguti, *mersonnianus* 1953

Genus *Metacamopia* Lebedev, 1972

28. *M. chorinemi* (Yamaguti, *thalmus* 1953) Lebedev, 1984

Family Axinidae Monticelli, 1903

Genus *Axine* Abildgaard, 1794

29. *A. hemirhamphae* Tripathi, 1959

Genus *Neoaxine* Price, 1946

30. *N. constricta* (Yamaguti, 1938) Price, 1946

Family Microcotylidae Taschenberg, 1879

Genus *Microcotyle* van Beneden & Hesse, 1863

31. *M. helotes* Sandars, 1944

Genus *Polylabris* Euzet & Cauwet, 1967

32. *P. mamaevi* Ogawa & Egusa, 1980

Family Diclidophoridae Cerfontaine, 1895

Genus *Helciferus* Mamaev, 1972

33. *H. tenuis* Mamaev, 1972

Genus *Heterobothrium* Cerfontaine, 1895

34. *H. tonkinense* Bychowsky & Nagibina, 1976
35. *H. phamvanluci* n. sp.

Genus *Papillochoricotyle* Mamaev, 1975

36. *P. ilishae* Mamaev, 1975

Family Gastrocotylidae Price, 1943

Genus *Pseudaxine* Parona & Perugia, 1890

37. *P. bychowskyi* (Lebedev, 1977) Bouguerche, Tazerouti, Gey & Justine, 2020
38. *P. trachuri* Parona & Perugia, 1889

Family Gotocotylidae Yamaguti, 1963

Genus *Cathucotyle* Lebedev, 1968

39. *C. cathuaui* Lebedev, 1968

Family Mazocraeidae Price, 1936

Genus *Mazocraeoides* Price, 1936

40. *Mazocraeoides* sp.

Genus *Heteromazocraes* Mamaev, 1981

41. *H. vicinus* (Mamaev, 1975) Mamaev, 1981

Family Plectanocotylidae Monticelli, 1903

Genus *Octoplectanocotyla* Yamaguti, 1937

42. *Octoplectanocotyla* sp.

Family Protomicrocotylidae Johnston & Tiegs, 1922

Genus *Vallisiopsis* Subhapradha, 1951

43. *V. sphyraenae* Yamaguti, 1968

Genus *Bilaterocotyloides* Ramalingam, 1961

44. *B. carangis* Ramalingam, 1961

Family Thoracocotylidae Price, 1936

Genus *Pricea* Chauhan, 1945

45. *P. multae* Chauhan, 1945

3.3. Characteristics of Monogenoidea species

The study showed that the species, genera and family composition of Monogenoidea in Cat Ba Island area is quite diverse, although many parasites are restricted to a single host species. Most of monogenean species in this study are only found from a single host (table 3.3).

Most monogeneans are ectoparasites, commonly found on the gill arches; however, some may also inhabit the oral cavity or gill chamber. Notably, *Paradiplectanotrema trachuri* is one of the few known endoparasitic monogeneans, discovered in the stomach of *Argyrosomus japonicus* in the Cat Ba Island area.

The investigation of the Monogenoidea composition in the Cat Ba Archipelago led to discovery of three new species to science (*Glyphidohaptor* sp., *Tetrancistrum* sp. and *Heterobothrium phamvanluci* sp. n.). In addition, 9 spesies were newly recorded for the Vietnamese Monogenoidea fauna (*Glyphidohaptor sigani, Tetrancistrum indicum, T. sigani, Axine hemirhamphae, Neoaxine constricta, Microcotyle helotes, Polylabris mamaevi, Pseudaxine bychowskyi* and *Octoplectano-cotyla* sp.). Several new host species were also documented.

Table 3.3. Summary of newly recorded monogenean species (simplified)

No.	Monogenean species	Host species	Evaluation
1	*Glyphidohaptor sigani*	*Siganus fuscescens*	New record
2	*Glyphidohaptor* sp.	*S. fuscescens*	New species
3	*Tetrancistrum indicum*	*S. canaliculatus*	New record
4	*T. sigani*	*S. fuscescens*	New record
5	*Tetrancistrum* sp.	*S. fuscescens*	New species
6	*Hemirhamphiculus similis*	*Hyporhamphus quoyi*	New host
7	*Lobotrema argyrosomi*	*Argyrosomus japonicus*	New host
8	*Axine hemirhamphae*	*Rhynchorhamphus georgii*	New record
9	*Neoaxine constricta*	*Strongylura strongylura*	New record
10	*Microcotyle helotes*	*Terapon theraps*	New record

No.	Monogenean species	Host species	Evaluation
11	*Polylabris mamaevi*	*S. canaliculatus*	New record
12	*Helciferus tenuis*	*Coilia rebentischii*	New host
13	*Heterobothrium tonkinense*	*Lagocephalus lunaris*	New host
14	*Heterobothrium phamvanluci n. sp.*	*L. spadiceus*	New species
15	*Papillochoricotyle ilishae*	*Ilisha megaloptera*	New host
16	*Pseudaxine bychowskyi*	*Alepes djedaba*	New record
17	*Octoplectanocotyla sp.*	*Trichiurus lepturus*	New record
18	*Vallisiopsis sphyraenae*	*Sphyraena obtusata*	New host

3.4. The description of monogeneans in the Cat Ba Island area

3.4.1. Heterobothrium phamvanluci sp. n.

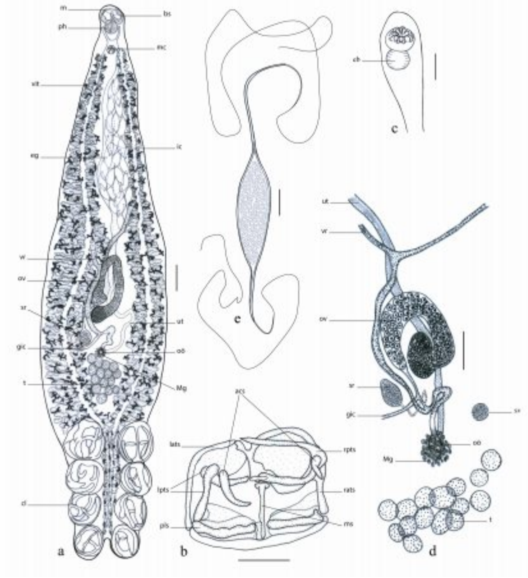
Host: *Lagocephalus spadiceus*

Site on host: Gills

Description: (based on 9 specimens, Fig 3.35A,B):

Body fusiform, dorsoventrally flttened, tapering anteriorly. Total length 3772–7011 (4977) µm. Maximum width at the level of ovary 1025–2165 (1659) µm (Fig. 3.35A-a). Peduncle absent; no separation of haptor from body proper. Haptor symmetric, 902–1591 × 738–1230 (1330 × 975) µm, bearing four pairs of clamps. Clamp size variable: fist pair largest, 262–476 × 262–508 (329 × 374) µm; fourth pair smallest, 139–262 × 230–312 (235 × 274) µm; middle pairs similar, 230–476 × 262–508 (284 × 336) µm. First pair turned 180° in horizontal plane.

Clamp structure similar type-shaped (Fig. 3.35A-b): posterolateral sclerites, anterior and posterior peripheral sclerites, accessory sclerites, and median sclerite. Axial and posterolateral peripheral sclerites asymmetrical; right posterolateral sclerite ring-shaped with handle; left crescentic-shaped. Anterior peripheral sclerites inward-curved; longer than posterior pair, nearly straight. Outer median sclerite loop slightly surpassing median line. Clamp walls with well-developed muscles. Mouth subterminal. Buccal suckers muscular, oval, 66–111 × 78–123 (91 × 102) µm. Pharynx oval, approximately same size as buccal sucker, 90–127 × 103–148 (110 × 128) µm. Oesophagus not observed. Intestine bifurcating immediately behind pharynx, numerous lateral branches terminating near haptor. Testes numerous, 24–40 (28), intercaecal, globular, located level with or posterior to oötype. Ejaculatory bulb present. Male copulatory tubular, muscular, diameter 70–103 (80) µm, corona with 8–9 hooks (Fig. 3.35A-c). Seminal vesicle globular, 69–98 × 102–131 (84 × 116) µm, posterior to ovary. Vas deferens descending sinuously from seminal vesicle, entering posterior end of ejaculatory bulb. Ejaculatory bulb 53–98 × 49–98 (76 × 74) µm.



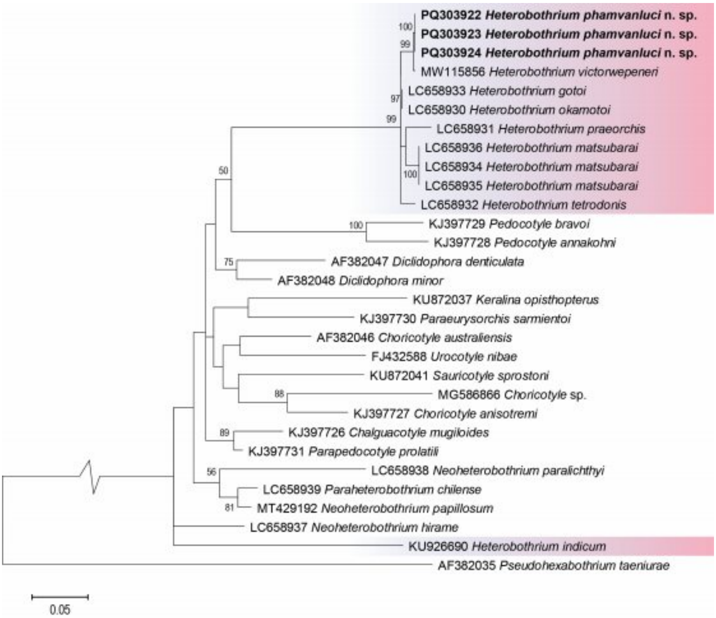
Hình 3.35A. ***Heterobothrium phamvanluci*** **n. sp.**

a. Total view; b. clamp; c. Ejaculatory bulb with arrangements of spines of the male copulatory organ; d. Reproductive organs; e. E, Egg. Scale bars: a, d, 200 µm; b, c, e, 50 µm.

Ovary tubular, 697–1296 × 147–213 (1113 × 193) µm, question-markshaped, intercaecal, located mid-region of body proper (Fig. 3.35A-d). Seminal receptacle oval, well-defied, 74–123 × 94–209 (90 × 135) µm, postovarian. Oötype oval, posterior to ovary, surrounded by Mehlis'glands. Vitellarium follicular, coextensive with caeca. Vitelline reservoir Yshaped, common duct joining oötype. Genito-intestinal canal connecting with vitelline reservoir duct. Uterus arising from dextral margin of oötype extending sinuously forward, opening at copulatory organ. Eggs in uterus with fiaments at both ends, 159–225 × 61–74 (191 × 67) µm.

Remarks

Heterobothrium phamvanluci n. sp. also has fewer testes than *H. yamagutii* (24–40 vs. 100–160), *H. praeochis* (up to 310), *H. tabetai* (90–140), *H. aljufailiae* (40–250), *H. iwatai* (50–170), and *H. tonkinense* (50–100). Furthermore, its body length (3772–7011) is smaller than those of *H. yamagutii* (7400–14,400), *H. shinagawai* (10,400), *H. tabetai* (11,170–12,940), *H. aljufailiae* (8820–18,130), and *H. iwatai* (8050–10,770). The haptor of *H. phamvanluci* n. sp. (902–1591 × 738–1230) is smaller than that of *H. shinagawai* (3900 × 4000). While *H. phamvanluci* n. sp. has a slightly larger body length than *H. tonkinense*, its haptor is smaller. Additionally, the fourth pair of clamps in *H. phamvanluci* n. sp. is the smallest, whereas all four pairs of clamps in *H. tonkinense* are similar in size. Moreover, in *H. phamvanluci* n. sp., the testes are located either level with or posterior to ootype¨ (posterior to the ovary), while in *H. tonkinense*, they are situated immediately behind seminal receptacle (level with the ovary).



Hình 3.35B. Maximum likelihood phylogram based on partial sequences of the 28S rDNA gene. GenBank accession number precedes species name. Newly generated sequence is in bold. *Pseudohexabothrium taeniurae* was used as an outgroup. Bootstrap confience values expressed as a percentage based on 1,000 replicates.

The 28S rDNA sequences obtained from *H. phamvanluci* n. sp. in this study (GenBank accession numbers PQ303922–PQ303924) were 1274–1276 bp long, with the extreme ends trimmed to 738 bp. These sequences were closely related to H. victorwepeneri (GenBank No. MW115855), with 99.75 % identity. Further comparisons with available sequence data for *Heterobothrium* spp. and other closely related genera within the family Diclidophoridae indicate that the sequences of *H. phamvanluci* n. sp. formed a monophyletic clade. These sequences cluster as sibling species with other member of the genus Heterobothrium, except for *Heterobothrium indicum* (GenBank No. KU926690) (Fig. 3.35B). In the phylogenetic tree, all diclidophorid species form a single lineage, excluding the outgroup taxon *Pseudohexabothrium taeniurae* (GenBank No. AF382035).

***3.4.2. Glyphidohaptor* sp.** (New species but not published yet)

Host: *Siganus fuscescens*

Site on host: Gills

Description (based on the 3 specimens):

Body fusiform, 702–850 µm long; widest at the ovarian region, 180–224 µm. Head lobes well developed. Pharynx 29–45 µm wide, cylindrical to elongated oval. Testes pear-shaped, 120–160 µm long and 37–65 µm wide; seminal vesicles small; prostatic cistern long and narrow, located on the dorsal surface of the male genitalia. Male copulatory organ (MCO) 53–75 µm long, tubular and curved, divided into two unequal parts, tapering at both ends. Appendages rod-shaped and blunt. Ovary pear-shaped; vaginal opening located on the anterior body; vaginal sac modified and slightly rigid; vaginal canal bent, extending to the oviduct; yolk glands dense. Haptor 75–80 µm long, 90–112 µm wide; ventral hook 33–47 µm long, 27–31 µm wide; dorsal hook 40–45 µm long, 32–35 µm wide; ventral bar 27–30 µm long; dorsal bar 33–40 µm long; marginal hook 11–13 µm long.

Remark:

The primary diagnostic feature distinguishing species within the genus *Glyphidohaptor* is the morphology of the genital complex, including the male copulatory organ (MCO) and its supporting appendages. The MCO is tubular, possesses a basal rim, and is enclosed within a variably developed sheath. The supporting appendages are rod-shaped, bearing plate-like projections along the proximal half.

MCO morphology differs among species: in *G. safiensis*, the MCO tube is slightly curved; in *G. phractophallus*, it is dome-shaped (U-shaped); *G. sigani* exhibits a posteriorly flared tube; while in *G. plectocirra*, the tube is straight. *Glyphidohaptor* sp. from Cat Ba Island has a broadly V-shaped curved MCO tube, intermediate between *G. safiensis* and *G. phractophallus*, but it differs in both the anterior and posterior tube structures. Specifically, *G. phractophallus* has two club-shaped ends, *G. safiensis* has a club-shaped end with a pointed tip, while the Cat Ba specimen possesses a uniformly shaped tube at both ends.

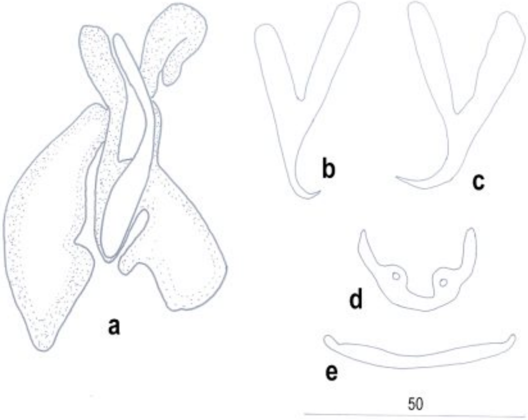


Figure 3.2A. *Glyphidohaptor* sp.
a. Male copulatory organ; b. Ventral hook; c. Dorsal hook;
d. Ventral bar; e. Dorsal bar (scale: µm)

The appendages also vary among species. In *G. safiensis*, they are rod-shaped and expand toward flattened tips; in *G. phractophallus*, they are flat and blade-like; *G. sigani* has rod-shaped appendages with forked tips; and *G. plectocirra* has pointed appendages. In contrast, the appendage of *Glyphidohaptor* sp. from Cat Ba is rod-shaped, unforked, unexpanded, and blunt at the tip. While all *Glyphidohaptor* species have plate-like projections along the posterior half of the appendage, *Glyphidohaptor* sp. exhibits the largest and most blade-shaped projection among them.

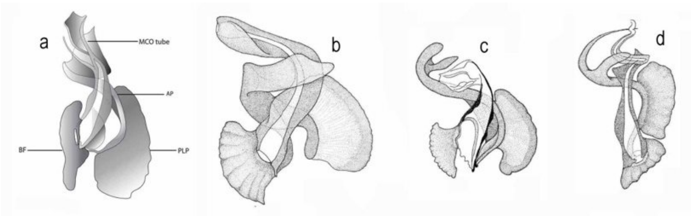


Figure 3.2C. The MCO of *Glyphidohaptor* spp. (Paperna, 1972)
a- *G. safiensis*; b- *G. phractophallus*; c- *G. sigani*; d- *G. plectocirra*

***3.4.5. Tetrancistrum* sp.** (New species but not published yet)

Host: *Siganus fuscescens*

Site on host: Gills

Description: (based on 5 specimens):

The body is leaf-shaped, with the widest region located in the middle and tapering at both the anterior and posterior ends. Body length ranges from 1,507 to 1,876 µm; the widest part measures 484–603 µm. The haptor tapers gradually toward the posterior end, measuring 124–141 µm in length and 107–138 µm in width. The head lobes are moderately developed and include a large group of cephalic glands positioned bilaterally and posterior to the pharynx. Eyespots are absent. The pharynx is elongate ovoid to pear-shaped, 73–85 µm long and 74–85 µm wide.

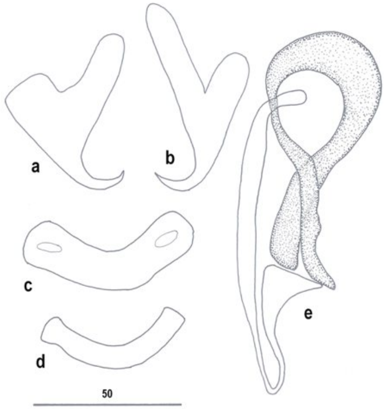


Figure 3.5A. *Tetrancistrum* sp.
a. Ventral hook; b. Dorsal hook; c. Ventral bar;
d. Dorsal bar; e. Male copulatory organ

The testes are spherical, measuring 229–288 µm in length and 175–229 µm in width. The vas deferens was not observed. Two small prostate glands are present. The male reproductive system includes the MCO and associated appendages. The MCO is tubular and trumpet-shaped, slightly curved anteriorly; the posterior portion is folded into a J-shape and flared at the end. The MCO tube exhibits an uneven diameter (Figure 3.5A, B). The appendages are rod-shaped and situated externally to the MCO. The total length of the genital complex is 120–134 µm.

The ovary is relatively large, 157–206 µm long and 92–129 µm wide. The oötype receives the vaginal canal and vas deferens; the uterus is dilated. The vaginal opening is moderately sized. Both the anterior and posterior portions of the vagina are swollen, with a slightly sclerotized midsection. The vaginal canal is tortuous. Yolk glands are dense, discharging via three pairs of yolk ducts on each side, which then combine into a common yolk duct on each side.

Remark:

The genus *Tetrancistrum* Goto & Kikuchi, 1917 currently includes 17 described species across the Indo-Pacific region: 10 parasitizing species of the family Siganidae, 5 of Acanthuridae, and 2 of Lutjanidae (Kritsky et al., 2007b; Al Jufaili & Palm, 2017). The *Tetrancistrum* sp. specimens found parasitizing the rabbitfish (*Siganus fuscescens*) in this study exhibit a unique MCO structure compared to all other known species in the genus. The differences are seen both in the shape and size of the MCO (indicated by blue arrows in figures). However, due to the lack of additional morphological and molecular data, these differences are not yet sufficient to confirm this as a new species to science.

Notably, this *Tetrancistrum* sp. represents the largest body size recorded among all known species of the genus, including *T. indicum*. Its MCO is J-shaped and flared at the posterior end, resembling a saxophone. Although it shares a similar structure with *T. yamagutii*, which also has a J-shaped MCO, the differences are clear: *T. yamagutii* features a tubular MCO with a straight anterior part, uniform diameter, and slightly flared posterior portion, and has a smaller average MCO length (97 µm). In contrast, the MCO in our specimen's averages 112 µm, has a slightly ventrally curved anterior region, and an uneven tube diameter.

CONCLUSION AND RECOMMENDATION

Conclusion

(1) Regarding the status of monogenean infections in marine fish species in the Cat Ba Island area:

- A total of 1,066 individuals representing 82 marine fish species from 36 families and 18 orders were examined. Among them, 54.9% (45 out of 82 species) were found to be infected with monogeneans.

- The average infection intensity was 11.2 worms per fish, ranging from 1 to 240 worms per fish. A total of 3,260 monogenean specimens were collected, including 2,792 monopisthocotylean and 468 polyopisthocotylean specimens. The average infection intensity of monopisthocotyleans (13.47 worms/fish) was significantly higher than that of polyopisthocotyleans (5.68 worms/fish) ($p < 0.01$).

- Seasonal variation did not significantly influence the prevalence or intensity of monogenean infections in wild marine fish populations in the Cat Ba Island area.

(2) Regarding the species composition of monogeneans (SLĐC) in marine fish species in the Cat Ba Island area:

- A total of 45 monogenean species were identified, belonging to 29 genera, 13 families, and 2 orders. The genera *Haliotrema* and *Ligophorus* were the most diversity, with 5 and 4 species recorded, respectively.

- One new species to science has been described and published (*Heterobothrium phamvanluci* sp. n.), and two additional species (*Glyphidohaptor* sp. and *Tetrancistrum* sp.) have been described but are not yet formally published.

- Nine species were newly added to the known monogenean fauna of Vietnam: *Glyphidohaptor sigani*, *Tetrancistrum indicum*, *T. sigani*, *Axine hemirhamphae*, *Neoaxine constricta*, *Microcotyle helotes*, *Polylabris mamaevi*, *Pseudaxine bychowskyi*, and *Octoplectanocotyla* sp. Additionally, six new host records were documented.

Recommendation

(1) Taxonomic Completion and Publication: Complete the morphological descriptions and conduct additional molecular analyses for *Glyphidohaptor* sp. and *Tetrancistrum* sp. to formally describe and publish them as new species in peer-reviewed taxonomic journals.

(2) Further Investigations: Extend research to cover gaps in data regarding the composition and infection intensity of monogeneans in economically important marine fish species, particularly those under aquaculture, to support disease control and management strategies in mariculture.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1) Hoang-Ha Thi Nguyen, Ha Van Nguyen, Hoang Van Hien, Nguyen Ngoc Chinh, Vinh Thi Thanh Truong, Van Van Kim, Truong Dinh Hoai, Phuc Pham Duc, Stephen E. Greiman, Hung Manh Nguyen (2024). Marine fish parasites in the Cat Ba Archipelago, Vietnam: the results of 2010-2023 field surveys. Systematic Parasitology, 101: 20. https://doi.org/10.1007/s11230-023-10145-w

2) Hoang-Ha Thi Nguyen, Ha Van Nguyen, Hung Manh Nguyen (2024). New record and redescription of *Microcotyle helotes* Sandars, 1944 (Monogenea: Microcotylidae) in Viet Nam. Academia Journal of Biology, 46(2): 1-6. https://doi.org/10.15625/2615-9023/19870

3) Van Van Kim, Hoang-Ha Thi Nguyen, Vinh Thi Thanh Truong, Lua Thi Dang, Manh Duc Vu, Nguyen Ngoc Chinh, The Duc Nguyen, Quan Van Nguyen, Hung Manh Nguyen (2025). A new species of *Heterobothrium* (Monogenea: Diclidophoridae) from half-smooth golden pufferfish *Lagocephalus spadiceus* in the Gulf of Tonkin, Vietnam, with an identification key for the genus. Regional Studies in Marine Science, 81: 104007. https://doi.org/10.1016/j.rsma.2024.104007