RESEARCH ARTICLE

Occurrence and Identification of Acanthocephalan Parasite *Rhadinorhynchus* sp. in Small Pelagic Fish from Various Marine Stocks in Leyte, Philippines

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ABSTRACT

This study demonstrates the first identification and characterization of acanthocephalan parasites (thorny-headed worms) infecting small pelagic fish stocks sold in Leyte, Philippines. A total of 340 small pelagic fish, representing eight distinct species across four families, were purchased from the Baybay City Public Market, with the fish collected from the territorial waters of Baybay City, Albuera, Inopacan, Hilongos, and Bato. The fish samples were aggregated into 34 pools (n = 10 fish per pool) for gastrointestinal examination. Five (14.70%) of the 34 pools tested positive for acanthocephalans, with evident infections in the scombrid species *Katsuwonus pelamis* and *Auxis rochei*. Morphometric characterization of male and female worm morphotypes indicates that the parasite is a *Rhadinorhynchus* sp. and is more similar to *Rhadinorhynchus ganapatti*. Bayesian estimation of pooled prevalence using the Gibbs sampler was employed, providing prevalence estimates for a fixed pool size and testing the uncertain sensitivity and specificity. The model yielded a posterior median individual prevalence of 0.4401 (95% PI: 0.0436-0.9304), indicating a low-intensity, latent infection directly influenced by poor level sensitivity (0.1562) and specificity (0.5727). Despite its relatively low infection rate, its public health implications can never be underestimated, especially since Filipinos have a deeply rooted culinary tradition of eating raw fish known as "kinilaw." The findings highlight a critical need for localized food-safety interventions, immediate post-harvest evisceration, and molecular-based surveillance to profile the parasite's occurrence across Philippine pelagic fisheries.

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1. INTRODUCTION

The Philippine Sea is considered as one of the epicenters of marine biodiversity, boasting numerous marine species that utilize it as a vital habitat or migration pathway. It is part of the global maxima of marine biodiversity in the Indo-Malay-Philippines archipelago (Carpenter and Springer 2005). It is no wonder that the marine fish industry in the country significantly contributes to national food security and macroeconomy, providing livelihoods for resident fisherfolk and generating income for Local Government Units. According to the 2023 Philippine Fisheries Profile, the national production volume/performance of the fisheries industry amounts to 4.26 million metric tons, with the Municipal Fisheries Production Volume (marine and inland) by Region VIII contributing an estimated 68,089.53 metric tons (BFAR-DA 2023). Most fisherfolk in the region, capture small pelagic fish species such as scads, sardines, and mackerels for their own consumption or sold at fresh landing sites and larger market locations (Clores 2024).

Because of the high density and diverse community of marine life in these areas, parasite transmission are inevitable. Marine fish are prone to various ectoparasite and endoparasitic infections, affecting directly or indirectly their growth, reproduction, and survivability (Villahermosa et al. 2022). One of the most common parasite that infects

small pelagic fish are the acanthocephalans, commonly known as thorny-headed-worms or spiny-headed worms. These obligate endoparasites possess thorns or spines on their anterior body parts, which serve as holdfast devices. The prominent, noticeable feature is the retractable proboscis, whose arrangement and number of hooks could be an efficient tool for species identification. Adult parasites thrive and reproduce exclusively in the host's intestine using their proboscis as the anchorage system. The parasite adopts a symbiotrophic lifestyle, where its nutrition is dependent on the host's intestinal nutritional contents. These parasites follow a complex life cycle, where the adult worms reproduce within the definitive host, and their embryonated eggs are shed into the environment via feces. Once in the ocean, these eggs are ingested by crustaceans/planktons – thereby entering the pelagic food web when the fish consume these crustaceans (Amin et al. 2011; Margulis and Chapman 2009).

Aside from their ecological implications, certain acantonchephalan species are zoonotic. Humans acquire the infection through consumption of raw, inadequately cooked, or traditionally preserved fish products (Buchmann and Karami 2024; CDC 2019). The local Filipino populations face a distinct risk of exposure due to the traditional culinary practices of consuming raw fish cured in vinegar, lemon, and other spices, known as "kinilaw." Acanthocephaliasis in humans range from acute abdominal pain, diarrhea, nausea, and localizaed tissue inflammation (Mathison et al. 2021). Additionally, acanthocephalans serve as valuable bioindicator for the presence and determination of heavy metal levels in aquatic ecosystems. They can accumulate more metals than their host fish, making them a metal biosink (Paller et al. 2016).

While multiple reports have documented the acanthocephalan occurrence in Philippine fishes, from freshwater species such as Nile tilapia (Munda and Estaño, 2020; Paller et al. 2016) to marine teleosts like tunas and rabbitfishes (Pramardika et al. 2024; Villahermosa et al. 2022), there is an evident data gap in its occurrence in the Eastern Visayas. Despite the importance of the parasite infection, it remains understudied among fish stocks in the region undermining local food safety surveillance. To address this gap, this study presents the first report identifying and characterizing acanthocephalan parasites collected from small pelagic fish from marine stocks around the region, providing baseline information about the potential bio-economic implications of the infection.

2. MATERIALS AND METHODS

2.1 Study area and sampling methods

The study was carried out in Baybay City (10.6521°N, 124.8525°E), Leyte, Philippines. This locality has an emerging fish industry, spanning a vast marine and freshwater area. The industry is centered on both aquaculture (tilapia farming) and capture fishing (Sabalza 2019). Since Baybay City is a big city (92 barangays) the demand for fish and fishery products is substantial, thus, aside from the small pelagic fish captured in Baybay City waters, fish vendors tend to import fish captured from different territorial waters from neighboring municipalities such as Albuera (10.9000°N, 124.7188°E), Bato (10.3291°N, 124.8293°E), Inopacan (10.5096°N, 124.8403°E), and Hilongos (10.3733°N, 124.7487°E). Hence, majority of the small pelagic fish supply comes from these five marine stocks (Figure 1).

To demonstrate the presence and degree of acanthocephalan infection in fish harvested from the marine waters of Baybay City and other neighboring municipalities, the sampling required a large population, which was nearly impossible to accommodate individually. To achieve a representative and statistically sound sampling, the fish sample population was aggregated into groups or pools that served as representatives. This approach further aided in the identification of animals infected by the parasite, demonstrated the status of infection at the site level, and provided an economic estimate of the prevalence occurring within the population without compromising the results (Chen and Swallow 1990; Worlund and Taylor 1983). Thus, a pooled sampling method was employed in the study. Small pelagic fish species were purchased from different and randomly selected fish vendors in Baybay City Public Market.
During the conduct of the study, 90 fish vendors occupied the numbered stalls at the Baybay City Public Market, selling various fishery products. To systematically select participating vendors, the number of pools to be tested served as the baseline for generating random numbers to select fish stalls for inclusion in the study. The list of random numbers was generated using the "Random Number Sampling" utility available online at AusVet EpiTools (Sergeant 2015). The specific input values required for the sampling calculator are outlined in Table 1. Among the 90 active vendors, only 45 were consistently selling small pelagic fish species; from this target sub-population, 34 sample stalls were randomly selected and included in the study. Additionally, to

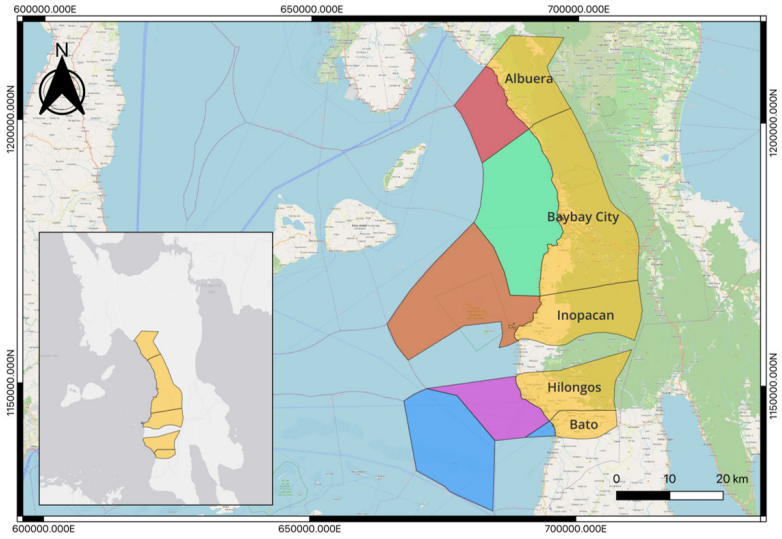

Figure 1. Map of the study area showing the five localities (yellow shaded land masses) and their respective territorial waters/fishing grounds: Albuera (reddish pink), Baybay City (pastel green), Inopacan (light brown), Hilongos (lilac), and Bato (sky blue). The inset map (bottom left) highlights the location of these combined municipalities within the region (map generated using QGIS 3.42.1 – Münster).

Table 1. Input values for random number sampling at AusVet Epitools.

Input values	
Sample size :	34
Sampling with/without replacement?	
Sampling with replacement	
· Sampling without replacement	
Sort output?	
· Sorted	
Unsorted	
Random number source	
· Specified range	
Upload list	
Enter minimum value for desired range :	45
Enter maximum value for desired range :	90

standardized the selection process, fish samples were screened based on interviews and verbal feedback from the fish vendors. This preliminary identification was then validated using established fishery biology critera and local taxonomic guides, including morphological traits (body shape, coloration, and fin structures), ensuring that only the small pelagic fish species were sampled.

2.2 Sample size determination

The sample size was calculated using the AusVet Epitools – "Sample size for Pooled Sampling in a Large Population" adapted from the methods of Cowling et al. (1999), implemented using R software (version 3.5.3). The input values include: *Size of pools* = 10; *Test sensitivity* = 90% (0.9); *Desired-herd sensitivity* = 95% (0.95); and the *Design (target) prevalence* = 1% (0.01). The values were encoded using the Ausvet EpiTools "Pool Sample Size Calculation" online calculator. Thus, for a pool size of 10, a minimum of 34 pools, each composed of 340 different small pelagic fish, were required and tested to provide a 95% probability of detecting the infection among small pelagic fish from the marine stocks of Leyte Island (Table 2).

2.3 Sample collection

A preliminary inventory of common small pelagic fish sold at Baybay City Public Market was conducted. Prior to collection, each participating fish vendor was informed regarding the study. Following their consent, a brief interview was conducted regarding the availability of small pelagic fish species typically available from September to December, accounting for seasonal variations in fish catch based on the vendors' commercial experience. To determine the specific fish species and the corresponding number of pools to be collected during each sampling period, a "drawing-by-lots" method was employed. Sampling was conducted three times a week to minimize sampling bias and maximize population representation.

For the actual collection, approximately one kilogram of small pelagic fish was purchased directly from each randomly selected, consented

Table 2. Sampling information and pool allocation number of the collected small pelagic fish from different stalls and origin.

Pool Allocation Number	Stall No.	Fish Origin	Fish Species	Common Name	Pool Size
1	47	Hilongos	*Mene maculata*	Moonfish	10
2	48	Inopacan	*Sardinella lemuru*	Sardine	10
3	50	Baybay City	*Selar crumenophthalmus*	Big-eyed scad	10
4	51	Baybay City	*Selaroides leptolepis*	Yellow-striped scad	10
5	52	Baybay City	*Sardinella lemuru*	Sardine	10
6	53	Bato	*Katsuwonus pelamis*	Skipjack tuna	10
7	54	Inopacan	*Auxis rochei*	Bullet tuna	10
8	57	Baybay City	*Rastrelliger faughni*	Island mackerel	10
9	58	Baybay City	*Selar crumenophthalmus*	Big-eyed scad	10
10	59	Baybay City	*Decapterus macrosoma*	Roundscad	10
11	60	Baybay City	*Decapterus macrosoma*	Roundscad	10
12	61	Baybay City	*Decapterus kurroides*	Red-tailed	10
13	62	Inopacan	*Sardinella lemuru*	Sardine	10
14	64	Baybay City	*Decapterus macrosoma*	Roundscad	10
15	65	Baybay City	*Selar crumenophthalmus*	Big-eyed scad	10
16	69	Inopacan	*Selaroides leptolepis*	Yellow-striped scad	10
17	70	Baybay City	*Katsuwonus pelamis*	Skipjack tuna	10
18	71	Baybay City	*Auxis rochei*	Bullet tuna	10
19	72	Baybay City	*Selaroides leptolepis*	Yellow-striped scad	10
20	73	Hilongos	*Sardinella lemuru*	Sardine	10
21	74	Baybay City	*Katsuwonus pelamis*	Skipjack tuna	10
22	75	Baybay City	*Selar crumenophthalmus*	Big-eyed scad	10
23	76	Inopacan	*Decapterus macrosoma*	Roundscad	10
24	77	Inopacan	*Sardinella lemuru*	Sardine	10
25	78	Inopacan	*Katsuwonus pelamis*	Skipjack tuna	10
26	80	Baybay City	*Sardinella lemuru*	Sardine	10
27	83	Inopacan	*Decapterus macrosoma*	Roundscad	10
28	84	Bato	*Decapterus kurroides*	Red-tailed	10
29	85	Bato	*Katsuwonus pelamis*	Skipjack tuna	10
30	86	Baybay City	*Selaroides leptolepis*	Yellow-striped scad	10
31	87	Baybay City	*Selar crumenophthalmus*	Big-eyed scad	10
32	88	Bato	*Decapterus macrosoma*	Roundscad	10
33	89	Baybay City	*Sardinella lemuru*	Sardine	10
34	90	Albuera	*Selaroides leptolepis*	Yellow-striped scad	10
					N = 340

vendor. The samples were stored immediately in a large styrobox containing ice to prevent degradation of the fish samples during transport. At the Diagnostic Laboratory of the Faculty of Veterinary Medicine, Visayas State University, fish identification and parasite detection/ isolation were conducted. Fish samples were taxonomically identified first using the FishBase online information system (Froese and Pauly 2025) and was sent for validation to the Department of Biological Sciences which confirmed the inclusion of eight unique species belonging to four distinct families (Table 2).

2.4 Parasite isolation and processing

The collected fish samples were examined immediately; only intact and apparently healthy fish were included in this study. The weight of each fish was measured using a digital weighing scale (in grams), while their total length was measured using a ruler (in centimeters). After checking, a midline incision was made in the abdomen using a scalpel blade (No. 22), starting from the anal opening to the pelvic fin. The abdominal cavity was examined, and the visceral organs were removed for easy inspection (Figure 2A-C). The intestinal organs were opened and cleaned

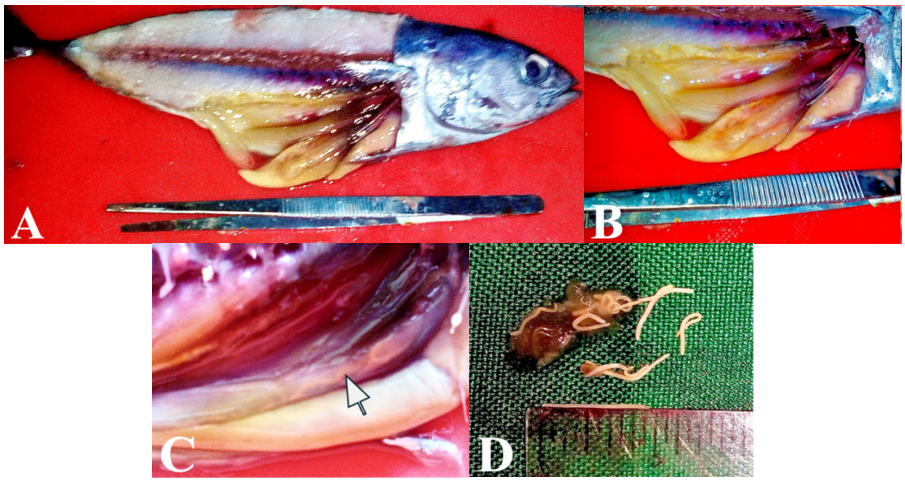

Figure 2. Dissection and collection of acanthocephalan parasites from sampled fish *Auxis rochei*; A) Dissection of the fish sample, B) Examination of the visceral organs, C) Closer view of the intestine with evident worm – pointed by a white arrow, D) Collected acanthocephalan parasites.

with distilled water. Detected acanthocephalans are harvested (Figure 2D) and submerged in cold distilled water for one hour to evert the proboscis for genus-level identification (Justine et al. 2012; Amin et al. 2011). Following parasite collection, the remaining fish samples were safely discarded as biological waste through deep burial. To prevent environmental contamination, all wastewater generated from cleaning of the discarded fish and processing samples was treated with a 10% bleach prior to regulated disposal. Finally, the isolated acanthocephalans were placed in a labeled vial containing 80% ethanol for long-term preservation, and the parasites were processing and mounted as described by Briones et al. (2015).

2.5 Morphological examination

The mounted parasites were examined morphologically at 40x and 100x total magnification under light microscopy (Leica DM500). The acanthocephalan parasites were identified based on their morphological characteristics using the standard diagnostic keys and morphometric structures, including, but not limited to, the trunk spines and proboscis armature, as described by Pramardika et al. (2024), Amin et al. (2019), and Briones et al. (2015). Taxonomic identification was limited to the genus level, though close morphological similarities to specific species were noted and reported as probable evidence of identity. Structural measurements were carried out using a calibrated ocular micrometer and ImageJ software (ver 1.8.0172) (Ferreira and Rasband, 2012), which was also utilized to derive scale bars (µm). Additionally, the determination and quantification of the acanthocephalan infection were limited exclusively to the detection of adult parasites within the host's gastrointestinal tract.

2.6 Data and statistical analyses

The presence of the parasite in fish samples was coded as "1" for positive and "0" for negative. The gathered data were managed and cleaned using Microsoft Excel. Descriptive statistics were also performed using the aforementioned software. Ausvet EpiTools (https://epitools.ausvet.com.au/) was used in various analyses (i.e., random number sampling, sample size for pooled sampling, pool sample size calculation, and Bayesian estimation using Gibbs sampler). R software (version 3.5.3) was used in the determination of pooled samples. The apparent prevalence of acanthocephalan *Rhadinorhynchus* sp. infection was computed using the formula (Thrusfield 2018):

$$(a)\ Proportion\ positive\ = \frac{Number\ of\ pool\ infected}{Total\ number\ of\ pool\ sampled} \times 100$$

The estimated confidence intervals for the proportion positive were also calculated using standard methods at a 95% confidence level for an infinite population (Thrusfield 2018). The calculation was based on the formula:

$$(b)\ SE\ (\hat{p}) = \sqrt{\frac{\hat{p}\ (1-\ \hat{p})}{n}}$$

Where $SE\ (\hat{p})$: standard error of the sample proportion, $\hat{p}$: sample proportion, and n for sample size. The confidence interval 95% was constructed

using the upper and lower limit of the interval defined by $\hat{p} = \pm 1.96\ x\ SE\ (\hat{p})$.

2.7 Bayesian estimation of pooled prevalence using the Gibbs sampler

Since the prevalence of the acanthocephalan parasite in the area remains unknown, a Bayesian estimation of pooled prevalence was performed using the Gibbs sampler. This method can provide prevalence estimates for a fixed pool size and tests with uncertain sensitivity and specificity, as observed in the study. The true values for sensitivity and specificity were not precisely known, but this can be estimated using Beta probability distributions (Sergeant, 2015).

The pooled prevalence was calculated using an online calculator adapted from method 7 of Cowling et al. (1999), which was run using R software. The application of Bayesian methods in this scenario was based on the description made by Joseph et al. (1995). This was based on the Gibbs sampler, an approach using the Markov Chain Monte Carlo method, in which random variables were generated from complex situations available (Gelfand and Smith 1990). Repeated simple calculations were performed by an intensive computer program to generate easy-to-trace analytical results in an iterative process, resulting in the desired characteristics enabled by the properties of Markov chains, as described by Tanner (1993).

Input values, such as pool size, the number of pools tested, and the number of positive pools, were positive integers, and the latter must be less than the number to be tested. The Alpha and Beta parameters for prior Beta distributions for true prevalence, test sensitivity, and specificity were also required. Expert knowledge or previous data on true prevalence, test sensitivity and specificity can be used as inputs for the Gibbs sampler utility. Since prior information was unavailable, a plausible approach was to set alpha = beta = 1. This resulted in a uniform (uninformed) distribution with equal probability of all values between 0 and 1 to occur (Sergeant 2015). Estimation of sensitivity and specificity was conducted at the pool level rather than the individual level, as the study involved pooled testing. Additional inputs necessary to simulate with the Gibbs sampler include the number of iterations and the number of iterations to be discarded. In the study, 25000 iterations were simulated, and 5000 were discarded. These enable the convergence of the model and its lower and upper limits, and provide a summary of output distributions (Sergeant 2015) (Table 3).

Table 3. Input values for Bayesian estimation of pooled prevalence using Gibbs sampler.

Input values		
Test results		
Size of pools	10	
Number of pools tested	34	
Number of pools positive	5	
Alpha and Beta parameters for prior distribution		
	Alpha	Beta
Prior prevalence	1	1
Test sensitivity	1	1
Test specificity	1	1
Simulation details		
Number of iterations	25000	
Number to discard	5000	
Lower CL for output	0.05	
Upper CL for output	0.95	

3. RESULTS

A total of 340 small pelagic fish, belonging to four families, were aggregated into 34 pools with 10 individual fish each and examined for acanthocephalan infection. Out of the eight small pelagic fish species, only the *Auxis rochei* (Bullet tuna) and *Katsuwonus pelamis* (Skipjack tuna) were positive for acanthocephalan infection (Table 4). A total of eighteen acanthocephalan parasites were recovered from the intestines of the fish. The collected parasites were grouped into two morphotypes based on shared morphometric characteristics, with morphotype 1 being male and morphotype 2 being female.

3.1 *Rhadinorhynchus* sp. morphotypes characterization

3.1.1 Morphotype 1: Male

The body/trunk has less evident "pseudo-segmentation" on the anterior and posterior regions and has a uniformly cylindrical shape. The total body length is 11 mm. Cuticular spines were absent dorsally. On the ventral region, one cuticular spine was present. The proboscis is fully everted and measures 387.78 µm in length by 33.75 µm in width (mid-portion). Longitudinal hooks row 18 - 20 with 38 to 40 hooks per row. Anterior hooks with a 10-12 circular arrangement. Basal hooks with 18-20 circular arrangement (Figure 3A). The neck opening has a length of 64.96 µm, and the proboscis receptacle is

Table 4. Summary of small pelagic fish examined for acanthocephalan parasite infection.

Fish species	Family	Average weight (g)	Average length (cm)	Total	Number of pools positive (%)
Mene maculata	Menidae	23.00 ± 0.00	10.90 ±0.00	10	0
Sardinella lemuru	Clupeidae	28.86 ± 12.25	13.64 ±1.78	70	0
Selar crumenophthalmus	Carangidae	43.20 ± 14.41	14.28 ±0.50	50	0
Selaroides leptolepis	Carangidae	31.90 ± 3.99	13.42 ±0.82	50	0
Katsuwonus pelamis	Scombridae	157.8 ± 30.06	21.86 ±0.89	50	80
Auxis rochei	Scombridae	49.00 ± 2.50	14.55 ±0.45	20	50
Rastrelliger faughni	Scombridae	57.00 ± 0.00	15.00 ±0.00	10	0
Decapterus macrosoma	Carangidae	42.45 ± 21.49	14.72 ±3.06	60	0
Decapterus kurroides	Carangidae	54.50 ± 37.50	15.50 ±5.10	20	0

1118.44 µm in length (Figure 3B). There were two arranged testes. The anterior testis measures 178.09 µm long and 82.40 µm wide. The posterior testis measured 155.33 µm in length and 86.67 µm in width (Figure 3C). Four elongated cement glands were observed in continuous arrangement (measurement ranges from 140 µm to 162.08 µm long) from the anterior to the posterior. The bursa is present in the most posterior part (34.64 µm long and 51.63 µm wide) (Figure 3D) (Pramardika et al. 2024; Amin et al. 2019).

3.1.2 Morphotype 2: Female

The body wall/trunk exhibits an evident "pseudo-segmentation" presentation, as seen from the anterior third to the posterior portion of the body. The total body length is 14.75 mm. The proboscis is partially retracted, measuring 155.05 µm in length and 27.51 µm in width (mid-portion). Longitudinal rows of hooks were 16 to 20, with 8 to 10 hooks in each row. Anterior and basal hooks comprise hooks 9 to 12 and 7 to 14, respectively (Figure 4A). The neck opening has a length of 57.30 µm, and the proboscis receptacle measures 974.88 µm in length; cuticular spines are absent dorsally, but 8 ventral spines were observed (Figure 4B). For the reproductive system, the uterus was long, with the presence of the vagina and the subterminal gonophore (Figure 4C-D) (Pramardika et al. 2024; Amin et al. 2019).

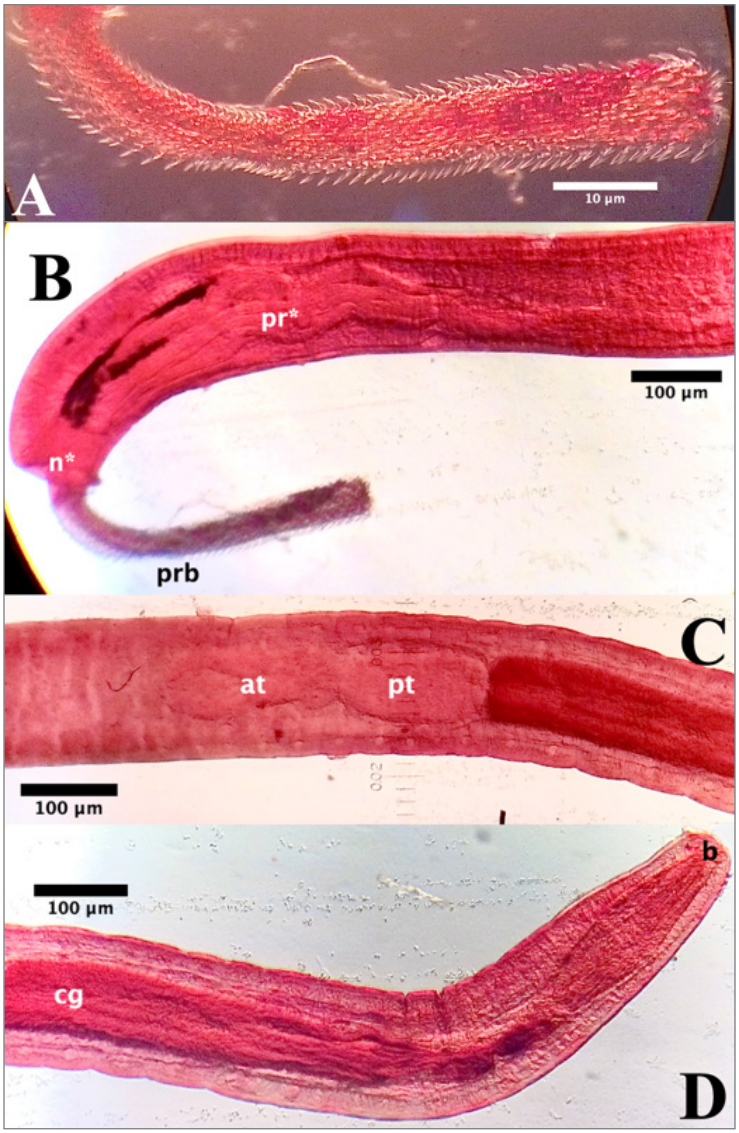

Figure 3. Acanthocephalan parasite *Rhadinorhynchus* sp. Morphotype 1: Male. A) Fully everted proboscis armature with evident anterior and posterior hooks (scale bar 10 µm), B) Proboscis, neck, and anterior trunk, C) Middle third of the trunk with two arranged oblong shaped testes – anterior and posterior, D) Posterior half of the trunk with evident elongated cement glands (B, C, D; scale bar 100 µm). Labels: at – anterior testes; pt – posterior testes, prb– proboscis, n* – neck, pr* – proboscis receptacle, cg – cement gland, b – bursa.

3.2 Prevalence

Of the 340 small pelagic fish examined, aggregated into 34 pools with 10 fish per pool, five pools were positive for *Rhadinorhynchus* sp., yielding an overall pool prevalence of 14.70%. These infections were localized to Pool 18, which comprised *Auxis rochei,* and to Pools 6, 17, 25, and 29, which all comprised of *Katsuwonus pelamis*. Specifically, the proportion positive pools ranges from 10% (Pools 17, 18, 25, and 29: one infected fish per pool) to 20% (Pool 6: two infected fish per pool). These findings imply a low-intensity, latent infection state within the local population, indicating that while the parasite is ecologically established, it maintains a low baseline presence that does not manifest in widespread or high-density burdens; alternatively, it is possible that the acanthocephalans present in the other fish specimens were predominantly in the larval stage, thereby leading to missed identification and detection under our "adult-focused diagnostic criteria."

3.3 Bayesian estimation of individual-level prevalence from pooled samples

The Bayesian analyses yielded a posterior mean prevalence of 0.4401 (95% PI: 0.0436-0.9304). Given that five out of the 34 examined pools tested positive, the maximum posterior probability that the group of small pelagic fish is infected reached 1.00 (Table 5).

The posterior distribution of the prevalence derived from this calculation is summarized in Figure 5A. The histogram indicates that most of the pools testing positive for the infection represent true positives, as a high proportion

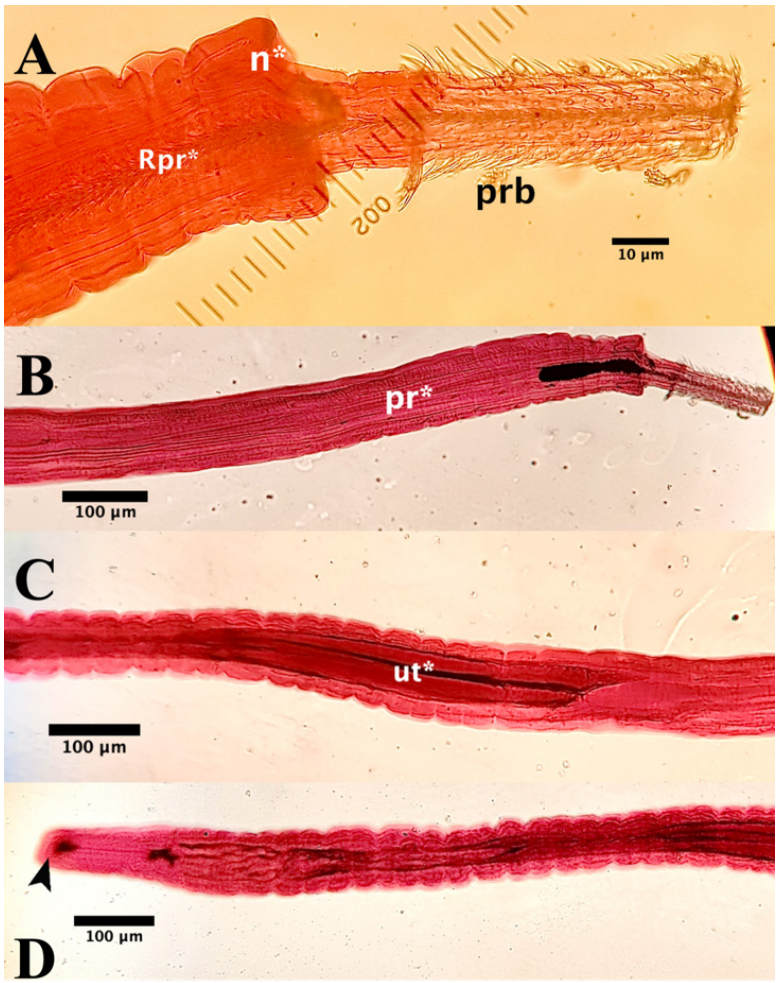

Figure 4. Acanthocephalan parasite *Rhadinorhynchus* sp. Morphotype 2: Female. A) Partially retracted proboscis armature with anterior and posterior hooks (scale bar 10 µm), B) Proboscis, neck, and anterior trunk, C) Middle and posterior trunk with evident elongated uterus, D) Posterior most portion of the parasite (B, C, D; scale bar 100 µm). Labels: prb – proboscis, n* – neck, Rpr* – retracted proboscis, pr* – proboscis receptacle, ut* – uterus, black arrow head – vagina and subterminal gonophore.

Table 5. Posterior probability distribution for prevalence, sensitivity and specificity of acanthocephalan parasite infection in small pelagic fish in Leyte.

Summary results	Prevalence	Sensitivity	Specificity
Minimum	1.00E-04	1.00E-04	1.00E-04
5 %ile	0.0436	0.0474	0.0599
Median	0.4401	0.1562	0.5727
95 %ile	0.9304	0.3192	0.9588
Maximum	1	0.9988	0.9999
Mean	0.4446	0.1609	0.5501
SD	0.2792	0.1052	0.2904
Iterations	20000	20000	20000

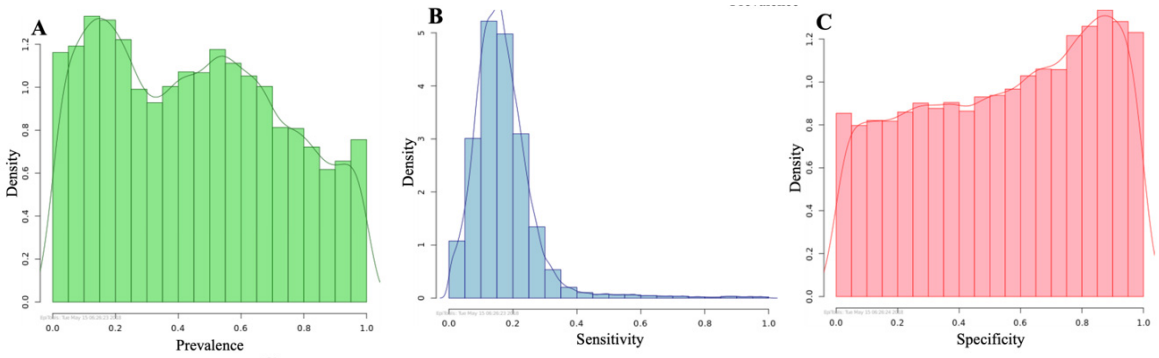

Figure 5. A) Posterior distribution of animal-level prevalence, B) Posterior distribution of sensitivity, C) Posterior distribution of specificity.

of the model iterations consistently classified these pools as positive. The posterior distribution also suggests that within any single positive pool, it is highly probable that a maximum of four out of the ten fish examined were truly infected.

3.4 Estimate of pool sensitivity and specificity

Of the 34 pools examined, 29 (85.29%) tested negative, while 5 pools (14.70%) were positive. The initial pool-level sensitivity of the fish samples was estimated at 0.1562 (95% CI: 0.0474-0.3192; Figure 5B), whereas the pool-level specificity was 0.5727 (95% CI: 0.0599-0.9588; Figure 5C). Using the Bayesian approach, the final diagnostic performance metrics were optimized by incorporating posterior distributions of animal-level prevalence. Based on this model, the diagnostic protocol demonstrated a posterior probability of 0.9988 (95% PI: 0.0474-0.3192), indicating that an infected pool would yield a positive result, and a 0.9999 probability (95% PI: 0.0599-0.9588) that an uninfected pool would correctly test negative (Table 5).

This "near-perfect" posterior specificity (99.99%) suggests an exceptionally low probability of false-positive outcomes for uninfected fish samples. It was also noted that the apparent prevalence of the infection (*Ap* = 14.71%) was slightly higher than the calculated true prevalence (*tp* = 13.98%). These calculations indicate the degree of infection in the pooled sample, which is low as hypothesized and is affected by the sensitivity and specificity of the test.

4. DISCUSSION

4.1 Acanthocephalan parasite *Rhadinorhynchus* sp.

Morphological analyses of the isolated acanthocephalans revealed two distinct morphotypes of *Rhadinorhynchus* sp., both of which exhibit close structural affinity to *Rhadinorhynchus ganapatti*. Locally, the reports by Pramardika et al. (2024) and Briones et al. (2015) represent the only known studies on the presence of these specific endoparasites in the Philippines, both of which similarly isolated the parasite from skipjack tuna (*Katsuwonus pelamis*). Consequently, the findings of this study constitute the first formal report of *Rhadinorhynchus* sp. in bullet tuna (*Auxis rochei*) and *K. pelamis* harvested within Central Philippine marine waters (Leyte Island), establishing a new geographic location record for this genus of acanthocephalans.

Among the eight small pelagic fish species across the 34 pools, *Rhadinorhynchus* sp. infections were evident in *K. pelamis* (pools 6, 17, 25, and 29) and *A. rochei* (pool 18). Both of these fish species belong to the family Scombridae. While available published reports suggests the *K. pelamis* serves as a highly specific host for *Rhadinorhynchus* sp. (Pramardika et al. 2024), the collection of the parasite in *A. rochei* coincides with the regional findings of Kleinertz et al. (2016), in Balinese waters, Indonesia. The presence of *Rhadinorhynchus* sp. in both fish species indicates that they do not display strict single-species host specificity, instead they have the capacity for cross-species transmission within these pelagic teleosts.

4.2 Biological drivers of infection

Amin et al. (2019) indicated that *Rhadinorhynchus* sp. infection in pelagic fish can be attributed to their distinct ecology as migratory and cosmopolitan species. The *K. pelamis* and *A. rochei* are both opportunistic predators with diets comprise of varied crustraceans such as copepods, isopods, amphiphods, or even smaller fish species. With the life-cycle of the parasite, these crustaceans serves as intermediate hosts (IH) carrying the cystacanth larvae (infective stage), the transmission of the parasite is

dependent in these food-web dynamics.

With the Eastern Visayas having a rich and diverse marine ecosystem, it provides a good environment for these IH crustaceans to thrive and be available in the parasite transmission. The positive fish samples identified in this study can be traced to the local fishing grounds of Bato (pools 6 and 29) Baybay City (pools 17 and 18), and Inopacan (pool 25). The presence of *Rhadinorhynchus* sp. from these separate landing sites confirms the widespread and ecologically established transmission dynamics of the parasite throughout the coastal areas of Leyte.

4.3 Diagnostic constraints

While the parasite infection is established in this study, it is worth noting that one critical factor must be considered when determining infection rates in small pelagic fish, mostly of the marketed scombrids were juveniles. The presence of acanthocephalan parasites depends on the ontogenetic dietary shifts of the fish host. As the fish matures, its feeding preference changes from crustaceans to larger teleost fish or copepods. If the fish transitioned its diet from the IH crustaceans, the existing adult parasite burden will naturally lower or be eliminated over time. But if the larger prey serve as transport hosts that harbor the larvae, then the parasite burden could persist as the fish matures (Buchmann and Karami 2024; CDC 2019).

Our study has documented infections in a specific group of fish (scombrids), and diagnosis is limited to light microscopy for detecting adult acanthocephalans in the intestinal lumen; the larval stages of the parasite, which may reside in other examined fish may have remained completely undetected. These larval stages mostly lack a fully developed proboscis, making them difficult to identify visually. Thus, advanced molecular profiling is strongly warranted in future surveillance programs to determine the parasite's occurrence in other small pelagic fish.

4.4 Public health implications

Certain marine acanthocephalans have the potential to cause zoonotic disease (acanthocephaliasis), while others are considered incidental or non-pathogenic to humans (Buchmann and Karami 2024). Infection usually occurs through the consumption of raw or undercooked fishery products containing the parasite. This risk is relevant in the Philippines because of the deep-rooted traditional culinary practices, with "kinilaw" as the most common. It presents a "distinct cultural exposure route." To reduce this transmission risk, various post-harvest handling and preparation guidelines can be implemented, including thorough evisceration to avoid post-mortem migration of the larvae into the musculature, thorough cooking of the fishery products, and deep-freezing of the freshly caught fish to neutralize the viable or latent larval stages.

5. CONCLUSION

This study provides definitive baseline evidence of *Rhadinorhynchus* sp. infection among economically important small pelagic fish caught from the marine waters of Leyte Island. Despite the individual fish-level prevalence being low and with the infection restricted to scombrid hosts (*K. pelamis* and *A. rochei*), the current findings clearly demonstrate the establishment and cosmopolitan distribution of acanthocephalans in fish, provided that appropriate hosts are present. As a fishborne parasite with zoonotic potential, its public health implications should never be underestimated, as the raw-fish-eating habit, such as the delicacy "kinilaw," has become a culinary tradition among Filipinos. Concurrently, with the documented capacity of different genera of acanthocephalans as ecological bioindicators of environmental pollutants (e.g., heavy metals), the genus *Rhadinorhynchus* holds considerable promise as a localized bioindicator for pollution monitoring in regional fishing grounds. Future research utilizing molecular diagnostics is strongly warranted to detect larval stages of the parasites across other small pelagic stocks as well as to establish proper food-safety guidelines covering fishborne marine parasites.

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AUTHOR CONTRIBUTIONS

Sumagang JCG: Conceptualization, Methodology, Investigation, Formal analysis, Writing-original draft; **Balala LM**: Conceptualization; **Bagot MA**: Conceptualization, Methodology, Writing-review & editing; **Dautil JP**: Conceptualization,

Methodology, Writing-review & editing**; Lumain JPLM**: Validation, Writing-draft preparation, review & editing; **Lañada EB**: Supervision, Conceptualization, Validation, Writing-review & editing

CONFLICTS OF INTEREST

The authors have no competing interest to declare.

ETHICS STATEMENT

The Faculty of Veterinary Medicine Research Committee has confirmed that ethical approval is not required for the conduct of the study. The fish samples were purchased directly from the fish vendors.

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