

(Original Research)

Morphometry-based Risk Analysis for *Angiostrongylus* spp. in Snail Intermediate Hosts in a Rural Area in Mulanay, Quezon Province, Philippines

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Abstract

Background: Neuroangiostrongyliasis is an emerging foodborne zoonosis in the Philippines and remains understudied in the country. This study surveyed the distribution and morphometry-based risk for *Angiostrongylus* spp. in snail hosts in Brgy. Matataja, Mulanay, Quezon Province. **Methods:** Snails (n=138) were collected from January to April 2025. Shell morphometry was recorded prior to dissection. Principal Component Analysis (PCA) was first used to reduce trait multicollinearity in measured morphometry. Logistic and linear regression analyses were then used to evaluate morphometry-based infection risk and larval capacity in host species, respectively. **Results:** Total prevalence was 15.90% (n=22). *Lissachatina fulica* showed higher prevalence (60.90%) with moderate intensity (6.57 ± 6.69), while *Pomacea canaliculata* exhibited lower prevalence (8.4%) but exhibited extreme aggregation ($k=0.02$, $D=0.972$). Species-specific modeling identified shell size (PC1) as a risk predictor only for *L. fulica* ($p=0.027$), while size limited carrying capacity across both species ($p<0.05$). **Conclusion:** This is the first report of *Angiostrongylus* sp. in Quezon Province to our knowledge. The significant influence of shell morphometrics on larval burden highlights the importance of host physical traits in parasite transmission dynamics and the need for localized public health surveillance.

Keywords

Angiostrongylus spp., Snail hosts, Surveillance, Foodborne helminth

1. Introduction

Angiostrongylus spp. is a group of parasitic metastrongylids parasitizing mammals as their definitive hosts [1]. Two known species, *A. cantonensis* and *A. malaysiensis*, belong to the group commonly referred to as the rat lungworms due to their similar biology [2,3]. Snails are some of the main intermediate hosts of *Angiostrongylus* spp [3]. *A. cantonensis* incidentally infects humans [4]. It is transmitted after consuming improperly cooked snails, such as *P. canaliculata* snails, and may even be present as larvae in seafood or contaminated crops [5,6,7]. Moreover, the invasive Giant African Land Snail, *L. fulica*, widespread throughout Luzon, is also considered to be a primary intermediate host of *A. cantonensis* [8,9]. *Angiostrongylus* spp. have been found in several areas in Luzon Island, particularly Laguna, Nueva Ecija, and Metro Manila [10,11,12]. The prevalence of *Angiostrongylus* spp. in its definitive and intermediate hosts and possible spatial patterns have been studied in rural areas where the parasite has been reported [13,14].

Few studies have reported the presence of *Angiostrongylus* spp. in Metro Manila and the suburbs [15,16] but indicate its incidence in residential areas. Cases of eosinophilic meningoencephalitis, the primary disease associated with *A. cantonensis*, has been documented in the country [17]. As such, there remains a need to conduct research contributing to *Angiostrongylus* spp. surveillance amid its growing emergence across the Asia-Pacific region [18,19,20,21,22,23,24].

2. Materials and Methods

2.1. Study Site

The study was conducted in Brgy. Matataja, Mulanay, Quezon Province. It is located at 13°29' N, 122°29' E, and is characterized by rugged, arable terrain with patches of forests. Most of the barangay is used for agriculture, with several rice fields found along the peripheral plains (Fig. 1). The presence of agricultural and residential lands

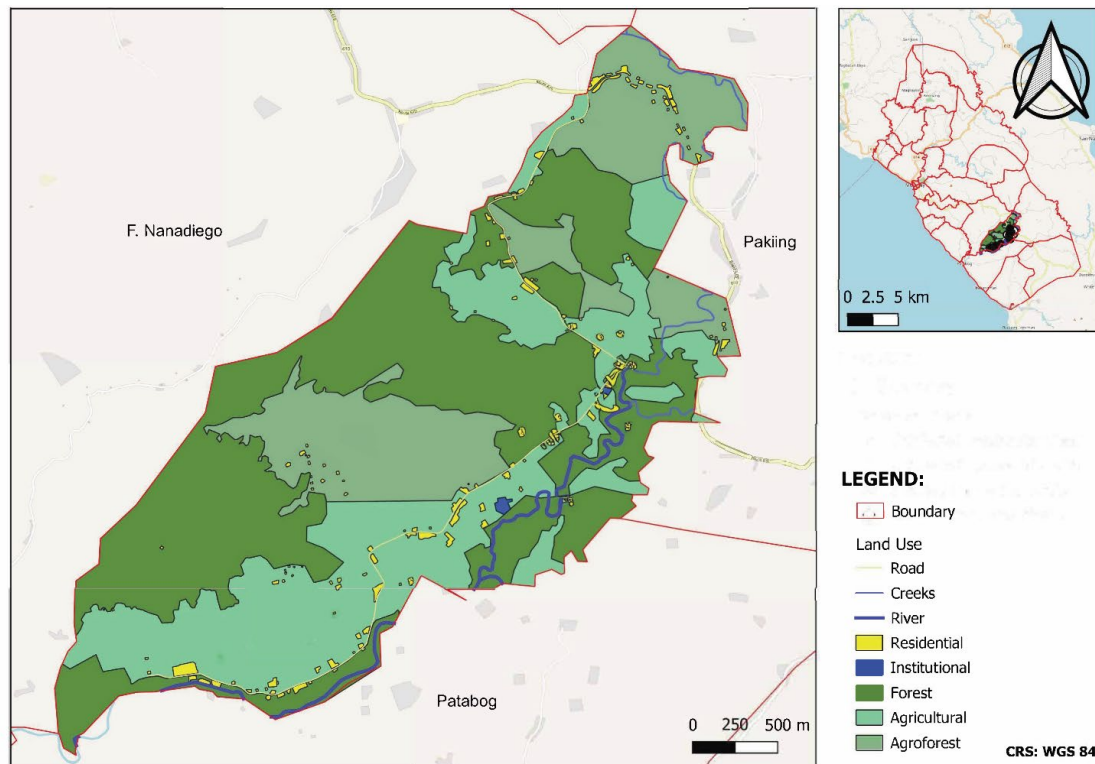


Figure 1. Map of Barangay Matataja, Mulanay Quezon Province with land use gradient.

This study aimed to detect the presence of *Angiostrongylus* spp. in Mulanay, Quezon Province, and investigate their host-parasite transmission dynamics by first determining its prevalence and species-level aggregation patterns. Secondly, the study sought to profile the influence of host morphometry on infection risk and host larval carrying capacity through a combination of Principal Component Analysis (PCA) and a two-step probabilistic modeling approach, identifying how host attributes, such as shell size and shape, drive parasite distribution while accounting for high overdispersion and stochastic environmental factors in larval burdens.

and their proximity to each other make the area suitable to support the life cycle of *Angiostrongylus*.

Sampling sites were selected based on the following purposive criteria: (1) a history or culture of snail and other gastropod consumption; (2) a rural community with agricultural and residential land; (3) presence of snails and other gastropods in bodies of freshwater and land; and (4) evidence of close interactions between humans, gastropods, and rodents. Collection was conducted in three phases between January and April of 2025. The collection was done by handpicking from 6:00 AM to 10:00 AM. Opportunistic visual search was employed in residential areas, rice fields,

disposals, forest patches, and waterways, following previous surveillance studies [25,13,26,27,28], with a 10 minute maximum time allocation per collection site to ensure broad coverage of the study area. The snails were placed in disposable polypropylene containers. Freshwater snails were submerged under a 1:3 ratio of source water and distilled water for transport and kept in groups of three (3) to five (5) per container based on size to avoid crowding stress. Terrestrial snails were stored individually after spraying with distilled water to moisten the containment. The samples were then collectively stored in a polypropylene crate labelled with the appropriate biosafety level indicator before transport. Commercial chemical baits were not used to avoid accidentally killing the gastropod hosts [29]. Informed consent of the local government unit and the local community, as well as necessary permits (UPLB-2024-066) were obtained prior to data collection. Data were kept under strict regulations following Republic Act No. 10173 or the Data Privacy Act of 2012.

2.2. Sample Processing

2.2.1 Intermediate Host Identification and Morphometry

The linear morphometrics of each snail were measured using a physical caliper. The following linear morphometric traits and their corresponding labels are as follows: shell length (SL); shell width (SW); aperture length (AL); aperture width (AW); penultimate whorl width (PWW); body whorl length (BWL). The snails were classified and grouped based on similarity in size and appearance. Specimens were then identified to the lowest taxonomic level determinable through established morphologic keys [30,31,32,33]. Shells of voucher specimens were kept for future reference.

2.2.2. Identification of *Angiostrongylus* spp. Larvae

Squashing. The snails were extracted from the shell by carefully cutting and removing the shell body whorl, and then delicately pulled out using Kelly forceps. The snails were dissected, with the respiratory system organs such as the lungs, gills (for freshwater snails), and pulmonary sacs. Squash mounts were prepared by pressing the organ between two clear glass slides until a

thin film was achieved and then examined microscopically.

Artificial Digestion. Snail samples were also artificially digested using 0.1% artificial gastric juice (AGJ) solution. Initial preparation included mixing 1g of pepsin powder, 10 mL of concentrated HCl, and distilled water to a final volume of 1000 mL. Samples were cut into small pieces and mixed with 50mL of AGJ. These were placed on a hot plate and magnetically stirred at 37 °C for 1-2 hours [10,13,34]. The mixtures were then filtered through a 500 µm sieve and transferred into 50 mL conical tubes. The digesta were then centrifuged at 4,500 rpm for 10 minutes and then decanted. The resulting pellets were then preserved under 95% ethanol until further microscopic examination.

Microscopic Identification. The morphological descriptions of *Angiostrongylus* spp. based on previously published literature were used for microscopic identification of the larvae [35,36,37,39].

2.3. Data Analysis

The prevalence of *Angiostrongylus* spp. was calculated by getting the proportion of the infected snails over the total number of snails collected. Further, mean intensity was calculated as the total number of parasites recovered divided by the number of infected snails hosts only with uninfected snail hosts excluded from the calculation.

A Shapiro-Wilk test was conducted across all data to assess normality. The relationship between host species was assessed via Fisher's exact test for prevalence and Kruskal-Wallis test for mean intensity, respectively. Distribution was first characterized by determinants of parasite aggregation (k) and Poulin's Discrepancy Index (D) computed via a Gini-style algorithm for high zero-inflation. A p-value of <0.05 indicated a significant result for all subsequent analyses.

To address multicollinearity among correlated measurements, Principal Component Analysis (PCA) was used to reduce the individual morphometric trait variables into two principal components corresponding to shell size (PC1) and shell shape (PC2), which served as predictors in a two-stage modelling framework. First, logistic regression was used to model infection risk in host

species via estimating the probability of infection as a function of shell size, evaluated via Likelihood Ratio Test (LRT) and expressed as odds ratios (OR). Second, negative binomial regression was used to model host larval carrying capacity through modeling parasite intensity as a function of shell size and shape. All statistical analyses, modelling, and visualizations were performed using RTools version 4.5 packages and operated via RStudio version 4.4.2. Copies of all R scripts were kept for reproducibility.

3. Results and Discussion

3.1. Morphological Detection Reveals First Documented Report of *Angiostrongylus* spp. in Quezon Province, Philippines

A total of 138 snail samples were collected (Fig. 2). Among the collected snails, five species were identified: *L. fulica* (n=23), *P. canaliculata* (n=107), *R. ovum* (n=4), *Helicostyla* sp. (n=2), and *Melanoides* sp. (n=2) (Fig. 2A). Only *L. fulica* and *P. canaliculata* tested positive for the presence of

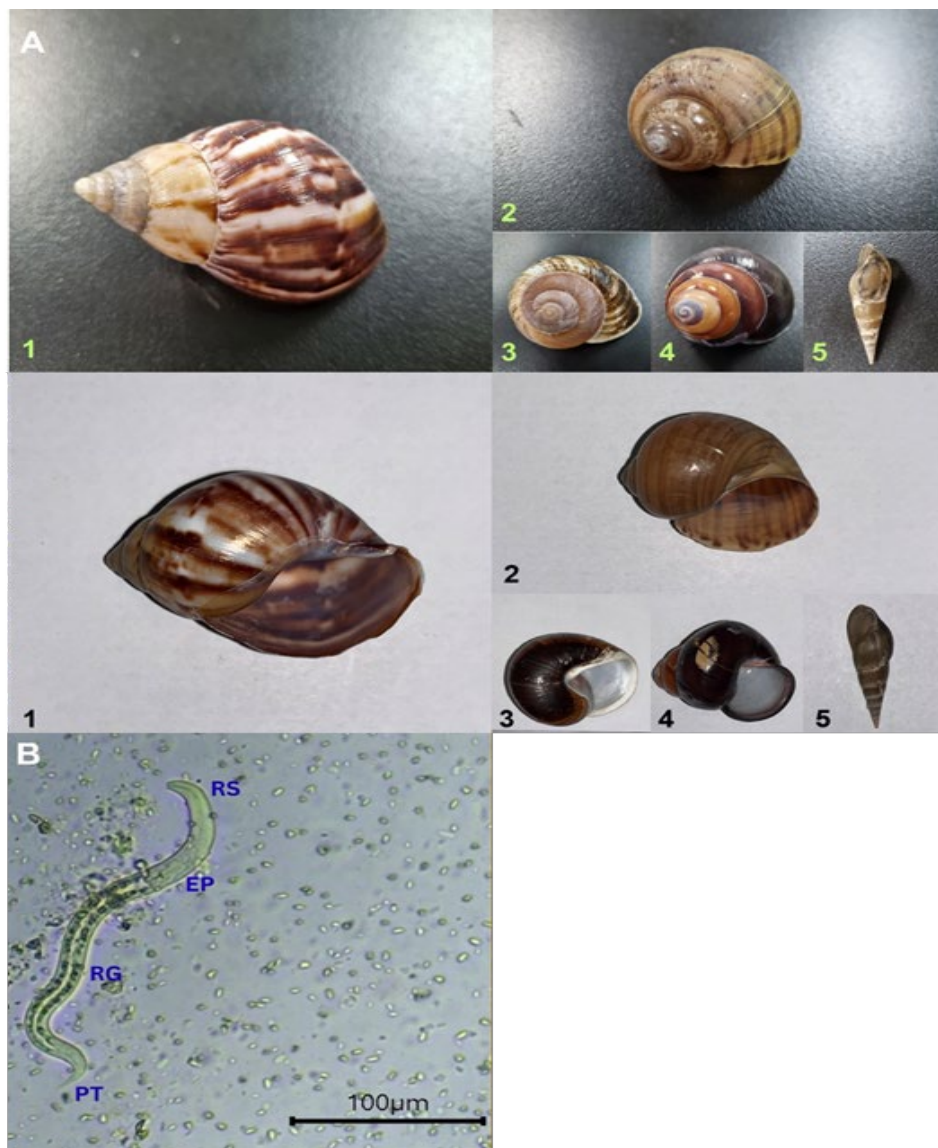


Figure 2. Detection of *Angiostrongylus* spp. larvae in snail hosts. (A) Snail species collected from Brgy. Matataja, Quezon province in dorsal and ventral views: (1) *L. fulica*, (2) *P. canaliculata*, (3) *R. ovum*, (4) *Helicostyla* sp., (5) *Melanoides* sp. (B) First stage (L1) *Angiostrongylus* sp. larva from filtered *L. fulica* artificial digesta with visible anterior rod-like structure (RS), esophagus (EP), intestinal cells showing reflective granules (RG), and a terminally located pointed tip (PT) identified using descriptions by Howe *et al.*, 2019 [36]; 100x magnification.

larval nematode clusters and as such, were the only species subsequently analyzed. Moreover, the disparity in sample sizes between the two species may reflect their relative local abundance during collection within the study area. Further, the smaller number of *L. fulica* collected may be attributed to its reduced activity and foraging during the dry season, as the species is typically more abundant and active during the wet season.

Angiostrongylus spp. larvae were identified by a distinct pointed tip (PT) at the end of their tails found across all stages [35,43]. The approximately 300 μm L₁ *Angiostrongylus* spp. larvae were identified by a rod-like structure (RS) found immediately posterior to its buccal cavity, a rhabditoid esophageal bulb (EP), and reflective granules (RG) found in its intestinal region (Fig. 2B) [6,36]. The distinct electrophoretic bands approximately 565 bp in length that matched the positive control of *Angiostrongylus* sp. (Fig. 2C) confirm the identity of the nematodes as under the genus *Angiostrongylus* [40]. To our knowledge, this finding consequently indicates the first

forest canopies while *Helicostyla* sp. in trees [41,42]. Thus, limiting chances of exposure. In contrast, the invasive *L. fulica* are habitat generalist, thereby increasing likelihood in contracting *Angiostrongylus* spp. larvae through rat feces [7,28]. Further, *A. cantonensis* larvae have been previously reported, albeit in low prevalence, in *Melanoides* sp. in Mindanao [13] in contrast to what has been recorded in the current study. However, the low sample size of these three may also have contributed to the seemingly absence of infection snail species.

3.2. High *Angiostrongylus* Prevalence in *L. fulica* and Extreme Aggregation in *P. canaliculata* Indicate Divergent Transmission Strategies

The total prevalence of *Angiostrongylus* spp. infection in Brgy. Matataja is 17.69 [11.56 – 25.35] with an overall mean intensity of 11.48 ± 18.91 (Table 1). Prevalence is significantly higher in *L. fulica* at 60.87 [40.79-77.84]) than in *P. canaliculata* at 8.4 [3.2-13.7]) ($p=0.000000152$,

Table 1. Summary of parasitological indices and aggregation parameters for *Angiostrongylus* spp. in *Lissachatina fulica* and *Pomacea canaliculata*.

Species	N	Prevalence, % [95% CI]	Mean Intensity $\bar{x} \pm \text{SD}$	Mean Abundance $\bar{x} \pm \text{SD}$	VMR	k	D
<i>Lissachatina fulica</i>	23	60.9 [40.9-80.8]	6.57 ± 6.69	4.00 ± 6.10	9.30	0.456	0.655
<i>Pomacea canaliculata</i>	107	8.4 [3.2-13.7]	19.11 ± 28.34	1.61 ± 9.43	55.38	0.020	0.972
<i>Ryssota ovum</i>	4	-	-	-	-	-	-
<i>Helicostyla</i> sp.	2	-	-	-	-	-	-
<i>Melanoides</i> sp.	2	-	-	-	-	-	-
Overall	138	17.69 [11.56 – 25.35]	11.50 ± 18.91	2.03 ± 8.96	-	-	-

documented detection of *Angiostrongylus* spp. in Mulanay and subsequently, Quezon Province. As such, further surveillance is needed to assess the reach of the parasite, its local ecology, and its zoonotic risks.

No infections were found in *R. ovum*, *Helicostyla* sp., and *Melanoides* sp. There were no previous reports surveying the terrestrial snails, *R. ovum* and *Helicostyla* sp., for *Angiostrongylus* spp. infection. However, it can be hypothesized that there is a lower likelihood for these native snail species to have *Angiostrongylus* spp. infection as they rely mostly in undisturbed habitats – with *R. ovum* usually confined in moist

$V=0.5246$), identifying *L. fulica*, a terrestrial snail, as a primary intermediate host of *Angiostrongylus* spp, which aligns with the current understanding of *Angiostrongylus* spp. infection in gastropod hosts, where prevalence tends to be higher in terrestrial mollusks than in their freshwater counterparts. This may be attributed to low overlap between the habitats of the definitive rat host and the intermediate snail host, consequently reducing the likelihood that a freshwater snail, such as *P. canaliculata*, to be exposed to rat feces with the larvae [7,28]. In contrast, mean intensity was higher but more variable in *P. canaliculata* (19.11 ± 28.34), than in *L. fulica* (6.57 ± 6.69) though not statistically significant ($\chi^2=0.14493$,

df=1, $p=0.7034$). This suggests that both species are equally probable to harbor for *Angiostrongylus* larvae. The remarkably high variance ($SD=28.34$) in *P. canaliculata* indicates that the larval burden is not uniform in the population, hinting at the presence of extremely high-intensity individuals. Abbreviations: VMR, Variance-to-Mean Ratio. k denotes the parameter of aggregation, where $k < 1$ indicates high aggregation. D denotes Poulin's discrepancy index, where $D=1$ denotes the highest possible parasite aggregation in its host population.

Aggregation analysis revealed that the distribution of *Angiostrongylus* spp. larvae in *L. fulica* is moderately aggregated ($D=0.655$ [0.472 - 0.770], $k=0.456$), indicating a stable host-parasite equilibrium. The evenness of distribution allows the parasite to be ubiquitous across the terrestrial landscape that facilitates endemic transmission of the larvae from the snail to the rat host, aligning with the current understanding of *L. fulica* as a primary intermediate host in the parasite's life cycle [17,44]. However, bootstrap comparison reveals a significantly higher degree of aggregation in *P. canaliculata* is observed ($D=0.972$ [0.941 - 0.986], $k=0.02$) at 95% confidence, where the parasitic population is sequestered within a few snails despite exhibiting lower prevalence (Fig. 3). This may suggest that *P. canaliculata* has a higher potential of spreading the disease, which adds to the risk of it being eaten

as a delicacy [46,47]. The differences in the aggregation of the larvae in these two snail species may partly be attributed to the differences in their habitat. Environmental heterogeneity, including the spatial patchiness of parasite infective stages in terrestrial and aquatic habitats, may also influence aggregation patterns by creating unequal exposure among individual hosts with aquatic habitat facilitating higher chances of exposure. However, this hypothesis was not directly evaluated in the present study and warrants further investigation.

These results highlight key differences in the underlying infection strategies between the two species. However, this does not diminish the risk of *L. fulica* as a vector for angiostrongyliasis due to its proximity to residential land and its potential to contaminate areas or objects with its mucus secretions containing infective *Angiostrongylus* spp. larvae [48]. As such, host-parasite interactions between *Angiostrongylus* spp., their definitive and intermediate hosts, and their environment, warrant further investigation.

3.3. Host Size Decreases Infection Probability in *L. fulica* but Scales with Community-Wide Larval Capacity

Principal component analysis (PCA) of the six morphometric traits revealed that shell size (SL, SW, AL, AW, and BWL) accounted for 82.6% of

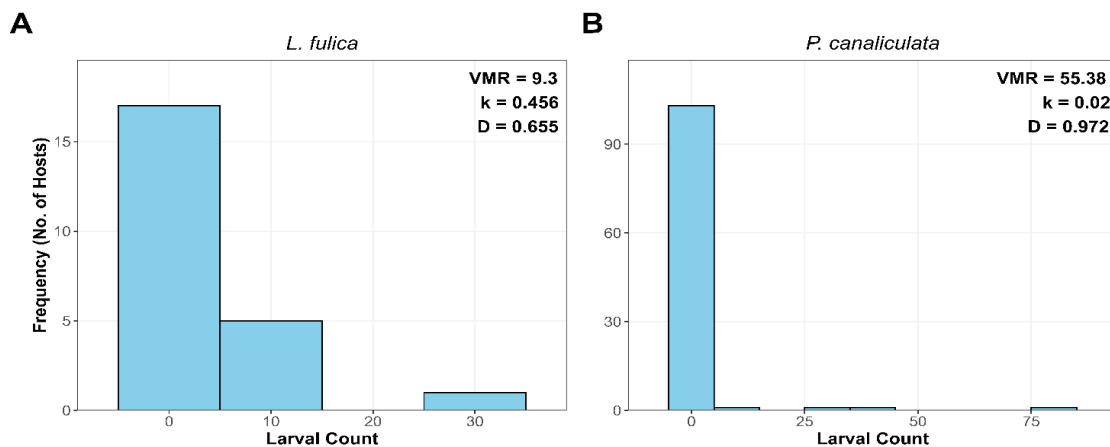


Figure 3. Larval frequency distribution and aggregation of *Angiostrongylus* spp. Values represent the frequency of larval counts (intensity) for (A) *L. fulica* and (B) *P. canaliculata*. Aggregation indices are provided for each species: Variance-to-Mean Ratio (VMR), Negative Binomial dispersion parameter (k), and Poulin's Discrepancy Index (D). The characteristic "L-shaped" distribution in both panels indicates high parasite overdispersion, particularly in *P. canaliculata* ($D=0.97$).

explained variance (PC1). Shell shape (PWW) accounts for 13.4% (PC2) (Supplementary Fig.1). These principal components were subsequently utilized as morphometric predictors for risk profiling across both species (Fig. 4).

71% as it reaches adulthood. This shift aligns with previously established theories on growth-immunity tradeoffs, where younger snails likely prioritize growth at the expense of immune development, which is then recompensated as the snails accrue body mass [49, 50]. Ultimately, the

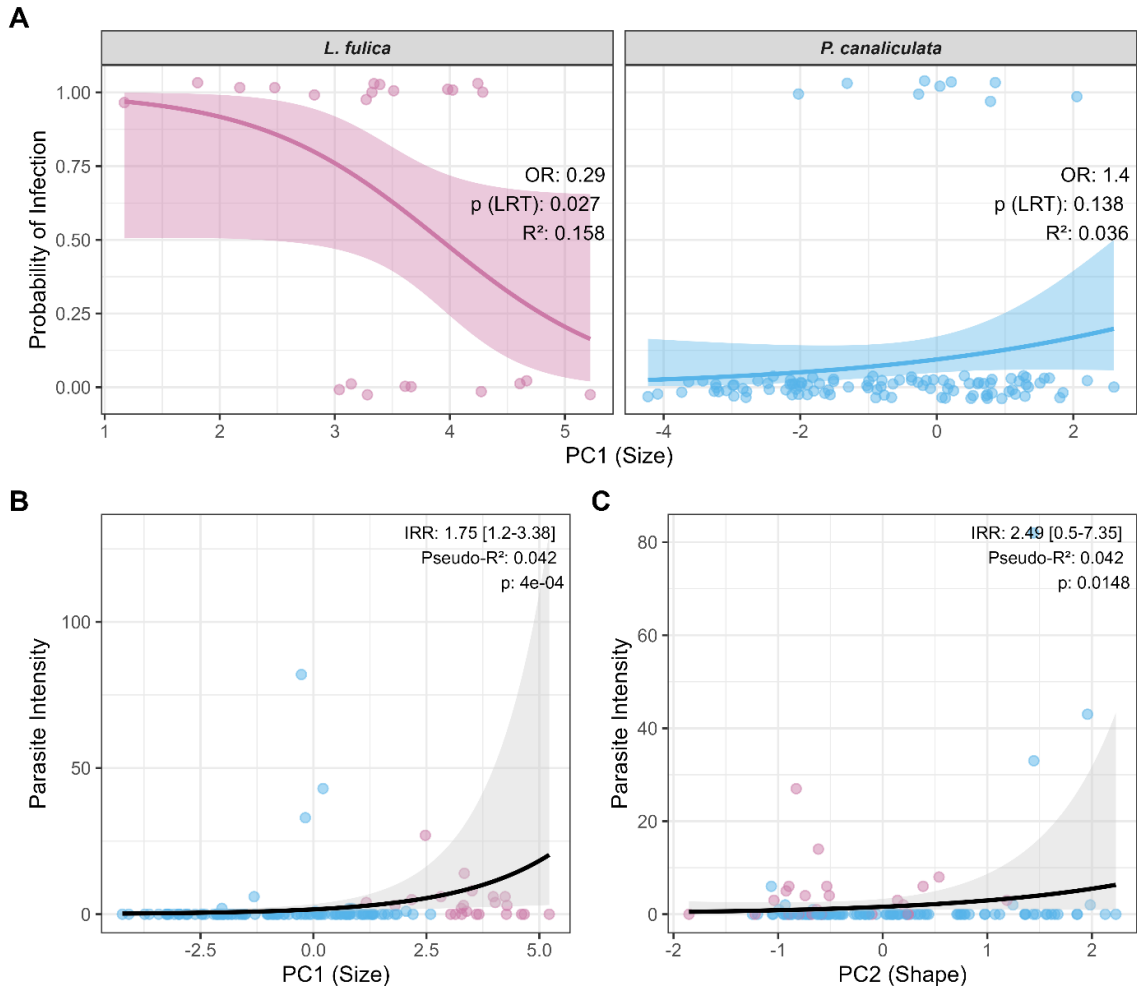


Figure 4. Morphometric drivers of *Angiostrongylus* spp. larval prevalence and intensity in intermediate hosts. (A) Odds ratios (OR) represent the probability of infection via prevalence as a function of PC1 (Size) and PC2 (Shape). Points to the right of the dashed vertical line (OR > 1.0) indicate an increased likelihood of infection per unit increase in the PC score. (B) Linear regression of larval burden (Intensity) against PC scores for infected individuals. The relationship illustrates how host size and shape influence total larval capacity; datapoints colored in pink and blue correspond to *L. fulica* and *P. canaliculata*, respectively. Together, these panels demonstrate whether morphometric traits act as a filter for initial infection (A) or as a determinant of the final parasite load (B). McFadden's pseudo- R^2 values were used to denote model fitness for all tests.

Logistic regression indicates PC1 (shell size) significantly influences infection prevalence in *L. fulica*, with smaller individuals showing higher infection probability (Odds ratio, OR=0.29, p=0.027) (Fig 4A). This inverse relationship indicates that the odds of *Angiostrongylus* spp. infection in *L. fulica* are reduced by as much as

lower probability of infection in larger *L. fulica* snails suggests that these may have reached an energetic threshold that allows for both higher body mass and a more established immunity against *Angiostrongylus* larvae. In contrast, shell size was not a significant predictor of infection likelihood in *P. canaliculata* (OR=1.4, p=0.138).

This suggests that, unlike *L. fulica*, which exhibits a distinct body mass-dependent susceptibility, *P. canaliculata* snails are likely driven by stochastic environmental factors independent of their development, implying uniform vulnerability to *Angiostrongylus* spp. infection regardless of age or ontogenetic stage.

Model fit further validates these species-specific patterns. Morphometric predictors explained a notable portion of variation in *L. fulica* infection (pseudo- $R^2=0.158$) but were negligible in *P. canaliculata* (pseudo- $R^2=0.036$) (Fig. 4A). Moreover, this disparity is further highlighted by the significantly higher prevalence in *L. fulica* (60.87%) compared to *P. canaliculata* (8.4%) ($p < 0.0001$), suggesting that internal metabolic constraints only manifest as primary drivers of infection when transmission events occur frequently enough to reveal differences in host resistance. In *L. fulica*, the terrestrial overlap between rat definitive hosts likely intensifies this pressure, whereas stochastic exposure appears to override the growth-immunity tradeoff in *P. canaliculata*, further implying that internal biological defenses are secondary to incidental encounters [49,50]. This distinction warrants further study.

However, negative binomial regression reveals that shell size (PC1) is a highly significant predictor of parasite load ($p=0.0004$, IRR 1.75, 1.2-3.38]), indicating that larval burden increases by approximately 75% per unit increase in shell size regardless of species (Fig. 4B). Larger snails are likely to provide a greater carrying capacity for *Angiostrongylus* spp. larvae through increase tissue volume and prolonged cumulative exposure over time. Nevertheless, morphometric predictors explained only a small proportion of the variation in larval burden (pseudo- $R^2=0.042$). Although shell shape (PC2) was statistically significant (IRR=2.49, 95% CI=0.50–7.35, $p=0.0148$), its wide confidence interval indicates considerable uncertainty in the estimated effect (Fig. 3C). These findings suggest that while host size may influence the theoretical carrying capacity for larvae, it is a relatively weak determinant of infection intensity. Instead, variation in larval burden is likely driven predominantly by ecological and transmission-related factors, including spatial heterogeneity in contamination by rat feces, differences in exposure to infective larvae, environmental conditions

influencing larval survival, and local transmission dynamics, rather than host morphology alone.

4. Conclusions

The foodborne parasite *Angiostrongylus* spp. was found to have high prevalence in Brgy. Matataja, Mulanay, Quezon Province. To our knowledge, this study provides the first documented morphological detection of *Angiostrongylus* spp. in Mulanay, Quezon Province, identifying Brgy. Matataja as a newly documented endemic focus. The findings of this study reveal divergent transmission profiles: *L. fulica* contributes to persistent endemic transmission and a high-prevalence vector governed by growth-immunity tradeoffs where the likelihood of infection decreases as the snail grows. Conversely, *P. canaliculata* functions as an incidental “superspreader” with a lower prevalence but exhibits extreme aggregation and a uniform infection risk regardless of size or ontogenetic stage. Host size serves as a theoretical carrying capacity rather than a major contributor of larval burden. These results suggest that environmental exposure overrides intrinsic constraints such as morphometry and metabolic tradeoffs, especially in *P. canaliculata*. However, due to limited resources and site accessibility, this study was limited to dry-season sampling. Future studies encompassing both dry and wet seasons would further understanding of seasonal variation in parasite transmission and infection patterns.

Nevertheless, based on the findings of this study, a targeted surveillance and public education on *Angiostrongylus* spp. and their associated disease is recommended. Further, rat control is recommended in agricultural areas to reduce the likelihood of fecal shedding containing *Angiostrongylus* spp. larvae that may be ingested by *P. canaliculata*, while proactive sanitation and pest control in residential areas should be prioritized to mitigate mucus-mediated transmission. The stochastic nature of *P. canaliculata* implies that snails of all sizes are considered hazardous and potential vectors for *Angiostrongylus* spp. larvae. Therefore, local government health offices are encouraged to strictly promote and disseminate standard preparation and handling protocols for snails harvested as delicacies. Finally, future research integrating rat definitive host surveillance is

highly recommended to further investigate possible fluctuations in aggregation patterns and other risk factors.

5. Abbreviations

PCA, principal component analysis; VMR, variance-to-mean ratio; IRR, incidence rate ratio; SL, shell length; SW, shell width; AL, aperture length; AW, aperture width; PWW, penultimate whorl length; BWL, body whorl length; AGJ, artificial gastric juice; LRT, likelihood ratio test; PC1, principal component 1; PC2, principal component 2; OR, odds ratio.

Supplementary Material

Supplementary materials are as follows:

1. Supplementary Fig. 1. PCA loading biplot of the six individual morphometric traits.
2. Supplementary File 2. Zip file containing all R scripts used for data analysis.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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