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Egg morphology, dispersal, and transmission in acanthocephalan parasites: integrating phylogenetic and ecological approaches

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**Egg morphology, dispersal, and transmission in
acanthocephalan parasites: integrating phylogenetic and
ecological approaches**

A Thesis Presented in Partial Fulfillment of the Requirements for the
Degree of Master of Science

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Introduction

Parasites are an important aspect of ecological communities, because they contribute to the overall biodiversity and structure of a community (Poulin, 2010a). The current estimation of parasite species indicates that parasites significantly contribute to the biodiversity of a community ($\approx 40\%$ of known species, Dobson, et al., 2008). Recent studies suggest that the number of existing helminth parasites is double the number of existing vertebrate hosts (Poulin & Morand, 2000; Dobson, et al., 2008). Also, parasites can alter community structure and function due to the behavioral impacts they have on their hosts (Mouritsen & Poulin, 2005; Wood, et al., 2007). Therefore, the study of parasite-host interactions is necessary to understand the mechanisms involved in sustaining the structure of natural communities.

Acanthocephalans, more commonly referred to as spiny- or thorny-headed worms, are trophically transmitted endoparasites (Kennedy, 2006). The taxonomic classification of the Acanthocephala has yet to be determined. The Acanthocephala is considered a phylum of its own by some researchers (Amin, 2013) and a clade within the Syndermata (syn. Rotifera) by others (Smales, 2015). The Acanthocephala includes 1,298 valid species (Amin, 2013; Smales, 2015), which are contained in four classes: Palaeacanthocephala (845 species, 65%), Eoacanthocephala (255 species, 20%), Archiacanthocephala (189 species, 15%) and Polyacanthocephala (4 species, $<1\%$). The Palaeacanthocephala infect amphibians, birds, fishes, mammals, and reptiles as definitive hosts. Common definitive hosts for the Archiacanthocephala are birds and mammals. The Eoacanthocephala infect fishes and infrequently reptiles and amphibians as definitive hosts. Common definitive hosts for the Polyacanthocephala are reptiles and fishes (Amin, 1987).

Epizootiology is the spread of parasites through a host population (Nickol, 1985). In acanthocephalans, the general cycle of transmission, shown in Figure 1, uses an arthropod intermediate host and a vertebrate definitive host (Kennedy, 2006). Mature eggs are expelled

from the definitive host into the environment, which are then consumed by an intermediate host. Once ingested by the intermediate host the acanthor, or larval stage of the parasite, emerges from the egg and progresses through a series of developmental stages. The final stage of development (cystacanth) within the intermediate host is infectious to the definitive host. Transmission to a definitive host occurs when the predator consumes an intermediate host containing a cystacanth. The parasites reach sexual maturity within the definitive host and mature eggs are released into the environment by the definitive host where they can be consumed by an intermediate host (Nickol, 1985).

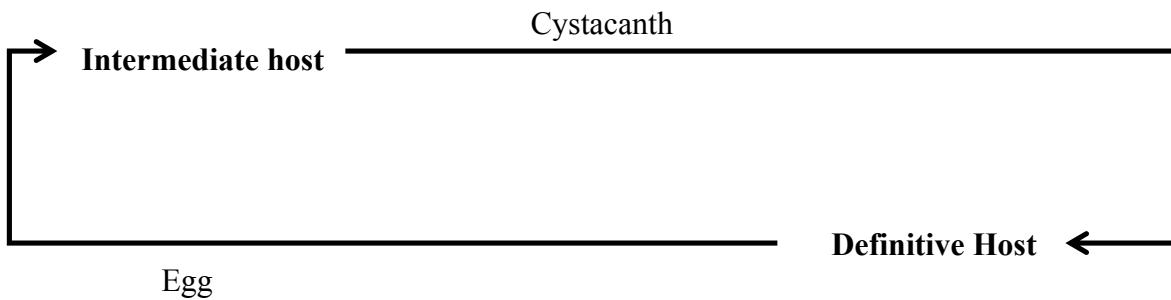


Figure 1. Schematic diagram of acanthocephalan life cycle

I examined egg morphology, dispersal, and transmission in acanthocephalan parasites using phylogenetic and ecological approaches. I assessed variation in multiple aspects of acanthocephalan egg morphology, specifically shape and size, and demonstrated that these traits exhibit significant variation among and within classes. I also studied the evolution of egg fibrils within the Acanthocephala using the comparative method (Harvey & Pagel, 1991), and demonstrated that fibrils are likely homoplasies. Finally, I used laboratory experiments to examine factors associated with transmission of the acanthocephalan parasite *Acanthocephalus dirus* (Van Cleave, 1931) to its intermediate host *Caecidotea intermedius* and demonstrated that the presence of egg fibrils appears to favor transmission to the intermediate host through multiple routes.

CHAPTER 1: Egg morphology as a variable trait in the Acanthocephala

ABSTRACT

Eggs of the Acanthocephala vary in morphology among and within taxonomic classes. To date, studies examining morphological variation have focused on the number of inner and outer membranes present, which can vary between three and five. This study focused on variation in multiple aspects of acanthocephalan egg morphology (shape, length, fibril presence, and polar prolongation presence) and demonstrated that these traits exhibit significant variation among and within classes. Given the diverse ecological factors that different acanthocephalan taxa encounter, I propose that some of this variation may be influenced by local selection pressures (e.g. habitat type, host biology). Finally, since variation in egg size and shape has been used as a trait in taxonomic keys, I examined the relative effectiveness of using egg shape and egg size as species identifiers. This analysis revealed that egg shape appears to be a more reliable indicator of species identity than egg size.

INTRODUCTION

Acanthocephalans are endoparasites that infect arthropods as intermediate hosts and vertebrates as definitive hosts and are found in diverse habitats (freshwater, marine, terrestrial) throughout the world (Kennedy, 2006). Prior to infecting intermediate hosts, acanthocephalan eggs are released into the environment, by the definitive host, where they are transmitted to the intermediate host when the host feeds. Given the variation in habitat and host type, it is likely that selection pressures associated with these factors have shaped the evolution of egg morphology. Consistent with this prediction, several studies show that significant variation in egg morphology, specifically shape and size, occurs in acanthocephalans (Marchand, 1984;

Peters, et al., 1991; Taraschewski & Peters, 1992; Taraschewski, et al., 1992; Taraschewski, 2000; Nikishin, 2001). In addition, this type of variation (shape, size, polar prolongation of the fertilization membrane) is incorporated into several taxonomic keys (e.g., Van Cleave, 1916, 1918b, 1919; Amin, 1987; McDonald, 1988; Amin, et al., 2008).

Despite the type of comparative evidence described above, the potential for egg morphology to be a trait that evolves in response to environmental factors has rarely been explored in acanthocephalans. Studies addressing this variation have typically focused on variation in the number of membranes present, which can vary between three and five (Marchand, 1984; Peters, et al., 1991; Taraschewski & Peters, 1992; Taraschewski, et al., 1992; Taraschewski, 2000; Nikishin, 2001). I examined the pattern of variation in the shape, size, egg fibrils, and membrane polar prolongation of acanthocephalan eggs using a representative sample of 165 valid taxa.

The Acanthocephala includes 1,298 valid species (Amin, 2013; Smales, 2015), which are contained in four classes: Palaeacanthocephala (845 species, 65%), Eoacanthocephala (255 species, 20%), Archiacanthocephala (189 species, 15%) and Polyacanthocephala (4 species, <1%). Common hosts that occur in each of the classes are as follows: Palaeacanthocephala – amphibians, birds, fishes, mammals, and reptiles; Archiacanthocephala - birds and mammals; Eoacanthocephala - fishes and infrequently reptiles and amphibians; Polyacanthocephala - reptiles and fishes (Amin, 1987). I reviewed the available literature, including all studies in which I could locate dimensions of eggs, to document the pattern of variation in egg shape and size, as well as presence of a fibril membrane and polar prolongation of the fertilization membrane.

MATERIALS AND METHODS

Defining Aspects of Egg Morphology

Egg morphology in acanthocephalans, and other taxa, is typically estimated using measures of length and width to describe egg size and qualitative descriptions (e.g. ellipsoid, slender) to describe shape. In this study, egg length is measured as the distance between the top and bottom apex of the outermost membrane (Figure 1) and egg width is measured at the widest part of the egg, which corresponds with the mid-point of egg length (Figure 1). Egg shape is determined by the calculation of egg length to egg width ratio (known as an elongation ratio, Deeming & Ruta, 2014; or mean eccentricity, Sengupta, et al., 2011). In some cases, there is a pinching in of a membrane at both the anterior and posterior ends of the egg, which results in the rounding of the membrane at the two ends. This polar prolongation is frequently seen in the fertilization membrane of the egg, but has been illustrated in the outermost membrane in a few species (see Špakulová, et al., 2011). In addition to size and shape, there is variation in the number of membranes present (three to five) and the presence of a fibril membrane in acanthocephalan eggs.

Documenting Variation

Information on egg shape (and size) of 165 acanthocephalan species was obtained from the literature for a representative sample of acanthocephalans. Literature searches were conducted with the databases JSTOR, Science Direct, and Biological Abstracts using several search terms (e.g., acanthocephalans, life cycle, egg morphology, egg shape, egg fibrils, new species) to identify papers that included taxonomic descriptions of any acanthocephalan species. In addition, papers cited in other studies that reviewed variation in egg morphology in acanthocephalans at a smaller scale were included (Marchand, 1984; Peters, et al., 1991;

Taraschewski & Peters, 1992; Taraschewski, et al., 1992; Taraschewski, 2000; Nikishin, 2001).

For a taxonomic description to be used in the study, measures of both egg length and egg width had to be present. Additional information concerning egg morphology (membrane number, shape description, presence of fibril membrane, and presence of polar prolongations of the fertilization membrane) was also documented.

To document the pattern of variation in egg morphology, I compared qualitative shape descriptions used in the literature and frequency plots of egg shape and size for members of each of the classes that contained data (Archiacanthocephala, Eoacanthocephala, Palaeacanthocephala). Measures of coefficient of variation (CV) were used to document the level of variation within classes. The CV measures variability among different groups accounting for the population mean (Gotelli & Ellison, 2013). Using the 165 acanthocephalans, I also determined whether there was a relationship between egg length (μm) and egg shape in each of the three major classes.

Given that egg shape descriptions and egg size have been included in taxonomic studies of acanthocephalans, I also examined which of the two traits was the more reliable indicator of species identity. To achieve this goal, I examined the level of variation in each trait using within-species comparisons. For this analysis, information from the same study on size and shape in multiple populations was identified for three species (*Acanthocephalus dirus*, Amin, 1984; *Moniliformis moniliformis*, Suriano, et al., 2000; *Southwellina hispida*, García-Varela, et al., 2012). For these taxa, I calculated values of the CV for egg length and elongation ratio. I then visualized the relationship between the two measures by plotting the CV values for egg length against the CV values for elongation ratio. If the two measures are equally viable as species identifiers then the plotted values should fall on or close to a line with a slope of 1 (i.e., the

within-species variation would be similar within each species). In contrast, if the CV values differ between the variables then the plotted values would deviate from the line. Deviations that are consistent in direction (e.g., all above the line) indicate that one measure is consistently more variable than the other. I also calculated the ratio of CV values (size CV / shape CV) and compared these values to an expected value of 1 using a one-sample t-test.

RESULTS

I collected information on egg morphology for 165 species, which represents approximately 13% of the total number of acanthocephalan species. For the group included in this study, there were 126 members of the Palaeacanthocephala (76%), 23 members of the Eoacanthocephala (14%), 16 members of the Archiacanthocephala (10%) and no members of the Polyacanthocephala (0%). These percentage values are relatively consistent with the distribution of species among acanthocephalan classes (see Introduction, Amin, 2013; Smales, 2015). All egg morphology information collected, including egg membrane number, average egg length, elongation ratio, shape description, presence of fibril membrane and polar prolongation of the fertilization membrane, can be seen in Appendix 1.

Table 1 summarizes qualitative egg shape descriptions in the Acanthocephala. Of the 165 species examined, egg shape was described in the literature for 55 species (33%). Within each class, shape descriptions were available for 12.5% of the Archiacanthocephala (2/16), 61% of the Eoacanthocephala (14/23) and 31% of the Palaeacanthocephala (39/126). Egg descriptions for members of the Archiacanthocephala were all ellipsoid, whereas egg descriptions for members of the Eoacanthocephala and Palaeacanthocephala varied. Eggs from members of the Eoacanthocephala were most frequently described as ‘ovoid’ or ‘oval’. In contrast, eggs from

members of the Palaeacanthocephala were most frequently described as ‘fusoid’ or ‘fusiform’ (Table 1).

Figure 2 shows egg shapes associated with common elongation ratios (Figure 2a) and summarizes egg length (μm) and egg shape (elongation ratio) variation in the Acanthocephala (Figure 2b). The figure illustrates that there is considerable variation in both shape and size among acanthocephalan eggs and that the pattern of variation differs between the two measures (Figure 2b). In the Archiacanthocephala, egg shape measurements are clustered together in five size classes and have a CV of 0.18. Whereas, egg length measurements in the Archiacanthocephala are spread over nine size classes and have a CV of 0.33. In the Eoacanthocephala, egg shape measurements span twelve size classes and a CV of 0.27. Eoacanthocephalan egg length measures have a CV of 0.49 and are separated in to two different clusters with the smaller size cluster having a higher frequency than the larger size cluster. The Palaeacanthocephala displays the greatest variation in egg shape with a CV of 0.35. Egg length measurements in the Palaeacanthocephala have a CV of 0.35 and the size frequencies appear to be bi-modal with the peaks occurring at $70\mu\text{m}$ and $110\mu\text{m}$ (Figure 2b).

To examine whether there was a relationship between egg size (length, μm) and egg shape (ER) in each of the three major classes of the Acanthocephala, I plotted egg size (length, μm) versus egg shape (ER) for each species of the three major classes (Figure 3). In the Archiacanthocephala, the correlation coefficient for egg size and egg shape was 0.2 ($P > 0.05$). The Eoacanthocephala had the lowest correlation coefficient of the three classes, at -0.11 ($P > 0.05$). The correlation coefficient for egg length and egg shape in the Palaeacanthocephala was 0.46 ($P < 0.001$). Thus, egg length and egg size were correlated in the Palaeacanthocephala, but not in the Archiacanthocephala and the Eoacanthocephala.

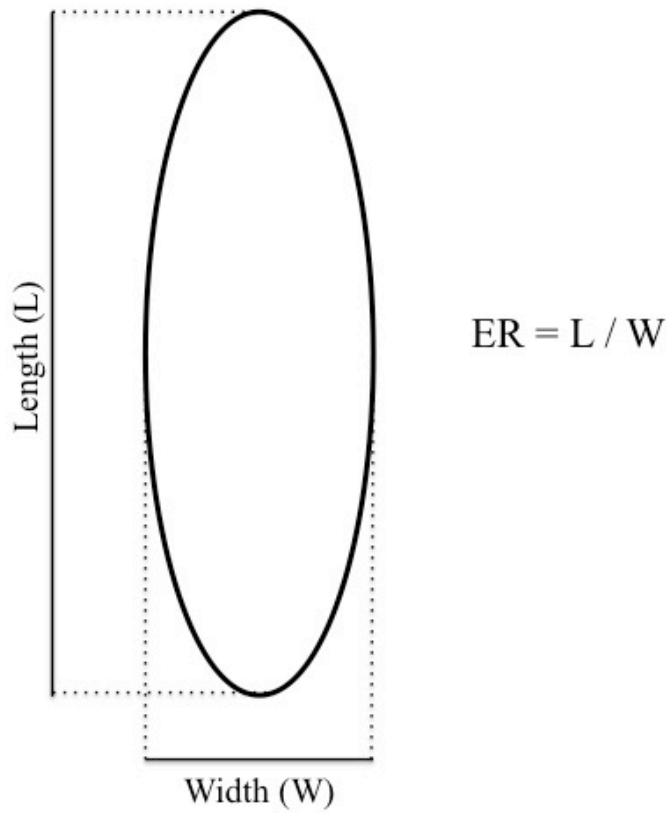


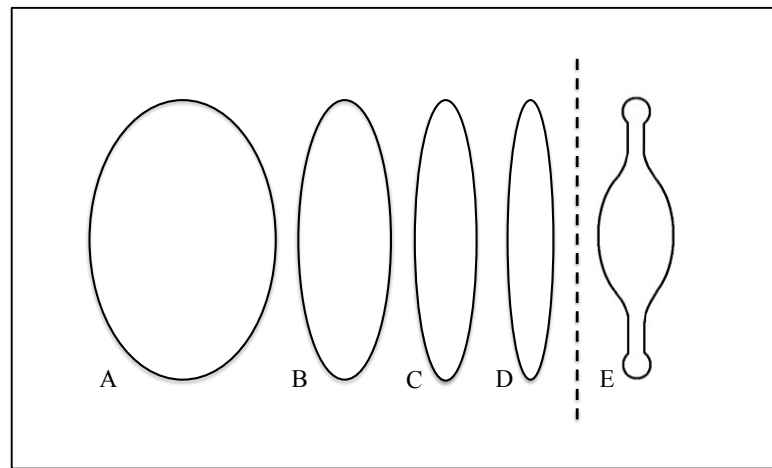
Figure 1. System used to measure egg size (length, width) and egg shape (elongation ratio = ER).

For the example shown $ER = 3.00$.

Table 1. Qualitative shape descriptors most frequently used to describe eggs in the Acanthocephala. The number of taxa that belong to each class out of the 165 used in this study and the numbers of taxa with shape descriptions are indicated. Bold designates the most frequently used shape descriptor per class. Species included are representative of the major classes of the Acanthocephala (Archiacanthocephala, Eoacanthocephala, and Palaeacanthocephala).

Class	No. of Taxa (%)	No. in Category	Shape Descriptors
Archi-	16(10)	0	- Ovoid (oval, elongated oval, elongate ovoid)
		2	- Ellipsoid (elliptical, ellipsoidal, elongate ellipsoid)
		0	- Fusoid (fusiform, elongate fusiform, slender fusiform)
		0	- Other (oblong, elongate, elongated, slender, spindle-shaped)
Eo-	23(14)	7	- Ovoid (oval, elongated oval, elongate ovoid)
		3	- Ellipsoid (elliptical, ellipsoidal, elongate ellipsoid)
		2	- Fusoid (fusiform, elongate fusiform, slender fusiform)
		2	- Other (oblong, elongate, elongated, slender, spindle-shaped)
Palae-	126 (76)	10	- Ovoid (oval, elongated oval, elongate ovoid)
		8	- Ellipsoid (elliptical, ellipsoidal, elongate ellipsoid)
		13	- Fusoid (fusiform, elongate fusiform, slender fusiform)
		8	- Other (oblong, elongate, elongated, slender, spindle-shaped)

a)



b)

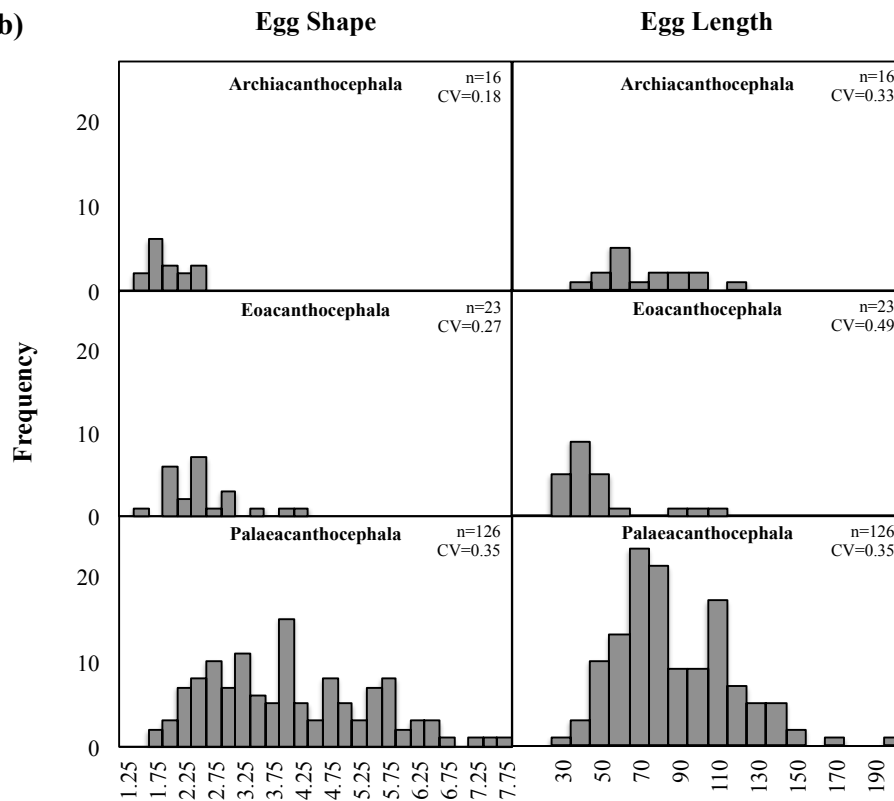


Figure 2. a) Egg shapes corresponding with common elongation ratios, egg length is standardized in the image to emphasize shape. Elongation ratios are: A=1.50, B=3.00, C=4.50, D=6.00, E=3.60 with polar prolongations. **b)** Quantitative frequency distribution of egg shape (elongation ratio) and egg length (μm) for acanthocephalan taxa ($n=165$). Species included are representative of the major classes of the Acanthocephala (Archiacanthocephala, Eoacanthocephala, and Palaeacanthocephala).

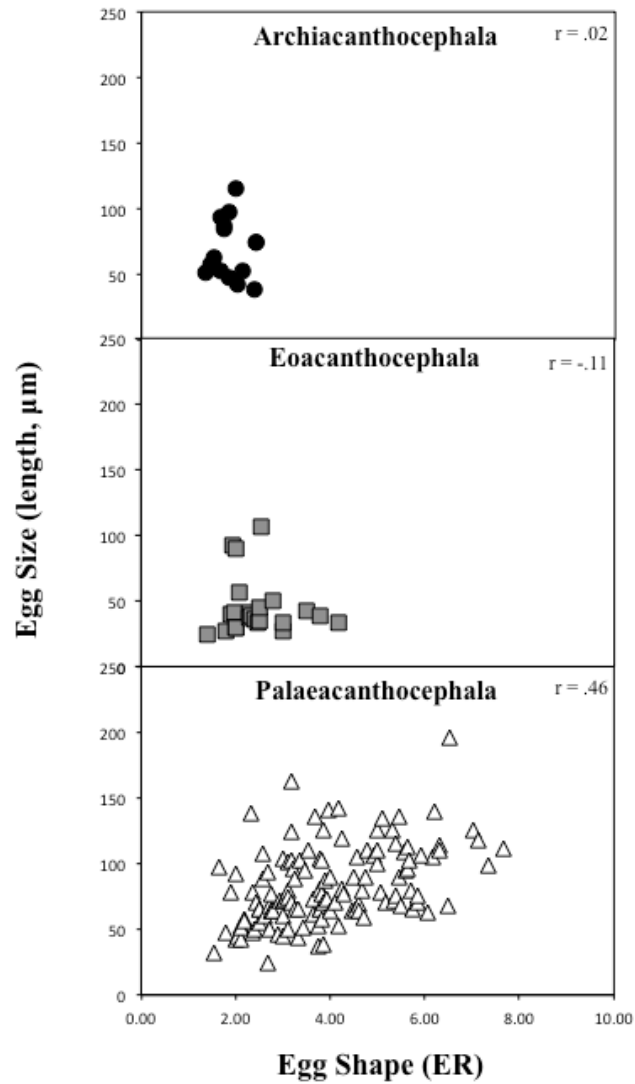


Figure 3. Relationship between egg size (length, μm) and egg shape (ER) in three classes of the Acanthocephala (Archiacanthocephala - 16 species, Eoacanthocephala - 23 species, and Palaeacanthocephala - 126 species). Correlation coefficients are reported for each class.

To determine the within-species variation in measures of egg shape and egg length, I plotted the CV values for each variable and compared the locations of the plotted values to a slope of 1 (dashed line, Figure 4). As can be seen in the figure, values deviated from the dashed line in a consistent manner. These values were located above the line indicating that there was a higher level of within-species variation in egg length than egg shape. Consistent with this pattern, the ratio of CV values differed from the expected value of 1 (one sample t-test, $t = 9.2$, $df = 2$, $P = 0.01$). Thus, elongation ratio is likely a more robust indicator of species identity than egg length.

DISCUSSION

This study is the first to document pattern of variation in multiple aspects of egg morphology in the Acanthocephala (Appendix 1). The results obtained indicate that there is considerable variation in both egg shape and size among the major acanthocephalan classes (Figure 2). Egg shape and length variation are smallest in the Archiacanthocephala (CV of 0.18, 0.33 respectively). The greatest variation in egg shape was displayed in the Palaeacanthocephala (CV of 0.35), whereas the greatest variation in egg length was displayed in the Eoacanthocephala (CV of 0.49). Variation in egg size and shape were correlated in the Palaeacanthocephala but not in the Eoacanthocephala or Archiacanthocephala (Figure 3) and members of the Palaeacanthocephala are found in a wide variety of aquatic and terrestrial habitats (Nikishin, 2001). This type of diversity may indicate that local selection pressures influenced the evolution of egg morphology in acanthocephalans (Marchand, 1984; Peters, et al., 1991; Taraschewski & Peters, 1992; Taraschewski, et al., 1992; Nikishin, 2001). The correlation identified between egg length and shape in the Palaeacanthocephala may also indicate that selection has acted in different ways in the three classes studied. Future studies are required to determine the relative importance of ecological and evolutionary factors to egg shape variation in acanthocephalans.

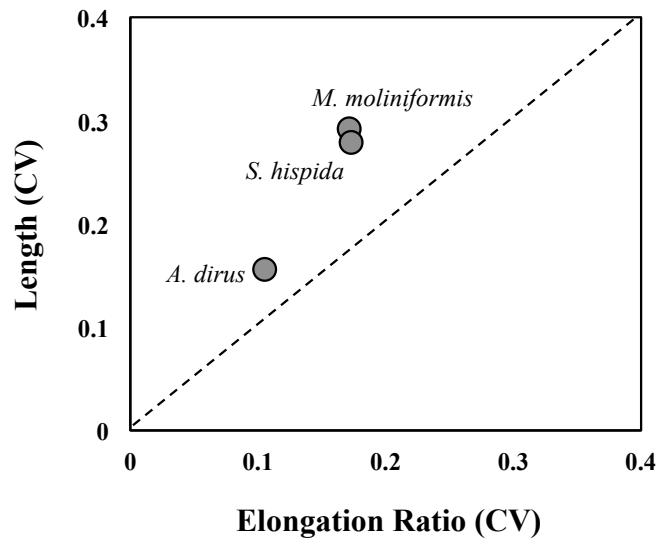


Figure 4. A comparison of within-species variation in egg shape and egg size. Data shown are for three species, which represent two classes (Palaeacanthocephala - *A. dirus* and *S. hispida*, Archiacanthocephala - *M. moniliformis*). Values above the dashed line indicate greater within-species variation in one trait. Based on this plot, egg length is consistently more variable than egg shape.

This study also revealed that published descriptions of egg shape can be inconsistent. For example, eggs with almost identical dimensions are described as elliptical in one study and fusiform in another (*Acanthocgyris tripathi*, 29 x 14.5mm, Rai, 1967; *Floridosentis mugilis*, 30 x 15mm, Suriano, et al., 2000). There are also inconsistencies with the terminology used to describe egg shape in the Acanthocephala. Shape descriptors like ellipsoid, fusiform, and elongate fusiform have been used frequently to describe egg shape in acanthocephalans. In these descriptions, fusiform has larger length to width ratios than ellipsoid eggs. All three of these descriptors can be applied effectively to the Acanthocephala because the eggs are symmetrical in both axes (vertical and horizontal). Another descriptor that is often used is 'ovoid' (or 'oval'), which is problematic because it refers to a shape that is near spherical (or globoid) with an asymmetry at its short axis (e.g., chicken egg, Paganelli, et al., 1974). Acanthocephalan eggs, unlike eggs in several other taxa, are generally symmetrical on both axes (e.g., Uznanski & Nickol, 1976; Barger & Nickol, 1998; Amin, et al., 2009; Arredondo & Gil de Pertierra, 2009; Amin, et al., 2011, 2014). It should be noted that using egg descriptions and measurements from the literature might be imprecise due to different preservation techniques and the egg's developmental stages. Given these constraints, relative measures (egg shape) are more likely to be consistent than absolute measures (egg length).

In the literature, egg size and shape have been used in combination with other traits, to identify acanthocephalans (e.g., Van Cleave, 1916, 1918b, 1919; Amin, 1987; Amin, et al., 2008; McDonald, 1988). The analysis of within-species variation in egg size and egg shape shows that egg shape is likely a more robust indicator of species identity than egg size because it was less variable among populations (Figure 4). Consistent with this relationship, it appears that egg size exhibits plasticity in response to host traits (e.g., Amin, 1984). In addition, competition among

adult acanthocephalans can influence body size (Dezfuli, et al., 2002), which is correlated with egg size (Poulin, et al., 2003). Given this type of variation, I suggest that egg shape (using elongation ratio) should be given priority in taxonomic descriptions over egg size in the Acanthocephala.

Appendix I. Egg characteristics of the 165 acanthocephalan species included in the study compiled from the literature**. Egg membrane number was included for reference since it is often reported with size measurements. M= membrane number, L= egg length (μm), ER= elongation ratio, F=fibril membrane, P= polar prolongation of middle membrane * denotes references that provided original shape description. In 12 of the species shown, data were recovered from studies in which synonyms were reported (*A. dirus* [syns. *A. jacksoni*, *A. parksidei*]; *C. longilemniscatus* [syn. *C. peposacae*]; *D. chandleri* [syn. *R. tenuicornis*]; *G. medius* [syn. *R. medius*]; *I. dimorpha* [syn. *S. dimorpha*]; *M. africanus* [syn. *E. segmentatus*]; *N. nudus* [syn. *D. nudus*]; *O. pardalis* [syn. *E. pardalis*]; *P. bazae* [syn. *E. bazae*]; *P. brevis* [syn. *A. brevis*]; *P. bulbocaudatus* [syn. *E. bulbocaudatus*]; *P. caspanensis* [syn. *A. caspanensis*]). **Information on *P. laevis* was provided through personal communication with Sophie Labaude.

Class	Genus	Species	M	L	ER	Shape Description	F	P	Reference(s)
Archi-	<i>Macracanthorhynchus</i>	<i>M. hirudinaceus</i>	4	98	1.85	-	-	-	Kates, 1943
		<i>M. ingens</i>	3	94	1.66	Elliptical	-	-	von Linstow, 1879*; Richardson, 2014
	<i>Mediorhynchus</i>	<i>M. africanus</i>	-	87	1.74	-	-	-	Southwell & Macfie, 1925
		<i>M. centurorum</i>	4	55	1.53	-	-	-	Nickol, 1969, 1977
		<i>M. colluricinclae</i>	-	51.5	1.34	-	-	-	Smales, 2002b
		<i>M. grandis</i>	4	47.5	1.86	-	-	-	Van Cleave, 1924; Schmidt, 1973a
		<i>M. orientalis</i>	-	53	2.12	-	-	-	Smales, 2002b
		<i>M. papillosus</i>	-	42.5	2.02	-	-	-	Van Cleave, 1918b
		<i>M. robustus</i>	-	38	2.38	-	-	-	Van Cleave, 1918b; Smales, 2002b
		<i>M. textori</i>	-	62.5	1.54	-	-	-	Smales, 2002b
		<i>M. wardi</i>	-	52	1.68	-	-	-	Smales, 2002b
	<i>Moniliformis</i>	<i>M. cestodiformis</i>	4	85	1.73	-	-	-	Van Cleave, 1924
		<i>M. clarki</i>	4	75	2.42	-	-	-	Van Cleave, 1924; Crook & Grundmann, 1964
		<i>M. dubius</i>	4	116	2.00	Elliptical	-	-	Moore, 1946*
		<i>M. moniliformis</i>	4	75	2.42	-	-	-	Van Cleave, 1924
<i>Oligacanthorhynchus</i>	<i>O. pardalis</i>	-	58	1.45	-	-	-	Southwell & Macfie, 1925	
Eo-	<i>Acanthogyrus</i>	<i>A. tripathii</i>	-	29	2.00	Elliptical	-	-	Rai, 1967*
	<i>Floridosentis</i>	<i>F. mugilis</i>	-	30	2.00	Fusoid	-	-	Suriano, et al., 2000*
	<i>Gracilisentis</i>	<i>G. gracilisentis</i>	-	38	3.80	Spindle-shaped	-	-	Van Cleave, 1919*
	<i>Neoechinorhynchus</i>	<i>N. agilis</i>	4	33.5	2.48	Elliptical	-	-	Van Cleave, 1919*
		<i>N. australis</i>	-	33	3.00	-	-	-	Van Cleave, 1931
		<i>N. bryanti</i>	-	37.2	2.30	Elongate, ovoid	-	-	Smales, 2013*
<i>N. crassus</i>		-	35	2.50	-	-	-	Van Cleave, 1919	

Class	Genus	Species	M	L	ER	Shape Description	F	P	Reference(s)
Eo-	<i>Neoechinorhynchus</i>	<i>N. cristatus</i>	4	56	2.07	-	-	-	Uglem, 1972
		<i>N. cylindratus</i>	-	50	2.78	-	-	-	Van Cleave 1919, 1921, 1924
		<i>N. emydis</i>	3	25	1.39	Oval	-	-	Van Cleave, 1919*, 1924*; Hopp, 1954*
		<i>N. rutili</i>	3	27	1.80	Oval	-	-	Van Cleave, 1919*; Merritt & Pratt, 1964
		<i>N. saginatus</i>	4	45	2.50	-	-	-	Uglem & Larson, 1969
		<i>N. schmidti</i>	-	36	2.40	Ovoid	-	-	Barger, et al., 2004*
		<i>N. spiramuscularis</i>	-	33.5	4.19	Fusiform	-	Y	Amin, et al., 2014*
		<i>N. tenellus</i>	-	41	2.28	-	-	-	Van Cleave, 1919
	<i>Octospinifer</i>	<i>O. macilentus</i>	3	38.5	2.33	Elliptical	-	-	Van Cleave, 1919; Harms, 1965*
	<i>Octospiniferoides</i>	<i>O. chandleri</i>	-	42	3.50	Slender	-	-	Scholz, et al., 1996*
	<i>Pallisentis</i>	<i>P. nagpurensis</i>	3	92	1.92	Oval	-	-	George & Nadakal, 1973*
		<i>P. panadei</i>	-	90	2.02	Elongated oval	-	-	Rai, 1967*
		<i>P. rexus</i>	4	107	2.55	-	-	-	Wongkham & Whitfield, 2004
	<i>Paulisentis</i>	<i>P. fractus</i>	-	41	1.95	Ovoid	-	-	Cable & Dill, 1967*
	<i>Raosentis</i>	<i>R. thapari</i>	-	39.5	1.88	-	-	-	Rai, 1967
<i>Tanaorhamphus</i>	<i>T. longirostris</i>	-	27	3.00	-	-	-	Van Cleave, 1919	
Palae-	<i>Acanthocephaloides</i>	<i>A. propinquus</i>	-	57.5	3.83	-	-	Y	Kvach & Oğuz, 2010
	<i>Acanthocephalus</i>	<i>A. bufonis</i>	-	77.5	2.74	Ellipsoidal	Y	-	Smales, 2005*
		<i>A. caspanensis</i>	-	63.75	2.75	-	-	-	Smales, 2007
		<i>A. clavula</i>	-	105	4.57	-	-	-	Amin, et al., 2008*
		<i>A. correalimai</i>	-	63.75	2.77	-	-	-	Smales, 2007
		<i>A. dirus</i>	4	99	7.33	-	Y	Y	Amin, 1984; Bullock, 1962
		<i>A. falcatus</i>	-	80.5	4.24	Oval	-	-	Amin, et al., 2008*
		<i>A. lucii</i>	-	62.4	6.06	Elongate Fusiform	-	-	Amin, et al., 2011*
		<i>A. ranae</i>	3	96	5.65	Fusiform	-	-	Van Cleave 1919; Amin, et al., 2008*; Heckmann, et al., 2011
		<i>A. rhinensis</i>	-	90	5.45	Slender Fusiform	-	Y	Amin, et al., 2008*
		<i>A. saopaulensis</i>	-	95.7	3.22	Ellipsoidal	Y	-	Smales, 2007*
<i>A. saurius</i>	-	43	3.31	-	-	-	Smales, 2007		

Class	Genus	Species	M	L	ER	Shape Description	F	P	Reference(s)
Palae-	<i>Acanthocephalus</i>	<i>A. tahlequahensis</i>	-	72	5.33	-	Y	Y	Oetinger & Buckner, 1976
		<i>A. ula</i>	-	65	3.25	-	-	-	Smales, 2007
	<i>Andracantha</i>	<i>A. mergi</i>	-	47	2.35	Oval	-	-	McDonald, 1988*
		<i>A. phalacrocoracis</i>	-	92	2.00	Oval	-	-	McDonald, 1988*
	<i>Arhythmorhynchus</i>	<i>A. brevis</i>	3	76	3.26	-	-	Y	Van Cleave, 1916, 1918b
		<i>A. pumiliostris</i>	3	77	4.28	-	-	Y	Van Cleave, 1916, 1918b
	<i>Bolbosoma</i>	<i>B. capitatum</i>	-	136	5.44	Fusiform	-	Y	Pinto, et al., 2004*
	<i>Cathayacanthus</i>	<i>C. spinitruncatus</i>	-	64.5	4.45	Oblong	-	Y	Amin, et al., 2014*
	<i>Centrorhynchus</i>	<i>C. albidus</i>	-	42	2.10	-	-	-	Lunaschi & Drago, 2010
		<i>C. asturinus</i>	-	55	2.50	-	-	-	Southwell & Macfie, 1925
		<i>C. chabaudi</i>	-	60	2.61	-	-	-	Smales, et al., 2017
		<i>C. clitorideus</i>	-	42.5	2.02	-	-	-	Smales, et al., 2017
		<i>C. crotophagicola</i>	-	50	2.38	-	-	-	Lunaschi & Drago, 2010
		<i>C. gendrei</i>	-	32.5	1.55	-	-	-	Smales, et al., 2017
		<i>C. guira</i>	-	58	2.19	Elongated	-	-	Lunaschi & Drago, 2010*
		<i>C. kuntzi</i>	-	50.5	2.73	-	-	-	Lunaschi & Drago, 2010
		<i>C. mariauxi</i>	-	45	2.05	-	-	-	Smales, et al., 2017
		<i>C. microcephalus</i>	-	57	2.19	-	-	-	Lunaschi & Drago, 2010
		<i>C. polymorphus</i>	-	47	1.81	-	-	-	Lunaschi & Drago, 2010
		<i>C. sarehae</i>	-	52.3	2.11	Ovoid	-	-	Smales, et al., 2017*
		<i>C. spinosus</i>	-	51	2.13	-	-	-	Van Cleave, 1924
	<i>Corynosoma</i>	<i>C. australe</i>	-	103.5	3.00	-	-	-	Sardella, et al., 2005
		<i>C. cetaceum</i>	-	162.5	3.19	-	-	Y	Sardella, et al., 2005
		<i>C. enhydri</i>	-	138.5	2.33	-	-	-	Neiland, 1962
		<i>C. falcatum</i>	-	81	3.77	-	-	Y	Van Cleave, 1953
		<i>C. hadweni</i>	-	104	3.78	-	-	Y	Van Cleave, 1953
		<i>C. longilemniscatus</i>	-	66	3.77	-	-	-	McDonald, 1988
<i>C. peposacae</i>		-	66	3.77	-	-	-	McDonald, 1988	
<i>C. semerme</i>		-	90	4.00	-	-	-	McDonald, 1988	

Class	Genus	Species	M	L	ER	Shape Description	F	P	Reference(s)
Palae-	<i>Corynosoma</i>	<i>C. strumosum</i>	-	90	4.74	-	-	-	McDonald, 1988
		<i>C. sudsuche</i>	-	68	2.72	-	-	-	McDonald, 1988
		<i>C. validum</i>	-	103	3.81	-	-	-	Van Cleave, 1953
		<i>C. villosum</i>	-	119	4.25	-	-	Y	Van Cleave, 1953
	<i>Dolfusentis</i>	<i>D. chandleri</i>	-	70	5.83	-	-	Y	Van Cleave, 1918a, 1919
	<i>Echinorhynchus</i>	<i>E. coregoni</i>	-	71	3.84	-	-	Y	Van Cleave, 1919, 1921, 1924
		<i>E. gadi</i>	4	76	5.85	-	-	-	Van Cleave, 1924
		<i>E. lageniformis</i>	4	90	4.50	-	Y	Y	Olson & Pratt, 1971
		<i>E. salvelini</i>	-	140	6.22	-	-	Y	Van Cleave, 1919
		<i>E. truttae</i>	3	125	5.00	-	-	Y	Awachie, 1966
	<i>Fessisentis</i>	<i>F. fessus</i>	4	117.5	7.12	Elongate	-	Y	Van Cleave, 1931; Nickol, 1972*
		<i>F. vancleavei</i>	4	75.5	5.39	-	-	Y	Buckner & Nickol, 1978
	<i>Filicollis</i>	<i>F. anatis</i>	3	64.9	3.31	-	-	-	McDonald, 1988
		<i>F. trophimenkoi</i>	-	101	3.11	-	Y	-	McDonald, 1988
	<i>Filisoma</i>	<i>F. indicum</i>	-	52.5	3.75	Elliptical	-	Y	Amin, et al., 2014*
		<i>F. longcementglandatus</i>	-	46.5	2.91	Fusiform	-	Y	Amin & Nahhas, 1994*
	<i>Gorgorhynchoides</i>	<i>G. indicus</i>	-	95	3.45	Oval	-	-	Bhattacharya & Banerjee, 2003*
	<i>Gorgorhynchus</i>	<i>G. medius</i>	-	75	3.13	-	-	-	Van Cleave, 1918a
	<i>Ibirhynchus</i>	<i>I. dimorpha</i>	-	98	1.63	-	-	Y	Schmidt, 1973b
	<i>Isthmosacanthus</i>	<i>I. fitzroyensis</i>	-	107.5	2.56	-	Y	Y	Smales, 2012
	<i>Koronacantha</i>	<i>K. mexicana</i>	-	69	4.60	Elongate Fusiform	-	Y	Monks & Perez-Ponce de Leon, 1996*
		<i>K. pectinarius</i>	-	76.5	3.83	Elongate Fusiform	-	Y	Monks, et al., 1997*
	<i>Leptorhynchoides</i>	<i>L. thecatus</i>	4	87.5	3.89	Spindle-shaped	Y	Y	DeGuisti, 1949*; Barger & Nickol, 1998
	<i>Neorhadinorhynchus</i>	<i>N. nudus</i>	-	38.5	3.85	Fusiform	-	Y	Hassanine, 2006*
	<i>Plagiorhynchus</i>	<i>P. allisonae</i>	-	142	4.19	-	-	Y	Smales, 2002a
		<i>P. cylindraceus</i>	3	77.9	2.35	Oval	-	-	McDonald, 1988*
		<i>P. gracilis</i>	3	65	2.50	Oval	-	-	McDonald, 1988*
<i>P. menuræ</i>		-	124	3.18	-	-	Y	Smales, 2002a	
<i>Polymorphus</i>	<i>P. actuganensis</i>	-	113.5	6.31	-	-	-	McDonald, 1988	

Class	Genus	Species	M	L	ER	Shape Description	F	P	Reference(s)
Palae-	<i>Polymorphus</i>	<i>P. cincli</i>	-	115.5	5.37	-	-	-	McDonald, 1988
		<i>P. contortus</i>	-	105	6.18	-	-	-	McDonald, 1988
		<i>P. diploinflatus</i>	-	108.5	5.56	-	Y	-	McDonald, 1988
		<i>P. kostylewii</i>	-	125	7.02	-	-	-	McDonald, 1988
		<i>P. marilis</i>	-	111	7.66	-	-	P	Van Cleave, 1939; McDonald, 1988
		<i>P. meyeri</i>	-	102	5.67	-	-	-	McDonald, 1988
		<i>P. minutus</i>	3	45	3.00	-	Y	Y	Nicholas & Hynes, 1963; McDonald, 1988
		<i>P. obtusus</i>	-	70	3.18	Oblong	-	Y	Van Cleave, 1918b, 1924; McDonald, 1988*
		<i>P. paradoxus</i>	-	112.5	5.63	-	-	-	McDonald, 1988
		<i>P. phippii</i>	-	125	5.32	-	-	-	McDonald, 1988
		<i>P. pupa</i>	-	136	3.68	Elongate	-	Y	McDonald, 1988*
		<i>P. spindlatus</i>	-	102.5	3.36	Elongate	-	Y	Amin & Heckmann, 1991*
		<i>P. trochus</i>	-	79.5	4.68	-	-	Y	Van Cleave, 1945a
	<i>Pomphorhynchus</i>	<i>P. bulbocolli</i>	-	68	6.48	-	-	-	Van Cleave, 1919, 1924
		<i>P. laevis</i>	3	106.5	5.92	-	-	Y	Labaude, S. (personal communication)
		<i>P. patagonicus</i>	-	196	6.53	-	-	Y	Trejo, 1992
		<i>P. spindlitruncatus</i>	-	80	5.71	Fusiform Elongate	-	Y	Amin, et al., 2003*
		<i>P. tereticollis</i>	-	110.5	6.31	-	-	Y	Spakulova, et al., 2011
	<i>Porrorchis</i>	<i>P. bazaar</i>	-	78	1.90	-	-	-	Southwell & Macfie, 1925
		<i>P. bulbocaudatus</i>	-	60	3.00	-	-	-	Southwell & Macfie, 1925
	<i>Proflicollis</i>	<i>P. altmani</i>	3	65	2.50	Oval	-	-	Perry, 1942; McDonald, 1988*
		<i>P. arcticus</i>	-	140.5	3.96	Elliptical	-	-	McDonald, 1988*
		<i>P. botulus</i>	-	88	2.59	-	-	-	McDonald, 1988
		<i>P. formosus</i>	3	50	3.13	Elliptical	-	-	Van Cleave, 1918b*; Schmidt & Olsen, 1964
		<i>P. major</i>	-	93	2.70	Elongated Ovoid	-	-	McDonald, 1988*
	<i>Pseudoacanthocephalus</i>	<i>P. caspanensis</i>	-	63.75	2.75	-	-	-	Smales, 2007
		<i>P. lutzi</i>	4	71	2.45	Elliptical	Y	-	Smales, 2007; Arrendondo & Gil de Perterra, 2009*
		<i>P. rhampholeontos</i>	-	72	3.00	Ellipsoidal	Y	-	Smales, 2005*

Class	Genus	Species	M	L	ER	Shape Description	F	P	Reference(s)
Palae-	<i>Pseudocorynosoma</i>	<i>P. anatrium</i>	-	106	4.93	-	-	Y	Van Cleave, 1945b; McDonald, 1988
		<i>P. constrictum</i>	-	37.5	3.75	-	Y	Y	Van Cleave, 1918b; Keithly, 1968
	<i>Rhadinorhynchus</i>	<i>R. biformis</i>	-	78.2	5.05	-	-	-	Smales, 2014
		<i>R. cadenati</i>	-	72.5	3.92	-	-	-	Amin, et al., 2011
		<i>R. carangis</i>	-	66	4.52	-	-	-	Smales, 2014
		<i>R. cololabis</i>	-	66	5.74	-	-	-	Amin, et al., 2011
		<i>R. dollfusi</i>	-	125	3.85	-	-	-	Amin, et al., 2011
		<i>R. dorsoventrospinosus</i>	-	100	5.00	Fusiform	-	Y	Amin, et al., 2011*
		<i>R. dujardini</i>	-	70	4.12	-	-	-	Amin, et al., 2011
		<i>R. johnstoni</i>	-	72	2.94	Elongate	-	Y	Amin & Nahhas, 1994*; Amin, et al., 2011
		<i>R. keralensis</i>	-	110	3.55	-	-	-	Amin, et al., 2011
		<i>R. laterospinosus</i>	-	62	3.65	-	-	Y	Amin, et al., 2011
		<i>R. lintoni</i>	-	110	5.00	-	-	-	Amin, et al., 2011
		<i>R. ornatus</i>	-	73	3.04	Fusiform	-	Y	Van Cleave, 1918a; Amin, et al., 2009*, Amin, et al., 2011
		<i>R. pelamysi</i>	-	68.5	5.48	-	-	-	Amin, et al., 2011
		<i>R. pichelinae</i>	-	59.5	4.72	-	-	Y	Smales, 2014*
		<i>R. plagioscionis</i>	-	110	4.78	-	-	-	Amin, et al., 2011
		<i>R. pristis</i>	4	95	5.59	-	Y	Y	Van Cleave, 1918a, Amin, et al., 2011
		<i>R. seriolae</i>	-	70.5	5.18	-	-	Y	Amin, et al., 2011; Smales, 2014*
		<i>R. stunkardi</i>	-	135	5.09	-	-	-	Amin, et al., 2011
	<i>R. trachuri</i>	-	73	3.65	-	-	-	Amin, et al., 2011	
	<i>Sclerocollum</i>	<i>S. rubrimaris</i>	-	51.5	3.43	Fusiform	-	Y	Hassanine, 2006*
		<i>S. saudi</i>	-	24	2.67	Oval to Elongate	Y	Y	Al-Jahadali, et al., 2015*
	<i>Serrasentis</i>	<i>S. sagittifer</i>	4	102	3.19	Elongate Ellipsoid	-	-	Travassos, 1966*
	<i>Southwellina</i>	<i>S. hispida</i>	-	79.5	3.12	-	-	-	Schmidt, 1973b; García-Varela, et al., 2012
	<i>Tegorhynchus</i>	<i>T. brevis</i>	-	52	4.16	-	-	Y	Monks, et al., 1997
	<i>Trajectura</i>	<i>T. perinsolens</i>	-	64.5	4.61	-	-	-	Pichelin & Cribb, 2001
	<i>Transvena</i>	<i>T. annulospinosa</i>	-	64	4.00	Fusiform	-	Y	Pichelin & Cribb, 2001*

CHAPTER 2: Evolution of egg fibrils in the Acanthocephala: a comparative approach

ABSTRACT

Acanthocephalans are a diverse group of endoparasites comprised of ~1300 species that display an array of egg morphologies. These parasites have a life cycle that starts with free-living eggs that are expelled into the habitat, by a vertebrate definitive host, where they are consumed by an arthropod intermediate host. Some of these acanthocephalans have egg fibrils, which have been proposed to aid in transmission to intermediate hosts. These mechanisms of transmission may be associated with the habitat and feeding behavior of the intermediate host. In this study, the evolution of egg fibrils in the Acanthocephala was examined using phylogenetic mapping (using 18S rDNA). The results displayed five taxa with egg fibrils dispersed throughout the Palaeacanthocephala, which likely indicates that egg fibrils evolved independently in each species. These findings are consistent with the interpretation that the function of egg fibrils in transmission might be specific to each species and with the notion that aspects (behavior, morphology) of egg to host transmission are associated with local ecology.

INTRODUCTION

Acanthocephalans are tropically transmitted endoparasites found in diverse habitats that infect arthropods as intermediate hosts and vertebrates as definitive hosts (Kennedy, 2006). Transmission of acanthocephalan eggs to the intermediate host occurs when eggs are expelled into the environment by the definitive host where they are consumed by intermediate hosts. Given the variety of host types and habitats, acanthocephalans, like other helminths, are proposed to vary in their mechanisms of transmission (Nikishin, 2001). For example, eggs of

Leptorhynchoides thecatus possess fibrils, which may aid in transmission to the amphipod intermediate host by attaching eggs to vegetation in the water column where amphipods feed (Uznanski & Nickol, 1976). Eggs of *Pallisentis rexus* expand in size and become buoyant in the water column, which may increase the likelihood that they are encountered by their copepod intermediate host (Wongkham & Whitfield, 2004). Other studies have also shown that variation in egg morphology may be related to mechanisms of dispersal and transmission to the intermediate host (Dezfuli, 1996; George & Nadakal, 1973; Nikishin, 2001; Arredonodo & Gil de Pertierra, 2009). Here I used a comparative approach to examine one of these traits (egg fibrils) in the Acanthocephala.

In acanthocephalans, egg fibrils are long, slender filaments that arise from one of the egg's membranes (Monné & Hönig, 1954). Table 1 provides a summary of information available in the literature concerning the anatomy and life cycles of acanthocephalans that possess egg fibrils. Fibrils are found most frequently in hosts that reside in aquatic habitats. For example, five of the seven taxa presented in the table have aquatic intermediate hosts, while the other two taxa have terrestrial intermediate hosts (ants, termites). Within the aquatic taxa, there is variation in the type of intermediate host and the microhabitat occupied by the intermediate host. For example, some aquatic taxa infect intermediate hosts that feed solely in the water column or solely on the sediment, whereas others feed both in the water column and on the sediment. This variation in intermediate host microhabitat use may indicate that egg fibrils could aid in transmission differently depending on the type of intermediate host. There is also variation in the descriptions of egg fibrils. Some taxa are reported to have many, short fibrils, whereas others have only two, long fibrils. The variation in number and length of fibrils may indicate that the varying types of fibrils could result in different mechanisms of transmission. The variation

indicated in Table 1 is consistent with the interpretation that fibrils may have evolved independently in each taxon.

Diversity in egg fibril occurrence could be due to common ancestry, convergent evolution, or a combination of the two (Freeman & Herron, 2001). If fibrils were the result of common ancestry, all taxa with fibrils would likely be found clustered together on a phylogeny and they would be expected to be similar morphologically. This might indicate that fibrils evolved once and have a similar function in all taxa. In the case of convergent evolution, taxa with fibrils would likely be found in different areas on a phylogeny and may differ morphologically. This might indicate that fibrils evolved independently in each taxon. Finally, a combination of evolution due to common ancestry and convergent evolution could occur if a phylogeny indicated two or more groupings of taxa with fibrils. For example, if two groupings of multiple taxa occur in different areas of the phylogeny it might suggest that fibrils evolved twice. This study combined existing molecular and morphological data in the Acanthocephala to explore egg fibril evolution in relation to host biology.

MATERIALS AND METHODS

To examine the evolution of egg fibrils in the Acanthocephala, I constructed a phylogeny based on available molecular data for 32 taxa (31 acanthocephalans, one rotifer outgroup). Table 2 summarizes specimen information and GenBank (Bensen, et al., 2007) accession numbers used to obtain 18S rDNA sequences. The acanthocephalan species included represent the three major classes of the Acanthocephala. The 18S rDNA sequences were aligned using Clustal W in MEGA version 6 (Tamura, et al., 2013) and resulted in 1,871 characters. The sequences were analyzed as a combined rDNA data set. Tree searches were conducted with maximum likelihood (ML) using MEGA version 6 (Tamura, et al., 2013). Bootstrap resampling was used to assess the

relative reliability of the tree, using 500 bootstrap pseudoreplicates. I used character mapping (e.g. Harvey & Pagel, 1991) to examine the evolution of egg fibrils in the Acanthocephala. Data on acanthocephalan taxa that displayed fibril membranes were obtained from the published literature (Chapter 1, Appendix 1).

Table 1. Sample of acanthocephalan taxa with fibril descriptions and life cycle information.

Species	Fibril Description	DH	IH	IH Habitat	IH Location
<i>Rhadinorhynchus pristis</i>	Numerous filaments ¹	F	Krill	AQ	WC
<i>Leptorhynchoides thecatus</i>	Arranged parallel to fertilization membrane ²	F	Amphipod	AQ	WC/S
<i>Polymorphus minutus</i>	Many fibrils twisted in one direction ³	B	Amphipod	AQ	WC/S
<i>Pseudoacanthocephalus lutzi</i>	Many fibrils on fibrillar coat ⁴	A	Amphipod	AQ	WC/S
<i>Acanthocephalus dirus</i>	Two, slender tapering filaments ⁵	F	Isopod	AQ	S
<i>Acanthocephalus bufonis</i>	Fibril layer ⁶	A	Ants, Termites	T	L
<i>Pseudoacanthocephalus rhampholeontos</i>	Many fibrils on fibrillar coat ⁶	A	Ants, Termites	T	L

DH= definitive host, IH= intermediate host, F= fish, B=bird, A= amphibian, AQ= aquatic, T= terrestrial, WC= water column, S= sediment, L= land

¹ Marchand (1984), ² Uznanski & Nickol (1976), ³ Monné & Hönig (1954), ⁴ Arredondo & Gil de Perterra (2009), ⁵ Oetinger & Nickol (1974), ⁶ Smales (2005)

Table 2. Specimen information and GenBank accession numbers for species included in phylogenetic tree. Habitat of the species is represented under Hab., where Aqua. represents aquatic and Terr. represents terrestrial. * represents the outgroup (rotifer). Host type: B = bird, F = fish, M = mammal.

Hab.	Species	Family	Host (Type)	18S rDNA
Aqua.	* <i>Asplanchna sieboldi</i>	Asplanchnidae	Not applicable (free-living)	AF092434
	<i>Acanthocephaloides propinquus</i>	Arythmacanthidae	<i>Gobius buccichii</i> (F)	AY830149
	<i>Filisoma bucerium</i>	Cavisomidae	<i>Kphosus elegans</i> (F)	AF064814
	<i>Acanthocephalus dirus</i>	Echinorhynchidae	<i>Semotilus artomaculatus</i> (F)	AY830151
	<i>Acanthocephalus lucii</i>	Echinorhynchidae	<i>Perca fluviatilis</i> (F)	AY830152
	<i>Echinorhynchus gadi</i>	Echinorhynchidae	<i>Macrourus berglax</i> (F)	JX014222
	<i>Echinorhynchus truttae</i>	Echinorhynchidae	<i>Thymallus thymallus</i> (F)	AY830156
	<i>Koronacantha mexicana</i>	Illiosentidae	<i>Pomadasys leuciscus</i> (F)	AY830157
	<i>Koronacantha pectinara</i>	Illiosentidae	<i>Microlepidotus brevipinnis</i> (F)	AF092433
	<i>Floridosentis mugilis</i>	Neoechinorhynchidae	<i>Mugil cephalus</i> (F)	AF064811
	<i>Neoechinorhynchus crassus</i>	Neoechinorhynchidae	<i>Catostomus commersoni</i> (F)	AF001842
	<i>Neoechinorhynchus saginata</i>	Neoechinorhynchidae	<i>Semotilus artomaculatus</i> (F)	AY830150
	<i>Arythmorhynchus brevis</i>	Polymorphidae	<i>Nycticorax nycticorax</i> (B)	AF064812
	<i>Corynosoma australe</i>	Polymorphidae	<i>Phocarctos hookeri</i> (M)	JX442168
	<i>Corynosoma enhydri</i>	Polymorphidae	<i>Enhydra lutris</i> (M)	AF001837
	<i>Corynosoma magdaleni</i>	Polymorphidae	<i>Halichoerus grypus</i> (M)	EU267803
	<i>Polymorphus altmani</i>	Polymorphidae	<i>Enhydra lutris</i> (M)	AF001838
	<i>Polymorphus minutus</i>	Polymorphidae	<i>Anas platyrhynchos diazi</i> (B)	EU267806
	<i>Polymorphus trochus</i>	Polymorphidae	<i>Fulica americana</i> (B)	JX442173
	<i>Pseudocorynosoma anatrium</i>	Polymorphidae	<i>Bucephala albeola</i> (B)	EU267801
	<i>Pseudocorynosoma constrictum</i>	Polymorphidae	<i>Anas clypeata</i> (B)	EU267800
	<i>Southwellina hispida</i>	Polymorphidae	<i>Tigrisoma mexicanum</i> (B)	EU267807
	<i>Pomphorhynchus bulbocolli</i>	Pomphorhynchidae	<i>Cyprinus carpio</i> (F)	AF001841
	<i>Pomphorhynchus laevis</i>	Pomphorhynchidae	<i>Rutilus rutilus</i> (F)	JX014223
	<i>Pomphorhynchus tereticollis</i>	Pomphorhynchidae	<i>Morone saxatilis</i> (F)	AY423347
	<i>Leptorhynchoides thecatus</i>	Rhadinorhynchidae	<i>Lepomis cyanellus</i> (F)	AF001840
	<i>Rhadinorhynchus prisits</i>	Rhadinorhynchidae	<i>Gempylus serpens</i> (F)	JX014226
	<i>Serrasentis sagittifer</i>	Rhadinorhynchidae	<i>Platycephalus arenarius</i> (F)	JX014227
	<i>Transvena annulospinosa</i>	Transvenidae	<i>Anampses neoguinaicus</i> (F)	AY830153
Terr.	<i>Moniliformis moniliformis</i>	Moniliformidae	<i>Rattus rattus</i> (M)	Z19562
	<i>Macraacanthorhynchus ingens</i>	Oligacanthorhynchidae	<i>Procyon lotor</i> (M)	AF001844
	<i>Plagiorhynchus cylindraceus</i>	Plagiorhynchidae	<i>Turdus migratorius</i> (B)	AF001839

RESULTS

The mapping of egg fibril presence for each species included in the molecular-based phylogeny is shown in Figure 1. Egg fibrils were present in five species and were dispersed throughout the phylogeny, *Acanthocephalus dirus*, *Leptorhynchoides thecatus*, *Polymorphus minutus*, *Pseudocorynosoma constrictum*, and *Rhadinorhynchus pristis*. All five of these species are members of the class Palaeacanthocephala. In cases where there were more than one species representing a genus (e.g. *Acanthocephalus*, *Polymorphus*, *Pseudocorynosoma*), presence of egg fibrils was only indicated in one species. The phylogeny also indicates that the genera *Polymorphus* and *Pomphorhynchus* are not monophyletic. García-Varela, et al. (2013) proposed that *Polymorphus* was a polyphyletic genus when studying the evolutionary relationships of the family Polymorphidae. The bootstrap values for *Pomphorhynchus* (100) suggest that it is polyphyletic genus although it has not been suggested in previous studies.

DISCUSSION

The mapping of egg fibril presence for each species indicated several trends. First, the five taxa with egg fibrils are not found clustered together, which suggests that egg fibrils evolved independently in each taxon (Figure 1). Second, when more than one species represented a genus (e.g. *Acanthocephalus*, *Polymorphus*, *Pseudocorynosoma*) egg fibrils were only present in one of the species. This species-level difference is consistent with the interpretation that egg fibrils are homoplasies due to convergent evolution. Third, all taxa included in this study with egg fibrils are found within the Palaeacanthocephala. This taxonomic class is the most diverse class in terms of host type and habitat (e.g., Amin, 1987), which may have influenced the evolution of the different egg morphologies.

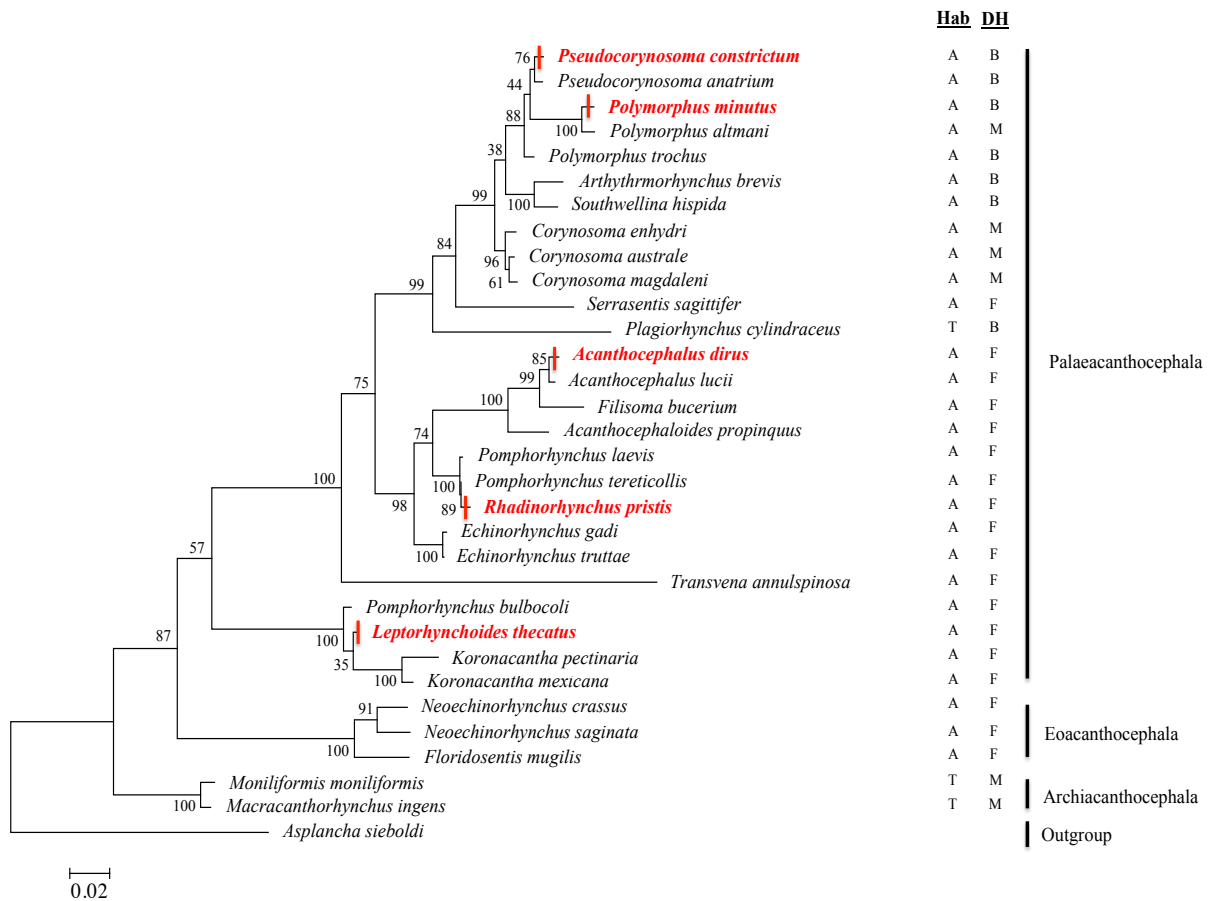


Figure 1. Phylogeny of fibril evolution using available 18S rDNA sequences (GenBank) of three of the four classes of the Acanthocephala. Egg fibril presence is indicated in red. Maximum likelihood tree (-ln 26,655.29) with branch length scaled to expected substitutions per site. Hab indicates habitat (A = aquatic, T = terrestrial). DH indicates the definitive host (B = bird, F = fish, M = mammal).

The results of the phylogenetic tree shown in Figure 1 are consistent with trees constructed in previous studies (García-Varela, et al., 2002; Verweyen, et al., 2011; García-Varela, et al., 2013). García-Varela, et al. (2002) constructed a molecular phylogeny including 19 acanthocephalans, in which egg fibril presence is known for 14 of the species (74%). Only one of these species, *L. thecatus*, possesses egg fibrils (García-Varela, et al., 2002). Verweyen, et al. (2011) constructed a molecular phylogeny including 35 acanthocephalans, in which egg fibril presence is known for 27 species (77%). Five of these species, the same five shown in Figure 1, possess fibrils. The five species are found dispersed within the Palaeacanthocephala, which is consistent with Figure 1 (Verweyen, et al., 2011). García-Varela, et al. (2013) constructed a molecular phylogeny of the family Polymorphidae including 23 species, in which egg fibril presence is known for 15 species. Two of these species, *P. constrictum* and *P. minutus*, possess fibrils. These two species are separated within the Polymorphidae, which is consistent with the conclusions in this study (García-Varela, et al., 2013). Thus, there is evidence of convergent evolution for egg fibrils from several different phylogenies.

Egg fibrils in the Acanthocephala have been proposed to aid transmission to intermediate hosts through multiple mechanisms. These mechanisms include the timing of egg fibril release, attachment to substrates, and enhancing establishment success inside the intermediate host (see Chapter 3). Therefore, it is reasonable to suggest that egg morphology is related to transmission in the Acanthocephala and that this relationship is driven by local ecology (i.e., habitat, host biology). The five taxa with egg fibrils, included in this study, were all found within the Palaeacanthocephala and all have aquatic hosts. Three of the taxa, *L. thecatus*, *P. minutus*, and *P. constrictum*, have amphipod intermediate hosts, whereas *A. dirus* infects isopods and *R. prisits* infects krill. In aquatic habitats, fibrils may aid in transmission by attaching to vegetation where

the intermediate host feeds. Egg fibrils may also aid in establishment inside the intermediate host by attaching to the intestines once the egg has been consumed. Both of these mechanisms are shown to be likely occurring in the acanthocephalan *A. dirus* (see Chapter 3).

While the data shown here appears to indicate that egg fibrils are homoplasies, it should be noted that not all acanthocephalans that possess fibrils were included in the phylogeny. As shown in Table 1, there are terrestrial taxa within the Acanthocephala that have egg fibrils (*A. bufonis*, *P. rhampholeontos*). Both *A. bufonis* and *P. rhampholeontos* are members of the Palaeacanthocephala and have amphibians as definitive hosts and ants and termites as intermediate hosts (Smales, 2005). The proposed benefits of fibrils in aquatic species (i.e. attachment to substrates, increasing establishment success) could also be applicable to terrestrial species. Fibrils could aid in attachment to substrates in the habitat and decrease the likelihood that the eggs are disturbed by environmental factors (e.g. wind). Fibrils could also increase establishment success in terrestrial hosts, in the same manner as in aquatic hosts, by attaching to the intestine wall, hence slowing passage through the gut of host that could increase the likelihood of infection.

In addition to the suggested evolution of egg fibrils in the Acanthocephala, other morphological traits of helminth eggs appear to be shaped by local ecology (Jarecka, 1961; Combes, et al., 1994). For example, in cestode tapeworms there is variation in egg morphology (shape, clumping behavior, and weight). In regards to weight, some tapeworm eggs are considered heavy and sink quickly through the water column, whereas other eggs are light and remain in the water column (Jarecka, 1961). The heavy eggs are found in species that have ostracod intermediate hosts that feed on the sediment, whereas the light eggs are found in species, which have copepod intermediate hosts that feed in the water column. The variation in egg

morphology described in tapeworms has also been proposed to be driven by host biology and feeding preference (Jarecka, 1961). Similarly, in the larval stage (cercariae) of some trematodes, behavioral mechanisms occur that increase encounter rates with target hosts (Combes, et al., 1994). For example, in some species cercariae cluster together in ways which appear larger and more attractive to their hosts. In other species, cercariae possess a tail that moves similar to a worm, in a way that is attractive to the target host. This type of variation in behavior has also been proposed to be associated with the variety of hosts the cercariae infect (Combes, et al., 1994). Thus, local selection pressures may have shaped several morphological traits associated with transmission to target hosts in multiple types of helminth parasites.

CHAPTER 3: Egg fibrils and transmission in the acanthocephalan parasite

Acanthocephalus dirus

ABSTRACT

Acanthocephalans are endoparasites that infect arthropods as intermediate hosts and a diverse array of vertebrates as definitive hosts. For transmission to the intermediate host to occur, acanthocephalan eggs must be consumed along with food in the intermediate host's habitat. Some acanthocephalans have egg fibrils that could play a role in this transmission. These fibrils are filaments that arise from the egg's fibrillar coat. To date, studies have proposed several, possible functions of fibrils in transmission to the intermediate host, but there is little experimental evidence to support the proposed functions. Studies have also indicated that fibril morphology appears to be species-specific and may be shaped by local factors (e.g. host biology, habitat). I examined the potential role of egg fibrils in dispersal and transmission of the acanthocephalan *Acanthocephalus dirus*, which infects a stream-dwelling isopod host (*Caecidotea intermedius*). I used lab-based experiments to examine the timing of fibril release, the role of fibrils in egg attachment to substrates and the role of fibrils in infection success (prevalence, intensity). Results showed that fibrils attached to substrates upon release and that the timing of this release did not occur for several days. This would allow eggs to sink through the water column to the sediment, which is occupied by the intermediate host, before attaching to a substrate. In trials in which *C. intermedius* were provided with access to either eggs with fibrils or eggs without fibrils, infection prevalence increased when fibrils were present. I suggest that the presence of egg fibrils could favor transmission to intermediate hosts through multiple routes,

i.e. timing of release, attachment to substrates in the microhabitat of the intermediate host, and establishment in the intermediate host.

INTRODUCTION

Trophically transmitted parasites infect multiple hosts (Crompton, 1975; Poulin, 2010b), which require them to be able to exist in multiple habitats, i.e. inside their hosts and in the habitats of their hosts. Given the variety of host types and habitats, parasites may vary in their mechanisms of transmission (e.g. tapeworms; Jarecka, 1961). For example, in egg to intermediate host transmission trematode eggs can be sessile or motile. When eggs are sessile they are passively ingested by an intermediate host. In contrast, when eggs are motile they can actively search for an intermediate host (Combes, et al., 1994; Esch & Fernandez, 1994). Similarly, in intermediate to definitive host interactions, some parasites increase the frequency of transmission to definitive hosts by modifying their intermediate host's behavior (Moore, 1983, 2002; Poulin, 2010b; Hughes, et al., 2012). Most research to date has focused on the transmission from the intermediate to definitive hosts. Here, I examined factors associated with transmission of the free-living stage of an acanthocephalan parasite to its intermediate host.

Acanthocephalans are trophically transmitted parasites that infect arthropods as intermediate hosts and a variety of vertebrates as definitive hosts. These parasites are found in diverse habitats (aquatic, semiaquatic, terrestrial) throughout the world (Kennedy, 2006). Several studies have shown that variation in egg morphology appears to be related to mechanisms of dispersal of the free-living stage as well as factors in transmission to the intermediate host (Dezfuli, 1996; Barger & Nickol, 1998; George & Nadakal, 1973; Nikishin, 2001; Wongkham & Whitfield, 2004; Arredonodo & Gil de Pertierra, 2009). For example, eggs of acanthocephalan *Pallisentis rexus* expand in size and become buoyant in the aquatic habitat, which increases the

likelihood that they are encountered by their copepod intermediate host (Wongkham & Whitfield, 2004). In several cases, acanthocephalan eggs possess fibrils, which are long, slender filaments that arise from the egg's fibrillar coat (Monné & Hönig, 1954; Chapter 1; Chapter 2). These fibrils have been proposed to play multiple roles in transmission, which include attaching to vegetation consumed by intermediate hosts and facilitating establishment inside the intermediate host (Oetinger & Nickol, 1974; Uznanski & Nickol, 1976; Barger & Nickol, 1998). I examined the potential significance of each of these pre- and post-ingestion mechanisms in the acanthocephalan *Acanthocephalus dirus* (Van Cleave, 1931).

I examined three specific ways that egg fibrils could increase transmission success to intermediate hosts: timing, attachment, and establishment. First, the timing of outer membrane degradation and fibril release has been suggested to increase the likelihood that acanthocephalan eggs disperse into the microhabitat occupied by the intermediate host (Uznanski & Nickol, 1976; Taraschewski & Peters, 1992; Taraschewski, 2000). Figure 1 demonstrates how variation in the timing of fibril release could benefit the parasite (e.g. Uznanski & Nickol, 1976; Barger & Nickol, 1998; Taraschewski & Peters, 1992). Second, fibrils can attach to substrates, which have been proposed to aid in transmission by increasing the likelihood that eggs remain in the microhabitat of the intermediate host, and attach to food items within that microhabitat (Uznanski & Nickol, 1976; Taraschewski & Peters, 1992). Third, the attachment properties of egg fibrils could potentially aid in transmission by slowing passage through the intestines of the intermediate host; hence, increasing establishment success (Oetinger & Nickol, 1974).

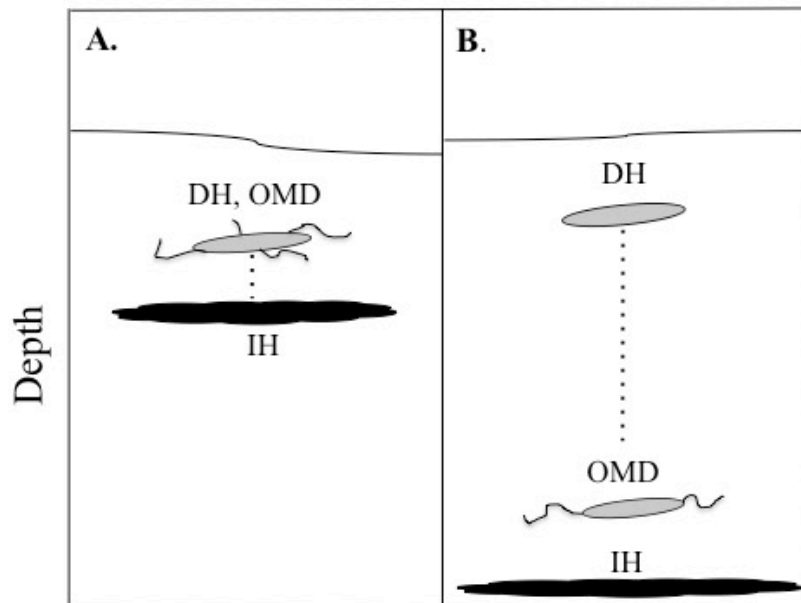


Figure 1. Alternative scenarios of egg fibril release and transmission to intermediate hosts (IH) in aquatic systems (eggs are shown in grey; lines emerging from the egg indicate fibrils; dotted lines indicate the eggs trajectory; algal mats, either floating in the water column or laying on the sediment, are shown in black). In scenario A, the egg's outer membrane degrades (OMD) inside the definitive host (DH), which allows fibrils to be present before the eggs are expelled into the habitat. This could benefit transmission in cases where intermediate hosts (IH) fed in the water column by allowing the eggs to attach to vegetation suspended in the water column. In scenario B, eggs are expelled by the definitive host before the outer membrane degrades, which could benefit transmission in cases where the intermediate host fed on the sediment by allowing the eggs to attach to the sediment.

The acanthocephalan *A. dirus* infects the stream dwelling isopod, *Caecidotea intermedius*, as its intermediate host and stream fishes as definitive hosts (Muzzall & Rabalais, 1975; Camp & Huizinga 1980; Sparkes, et al., 2004). Infection of juvenile *C. intermedius* occurs when *A. dirus* eggs are consumed along with food located on the sediment (Kopp, et al., 2011). Eggs of *A. dirus* possess fibrils, which are released from the egg when the outer membrane degrades (Oetinger & Nickol, 1974). Here, I used laboratory-based experiments to examine the potential role of *A. dirus* fibrils in dispersal and transmission to *C. intermedius*. To address this goal, I examined the following specific questions: 1. Is the timing of fibril release delayed in a manner that is likely to result in dispersal of the eggs into the habitat of the sediment-dwelling host? 2. Do *A. dirus* fibrils attach to substrates in the environment? 3. Does the presence of *A. dirus* fibrils increase the likelihood of establishment in the intermediate host?

MATERIALS AND METHODS

Site of Study

All organisms were collected from Buffalo Creek, located 60 km northwest of Chicago in Lake County, IL, USA. In this site, *A. dirus* development is relatively synchronous with infection of juvenile *C. intermedius* occurring during summer (Sparkes, et al., 2004). In *C. intermedius*, *A. dirus* develop from the acanthor stage to the cystacanth stage, which is infective to the definitive hosts, in two to three months. *Lepomis cyanellus* (green sunfish) and *Semotilus atromaculatus* (creek chub) are infected with *A. dirus* cystacanths from winter through the spring (Sparkes, et al., 2004, 2006; Bierbower & Sparkes, 2007). In these months, *A. dirus* infections have a prevalence of 61% and a mean intensity of one (Kopp, et al., 2011). Gravid female *A. dirus* are present in *L. cyanellus* and *S. atromaculatus* during late spring and early summer (Kopp, et al., 2011).

Timing of Fibril Release

To determine whether the timing of fibril release is likely to result in dispersal of the eggs into the habitat of sediment-dwelling host, I examined the timing of fibril release in mature *A. dirus* eggs. One treatment group was tested in stream water and the other was tested in filtered stream water. To identify the expected time course of release, I exposed eggs to stream water (the typical condition in nature). I also examined the timing of fibril release in stream water that had been filtered to remove microbes, in order to determine if the timing was due to the presence of stream microorganisms (e.g. Oetinger & Nickol, 1974). Stream water was collected from Buffalo Creek (23 March 2016) and was either used in its natural form or filtered through Whatman Grade 1 filter paper and then through a polyethersulfone (PES) filter with a 0.45µm inclusion (Whatman, Puradisc 25mm). Mature eggs were obtained from gravid *A. dirus* females (collected from *L. cyanellus* and *S. atromaculatus*, 23 March 2016). From each *A. dirus* female (n = 31), 40µl of egg solution was pipetted on to two counting cell slides, one containing 40µl of stream water and the other containing 40µl of filtered stream water (50x20mm, plastic Sedgewick-Rafter Counting Cell slide). Slides were refrigerated to mimic environmental conditions (8 - 14°C) and monitored daily for seven days to determine the timing of fibril release. To monitor the timing of fibril release the starting point on the slide was decided at random and then the presence of fibrils was recorded for the first ten eggs observed (Nikon Eclipse E400, 200x). Data collected on day seven was used to determine if there was a difference between the proportions of eggs with fibrils released in stream and filtered water.

Fibril Release and Attachment

To determine whether *A. dirus* fibrils attach to substrates in the environment, I examined if mature eggs attached to a substrate in stream water before and after fibril release. Eggs were

obtained from *A. dirus* in *L. cyanellus* and *S. atromaculatus* on 23 March 2016 (number of *A. dirus* females = 13). For all samples, the egg solution (mature eggs and filtered stream water) was homogenized and 10 μ l was pipetted on a counting slide containing stream water (50 x 20 mm, 1ml, plastic Sedgewick-Rafter Counting Cell slide). Slides were refrigerated to mimic environmental conditions (8 - 14°C). Slides were monitored daily, the presence or absence of egg fibrils and egg attachments were recorded for the first ten eggs observed (Nikon Eclipse E400, 200x). An attempt was made to sample eggs at different locations on the slide to avoid counting the same egg more than once. To test for egg attachment, the slide's coverslip was slowly moved a 1 cm forward and backward. If the egg moved with the water it was considered unattached to the slide. In contrast, if the egg did not move with the water it was considered attached to slide. To determine whether there was a difference in the percent of eggs attached to the slide in the two groups ('fibrils' and 'no fibrils') a Wilcoxon signed-rank test (Conover, 1980) was used because data were not normal despite transformation (determined with Shapiro-Wilk test in R).

Establishment success in *C. intermedius*

To examine whether the presence of *A. dirus* fibrils increased the likelihood of establishment in the intermediate host, I exposed juvenile *C. intermedius* to leaves that contained either eggs with fibrils released or eggs without fibrils released. Both eggs with fibrils released and eggs without fibrils released were obtained from the same female *A. dirus*. To create the two groups, half of the eggs from the female were placed in stream water (to allow fibril release) and half of the eggs were placed in filtered water (to keep fibrils from being released). In both groups, mature *A. dirus* eggs were placed on a leaf disk in stream water, which would give fibrils released eggs and no fibrils released eggs the same likelihood of being encountered by *C. intermedius*.

To obtain uninfected juvenile *C. intermedius*, gravid females were collected and transported to the laboratory at DePaul University (16 April 2016, n = 45). Each *C. intermedius* was held in an individual container (80 x 120 mm) with conditioned leaves and stream water (200ml). Each day individual containers were aerated, by disturbing the surface of the water, and monitored for the presence of juveniles. When juveniles were present, the female was removed and the juveniles were left undisturbed, to mature for two months (30 families). To increase the likelihood that juveniles fed during the experiment, their food source was removed two days prior to the trials.

Table 1 summarizes the experimental design used for the study. In each trial, the egg solution (mature eggs and filtered stream water) was homogenized and pipetted onto a leaf disk in an individual container (35 x 10 mm, 5ml) with stream water (all leaves were conditioned in diH₂O). An extra set of mature eggs was taken from each parasite to estimate the number of eggs per milliliter of solution for each parasite (n = 30). To create the fibril released and not released egg treatments, half of the containers sat for four days, prior to the start of the experiment, to allow for egg outer membrane degradation and fibril release ('fibrils' group). The second half of the containers had mature eggs added the first day of the experiment, which did not allow enough time for fibril release ('no fibrils' group). One juvenile *C. intermedius* from a family was placed into each container. Juveniles fed on the leaf disks, containing *A. dirus* eggs, in their individual dishes for either two, three, or four days. The containers were aerated daily during the experiment. After the allotted days of feeding, the juveniles were transferred to new individual containers with stream water and conditioned leaves without *A. dirus* eggs (35 x 10 mm, 5ml). Each container was monitored and aerated daily for two weeks, after which time each individual was preserved (70% ethanol), measured (body length), dissected and the number of *A. dirus*

present was recorded. Overall parasite prevalence, the number of *C. intermedius* infected over the total number of *C. intermedius* in the study, and overall mean intensity, number of *A. dirus* infecting a *C. intermedius*, were calculated (Bush, et al., 1997). To determine whether there was a difference in overall parasite prevalence and overall mean intensity in the ‘fibrils’ and ‘no fibrils’ groups, I compared these variables using a paired t-test in which the experimental unit was either percentage or average (normality determined with Shapiro-Wilk test in R). To determine that all infections observed were due to experimental infections, 30 *C. intermedius* were randomly sampled from the remaining juveniles in each family to determine their infection status.

Table 1. Experimental design used to examine establishment success of *A. dirus* in *C. intermedius*. Half of the mature eggs from *A. dirus* females were used in each treatment (fibrils released, no fibrils released). Egg density refers to the volume of egg solution used in each trial. Days of feeding refer to the number of days *C. intermedius* fed on leaves containing *A. dirus* eggs.

Egg Treatment	Egg Density	Days of feeding
Fibrils Released	50µL	2
		3
		4
	20µL	2
		3
		4
No Fibrils Released	50µL	2
		3
		4
	20µL	2
		3
		4

RESULTS

Timing of Fibril Release

The percent of female *A. dirus* with at least one egg with released fibrils in stream water and filtered water each day are shown in Figure 2a. In *A. dirus* females, fibril release was observed starting on day one and continued to increase with each consecutive day in both stream and filtered water. There is a slight increase in the percent of females with eggs with fibrils in stream water than in filtered water. The percent of eggs with released fibrils in stream water and filtered water are shown in Figure 2b. There was a greater difference in the proportion of eggs with fibrils released in stream water than eggs with fibrils released in filtered water. The median number of eggs with fibrils released on day seven in stream water was 50 (0 - 70). In contrast, filtered water had a median of zero eggs with fibrils released on day seven. When analyzing the effects of the treatment groups on day seven, there was a difference in the proportion of eggs with fibrils released in stream water than eggs with fibrils released in filtered water (Wilcoxon signed-rank test, $T_{30} = 4.9$, $p < 0.005$).

Attachment to Substrate

The percent attachment of eggs for each sample with and without fibrils is shown in Figure 3. Eggs with fibrils released had a higher percent attachment than eggs without fibrils released (Wilcoxon signed-rank test, $T_{12} = 3.2$, $p < 0.005$). This difference translated into a mean attachment of 96.7% (± 6.8) for eggs with fibrils released and 7.2% (± 9.9) for eggs without fibrils released.

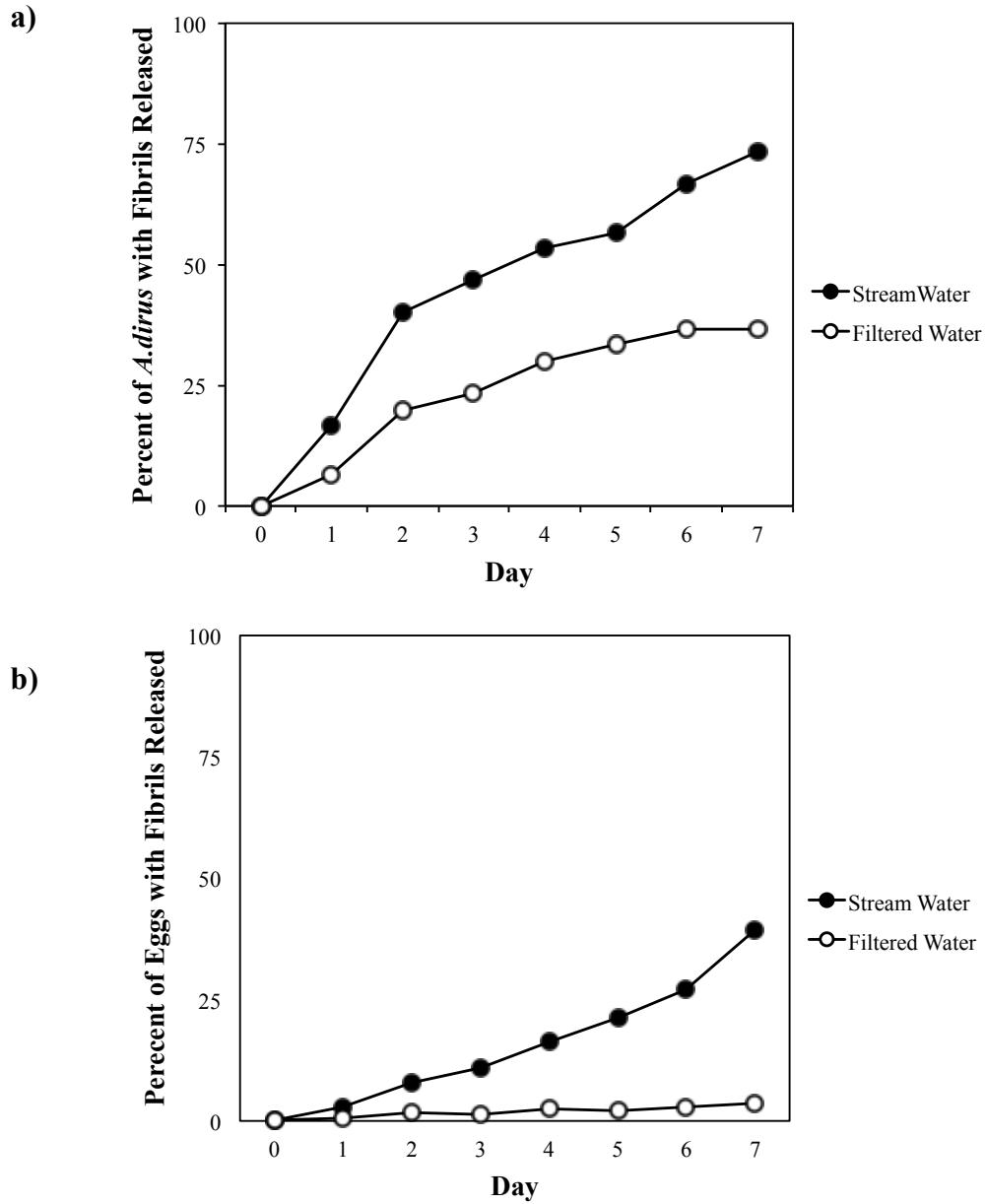


Figure 2. a. Percent of female *A. dirus* with eggs releasing fibrils in stream water (filled circle) and filtered water (unfilled circle). For each trial, eggs from the same female ($n = 31$) were treated with either stream water and filtered water. **b.** Percent of eggs with released fibrils in stream water (filled circle) and filtered water (unfilled circle). For each of the 31 females, 10 eggs were observed each day ($n = 310$).

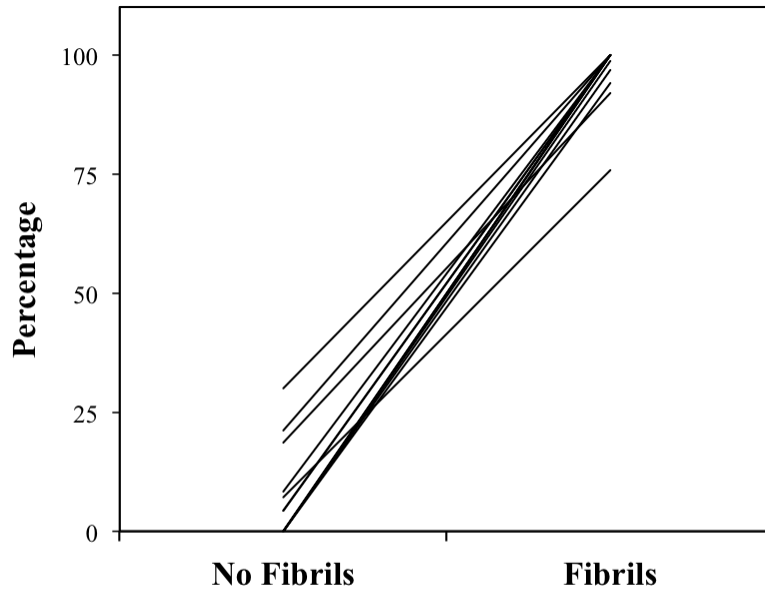


Figure 3. Relationship between the presence of fibrils and the percent of *A. dirus* eggs that attached to substrates. Individual lines represent eggs obtained from the same female (n = 13).

Establishment success in *C. intermedius*

Experimental infections were used to determine the effect of fibrils on establishment success in *C. intermedius*. Of the 360 *C. intermedius* used, 274 survived (76%). Estimates of the number of eggs dispensed in each treatment for each density indicate that the 20 μ l density had an average of 404 (\pm 22 standard error) eggs and the 50 μ l density had an average of 1009 (\pm 75 standard error) eggs. Randomly sampled juveniles from each family, not used in infection trials, were all uninfected indicating that infections present in the study were due to experimental exposure.

A summary of *A. dirus* prevalence and mean intensity for each density and the number of days *C. intermedius* were exposed to *A. dirus* eggs are shown in Table 2. Overall prevalence for the no fibril group was 41% and 55% in the fibril group, which resulted in a +14 difference (Table 2). Prevalence of *A. dirus* was greater in the fibrils group than the no fibrils group (paired t-test, $t_{29} = 3.0$, $p = 0.005$). Overall mean intensity for the no fibrils group was 3.1 and was 4.3 in the fibrils group, which resulted in a +1.2 difference (Table 2). Analysis of overall mean intensity in the no fibrils and the fibrils group indicated that there was not a difference between the groups (Wilcoxon signed-rank test, $V_{27} = 193.5$, $p < 0.1$). At the 50 μ l density, the mean intensity across the different days of feeding (2, 3, & 4) revealed a difference of +2.5 in the fibrils group, whereas the 20 μ l density showed no difference. Analysis of mean intensity for the 50 μ l density indicated that there was a difference between the no fibrils and fibrils eggs (Wilcoxon signed-rank test, $V_{24} = 234.5$, $p < 0.005$). Analysis of mean intensity for the 20 μ l density indicated that there was no difference between the no fibrils and fibrils group (Wilcoxon signed-rank test, $V_{24} = 182.5$, $p > 0.1$).

Table 2. Summary of *A. dirus* prevalence and mean intensity for each treatment group. Density refers to the volume of egg solution used in each trial and days refer to the number of days *C. intermedius* fed on *A. dirus* eggs. There was not a difference in *C. intermedius* body size in the fibrils and no fibrils group (paired t-test, $t_{29} = 1.49$, $p > 0.1$).

Density	Days	Prevalence (%)			Mean intensity		
		No Fibrils	Fibrils	Diff.	No Fibrils	Fibrils	Diff.
20	2	27	42	+15	2.9	2.2	-0.7
	3	52	56	+4	4.2	5.4	+1.2
	4	40	55	+15	4.6	4.0	-0.6
	Mean	40	51	+11	3.9	3.9	0.0
50	2	32	64	+32	1.8	3.1	+1.3
	3	50	50	0	2.7	5.9	+3.2
	4	48	61	+13	2.5	5.3	+2.9
	Mean	43	58	+15	2.3	4.8	+2.5
Overall	Mean	41	55	+14	3.1	4.3	+1.2

DISCUSSION

The results indicated are consistent with the notion that egg fibrils could increase transmission success to intermediate hosts by multiple mechanisms: timing of release, attachment to substrates, and establishment success. Delay in *A. dirus* fibril release may increase the likelihood that eggs disperse into the microhabitat (sediment) occupied by intermediate hosts prior to fibril release. The fibrils of *A. dirus* also influenced attachment, which could increase the likelihood that eggs remain in the microhabitat and are consumed by the intermediate host. In addition, the presence of fibrils increased both the prevalence and intensity of *A. dirus* infection. Collectively, these results indicate that egg fibrils in *A. dirus* likely play a role in transmission to intermediate hosts.

Given that fibrils attach to substrates in microhabitats occupied by intermediate hosts, a delay in the timing of fibril release would be beneficial when the intermediate host is sediment-dwelling versus a host that feeds in the water column (Figure 1). In this study, *A. dirus* fibril release occurred after eggs were expelled from the definitive host and this release was associated with environmental microorganisms. This could allow eggs to reach the sediment, where the intermediate host feeds, prior to releasing fibrils. In contrast, the acanthocephalan *Leptorhynchoides thecatus* has fibrils released before eggs are expelled from the definitive host, which could allow eggs to attach to vegetation that the intermediate host feeds on while sinking through the water column (Barger & Nickol, 1998). Thus, the timing of fibril release in acanthocephalans appears to be related to the factors associated with the host's habitat.

Variation in egg morphology appears to be consistent with different mechanisms of transmission in several taxa. For example, fibrils have been proposed to increase encounter rates with water column-dwelling hosts in other helminths (e.g. cestodes; Munson, 1972). Egg

buoyancy has also been proposed to increase transmission to intermediate hosts that feed in the water column. For example, the outer membrane of eggs of the acanthocephalan *Pallisentis rexus* expand, and become buoyant, increasing the time they spend floating in water column, where their copepod intermediate host feeds (Wongkham & Whitfield, 2004). Similarly in tapeworms, taxa with swimming hosts (e.g. copepods) have buoyant eggs, whereas taxa with benthic hosts have heavy, quickly sinking, eggs (Jarecka, 1961).

Oetinger and Nickol (1974) proposed one benefit of egg fibrils in acanthocephalans is that they could slow passage of eggs through the intestines of the intermediate host, hence increasing establishment success. The results obtained here are consistent with this hypothesis in that there was an increase in infection prevalence and mean intensity of *A. dirus* eggs with fibrils released (Table 2). However, there is a concern that the presence of fibrils might have increased establishment success by attaching the eggs to the leaf, rather than slowing passage through the intestines of *C. intermedius*. Given the chosen experimental design, I was unable to determine the number of eggs consumed by each individual. Consequently, it is unclear whether the presence of released fibrils could have had an effect on consumption by *C. intermedius*.

Given the potential benefits of egg fibrils in transmission to and establishment in intermediate hosts, it seems likely that the presence of fibrils would be beneficial in all acanthocephalans. However, fibrils appear to be present in less than 10% of taxa described in the literature (Chapter 1). A phylogeny of fibril evolution indicated that fibrils are likely homoplasies (Chapter 2). If fibrils evolved as a mechanism to increase establishment success in intermediate hosts, then they would likely be found in all acanthocephalans regardless of habitat (aquatic, semi-aquatic, terrestrial). Fibrils appear to be more common in aquatic habitats

(Chapter 2, Table 1), which may indicate that properties of the habitat may influence the evolution of fibrils in many cases.

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