Resolving evolutionary changes in parasite life cycle complexity: Molecular phylogeny of the trematode genus *Alloglossidium* indicates more than one origin of precociousness

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ABSTRACT

The evolutionary causes and consequences of changes in complex life cycles are of central importance in parasitology. However, data remain limited because in part, knowledge on phylogenetic relationships among species that differ in life cycle patterns remains scarce. We present a molecular phylogeny of the trematode genus *Alloglossidium*, which contains several species that display precocious (a.k.a., progenetic) life cycles (i.e., maturation in what is typically regarded as an intermediate host). The molecular phylogeny contrasts with previous morphological and life-history based phylogenetic hypotheses. In particular, a precocious life cycle wherein leeches are used as final hosts evolved early in the history of the genus. Among the remaining species, which are a separate clade, a three-host life cycle using ictalurid catfishes is ancestral. Furthermore, there are at least two additional independent evolutionary events that lead to a precocious life cycle where a catfish host is lost and a crustacean is used as a final host. We conclude with a discussion on how existing hypotheses on the evolution of precociousness, and parasite life cycle complexity in general, may or may not relate to the patterns observed in genus *Alloglossidium*.

1. Introduction

The addition or removal of a host from a parasite's life cycle is not a trivial evolutionary event; life cycle transitions can have major consequences for parasite transmission, behavior, physiology, development, and mating systems (e.g., Kasl et al., 2015). Moreover, complex life cycles involving trophic transmission are regarded as a major adaptive peak for parasitism (Lafferty and Kuris, 2002; Poulin and Randhawa, 2015). Consequently, the evolution of changes in life cycle complexity is a longstanding topic in parasitology (Poulin, 2007) and there is now renewed emphasis on elucidating the factors driving the selection for, and maintenance of, such complexity (Choisy et al., 2003; Parker et al., 2003; Hammerschmidt et al., 2009; Benesh et al., 2013, Parker et al., 2015a,b; Auld and Tinsley, 2015). However, studying the causes and consequences of complex life changes hinges on knowledge of the evolutionary order of life cycle transitions, which in itself, requires knowledge of phylogenetic relationships among species that differ in life cycle patterns. Unfortunately, such relationships are still not known among many parasitic taxa, especially within groups that display life cycle variation.

Digenean trematodes are notable for having some of the most complex life cycles, typically incorporating both free-living and parasitic developmental stages and almost always including both asexual and sexual reproduction (Cribb et al., 2003; Olson et al., 2003). Across the Digenea, a 3-host life cycle is the most commonly found pattern (Cribb et al., 2003, Olson et al., 2003, see Fig. 1A as an example). In contrast to this common 3-host pattern, however, some species exhibit what has been termed a "progenetic" life cycle (Cribb et al., 2003; Lefebvre and Poulin, 2005a). Hereafter, we refer to species exhibiting such patterns as "precocious" rather than progenetic to reflect the parasite's early onset of adult development (attaining sexual maturity) within what is typically deemed an intermediate host. Some of these precocious species have obligate 2-host patterns where it is presumed that the 3rd host, i.e., the previous final host, was lost, whereas other species are facultative in having a 2- or 3-host pattern (Lefebvre and Poulin, 2005b). It is these precocious life cycle variations that are the focus of our study. In particular, we are interested in the evolution of precocious life cycles within the digenean genus *Alloglossidium*.

The genus *Alloglossidium* Simer, 1929 is comprised of 18 nominal species (Table 1). It is a uniquely appropriate system with which to
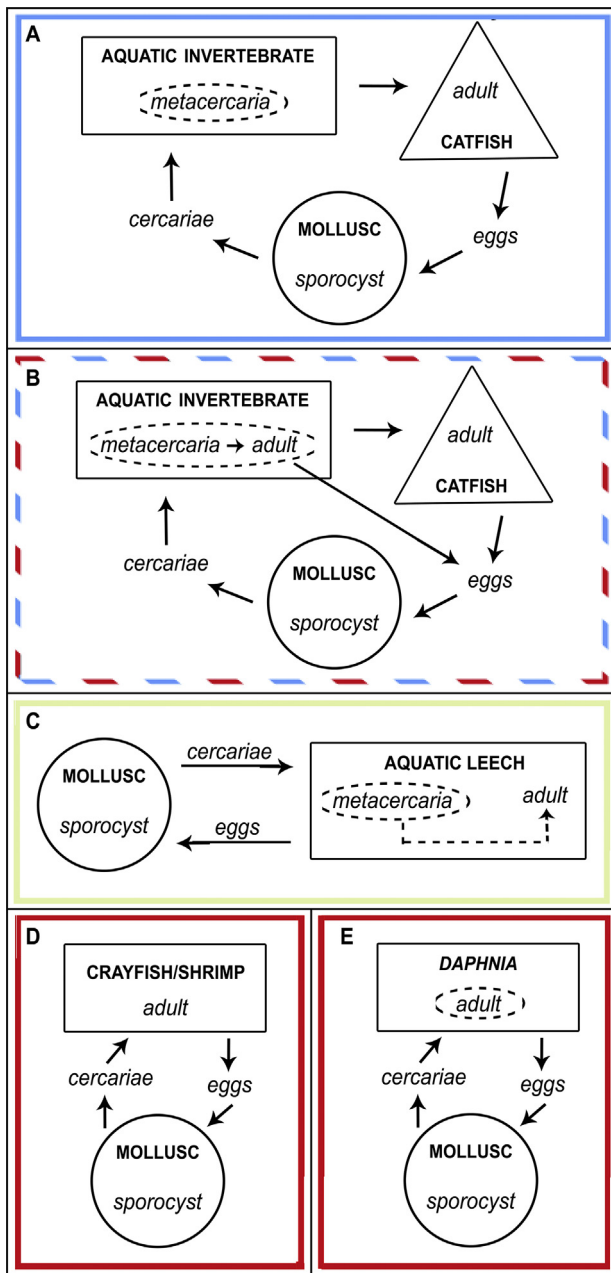
Fig. 1. Diagram of *Alloglossidium* life cycle patterns (see Table 1 for species associations). Hosts are denoted using bold uppercase type. Parasite life history stages are denoted using italicized lowercase type. A. In the obligate 3-host life cycle, the parasite is ingested by a molluscan 1st intermediate host, undergoes asexual reproduction leading to the release of larval cercariae. Cercariae subsequently penetrate an invertebrate 2nd intermediate host, developing into an encysted (indicated by dotted lines) metacercarial stage. Upon ingestion of the 2nd intermediate host by the final host (an ictalurid catfish), the parasite excysts, migrates to the intestines, and becomes sexually mature, thereby completing the life cycle. B. In the facultative precocious life cycle (found in *A. progeneticum*), the parasite becomes sexually mature while still encysted in a crayfish 2nd intermediate host (i.e. a fish host is not needed). However, encysted adults can still be trophically transmitted to an ictalurid host and is therefore considered a facultative 2- or 3-host life cycle. C–E show life cycles for species with obligate precocious life cycles. C. Species utilizing leeches as the final host maintain all life history stages (i.e., the encysted metacercarial stage), first encysting, then excysting and migrating to the gut within the same host individual to complete development. D. Species reported from crayfish and shrimp have lost the encysted metacercarial stage, instead persisting freely in the antennal glands of their respective hosts. E. *Alloglossidium anomophagis* infects the body cavity of *Daphnia* and becomes an adult while remaining encysted.

address questions regarding evolutionary changes in life cycle complexity because it contains species with 3-host life cycles and with precocious life cycles. Moreover, roughly 15% of the known precociously developing digenean species occur within this genus (Lefebvre and Poulin, 2005a). Five species have life cycles reflecting the typical 3-host digenean pattern where an ictalurid catfish is the final host (Table 1, Fig. 1A, but see discussion for caveats on undiscovered life history). One species, *Alloglossidium progeneticum*, is capable of facultatively incorporating either 2- or 3-hosts in its life cycle because it can precociously develop while still encysted in an "intermediate" crayfish host (Table 1, Fig. 1B). The remaining 12 species exhibit one of three obligate precocious life patterns classified by the type of final host (i.e., leech or freshwater crustacean) and the presence or absence of an encysted metacercarial stage (Table 1, see Fig. 1C–E for elaboration). Because of this life cycle variation found among species, the genus *Alloglossidium* was recognized early on as a potential model system with which to study developmental constraints and integrative biological components (e.g., the origin, stasis, and diversification) of complex life cycles (Brooks, 2003). While past studies have addressed the evolution of life cycle changes in this genus (discussed below), interpretations are limited in part due to the finding of cryptic species in the genus (e.g., Tkach and Mills, 2011) and exclusion of *A. anomophagis*.

The first two studies to address the phylogeny of species in the genus *Alloglossidium* defined interspecific relationships through logical deductions. Font (1980) suggested a 3-host life cycle was the ancestral condition for the genus due to the ubiquity of this pattern among digeneans. He deduced there was a subsequent split leading to the speciation of *Alloglossidium* maturing in crustacean hosts (through a progressive transition via the facultative precocious pattern) and to those species maturing in leeches. In contrast, Riggs and Ulmer (1983) postulated that the obligate 2-host pattern in leeches was the ancestral condition. They argued that leeches predated catfishes in the fossil record and that the similarity between the gut lumen of leeches and the intestines of catfishes enabled a host-switching event from leeches to catfishes. Species associated with crustacean final hosts were not addressed by Riggs and Ulmer (1983).

Previous method-based phylogenetic hypotheses were based on a combination of morphological and life history characters where the most recent analysis proposed a single transition from a 3- to 2-host crustacean life cycle pattern, followed by a host switching event from crustaceans to leeches (Fig. 2; Carney and Brooks, 1991; Smythe and Font, 2001; but see Brooks, 2003 for methodological corrections). However, incorporating the same life history traits to both reconstruct the phylogeny and assess their ancestral changes in life cycle pattern has the potential to confound the analysis. Ideally, when testing hypotheses related to trait evolution, a phylogeny constructed from data independent of the traits themselves would be preferred.

The aim of our study was to construct the first molecular-based phylogeny with which to study the evolutionary relationships within the genus *Alloglossidium*. This phylogeny subsequently provides an independent framework to assess the character evolution of life cycle pattern transitions. In particular, we compare the results of the molecular-based phylogeny to the existing deductive or morphology-based hypotheses on complex life cycle evolution in the genus *Alloglossidium*. In doing so, we elucidate those parameters essential to study the evolution of life cycle complexity, i.e., when a particular life cycle pattern developed and the number of transitions among possible patterns. Lastly, we discuss how existing hypotheses on the evolution of precociousness and parasite complex life cycles in general may or may not relate to the patterns observed in genus *Alloglossidium*.

2. Materials and methods

2.1. Taxon sampling and outgroup selection

Sampling of *Alloglossidium* species was conducted as part of a large-