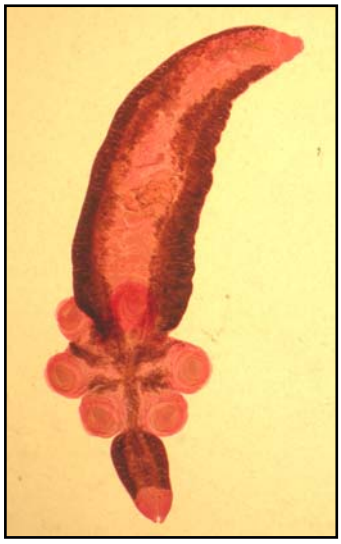
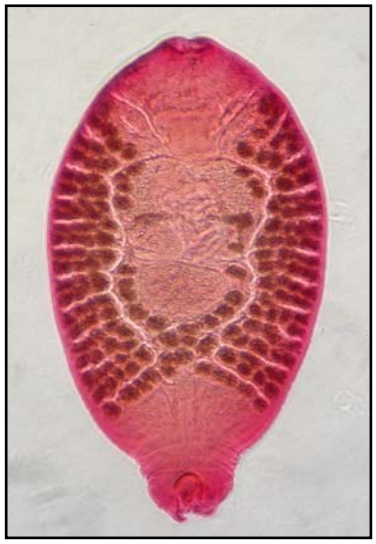
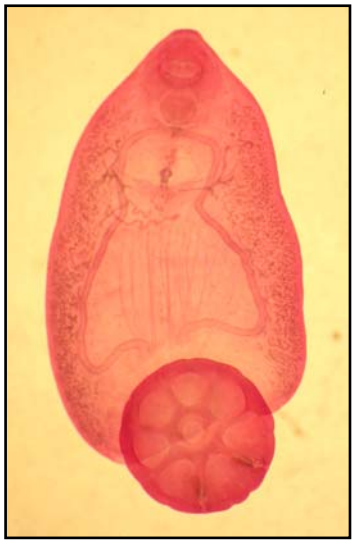
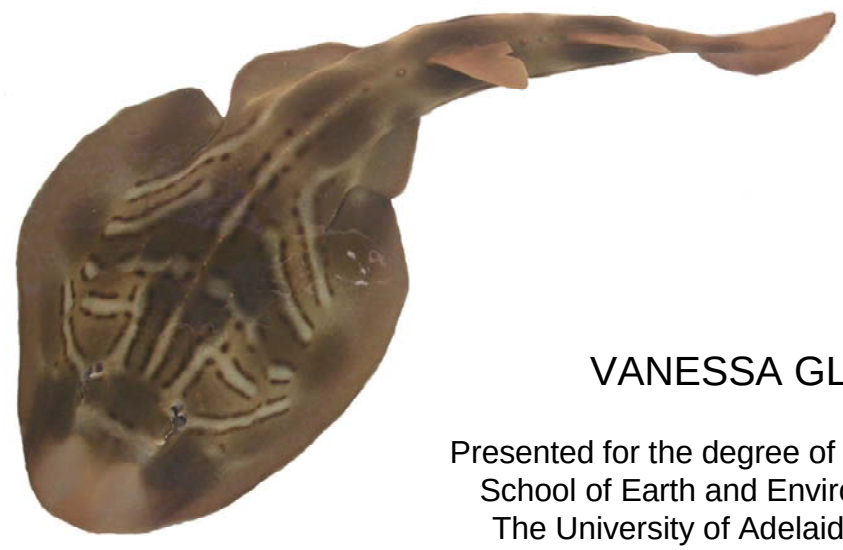
MONOGENEANS OF THE SOUTHERN FIDDLER RAY, *TRYGONORRHINA FASCIATA* (RHINOBATIDAE) IN SOUTH AUSTRALIA: AN EXCEPTIONAL MODEL TO COMPARE PARASITE LIFE HISTORY TRAITS, INVASION STRATEGIES AND HOST SPECIFICITY



VANESSA GLENNON

Presented for the degree of Doctor of Philosophy
School of Earth and Environmental Sciences
The University of Adelaide, South Australia

February, 2008

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Title page images. Top (left to right): *Branchotenthes octohamatus* (Hexabothriidae); *Pseudoleptobothrium aptychotremae* (Microbothriidae); *Calicotyle australis* (Monocotylidae). Bottom: *Trygonorrhina fasciata* (Rhinobatidae)
Photos: V. Glennon

DEDICATION

To my beloved parents Rose and Bryan Glennon

As a child you showed me the world, encouraged my dreams, quelled my fears and taught me to reach. Every achievement of mine is an achievement of yours.

PUBLICATIONS ARISING FROM THIS PhD

Glennon, V., Chisholm, L.A. and Whittington, I.D., 2005. *Branchotenthes octohamatus* sp. n. (Monogenea: Hexabothriidae) from the gills of the southern fiddler ray, *Trygonorrhina fasciata* (Rhinobatidae) in South Australia: description of adult and larva. *Folia Parasitologica* 52: 223–230.

Glennon, V., Chisholm, L.A. and Whittington, I.D., 2006. A redescription of *Calicotyle australis* Johnston, 1934 (Monogenea: Monocotylidae) from the type host *Trygonorrhina fasciata* (Rhinobatidae) off Adelaide, South Australia, including descriptions of live and silver stained larvae. *Systematic Parasitology* 63: 29–40.

Glennon, V., Chisholm, L.A. and Whittington, I.D., 2006. *Pseudoleptobothrium aptychotremae* Young, 1967 (Monogenea, Microbothriidae) redescribed from a new host, *Trygonorrhina fasciata* (Rhinobatidae) in South Australia with a description of the larva and post-larval development. *Acta Parasitologica* 51: 40–46.

Glennon, V., Chisholm, L.A. and Whittington, I.D., 2006. Three unrelated species, 3 sites, same host - monogenean parasites of the southern fiddler ray, *Trygonorrhina fasciata*, in South Australia: egg hatching strategies and larval behaviour. *Parasitology* 133: 55–66.

Glennon, V., Chisholm, L.A. and Whittington, I.D., 2007. Experimental infections, using a fluorescent marker, of two elasmobranch species by unciliated larvae of *Branchotenthes octohamatus* (Monogenea: Hexabothriidae): invasion route, host specificity and post-larval development. *Parasitology* 134: 1243–1252.

Glennon, V., Perkins, E.M., Chisholm, L.A. and Whittington, I.D. Comparative phylogeography reveals host generalists, specialists and cryptic diversity: hexabothriid, microbothriid and monocotylid Monogenea from Rhinobatidae in southern Australia. *International Journal for Parasitology* (in press).

TABLE OF CONTENTS

ABSTRACT

Trygonorrhina fasciata (Rhinobatidae) specimens naturally infected by three monogenean species were captured and maintained in marine aquaria to promote a continuous parasite load. Monogenean eggs recovered from aquaria provided larvae for descriptions and life history experiments. I describe the adult, larva and post-larval development of a new species of hexabothriid, *Branchotenthes octohamatus*, from the gills. This is the first monogenean larva described with only eight hooklets. This character may be useful to help resolve problematic relationships within the Hexabothriidae and offers insight into more general hypotheses about relationships within the Monogenea. I also redescribe the adult of *Calicotyle australis* (Monocotylidae) from the cloaca and describe the larva. The number and arrangement of larval ciliated epidermal cells and sensilla was revealed using silver nitrate. I redescribe *Pseudoleptobothrium aptychotremae* (Microbothriidae) adults from the skin of *T. fasciata*, representing a new host and locality record. Larval anatomy and post-larval development are also documented. The presence of six needle-like spicules in the larval haptor is confirmed, supporting an earlier theory that spicules are ancestral vestiges.

My studies revealed three different egg hatching, host finding strategies and larval 'types'. *Branchotenthes octohamatus* has a 'sit-and-wait' strategy, entirely dependent on mechanical disturbance to stimulate eggs to hatch. Larvae are unciliated, cannot swim, lack pigmented eyespots and show no photo-response but may survive for more than two days after hatching at 22 ºC. In contrast, eggs of *C. australis* hatch spontaneously with a strong diurnal rhythm in the first few hours of daylight when exposed to a LD12:12 illumination regime. Larvae are ciliated and can swim, have pigmented eyespots, are photo-positive and can remain active and survive for up to 24 h after hatching at 22 ºC. Eggs of *P. aptychotremae* may have a 'bet-hedging' strategy. Some eggs hatch spontaneously and rhythmically in an LD12:12 regime during the last few hours of daylight but their low hatching success rate suggests that other eggs may require a different cue provided by the host. Larvae are ciliated, can swim, lack pigmented eyespots, show no photo-response and remain active for only a few hours at 22 ºC.

Experiments using the fluorescent dye, 5(6)-carboxyfluorescein diacetate *N*-succinimidyl ester (CFSE) revealed *B. octohamatus* on gills of *T. fasciata* within 30 min of exposure to the host. This provides strong evidence that larvae invade the gills directly via the host's inhalant respiratory current and do not migrate after initial attachment elsewhere.

Five rhinobatid species (*Aptychotrema vincentiana, T. fasciata, Trygonorrhina* sp. A, *A. rostrata* and *Rhinobatos typus*), with overlapping distributions spanning west, south and east Australian coastal waters were surveyed for monogeneans at four locations between Fremantle, Western Australia and Stradbroke Island, Queensland. Genetic homogeneity, using the mitochrondrial gene Cytochrome b (cytb) and the nuclear marker, Elongation factor-1 alpha (EF1a), was observed for all *Branchotenthes* and *Calicotyle* specimens irrespective of collection locality or rhinobatid species. Genetic homogeneity was observed for *Pseudoleptobothrium* specimens collected in western and southern Australia. However, local genetic heterogeneity was apparent among *Pseudoleptobothrium* specimens collected from two sympatric host species in New South Wales. Analyses revealed a highly divergent clade, indicating a morphologically cryptic, ancestral species.

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At the outset of this degree, I was aware of the intellectual and temporal commitment it represented for me. I did not, however, fully appreciate the level of commitment that it would demand others to make on my behalf. To these people, I offer my heartfelt thanks.

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Chapters III – VII are published papers and Chapter VIII is now in press. Acknowledgements specific to these chapters are as follows:

CHAPTER III

We thank Eric Hoberg and Pat Pilitt at the United States National Parasite Collection (USNPC) for the loan of specimens. We are especially grateful to Delane Kritsky (Idaho State University) for examining specimens and offering valuable guidance. We also thank Ian Beveridge (University of Melbourne) for his expertise in Latin nomenclature. Funding for this research was provided by the School of Earth and Environmental Sciences of the University of Adelaide and an Australian Postgraduate Award (APA) to V. Glennon during her candidature and Australian Research Council (ARC) grant no. DP0557697 (2005–2007) awarded to Ian Whittington (IDW).

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CHAPTER VIII

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CHAPTER I

GENERAL INTRODUCTION

CHAPTER I

Two of the important questions that lie at the heart of evolutionary ecology are: "why are there so many species?" and "how do differences in life history traits evolve?" The first question, originally posed by Hutchinson (1959), pertains to the evolution of specialisation. It is a subject widely discussed in the literature where value-laden phrases abound. Specialisation has been termed an evolutionary 'dead end' or a 'blind alley' by some systematists, while others assert that a 'jack-of-all-trades can be master of none' (Berenbaum 1996). Although views may differ as to whether specificity for a particular resource or set of resources is beneficial in an evolutionary sense, its ubiquity in nature cannot be debated. However, degrees of specialisation vary considerably among organisms and the phenomenon is still to be fully understood. The second question: "how do differences in life history traits evolve?" seeks to identify the selective forces responsible for shaping the lives of organisms within their habitats. This requires not only an understanding of how principal life history traits vary between organisms but also an understanding of their respective environments.

Parasite-host systems provide a valuable platform to examine these questions. First, host specificity is a universal feature of parasitism, representing a key life history trait (Sasal *et al.* 2004) and second, the principal environment for a parasite is the host, so the ecological niche of a parasite is often easier to define than that of a free-living organism (De Meeûs *et al.* 1998). In particular, parasites with simple lifecycles involving a single host species are especially informative. These parasites allow principal life history traits to be quantified without the confounding effects of multiple hosts or asexually reproductive stages that may be present in parasites with complex lifecycles. Furthermore, when only one host species is involved in a parasite's lifecycle, patterns of specificity should be easier to determine (Desdevises *et al.* 2002). Parasites with direct lifecycles should theoretically also be more straightforward to culture for detailed laboratory investigation. Monogenean (platyhelminth) flatworms and their hosts comprise excellent models for the study of evolutionary questions. Not only do they fulfil the above criteria, they are also thought to be the most basal parasitic flatworm group (Lockyer *et al*. 2003), potentially offering great insight into the origins of parasitism. As such, there is

much to be gained from a comprehensive comparative study of parasites such as monogeneans from different families, occupying different sites on the same host species. Knowledge of how principal life history traits such as egg hatching, larval infection strategies and host specificity vary at this taxonomic level should facilitate a greater understanding of possible habitat-specific effects on the evolution of life histories.

In South Australia I have identified an ideal parasite-host system to undertake such a comparative study. The locally abundant southern fiddler ray, *Trygonorrhina fasciata* (Rhinobatidae), is host to three monogenean species from different families (Hexabothriidae, Microbothriidae and Monocotylidae), each occupying a different microhabitat (gills, skin and cloaca), respectively. These families are exceptional because unlike most monogeneans that parasitise teleosts (~95%) (Euzet and Combes 1998), hexabothriids, microbothriids and monocotylids parasitise only the chondrichthyan fishes (the sharks, rays and chimaeras), indicating a long history of association. Although united in the types of hosts they share, the specificity of these monogenean families for particular sites on the hosts, however, is dissimilar. Monocotylids have been recorded from sites as varied as the skin, gills, nasal tissues, cloaca, rectum, rectal gland, oviducts and even the inner wall of the body cavity (Chisholm and Whittington 1998); microbothriids have been recorded mainly from the skin (Young 1967) but also from gills and nasal tissue (Price 1963). In contrast, hexabothriids have only been recorded from the gills of their hosts (Boeger and Kritsky 1989). I have established that the southern fiddler ray keeps well in aquaria facilitating culture of parasites *in vivo* and providing a continuous supply of eggs and larvae for experimental work to investigate: adult and larval parasite taxonomy (Chapters III, IV,V); cues that promote egg hatching (Chapter VI), larval behaviour (Chapter VI) and larval infection strategies (Chapter VII).

Furthermore, monogeneans resembling two species from *T. fasciata* have also been reported on the eastern shovelnose ray, *Aptychotrema rostrata* (Rhinobatidae), in Queensland suggesting that this rhinobatid species may host the same suite of monogeneans. However, the geographic distributions of *T. fasciata* and *A. rostrata* are discontinuous. This implies either 1) that another rhinobatid species with a distribution that overlaps *A. rostrata* in the north and *T. fasciata* in the south may also be infected by these monogeneans, providing connections between host and parasite populations, and/or 2) that the monogenean species on *T. fasciata* may not

be the same as those on *A. rostrata* despite their apparent morphological similarities. Morphological comparisons, as well as molecular analyses, should help discriminate between specimens, thereby allowing the specificity of these monogenean species for a host species to be determined (Chapter VIII). By exploring connections between parasite morphology, molecular genetics, behaviour, host and site specificity, a greater understanding of the implicit nature of the parasite-host relationship may be achieved.

Notes on chapter style

Each of my data chapters has been written in a style suitable for publication. As such, the text reflects multiple authors. Chapters III – VII are already published and Chapter VIII is now in press. Each chapter can therefore be read as a stand alone paper but in sequence follow a logical progression, and together comprise my thesis. The order in which data is presented in each published chapter, conforms to that of the published paper. However, within the text of each chapter, tables and figures have been inserted and some formatting changes have been made to standardise stylistic differences between publishing houses.

Each data chapter is preceded by a statement of authorship detailing publication information and co-author contributions. Reprints of each published chapter are compiled in Appendix I in order of appearance in the thesis. The format of my thesis complies to that outlined under "Specifications for Thesis" on page 27 of *Academic Program Rules* (2008) produced by the Adelaide Graduate Centre.

CHAPTER II

LITERATURE REVIEW

CHAPTER II

Host specificity is considered one of the most important life history traits of parasites and refers to the restriction of parasite species to particular species of hosts (Rohde 1993; Sasal *et al.* 2004). According to Poulin and Mouillot (2003) "…it reflects better than any other parameter, the breadth of their ecological niche, and thus their exact position and role in the biosphere". Furthermore, the specificity of a parasite for a host will determine the likelihood of successful invasion into new habitats (Poulin and Mouillot 2003). Despite its recognised importance however, the evolutionary mechanisms that underlie specialisation leading to specificity are not fully understood in ecology (Desdevises *et al.* 2002).

Considerable variation exists in the level of host specificity exhibited by parasites. Some parasite species show preferences for a very narrow range of host species (specialists), whereas others infect a broader range (generalists) (Humphery-Smith 1989). However, even when host specificity may be low, parasites remain highly site or microhabitat specific, living and feeding in or on particular regions of the host (Adamson and Caira 1994). This site specificity is attributed to the fact that equivalent tissue sites of different host species are physiologically more similar than different organs or tissues in the same host species (Adamson and Caira 1994). The following pages review current theory surrounding the likely processes and mechanisms responsible for driving and maintaining specialisation as a principle life history trait among parasites. Specific reference, where appropriate, is made to the Monogenea.

How is specificity measured?

Of the parasitic platyhelminths, monogeneans are considered to be among the most host specific (Shulman 1961), with many species reported to be strictly host specific i.e. infecting a single host species (Whittington *et al.* 2000). However, the concept of specificity is a relative one. The term 'specialist' may be applied to species parasitising a single host genus or an entire family (Fox and Morrow 1981; Desdevises *et al.* 2002). Previously, host range, defined as the number of host species known to be infected by a parasite, was seen as a good estimator of parasite

specificity (Lymbery 1989). But for a measure of host specificity to be useful, the phylogenetic relationships of the hosts must be taken into account. For example, if two parasite species (e.g. A and B) each use four host species, they have an equivalent host range. However, if the hosts of species A are congeners, whereas the hosts of species B belong to the same or different families, then species A is much more specific than species B because it parasitises a single host genus (Poulin and Mouillot 2003). Furthermore, if parasite species A was highly prevalent in one host species but rare in the other three, whereas species B was equally common in all four of its host species, ecological differences with respect to host preferences or exploitation would be indicated (Poulin and Mouillot 2003). While numerous specificity indices have been developed (Rohde 1980; Adamson and Caira 1994; Desdevises *et al.* 2002; Rohde 2002; Caira *et al.* 2003; Poulin 2003), a single index that takes all evolutionary factors into account is yet to be devised (Sasal *et al.* 2004).

Obtaining the right measurement of specificity

The value of any specificity index will only ever be as good as the information used to derive it. For example, a lack of knowledge regarding the true host range of a parasite may lead to inaccurate or biased estimations of specificity (Brooks and McLennan 1993a). Because studies of specificity may be motivated by a variety of concerns (e.g. effects on human health (Combes 1990); biological controls or risk assessment following species introductions (Secord and Kareiva 1996); or host-parasite co-evolution (Klassen 1992)), variations in specificity may simply reflect differential sampling effort between parasitic groups. Furthermore, low prevalence and/or intensity may mean that some parasites remain unrecorded if insufficient numbers of hosts are examined, whereas others may be overlooked due to small size, cryptic colouration or microhabitat (Poulin and Morand 2000).

Inaccurate parasite species identifications may also confound evaluations of specificity. Parasite species descriptions are traditionally morphologically based, with differences between specimens sometimes small, representing “a subjective judgement on the part of the worker who has described them” (Cameron 1964). Desdevises *et al*. (2000) suggest that fewer species may exist than are currently described due to the misleading influence of high levels of phenotypic plasticity.

Conversely though, Chisholm *et al*. (2001a) point out that many investigators have taken a conservative approach, choosing not to erect new species when morphological differences are observed because of high levels of variation among individuals from the same species.

Among the Monogenea, reliable diagnostic characters are few as soft body proportions can vary markedly depending on the method of fixation. Factors such as host species, temperature and specimen age have also been identified as potential contributors to morphological variation (Kearn 1987; Rohde *et al.* 1992; Mo 1993; Desdevises *et al.* 2000). Such potentially high levels of 'within group' variation can invalidate discrimination 'between groups' during multivariate comparisons of morphometric data (Klingenberg 1996). Hard or sclerotised structures that are less likely to change during the fixation process are very useful in species identification for Monogenea but these are usually limited to sclerites on the haptor (attachment organ), the male copulatory organ and associated accessory structures and occasionally, elements of the vagina. Geometric morphometrics incorporating landmark data have been used successfully to quantify intraspecific variation among microscopic hooks of five species of taeniid tapeworms (Gubányi 1996) and may represent a useful tool in monogenean discrimination and systematics. Also adding to the difficulties of parasite identification are problems associated with accurate host identification, especially when host species are cryptic (Chisholm *et al.* 2001a).

According to Cameron (1964), in the absence of biological information, many species of parasite cannot be discussed objectively. A series of papers published in the journal *Nature* by Dawes and Griffiths (1958; 1959) and Llewellyn (1959) illustrates this well for the Monogenea. In short, Dawes and Griffiths proposed that the now sister taxon to *Calicotyle* (Monocotylidae), *Dictyocotyle coeliaca*, was a coelom-dwelling form of the cloacal parasite *C. kroyeri*, which had lost its original haptor on entering the body cavity and had regenerated a new 'pseudo-haptor'. Debate surrounding the issue only ceased when Kearn (1970) demonstrated the validity of each species based on features of the eggs and larvae. The distribution of ciliated cells and sensilla on the surface of larvae revealed by staining live specimens with silver nitrate has also been used to separate other monogenean taxa (Lambert 1980; Chisholm 1998: Chapter IV). However, the complexities associated with rearing and handling larvae and/or maintaining infected hosts, have meant that these avenues of research are often ignored. In addition to its

relevance in fundamental biology, information concerning progressive parasite development from the juvenile to the adult stage, should also serve to minimise confusion surrounding parasite identifications and hence assist in determining degrees of specificity for particular host species (see Chapters V and VII).

Morphology and molecules - helping to solve the mystery

Where traditional morphology-based identification techniques have been inconclusive, molecular techniques have played an important and increasingly frequent role in the identification and systematics of parasites over the last decade. Molecular techniques, particularly those based on nucleic acid amplification, allow fast and accurate parasite identification using minimal amounts of sample material (Monis *et al.* 2002). Analyses to determine interrelationships of the Monogenea based on molecular data have been undertaken at the level of class (e.g. Mollaret *et al.* 2000; Olson and Littlewood 2002), family (e.g. Chisholm *et al.* 2001b for the Monocotylidae; Whittington *et al.* 2004 for the Capsalidae) and within genera (e.g. Cunningham 1997; Cunningham and Mo 1997; Desdevises *et al.* 2000; Chisholm *et al.* 2001a; Matejusová *et al.* 2003). Most of these studies have been based on specific regions of the nuclear rDNA gene cluster that contain highly conserved regions, as well as regions that are more variable (Hillis and Dixon 1991). The small subunit (16-18S) rRNA nuclear gene shows a high degree of sequence conservation and is useful for examining ancient evolutionary events, e.g. pre-Cretaceous (Hillis and Dixon 1991). This slow rate of change allows the use of 'universal' primers in polymerase chain reaction (PCR) amplification (Cunningham 1997). The large subunit (23-28S) rRNA nuclear gene contains more rapidly evolving regions than the small subunit rRNA in addition to regions that evolve as slowly as those in the small subunit (Hillis and Dixon 1991). As such, the large subunit rRNA can be used to distinguish phylogenetic relationships among more closely related organisms, e.g. within phyla (Hillis and Dixon 1991). The non-coding internal transcribed spacer (ITS) regions of the rRNA gene cluster are more variable than the coding regions and have been used to distinguish between congeneric and morphologically identical parasites (Cunningham 1997). These ITS regions are flanked by conserved regions of the 18S, 5.8S and 28S genes, allowing amplification via PCR (Hillis and Dixon

1991). Other universal, nuclear markers, such as the protein-coding gene, Elongation factor 1-alpha (EF1a), have also been used to elucidate ancient, familial and generic relationships among taxa (Berney *et al.* 2000 and references therein).

For examining recently evolved lineages, mitochondrial DNA (mtDNA) has been used extensively. The utility of mtDNA relative to nuclear DNA is attributed to several unique properties including: high copy number; high mutation rate; neutrality; little to no recombination; and rapid lineage sorting due to haploidy (Benesh *et al.* 2006 and references therein). Among the mtDNA genes, Cytochrome b (cytb) is particularly useful for phylogenetic work (Farias *et al.* 2001), although problems have been identified that may limit its usefulness, such as variable rates of sequence evolution (reviewed by Ballard and Whitlock 2004), and the presence of nuclear paralogues or pseudogenes (reviewed by Bensasson *et al.* 2001). Molecular analyses incorporating multiple genes or an amalgam of nuclear and mitochondrial markers to corroborate findings are advised. However, while the advent of molecular technology has proven an invaluable tool in the resolution of many systematic issues, it is not a universal panacea, so a combination of morphological and molecular data is advocated (Chisholm *et al.* 2001a: Chapter VIII).

Why specialise?

Factors opposing the evolution of specialisation are not difficult to envisage. For example searching for the 'right' host can take time, so the infective stages of a 'choosy' parasite may run an increased risk of dying before finding a host than a less 'choosy' one (Fry 1996). Sasal *et al.* (1999) listed four costs of being a specialist; i) increased risk of extinction if the host species becomes rare or disappears; ii) smaller available niche space; iii) greater susceptibility to elimination by the host due to exposure to a single immune system; iv) increased risk of mortality upon entering the 'wrong' host. In theory, the broader the range of habitats an organism is able to exploit, the greater its evolutionary potential should be (Desdevises *et al.* 2002). Yet in spite of these perceived drawbacks, specialisation is widespread and, according to Whitlock (1996), its evolution is to be expected. He reasons that species are more likely to persist in a particular habitat if they evolve faster in that habitat and that species can have faster rates of fixation of specifically beneficial alleles and slower

rates of accumulation of deleterious alleles if they evolve in fewer habitats. This is because in habitat-restricted populations, a higher proportion of gene copies with habitat-specific effects will be exposed to selection, whereas for populations spread across a range of habitats, the strength of selection on loci with environment-specific effects will be weaker (Whitlock 1996). This idea, now widely supported (see Fry 1996; Kawecki 1997; 1998), challenged the traditional view that genetic trade-offs are essential for specialisation to occur. The earlier 'trade-off' hypothesis implied that alleles responsible for improving fitness in one habitat would have a negative impact on performance in other habitats, i.e. loci responsible for genetic variation in fitness between two habitats would show antagonistic pleiotropy (Futuyma and Moreno 1988). However, as Kawecki (1997) observed, several studies indicate that genetic fitness on different hosts may be affected by different sets of loci rather than by alternative alleles at the same loci, suggesting a certain degree of genetic independence.

Phylogenetic versus ecological host specificity

Studies investigating parasite specialisation have essentially focused on congruence of parasite-host phylogenies, i.e. co-evolution. The underlying assumption here is that parasite speciation is constrained by the host, i.e. where hosts go, parasites will follow (Gemmill *et al.* 2000). Indeed, with respect to their hosts a certain degree of evolutionary conservatism must be a feature of all parasites because a parasitic mode of life dictates the prior existence of a host (Humphery-Smith 1989). But as pointed out by Brooks and McLennan (1993a), although parasite evolution will be correlated in some historical way with host evolution, it will not necessarily be causally connected with it. There are no general correlative patterns of host specificity and parasite speciation, indicating that host specificity is a product of parasite adaptation, not speciation (Brooks and McLennan 1993a). Indeed, recent studies have shown that phylogenetic specificity may not accurately represent the temporal length of a relationship (Hoberg and Klassen 2002). Non-congruence of parasite-host phylogenies is a common feature attributed to 'host switching' or 'host capture' events that reflect a parasite's ability to adapt to changes in its resource base (Brooks and McLennan 1993a). Such host switching events are thought to be

connected with the origins of several monogenean taxa (Boeger and Kritsky 1997) and are also thought to have promoted adaptive radiation in this species-rich taxon (Brooks and McLennan 1991). The ability of parasites to 'switch hosts' suggests that determinants other than host evolutionary history control parasite specificity (Desdevises *et al.* 2002). Links between specificity and ecological factors have been identified in parasite-host systems, with some associations attributed more to ecological factors than to genetically controlled compatibility with the host (Desdevises *et al.* 2002). However, to separate the effects of history from the effects of ecology, phylogenetic information is essential (Sasal *et al.* 1999: Chapter VIII).

How is specificity shaped in the course of evolution?

Limited dispersal and limited adaptation have been offered as explanations for habitat restriction (Timms and Read 1999). For example, parasites may have a limited host range because they are isolated either geographically or ecologically from other potential hosts (Jaenike 1993). In this case, a proportionate degree of specialisation is not implied (Gemmill *et al.* 2000). Alternatively, specialisation may arise through adaptive processes (Timms and Read 1999). Ward (1992) proposed three categories to explain the evolution of host specificity via adaptation: i) features of the host (e.g. behaviour, anatomy, physiology) that demand specific adaptation in the parasite; ii) competition or predation associated with broader habitats; iii) mate location. However, others argue that many morpho-physiological features of parasites should be viewed as adaptations subsequent to specialisation, rather than being determinants of it (Adamson and Caira 1994). Separating causes from consequences can be difficult though, because a factor can be the result of specificity, i.e. via adaptation, while also constraining later specialisation (Futuyma and Moreno 1988). At a fundamental level, the habitat restrictions of parasites will, to a greater or lesser extent, reflect the habits and specificities of their free-living ancestors (Adamson and Caira 1994). For example, ectoparasitism in the Monogenea is believed to have arisen from opportunistic browsing by free-living progenitors on the skin of early fishes (Kearn 1998). Indeed, the evolutionary origin of parasitism within the Neodermata has recently been inferred phylogenetically by Park *et al*. (2007).

External factors such as host phenology will also influence specificity among parasites. According to Poulin (1997) ecological differences make some hosts more susceptible to parasite colonisation than others. Even if two hosts are physiologically equivalent, different phenologies may select for different species of parasite (Adamson and Caira 1994). Where transmission is linked to host behaviour that exposes the parasite to various 'potential' host species, host switching events leading to non-specific host-parasite associations are considered the general rule (Poulin 2007). However, high host specificity has also been observed among dispersal-prone parasites of intermingling hosts (Dick and Patterson 2007). Using a simple mathematical model, Ward (1992) demonstrated that specialisation should be associated with predictable resources, i.e. those that are stable through evolutionary time to minimise the risk of extinction. Adding weight to this, Norton and Carpenter (1998) observed more generalist parasite species when hosts were rare and suggested that the key to specificity may be relative host abundance. They proposed a hypothetical threshold in relative host abundance below which generalism is favoured. However, the impact of relative host abundance on specificity will reflect the capacity of parasites to disperse in space and time (Tompkins and Clayton 1999). Life history characteristics such as whether transmission occurs actively or passively and the lifespan of the infective stage(s) are integral to this (see Chapters VI and VII), as well as being inextricably linked to the maintenance of specificity through evolutionary time.

How is host specificity maintained?

To complete their lifecycle, all parasites depend upon successful transmission to the definitive host. Yet transmission is a goal beset with many obstacles and associated with high mortality of infective stages. Where transmission relies on the ingestion of infective stages (e.g. cestodes, nematodes), specificity may be determined and maintained passively via the feeding habits of the host (Adamson and Caira 1994). However, this is not the case for parasites with direct lifecycles such as monogeneans with active infective stages. The larvae of these parasites are faced with the challenge of finding, recognising and attaching to the definitive host if the lifecycle is to be completed. Yet larvae are tiny (~150 μm long), with a mean

swimming speed of ~4 mms^{-1} and have a relatively short window of viability from the time of hatching (lifespan < 48 h) (Whittington *et al.* 2000). While the odds would not seem to favour success, the prevalence of many Monogenea in nature bears testament to the fact that they do succeed! Mechanisms that serve to optimise transmission success might therefore be expected, and indeed do appear, to have evolved (see Chapter VI).

Egg hatching in monogeneans has been linked to light periodicity, variations in light intensity, chemicals in host mucus and tissue, as well as mechanical disturbance (Kearn 1981). These stimuli, believed to relate either to the general habits of the host or to the presence of a host in the vicinity of an egg, should be highly predictable (Llewellyn 1972; Adamson and Caira 1994). In terms of transmission success, the survival value of hatching with a diurnal rhythm might be to keep the parasite in the potential host's active space (Rea and Irwin 1994). For instance, if larvae hatch at times that coincide with periods of host inactivity, they may be more likely to reach their target. Alternatively, predation by filter feeders at night has been suggested as a possible driving force behind diurnal hatching in some species (Whittington and Kearn 1986; Ernst and Whittington 1996). Where hatching is stimulated by chemical signals from a host, larvae may be able to conserve valuable energy reserves until a host is nearby. Analyses of skin and mucus extracts from certain teleosts have revealed several chemical associations that may comprise a host-recognition system for monogeneans of teleosts (Buchmann and Lindenstrøm 2002). Among several monogenean species from elasmobranch hosts, urea has been identified as an important hatching factor (Whittington 1987a).

Once hatching has taken place, the task of finding and recognising a host and navigating a path in or over it, is greatly dependent on the behaviour of the larvae (see Chapter VI). Factors such as light intensity and direction (phototaxis), water currents (rheotaxis), gravity (geotaxis), shadows and disturbance have all been demonstrated to elicit behavioural responses among monogenean larvae and may increase their chances of contacting a host (Llewellyn 1972; Kearn 1980). Specialised sensory structures such as sensilla and photoreceptors, as well as ciliated locomotory cells, equip larvae with the means to detect and affect responses to such stimuli (see Chapters III and IV). However, these stimuli may be generated by any number of sources in the sea, so larvae need to 'know' when contact with the 'right' host has been made. For parasites such as monogeneans with active transmission

stages, it is thought that more specific cues may be used in host recognition because they can actively search for alternative hosts (Adamson and Caira 1994). For example, changes were noted in the swimming behaviour of *Rajonchocotyle emarginata* (Hexabothriidae) larvae (a gill parasite of *Raja* spp.) when in the presence of host tissue (Whittington and Kearn 1986). Additionally, Hirazawa *et al*. (2003) proposed that the pH of host mucus may be a factor in host recognition by *Heterobothrium okamotoi* (Diclidophoridae) larvae for their tiger puffer host, *Takifugu rubripes* (Tetraodontidae). Still, the degree to which a larva can refuse a host will again be influenced by the probability of it finding one within its lifespan (Adamson and Caira 1994). While studies have shown that larvae are capable of responding to stimuli such as those mentioned above, Pike (1990) expressed concern that levels of stimuli administered experimentally might be significantly different to those to which a parasite would be subjected under natural conditions. He therefore questioned their effectiveness in improving transmission rates in the absence of knowledge regarding the sensitivity of the parasite to these factors or indeed the likelihood that the parasite may respond to spurious signals. However, the possibility exists that the response of larvae to certain 'generic' stimuli (e.g. shadowing, currents) may relate to predator avoidance. As such, transmission success may be improved indirectly via increased survivorship. Although much remains to be understood about the machinations of infection, successful transmission is likely to be the result of an interplay of factors operating over different scales. Each signal to which a larva is capable of responding may help to fine-tune the process of successful host infection. However, to become established following host invasion, a parasite must also be capable of evading or exploiting a complex array of potential host immune defences. Lectins, complement factors, antibodies, acute phase proteins, lysozyme and anti-microbial peptides represent the most well known fish host molecules capable of binding to monogenean epitopes and eliciting adverse, as well as benign reactions (reviewed in Buchmann and Lindenstrøm 2002). Cellular receptors, such as Toll-like receptors that bestow even primitive organisms with the capacity to identify non-self molecules are also thought likely to contribute to host specificity, if present in hosts and/or parasites (Buchmann and Lindenstrøm 2002).

Maintaining site specificity

As the site of host invasion may not be specific to a precise location, larvae are also faced with the challenge of reaching the definitive site on the host (Llewellyn 1972: Chapter VII) . This may involve a period of migration from the initial point of contact, during which some developmental changes occur. For example, Kearn (1984) showed that juvenile *Entobdella soleae* (Capsalidae), a monogenean skin parasite of the common sole, *Solea solea*, migrate from the upper surface of their host to the lower surface (where sexual maturity is reached and larger size attained), by travelling forward either directly or obliquely with respect to the fish. The key to successful migration will obviously depend on larvae 'knowing' when the correct destination has been reached. Although the mechanics of larval invasion and subsequent migration are not well understood, it is clear that larvae must, in some way, be guided in their journey. Orientation towards or away from graded signals during migration requires an organism to be able to recognise and respond appropriately to a signal gradient (Sukhdeo and Sukhdeo 2002). Although behavioural orientation has been investigated among parasites, it has not been substantiated. Orientation responses have been demonstrated among adult parasites towards conspecifics (Kearn *et al.* 1993), but not from host signals during habitat selection (Kearn 1984). Fixed behaviours are thought to offer greater insight into the workings of this interesting process.

Fixed behaviours comprise two basic groups: releaser responses and rhythmical activities. They refer to genetically determined stereotyped behaviours that evolve under environmentally predictable conditions (Krebs and Davies 1997). Specifically, releaser responses are triggered by sign stimuli that may consist of a small subset of environmental features. Releaser responses are frequently seen in host-finding behaviours, such as repetitive up and down swimming of larvae (Kearn 1980: Chapter VI). With respect to parasite migration behaviour, the definitive host represents a predictable environment, both in terms of topography and biology. As these conditions are considered favourable for the evolution of releaser responses, it has been proposed that fixed behaviours may play an important role in parasite migration (Sukhdeo and Sukhdeo 2002). First though, it is necessary to determine whether larvae do in fact migrate (see Chapter VII). For example, the larvae of the monogeneans *Neoheterocotyle rhinobatidis* and *Merizocotyle icopae*

(Monocotylidae) attach directly to the gills and nasal tissues respectively of the shovelnose ray, *Rhinobatos typus* (Rhinobatidae), with no migratory phase (Chisholm and Whittington 2003). Due to their minute size, finding a post-larval parasite on the host immediately following settlement is extremely difficult. For instance, despite extensive investigation, nothing is known of the invasion route of *Calicotyle* spp. (Monocotylidae) and whether there is a migration to their definitive sites in the cloaca, rectum and rectal gland of their elasmobranch hosts (see Kearn 1987 for *C. kroyeri*), nor for *Benedenia rohdei* (Capsalidae) a gill parasite of the teleost *Lutjanus carponotatus* (Lutjanidae; see Whittington and Ernst 2002). Experimental infections using fluorescent labelling should, however, permit easier detection of newly invaded larvae on the host. Such a technique has been applied to examine settlement behaviour in the monogenean *Heterobothrium okamotoi* from the gills of the tiger puffer, *Takifugu rubripes* (see Chigasaki *et al.* 2000), and more recently, to investigate host recognition and post-larval survivorship of *H. okamotoi* (see Ohhashi *et al.* 2007). However, no attempt has been made thus far to use a fluorescent marker to study settlement or migratory behaviour of any monogenean from an elasmobranch (but see Chapter VII).

My study

My study seeks to determine the specificity of three monogenean species from three families (Hexabothriidae, Microbothriidae and Monocotylidae), each occupying a different microhabitat (gills, skin and cloaca), respectively, on the same host species, *Trygonorrhina fasciata* (Rhinobatidae), in South Australia. Morphology and molecules are used to verify adult parasite identifications in order to reveal the range of rhinobatid host species parasitised by these monogeneans in Australian waters. Progressive parasite development from larva to adult is studied to elucidate taxonomic queries. Cues that promote egg hatching, in addition to larval morphology, behaviour and longevity are studied to provide valuable information regarding infection dynamics and to offer insight into the factors responsible for shaping and maintaining host and site specificity through evolutionary time.

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CHAPTER III

BRANCHOTENTHES OCTOHAMATUS SP. N. (MONOGENEA: HEXABOTHRIIDAE) FROM THE GILLS OF THE SOUTHERN FIDDLER RAY, *TRYGONORRHINA FASCIATA* (RHINOBATIDAE) IN SOUTH AUSTRALIA: DESCRIPTION OF ADULT AND LARVA

Vanessa Glennon[1], Leslie A. Chisholm[1] and Ian D. Whittington[1, 2]

[1]Marine Parasitology Laboratory, School of Earth and Environmental Sciences, The University of Adelaide, North Terrace, South Australia 5005, Australia

[2]Monogenean Research Laboratory, Parasitology Section, The South Australian Museum, North Terrace, South Australia 5000, Australia

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Glennon, V. (Candidate)
Corresponding author: Collected ray species, maintained rays in aquaria, reared parasite larvae, undertook all experimental techniques, conducted all analyses, wrote manuscript and produced all figures.

Signed……..Date……

Chisholm, L.A.
Sought and won funding, supervised the direction of study, assisted the collection of rays and parasites and evaluated manuscript.

I give consent for V. Glennon to include this paper for examination towards the degree of Doctor of Philosophy.

Signed……..Date……

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CHAPTER IV

A REDESCRIPTION OF *CALICOTYLE AUSTRALIS* JOHNSTON, 1934 (MONOGENEA: MONOCOTYLIDAE) FROM THE TYPE HOST *TRYGONORRHINA FASCIATA* (RHINOBATIDAE) OFF ADELAIDE, SOUTH AUSTRALIA, INCLUDING DESCRIPTIONS OF LIVE AND SILVER STAINED LARVAE

Vanessa Glennon[1], Leslie A. Chisholm[1] and Ian D. Whittington[1, 2]

[1]Marine Parasitology Laboratory, School of Earth and Environmental Sciences, The University of Adelaide, North Terrace, South Australia 5005, Australia

[2]Monogenean Research Laboratory, Parasitology Section, The South Australian Museum, North Terrace, South Australia 5000, Australia

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Glennon, V. (Candidate)
Corresponding author: Collected ray species, maintained rays in aquaria, reared parasite larvae, undertook all experimental techniques, conducted all analyses, wrote manuscript and produced all figures.

Signed……..Date……

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Systematic Parasitology, vol. 63 (1), pp. 29-40

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CHAPTER V

PSEUDOLEPTOBOTHRIUM APTYCHOTREMAE YOUNG, 1967 (MONOGENEA: MICROBOTHRIIDAE) REDESCRIBED FROM A NEW HOST, THE SOUTHERN FIDDLER RAY, *TRYGONORRHINA FASCIATA* (RHINOBATIDAE) IN SOUTH AUSTRALIA WITH A DESCRIPTION OF THE LARVA AND POST-LARVAL DEVELOPMENT

Vanessa Glennon[1], Leslie A. Chisholm[1] and Ian D. Whittington[1, 2]

[1]Marine Parasitology Laboratory, School of Earth and Environmental Sciences, The University of Adelaide, North Terrace, South Australia 5005, Australia

[2]Monogenean Research Laboratory, Parasitology Section, The South Australian Museum, North Terrace, Adelaide, South Australia 5000, Australia

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Glennon, V. (Candidate)
Corresponding author: Collected ray species, maintained rays in aquaria, reared parasite larvae, undertook all experimental techniques, conducted all analyses, wrote manuscript and produced all figures.

Signed……..Date……

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I give consent for V. Glennon to include this paper for examination towards the degree of Doctor of Philosophy.

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Glennon, V., Chisholm, L.A. & Whittington, I.D. (2006). *Pseudoleptobothrium aptychotremae* Young, 1967 (Monogenea, Microbothriidae) redescribed from a new host, *Trygonorrhina fasciata* (Rhinobatidae) in South Australia with a description of the larva and post-larval development.
Acta Parasitologica, vol. 51 (1), pp. 40–46.

CHAPTER VI

THREE UNRELATED SPECIES, THREE SITES, SAME HOST – MONOGENEAN PARASITES OF THE SOUTHERN FIDDLER RAY, *TRYGONORRHINA FASCIATA*, IN SOUTH AUSTRALIA: EGG HATCHING STRATEGIES AND LARVAL BEHAVIOUR

Vanessa Glennon[1], Leslie A. Chisholm[1] and Ian D. Whittington[1, 2]

[1]Marine Parasitology Laboratory, School of Earth and Environmental Sciences, The University of Adelaide, North Terrace, South Australia 5005, Australia

[2]Monogenean Research Laboratory, Parasitology Section, The South Australian Museum, North Terrace, South Australia 5000, Australia

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Glennon, V. (Candidate)
Corresponding author: Collected ray species, maintained rays in aquaria, reared parasite larvae, undertook all experimental techniques, conducted all analyses, wrote manuscript and produced all figures.

Signed……..Date……

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Sought and won funding, supervised the direction of study and evaluated manuscript.

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CHAPTER VII

EXPERIMENTAL INFECTIONS, USING A FLUORESCENT MARKER, OF TWO ELASMOBRANCH SPECIES BY UNCILIATED LARVAE OF *BRANCHOTENTHES OCTOHAMATUS* (MONOGENEA: HEXABOTHRIIDAE): INVASION ROUTE, HOST SPECIFICITY AND POST-LARVAL DEVELOPMENT

Vanessa Glennon[1], Leslie A. Chisholm[1] and Ian D. Whittington[1, 2]

[1]Marine Parasitology Laboratory, School of Earth and Environmental Sciences, The University of Adelaide, North Terrace, South Australia 5005, Australia

[2]Monogenean Research Laboratory, Parasitology Section, The South Australian Museum, North Terrace, South Australia 5000, Australia

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Glennon, V. (Candidate)
Corresponding author: Sought and won funding, collected ray species, maintained rays in aquaria, reared parasite larvae, undertook all experimental techniques, conducted all analyses, wrote manuscript and produced all figures.

Signed……..Date……

Chisholm, L.A.
Sought and won funding, supervised the direction of study, assisted with ray dissections and evaluated manuscript.

I give consent for V. Glennon to include this paper for examination towards the degree of Doctor of Philosophy.

Signed……..Date……

Whittington, I.D.
Sought and won funding, supervised the direction of study and evaluated manuscript.

I give consent for V. Glennon to include this paper for examination towards the degree of Doctor of Philosophy.

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Glennon, V., Chisholm, L.A. & Whittington, I.D., (2007). Experimental infections, using a fluorescent marker, of two elasmobranch species by unciliated larvae of *Branchotenthes octohamatus* (Monogenea: Hexabothriidae): invasion route, host specificity and post-larval development.
Parasitology, vol. 134 (9), pp. 1243-1252.

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CHAPTER VIII

HEXABOTHRIID, MICROBOTHRIID AND MONOCOTYLID MONOGENEA FROM *TRYGONORRHINA FASCIATA* (RHINOBATIDAE) IN SOUTH AUSTRALIA: ARE THESE PARASITES HOST-SPECIALISTS, OR GENERALISTS?

Vanessa Glennon[1], Elizabeth M. Perkins [1], Leslie A. Chisholm[1] and Ian D. Whittington[1, 2]

[1]Marine Parasitology Laboratory, School of Earth and Environmental Sciences, The University of Adelaide, North Terrace, South Australia 5005, Australia

[2]Monogenean Research Laboratory, Parasitology Section, The South Australian Museum, North Terrace, South Australia 5000, Australia

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Glennon, V. (Candidate)
Corresponding author: Sought and won funding, collected parasite specimens, undertook all experimental techniques, conducted all analyses, wrote manuscript and produced all figures.

Signed……..Date……

Perkins, E.M.
Provided comprehensive training and guidance in molecular methods, assisted collection of parasite specimens and evaluated manuscript.

I give consent for V. Glennon to include this paper for examination towards the degree of Doctor of Philosophy.

Signed……..Date……

Chisholm, L.A.
Sought and won funding, supervised the direction of study, assisted collection of parasite specimens and evaluated manuscript.

I give consent for V. Glennon to include this paper for examination towards the degree of Doctor of Philosophy.

Signed……..Date……

Whittington, I.D.

Sought and won funding, supervised the direction of study, assisted collection of parasite specimens and evaluated manuscript.

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International Journal for Parasitology, vol. 38 (13), pp. 1599-1612

CHAPTER IX

GENERAL DISCUSSION

CHAPTER IX

My discovery that the southern fiddler ray, *Trygonorrhina fasciata* (Rhinobatidae), is parasitised by three monogenean species in South Australian waters has been fortuitous. The local abundance of this rhinobatid species that survives well in aquaria, coupled with the fact that monogeneans have direct lifecycles, has provided an excellent, tractable, parasite-host model for research. Using this exceptional model, my study has contributed fundamental knowledge to two broad areas of parasitological research: biology and taxonomy. It is the first study to examine a suite of life history traits for monogeneans from different families, occupying different sites on the same elasmobranch host species.

In the absence of sound taxonomy, biological observations have little meaning. As such, taxonomy has comprised an important part of my study. Indeed my thesis begins and ends on taxonomic matters. I have described a new species of hexabothriid, *Branchotenthes octohamatus*, from the gills of *T. fasciata* (Chapter III) and have also described its larva and charted its post-larval development. My study of the larva revealed that *B. octohamatus* has only eight (four pairs) hooklets in the larval haptor, currently representing a unique condition among hexabothriids, as all others described possess ten (five pairs) hooklets. My subsequent studies on post-larvae (Chapter VII) indicated that it is hooklet pair III (see Llewellyn 1963 for numbering) that is lost from *B. octohamatus*. As hooklet number and arrangement are important characters in monogenean systematics, this taxonomic discovery may be useful to help resolve phylogenetic relationships within the Hexabothriidae, as well as offering insight into more general evolutionary hypotheses about familial relationships within the Monogenea.

A redescription of *Calicotyle australis* (Monocotylidae) from its type host, *T. fasciata*, was necessary (Chapter IV). The original description by Johnston (1934) was based on a single parasite specimen from an unspecified site on a host collected off Glenelg in South Australia. The larva was undescribed, so I also studied larval morphology by examining live larvae and used silver nitrate stain to reveal the number and arrangement of ciliated epidermal cells and sensilla.

Pseudoleptobothrium aptychotremae (Microbothriidae) was described by Young (1967) from the skin of *Aptychotrema rostrata* (Rhinobatidae), in Moreton

Bay, Queensland. I redescribed *P. aptychotremae* from specimens I collected from the skin of *T. fasciata* in South Australia that were morphologically indistinguishable from specimens collected from the type host (Chapter V). This is a new host and locality record for *P. aptychotremae*. Additionally, I described the eggs and larva and confirmed the presence of six spicules in the larval haptor of *P. aptychotremae*, as is also recorded for the larva of the microbothriid, *Leptocotyle minor* (see Kearn 1965). However, subsequent molecular analyses of *Pseudoleptobothrium* specimens I collected from the skin of rhinobatids at four Australian coastal locations (Chapter VIII) demonstrated a deep genetic divergence among material collected from *A. rostrata* and *Trygonorrhina* sp. A, off New South Wales, indicating the presence of a morphologically cryptic, ancestral species. Interestingly, all the specimens I collected from *A. rostrata* off New South Wales and Queensland belong to this ancestral group, so it is very likely that Young's (1967) description of *P. aptychotremae* also from *A. rostrata* in Queensland was based on specimens belonging to this ancestral clade. Further genetic study is required to determine whether the two eastern *Pseudoleptobothrium* clades are reproductively isolated, therefore representing separate species. If this hypothesis is supported, then my redescription of *P. aptychotremae* from *T. fasciata* in South Australia will need to be revisited to see if morphological differences between the specimens from the different rhinobatid hosts can be identified. Beveridge (2007) revisited material of the anoplocephalid cestode *Progamotaenia zschokkei* from different host macropod genera after genetic variation had been revealed and identified six new *Progamotaenia* species based on genetic and morphological characters.

From my study, the presence of different *Pseudoleptobothrium* clades from *Trygonorrhina* sp. A in New South Wales may prove to be a valuable 'test case' for sympatric speciation among parasites and has, therefore, broad significance. According to Coyne and Orr (2004, p. 142), four criteria must be met to provide a plausible case for species to have arisen in sympatry: "1. The species must be largely or completely sympatric. 2. The species must have substantial reproductive isolation, preferably based on genetic differences. 3. The sympatric taxa must be sister groups. 4. The biogeographic and evolutionary history of the groups must make the existence of an allopatric phase *very unlikely*." Determining whether the two eastern *Pseudoleptobothrium* clades are reproductively isolated (Criterion 2) represents a priority. Until this is known, Criterion 3 will be difficult to satisfy, as

non-sister taxa, that originated allopatrically, can appear to be sister taxa if secondary contact has resulted in hybridisation (Coyne and Orr 2004). The need to satisfy Criterion 4 will therefore depend on the results of this future genetic research. Like many biological concepts that are based on free-living organisms, the typical definition of sympatry can be difficult to apply to parasites (McCoy 2003). Indeed some argue that constraints imposed by a parasitic lifestyle are likely to make sympatric speciation less likely for parasites than for free-living organisms (Brooks and McLennan 1993). Kunz (2002) defined sympatric speciation as 'the origin of new species in the same geographic area'. In line with this definition, a host switch, provided host species are sympatric, would constitute sympatric divergence for the parasite species as well. However, this might also be interpreted as allopatric speciation (McCoy 2003). Site shifts represent another possible mechanism for sympatric speciation among parasites (Brooks and McLennan 1993). Littlewood *et al.* (1997) investigated this theory by examining the genetic relationships of site-specific polystome monogenean species. They compared species infecting the same microhabitats in different host turtle species with those infecting different microhabitats in the same host species. Their results showed species occupying the same sites on different host species were more closely related than those occupying different sites on the same host species and concluded that speciation was unlikely to have occurred in the same host species (Littlewood *et al*. 1997). The scenario I have identified among *Pseudoleptobothrium* on *Trygonorrhina* sp. A, where both genetic clades are present and occupy the same site on the host, therefore represents a significant and unusual opportunity to investigate the evolution of reproductive isolation among truly co-occurring parasite populations.

Having identified and described my subjects from *T. fasciata* in South Australia (Chapters III, IV and V), I was eager to uncover the secrets of their life histories (Chapter VI). My studies of these monogenean species revealed three very different egg hatching and host finding strategies as well as three very different larval 'types'. *Branchotenthes octohamatus* from the gills has a 'sit-and-wait' strategy, dependent entirely on mechanical disturbance to stimulate eggs to hatch. Larvae that emerge are unciliated and cannot swim, lack pigmented eyespots and show no photo-response but may survive for more than two days after hatching at 22 ºC. In contrast, eggs of *C. australis* from the cloaca hatch spontaneously with a strong diurnal rhythm within the first few hours of daylight when exposed to a LD12:12

illumination regime. Hatched larvae are ciliated and can swim, have pigmented eyespots, are photo-positive and can remain active and survive for up to 24 h after hatching at 22 ºC. Eggs of *P. aptychotremae* from the skin may have a 'bet-hedging' strategy. Some eggs hatch spontaneously and rhythmically in an LD12:12 regime during the last few hours of daylight but their low hatching success rate suggests that other eggs may require a different cue provided by the host. Hatched larvae are ciliated and can swim, lack pigmented eyespots, show no photo-response and remain active for only a few hours at 22 ºC.

My comparison of life history traits among unrelated monogenean species from different sites on the same host species revealed major differences. However, as *B. octohamatus, C. australis* and *P. aptychotremae* are united in their choice of host species near Adelaide, should similar egg hatching strategies have been expected? Whittington (1987) reported similar hatching strategies for *Leptocotyle minor* (Microbothriidae) and *Hexabothrium appendiculatum* (Hexabothriidae), from the skin and gills respectively of the common dogfish, *Scyliorhinus canicula* (Scyliorhinidae). Eggs of these species hatched only in response to host skin secretions. Yet Kearn *et al*. (1992) observed differences in the hatching strategies of *Benedenia seriolae* (Capsalidae) and *Heteraxine heterocerca* (Heteraxinidae) from the skin and gills respectively of Japanese yellowtail, *Seriola quinqueradiata* (Carangidae). While eggs of both species hatched spontaneously, they did so at different times of the day. Other, separate studies have also shown different egg hatching strategies for the unrelated species *Acanthocotyle lobianchi* (Acanthocotylidae) from the skin (see Macdonald 1974), *Dictyocotyle coeliaca* (Monocotylidae) from the body cavity (see Kearn 1975) and *Rajonchocotyle emarginata* (Hexabothriidae) from the gills (see Whittington and Kearn 1986) of the cuckoo ray, *Leucoraja naevus* (Rajidae) (= *Raja naevus*). Explanations offered for the different strategies observed among these unrelated monogenean species infecting different sites on the same host species include the lack of a well-defined daily activity rhythm in the host (Whittington and Kearn 1986), and/or the sites of host invasion, the theory being that some sites may be more (or less) accessible to larval invasion at certain times of the day (Kearn *et al*. 1992).

I investigated the larval invasion site for *Branchotenthes octohamatus.* Unlike the free-swimming larvae of *C. australis* and *P. aptychotremae* that can actively 'search' for a host, *B. octohamatus* larvae are unciliated and cannot swim.

For infection to occur, *B. octohamatus* depends on a host approaching eggs and/or recently hatched larvae, making it a 'passive' host finding strategy. I used the fluorescent dye, 5(6)-carboxyfluorescein diacetate *N*-succinimidyl ester (CFSE) (Chapter VII) to facilitate visual identification of newly settled post-larvae on host tissue. This was the first use of this technique on a monogenean species with unciliated larvae and the first for any monogenean larvae infecting an elasmobranch host. CFSE-labeled post-larvae were found on the gills of *T. fasciata* within 30 min of exposure to the host, providing strong evidence that larvae invade host gills directly via the host's inhalant respiratory current and do not migrate on the host after initial attachment elsewhere. As discussed in Chapter VII, direct invasion of the gills via the host's inhalant respiratory current has been shown for *Diplozoon paradoxum* (Diplozooidae; see Bovet 1967), *Discocotyle sagittata* (Discocotylidae; see Paling 1969; Gannicott and Tinsley 1998) and also indicated for *Neoheterocotyle rhinobatidis* and *Merizocotyle icopae* (Monocotylidae; see Chisholm and Whittington 2003). Inspiration of eggs which become entangled on host gills and then hatch in the presence of host tissue, has also been proposed as a mechanism of infection for some monogeneans (e.g. *Microcotyle salpae* (Microcotylidae); see Ktari 1969). As *B. octohamatus* eggs are laid end-to-end forming long chains, inspiration of eggs by the host and subsequent entanglement on the gills is possible. However, unlike the eggs of *M. salpae* that hatch following contact with host gills (Ktari 1969), *B. octohamatus* eggs hatch in response to mechanical agitation (Chapter VI). Therefore, suspension of fully embryonated *B. octohamatus* eggs in the water column just prior to inspiration is likely to induce hatching before contact with gills occurs. However, if eggs are inspired and become caught on the gills during embryonation, then this could feasibly represent another mechanism of infection for *B. octohamatus*.

To date nothing is known about the path(s) taken by *Calicotyle* spp. to their definitive sites in the cloaca, rectum and rectal gland of their elasmobranch hosts. Kearn (1987) determined that *C. kroyeri* develops in the rectal gland of various *Raja* spp. (Rajidae) but despite intensive searches for post-larvae in scrapings from internal and external host sites, the invasion route could not be determined. How the larvae of *C. australis* reach their definitive site (i.e. do they have an internal or external path of migration to the cloaca?), therefore remains an intriguing question which could be answered in the future using the CFSE technique.

Despite a shared goal to infect the same host species, it is clear that no generalisations or predictions can be made about the egg hatching and host finding strategies that unrelated monogenean species will employ. Perhaps similar strategies are more likely to be found among related monogeneans, occupying the same or different sites on the same or different host species. For instance, eggs of the capsalid monogneans *Benedenia seriolae* (skin) from *Seriola quinqueradiata* (see Kearn *et al*. 1992), *B. lutjani* (skin) and *B. rohdei* (gills) from the yellow stripey, *Lutjanus carponotatus* (Lutjanidae, see Ernst and Whittington 1996), all hatch spontaneously and have ciliated larvae. Similarly, the monocotylid monogeneans *Neoheterocotyle rhinobatidis* (gills), *Troglocephalus rhinobatidis* (gills) and *Merizocotyle icopae* (nasal tissue) from *Rhinobatos typus* (Rhinobatidae), also hatch spontaneously and have ciliated larvae (Chisholm and Whittington 2000). I have found *C. australis* from *T. fasciata* to conform to this pattern for the Monocotylidae. However, given their phylogenetic affiliation, these similarities among closely related taxa are not overly surprising. Yet the pattern is not consistent. For example, among the gill-dwelling Hexabothriidae, egg hatching may be spontaneous (e.g. *Rajonchocotyle emarginata*), in response to host skin secretions (e.g. *Hexabothrium appendiculatum*), the presence of host tissue (e.g. *Squalonchocotyle torpedinis*), or following mechanical disturbance (e.g. *Branchotenthes octohamatus*), and ciliated, as well as unciliated larvae have been reported (Euzet and Raibaut 1960; Ktari and Maillard 1972; Whittington and Kearn 1986; Whittington 1987; Chapters III, VI and VII). These fascinating differences, in concert with other life history information, may allow adaptive traits arising from environmental selective pressures to be separated from the effects of phylogeny.

It is important to bear in mind that while ultimately successful, the contrasting strategies of the three monogenean species from *T. fasciata* in South Australia may not be equally 'efficient' at uniting larvae with their host (Chapter VI). Whether a parasite or a free-living organism, selection favours those who transmit the most copies of their genes to future generations (Rea and Irwin 1994). For a parasite, successful transmission to the host, followed by post-infection survival of a minimum number of individuals, is critical. However, a low individual infection success rate or high post-infection mortality may be offset by high fecundity, fast embryonation time, a short pre-patent period and/or greater longevity. Even where individual fecundity is low, shorter generation times may permit populations to

achieve high reproductive potential (Skorping *et al.* 1991). Different embryonation times at the same temperature have already been revealed for the three monogenean species from *T. fasciata* during my hatching experiments (Chapter VI). Differences in adult body size and egg laying strategies i.e., whether eggs are laid singly or in long chains, have also been identified. These traits have potential to influence egg output and may reflect parasite longevity. For instance, my development study showed that *B. octohamatus* appears to reach sexual maturity after 91 d at 21–25 ˚C at which time it measured ~5,400 µm TL (Chapter VII). However, adults of this species can measure up to 10,500 µm TL (Chapter III). For *B. octohamatus*, the fecundity advantage of large size may be a trade-off against delayed maturity (see Stearns 1992) and may indicate a long lifespan. During egg production, the appendages of adjacent *B. octohamatus* eggs fuse to form a chain and are retained within the uterus. Body size will therefore have a direct effect on the number of eggs comprising a chain and potentially how long they can be stored. This may be important if egg release is timed for certain periods of the day or night (e.g. *Zeuxapta seriolae* (Heteraxinidae; see Mooney *et al*. 2006). Such egg-laying rhythms may correspond to aspects of host phenology which help to increase the chances of larvae encountering a suitable host upon hatching (Kearn 1986). Further research on *B. octohamatus*, *C. australis* and *P. aptychotremae* is now required to place knowledge gained so far into context with other life history data.

The site occupied by a parasite on/in the host may have a significant part to play in post-infection survival and therefore also in shaping parasite life histories. For example, the threat of predation by cleaner fish is thought to have exerted strong selection pressure for a switch to more internal sites by some monogeneans during the course of evolution (Kearn 1994; Euzet and Combes 1998). Alternatively, parasite species occupying internal sites may be subjected to more potent host immune responses than less invasive species. Although the ectoparasitic monogeneans are considered to have only limited contact with the host immune system (Adamson and Caira 1994), as yet unidentified differences may exist in the degree to which *B. octohamatus*, *C. australis* and *P. aptychotremae* are subjected to host immune defenses in their respective microhabitats or through their diets. For example, *B. octohamatus* feeds on host blood, directly exposing the gastrodermis to host antibodies, complement factors and immunologically competent cells (Buchmann and Lindenstrøm 2002). *Calicotyle australis* and *P. aptychotremae* are

believed to feed on host epithelia, so they too will ingest potentially deleterious substances present in the epidermis including mucous cells (Buchmann and Lindenstrøm 2002). The severity of a host immunological reaction to parasitic infection may be linked to infection intensity and may therefore influence whether a parasite adopts a host 'specialist' versus 'generalist' strategy (Anderson 1982; Wakelin 1984).

The host species used by a parasite represents a fundamental life history characteristic and is the equivalent of resource specialisation in free-living organisms (Poulin 2007). However, this important information is generally unavailable for most parasite species. Of all potential host species, only a subset will ever be encountered by a parasite and of those encountered, not all will be suitable (Combes 1991). My study has confirmed the range of potential rhinobatid host species encountered by *B. octohamatus*, *C. australis* and *P. aptychotremae* in Australian waters. It has also revealed differences between these monogenean species with respect to their compatibility for the rhinobatids they may encounter as potential hosts (Chapter VIII).

Cytochrome b data confirmed that all *Branchotenthes* specimens recovered from the gills of rhinobatids surveyed are conspecific. This species has a geographic distribution that at least extends from Fremantle in Western Australia to Moreton Bay in Queensland across four rhinobatid species and is not, therefore, host specific. Similarly, all *Calicotyle* specimens collected from the cloaca of the rhinobatid species surveyed also appear to be conspecific, with a geographic range as extensive as that of *B. octohamatus*. While slight genetic divergence was observed between south-west and east Australian *C. australis* populations, the level of variation is extremely small compared to the outgroup (a *Calicotyle* species from México). Therefore, it appears unlikely that the two Australian *Calicotyle* populations on rhinobatids represent sister species at present. However, further investigation within the Bass Strait region is certainly warranted.

In contrast, a preference by *Pseudoleptobothrium* for *T. fasciata* over *A. vincentiana* within the south-west region of Australia has been indicated by my results. This monogenean was found on *T. fasciata* in Fremantle (WA) and Adelaide (SA) but was absent from *A. vincentiana* at these locations. Host preferences are also indicated for the two *Pseudoleptobothrium* populations on the east coast. As discussed previously, these two populations, that may be sister species, are loosely

correlated with host species (*A. rostrata* and *Trygonorrhina* sp. A). For *Pseudoleptobothrium*, it appears that more rhinobatid host species are encountered than exploited, whereas for *Branchotenthes* and *Calicotyle*, encountered rhinobatids are generally exploited. These differences may, in part, be the product of disparate abilities of the larvae to find and infect a host species. For example, my infection study has indicated that *B. octohamatus* enters the host passively via the host's inhalant respiratory current (Chapter VII). Parasites that enter the host through the mouth (ingested or otherwise) are generally predicted to be less host specific as this passive mode of transmission does not allow discrimination between encountered, potential host species (Poulin 2007). *Branchotenthes octohamatus* larvae are constrained by morphology to infect the host passively and my findings support the 'generalist' strategy predicted. However, *C. australis* larvae are highly motile. This motility suggests that a greater number of host species may be encountered than exploited (Poulin 2007), yet *C. australis* also appears to be a rhinobatid host generalist. This, again, may relate to the route of host infection. Although yet to be established, *C. australis* larvae may also enter the host via the mouth and then migrate internally to the rectal gland and cloaca. For example, the ciliated, active larvae of *Diplozoon paradoxum* stop swimming in a host's inhalant current and are carried passively into the buccal cavity (Bovet 1967). So while highly motile larvae are not morphologically constrained to adopt a passive host infection strategy, it cannot be presumed that they will not, and this may be the case for *C. australis*. The definitive site occupied by a parasite on the host is also relevant and may account for the differences observed between *C. australis* and *P. aptychotremae*, even though both of these species have motile larvae. *Pseudoleptobothrium aptychotremae* live on the skin of the host so it would seem fair to assume that larvae settle on the host surface directly. This liberty implies greater potential for pre-settlement host discrimination and may lead to fewer mistakes, resulting in higher host specificity.

In conclusion, my unique monogenean parasite-elasmobranch model has allowed me to investigate factors associated with egg hatching, larval morphology, larval behaviour and longevity to provide valuable information regarding infection dynamics at the egg, pre- and post-larval settlement stages. Progressive parasite development from larva to adult has been studied to clarify taxonomic queries and adult parasite taxonomy has been scrutinised (using morphology and molecular genetics) to determine host specificity. Molecular genetics has not only served to

address the important issue of host specificity for these monogenean parasites but has also increased our knowledge of biological diversity in Australian waters. Furthermore, connectivity between host and parasite populations, as well as the possible existence of latitudinal gradients in parasite-host distributions has been indicated. The fundamental taxonomic and biological knowledge gained by my study provides a foundation for further research to elucidate possible habitat-specific effects on the evolution of parasite life histories, and to gain a greater understanding of the parasite-host relationship. Comparative studies across taxa have much to contribute toward our understanding of parasite evolution. However, comparisons should not be limited to one or two life history traits as limited comparisons may reveal similarities but will not necessarily divulge their origin, i.e. whether due to an inherited trait, or a response to selective pressures imposed by the environment (Poulin 2007). An integrated approach, wherein a range of life history data is obtained, is therefore essential.

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Vanessa Glennon
Ph.D. student
Marine Parasitology Laboratory
Darling Building (DP418)
School of Earth and Environmental Sciences
The University of Adelaide
South Australia 5005
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