CHAPTER 1 INTRODUCTION

1.1 Introductory Remarks

Is a phylogenetic association present between Australian freshwater fish hosts and their monogenean parasites? If so, is this an example of coevolution, phylogenetic tracking or some other phenomenon? In this thesis I explore these questions.

Often the presence of a strong association between hosts and parasites has been loosely referred to as coevolution. However, coevolution as defined (page xvi) may not be the most appropriate term to use since other terms such as cospeciation and phylogenetic tracking may equally describe the association. The first association has a reciprocal host/parasite interaction; the second may or may not, while the third association has no reciprocal interaction. Phylogenetic associations are present when coevolution or phylogenetic tracking occurs while a phylogenetic association is absent when cospeciation without coevolution or phylogenetic tracking occurs (Blair *et al.*, 2001). Since studies of host-parasite associations rarely identify any host/parasite causal mechanisms (they are usually inferred or assumed), a general term such as 'phyletic association' is needed. Thus, coevolution, phylogenetic tracking and cospeciation are particular forms of phyletic association between unrelated organisms. Coevolution and phylogenetic tracking should only be used when the nature of the association is established or inferred, otherwise phyletic association should be used.

Studies on phyletic associations (coevolution, phylogenetic tracking and cospeciation) typically use comparisons of the phylogeny of the hosts with an independently derived phylogeny of the parasites. Where a single parasite species inhabits a single host species (i.e. is mono-host-specific), comparisons of phylogenies will provide information on the

extent of the phyletic association. However, it is often the case that a species of parasite inhabits more than one species of host. This can complicate interpretation of the results, but provides an opportunity to discuss phenomena such as host addition, host-switching, rates of cospeciation.

Studies on phyletic associations can be confounded by taxonomic problems and this is often an under-acknowledged problem. Taxonomic understanding of the host group, if vertebrates, is generally better than of the parasite group. Here, detailed taxonomic work on previously undescribed parasites is presented. This is based on both morphological and morphometric data. Mention will also be made of problems with the taxonomy of the hosts, especially hybrids. Another problem lies in assessing the degree of host-specificity of a particular parasite species. This problem has several dimensions to it. First is the difficulty of being sure that similar parasites from different fish are indeed the same species (or not). This relates to the problem of taxonomy and inter and intraspecific variation. The remaining dimensions/aspects concern sampling of hosts for parasites. For a single host species, how many individuals need to be collected at a single location and does more than one location need to be sampled? What properties of host individuals or species might influence the numbers of parasite individuals they harbour and hence the likelihood that all parasite species are sampled for that host species? Do inter-specific parasite interactions affect apparent host-specificity? The purpose of the first part of Chapter 1 is to introduce these ideas more fully and explain how they are relevant for the study reported here.

1.2 Phyletic Associations between Host-Parasite Phylogenies

The evolutionary association between a host and a parasite is referred to as coevolution when the population genetic interactions are such that a genetic change in one elicits a reciprocal change in the other, and should strictly be used to describe the evolutionary arms race scenario (Blair *et al.*, 2001). This association should also elicit cospeciation where speciation in the host, sooner or later, produces a speciation event in the parasite, producing congruence in their phylogenies. However, congruence in host and parasite phylogenies may also occur without one-for-one cospeciation.

Individual parasite species may infect several members of a host clade via the process of delayed speciation. Parasite speciation has often been shown to lag that of their host (eg. Brooks, 1987). This can be seen in ancyrocephalines of *Cichlidogyrus*. The explosive divergence of cichlid hosts, particularly *Haplochromis* species, in east African lakes has not produced a corresponding acceleration in speciation of their monogenean parasites (El-Naggar & Serag, 1985). If the parasite species phylogeny is congruent with the species clades within the host phylogeny, then many examples of host addition or host-switching may instead be a consequence of delayed parasite speciation.

Congruence between host and parasite phylogenies may also occur via phylogenetic tracking. Price (1980) takes the view that the short generation time, large populations (relative to those of the host), and isolated subpopulations of parasites combine to produce very high evolutionary potentials and that parasites may track evolutionary changes in the host or adapt to new conditions very quickly. This ability or need to track evolutionary changes in hosts may have led to the high host specificity found in certain parasite groups.

Phyletic associations (coevolution, cospeciation, phylogenetic tracking) between hosts and parasites are identified by examining the congruence between their phylogenetic patterns (cladograms or dendrograms). These independently derived phylogenetic patterns are usually produced from molecular or morphological data using phenetic or cladistic techniques. Previous species-level phylogenetic studies on monogeneans have used morphological characters coded into binary characters (Guégan & Agnèse, 1991; Klassen, 1992, 1994b; Klassen & Beverley-Burton, 1988; Wheeler & Beverley-Burton, 1989). A cladistic study using 62 species of ancyrocephaline monogeneans produced insufficient resolution among terminal taxa (Klassen, 1994a). Only 21 apomorphic character states were recognised for the 62 species of the ingroup (as 20 terminal taxa). Identifying sufficient character states would be especially difficult in congeneric species that show little morphological variation. Such an example is the species complex of *Gyrodactylus* where species identification is at the micro-morphometric level. At this level, species identification is often difficult (eg. *G. salaris*). Phylogenetic studies of monogeneans using molecular based methods (DNA) is still in its infancy (eg. Bruno *et al.*, 2001; Cable *et al.*, 2000; Cable *et al.*, 1999; Collins & Cunningham, 2000; Cunningham, 1997; Cunningham *et al.*, 2000; Cunningham & Mo, 1997; Cunningham *et al.*, 2001; Matejusová *et al.*, 2001a; Matejusová *et al.*, 2001b; Zietara *et al.*, 2000) and this approach is not examined in this study. Since molecular based studies are not examined here and there are generally a limited number of morphological characters available for cladistic studies of monogenean evolution at the species level, are there any alternatives for producing parasite phylogenies? The answer is yes and two alternatives are examined in the next section.

1.2.1 Host Phylogenies and Parasite Species Distribution on Host Species

The ideal model for considering host-parasite coevolution would be a set of closely related host species and a set of closely related parasite species exhibiting a strict one-host species to one-parasite species association. Rarely, however, is such a strict association (or specificity) encountered in nature including among monogenean parasites and their hosts (Morand *et al.*, 2002) (although see Section 1.5). Interestingly, however, congruence between the pattern of parasite association on hosts and host phylogeny can still be present. An examination of the distribution of parasite species (treating presence/absence of parasite species as characters) from six host species of Chaetodontidae, identified two most-parsimonious trees for the hosts (Morand *et al.*, 2002). One tree had exactly the same topology as the molecular tree of the hosts. The second tree gave a slightly different topology. A similar study on the association between 21 *Lamellodiscus* species (Diplectanidae) and their 16 sparid fish hosts (Desdevises *et al.* unpublished in Morand *et al.*, 2002) found almost no mono-host-specificity yet congruence of parasite association (presence/absence) on hosts with host phylogeny was present. Their conclusion was that "*species structure of monogenean communities does not seem to be the result of stochastic processes, rather that the evolutionary histories of both hosts and parasites may have a real importance in the species composition of communities*". Can this be explained as a possible case of delayed parasite speciation which may reflect the associations between species within the host phylogenetic clades? The use of parasite species distribution among host species for identifying phyletic associations is examined in Chapter 9.

1.2.2 Host Phylogenies and Host-Associated (induced?) Morphometric Variation of Parasite Haptoral Structure

To recognise evolutionary associations between hosts and parasites a high level of relevant signal must be present in the parasites' morphology. One way of identifying this association is to examine the interface between the host and parasite, this interface being the host gill structure and the monogenean haptoral sclerites. A physical change in the structure of the attachment site of the host requires a change in the parasite attachment structure. The posterior attachment organ and its associated sclerites are often adapted to fit a particular host attachment site (Chisholm *et al.*, 1998; Kearn, 1966; Llewellyn & Simmons, 1984; Simková *et al.*, 2001b). An association has also been reported between host species and morphometric variations of the haptoral sclerites in their monogeneans (Huyse & Volckaert, 2002). This host association was used to split *Gyrodactylus arcuatus* into several species (Geets *et al.*, 1999). These results would seem to imply that the mechanics and architecture of the haptoral sclerites are adaptations to host species and hence this variation may contain host phylogenetic signal. This would seem highly likely since the monogenean attachment organ and their host attachment site represent the interactive interface in any arms race or phylogenetic tracking. Thus the analysis of morphometric variation among parasite species may infer a parasite and/or host phylogeny.

Comparison between this kind of parasite-derived phylogeny and the host phylogeny derived from other sources of data has never been attempted in monogenean-host studies. Since morphometric variation of the haptor for a parasite species, as shown above, is often a function of their host species then parasite intraspecific-micro-morphometric variation may also infer a host phyletic association. To examine the host-

parasite phyletic associations at this level, the ideal situation would be to examine a parasite species or set of species with low host-specificity. This low specificity has to be due to delayed parasite speciation (see Section 1.2) without any host addition. Because little morphometric variability is expressed at the intra-specific level, how can a phylogeny be produced? These questions are examined in Chapter 9.

1.3 Cospeciation between Hosts and Parasites

Congruence of the association between species of hosts and species of parasites has often been attributed to synchronous cospeciation. This term describes those cases in which host speciation and parasite speciation are approximately contemporaneous (Model B, Figure 1.1. Delayed cospeciation might occur whereby speciation in the parasite may lag behind that of the host (Manter, 1955). In such circumstances, basal host lineages should have more associations attributable to cospeciation than derived host lineages (Model A, Figure 1.1) (i.e. basal lineages have parasite species of mono-host-specificity while derived lineages have parasites of low host specificity). Speciation in the host may also lag behind that of the parasite (Brooks & McLennan, 1993; Hafner & Nadler, 1988) (i.e. multiple congeners of parasite would occur on a single host species for both basal and derived lineages) (Model C, Figure 1.1). Thus all parasite species would be mono-host-specific. In Chapter 9 I test the hypothesis that infection of multiple host species by a single widespread parasite species may be the result of delayed cospeciation.

1.4 Parasite Taxonomy

In the previous section, I explored the possibility of using parasite distribution and morphometric variation for identifying host-parasite phyletic associations. However,

before this can be examined two issues need to be addressed: the taxonomy of the parasites and their host-specificity.

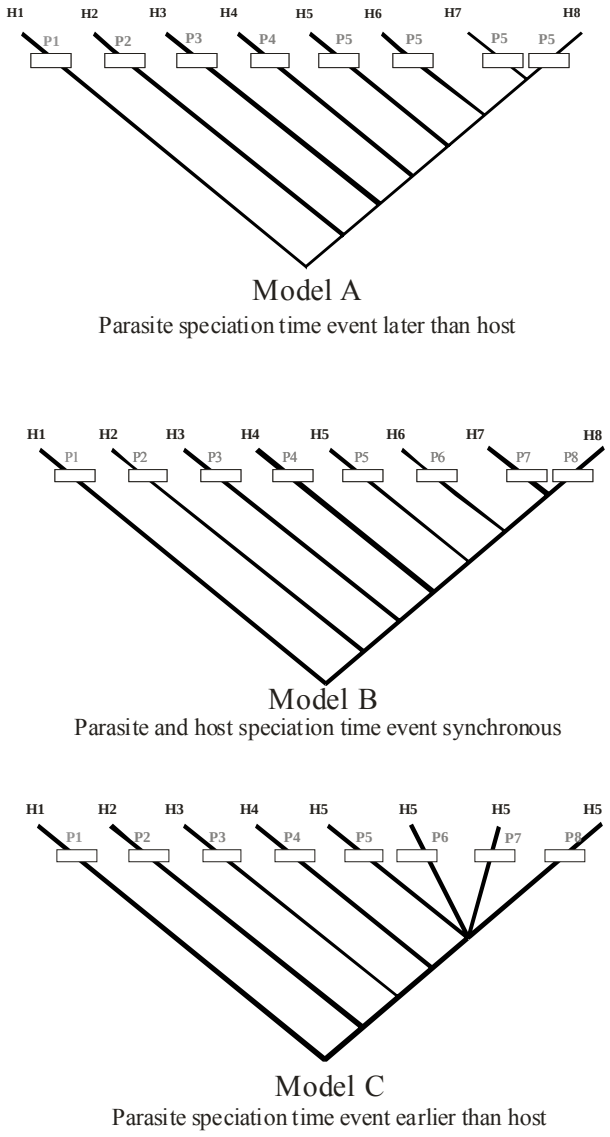


Figure 1.1. Models of cospeciation.
Model A: parasite speciation lags host speciation. Model B: parasite speciation is synchronous with host speciation. Model C: host speciation lags parasite speciation.

1.4.1 Taxonomy

An accurate taxonomic framework is essential for studies of evolutionary association. This applies at the level of genera and of species. What morphological features of a parasite are important in taxonomy? Should ancyrocephaline species be grouped into

genera using the characters of the copulatory apparatus or the haptoral structure or both? These two sets of morphological characters are often applied to define genera of Ancyrocephalinae to which species newly described in this thesis belong. Firstly, Bychowsky & Nagibina (1978) considered morphological characteristics of the haptor structure and position of vagina, vas deferens, ovary and testis important in separating genera of the subfamily and these characters have been the main basis for many taxonomic groupings of ancyrocephaline monogeneans.

Secondly, copulatory apparatus structure, which was not mentioned by Bychowsky & Nagibina (1978), is now having a greater role in defining genera than previously. It was recognised early in monogenean taxonomy that diagnostically important sexual characters have more taxonomic value than the sporadic occurrence of spines on, or differences in size or shape of certain haptoral parts (e.g. Mizelle & Hughes, 1938; Young, 1968). Young (1968) recognized the importance of minor variations in copulatory apparatus morphology and used these to group species of *Haliotrema.* However, he did not go so far as to separate these groups into different genera. The reclassification of the Nearctic genera of Ancyrocephalinae used minor variations in copulatory apparatus morphology and identified several copulatory apparatus types (Beverley-Burton & Suriano, 1980a, b; Beverley-Burton & Klassen, 1990; Suriano & Beverley-Burton, 1982). More recently, copulatory apparatus morphology has been used to classify the groups of *Haliotrema* species from the marine boxfish (Klassen, 1991). In this thesis I compare the two approaches to genus-level classification and this is addressed in Chapter 4.

In Chapter 5 I extend the analysis to examine parasite species variation on different hosts and validation of species described in Chapter 4. When defining species, characters of the haptor rather than of the copulatory apparatus tend to be used because the former are generally more easily identified and measured. Because haptoral characters are easily measured, a statistical approach can be used. Analytical tools such as Discriminant Function Analysis (DFA), Principal Component Analysis (PCA) and Multivariate Analysis (MANOVA) are often used to distinguish among species and genera using morphometric variation of characters. However, such methods have been little used in studies of monogenean species. Klassen (1991) used MANOVA and DFA to confirm species of *Haliotrema* described from boxfish while MANOVA and PCA were used to identify species of *Gyrodactylus* from salmonid hosts (Shinn *et al.*, 2000). I will apply a statistical approach, using MANOVA, PCA, DFA and Hierarchical cluster analysis (HCA), to confirm or refute an association between haptoral sclerite variation and the genus-level and species-level classification based on morphology.

1.5 Host-Parasite Specificity

Specificity in host-parasite associations is the level of restriction of parasites to certain host species. Although this degree of association differs between parasite species, it is often said to be high between monogenean parasites and their fish hosts (e.g. Poulin, 1992). A survey of 435 species of marine monogeneans found 78% were restricted to one host species, 89% were restricted to one genus, 96% to one family and 98% to one order (Rohde, 1993). Poulin (1992) found a lower level of host specificity for parasites of freshwater fishes of Canada (65.3% restricted to one host species). Two types of specificity are recognised. Parasite taxa that infect a single host taxon or closely related taxa are said to show phylogenetic host specificity (Rohde, 1993); parasites having a

wide host range but with certain host preferences, usually determined by the ecological requirements of the host, show ecological specificity (Rohde, 1993).

High host specificity would seem to imply a high level of phyletic association with the host group and hence a high congruence between host and parasite phylogenies is expected. Because high host-specificity is often assumed, monogenean parasites and their teleost hosts have been cited as ideal model systems for studying "coevolution" (Carney & Dick, 2000). However few studies have actually used monogeneans to study species-level associations and fewer have detected any "coevolution" or phyletic association between monogeneans and their fish hosts. A recent review of several publications and ongoing studies concerning monogenean communities (Morand *et al.*, 2002) has shown that a "cospeciation" pattern between parasites and host has rarely been demonstrated, although it has often been inferred (Beverley-Burton, 1995; Boeger & Kritsky, 1997; Van Every & Kritsky, 1992). This has been shown for hosts of Centrarchidae, especially species of *Lepomis* (Beverley-Burton & Klassen, 1990). Therefore, if high specificity does not reflect coevolution, is high host specificity apparent rather than real? In Chapter 7, I explore the nature of the monogenean distribution among their host species.

1.5.1 Host Sampling Considerations

The reported degree of host specificity is often a reflection of host sampling intensity. A sample of nine host specimens gives 95% confidence of recording a parasite species occurring in a population of hosts at a prevalence of 28% or more (Cribb *et al.*, 1994; Post & Millest, 1991). Sampling effort among host species can either generate spurious patterns or it can mask existing ones (Poulin, 1995, 2001a; Walther & Morand, 1998).

Low intensity sampling of host species and limited numbers of sample sites might tend to suggest a higher level of specificity for their parasites than if sampling intensity is higher. The level of sampling intensity required to detect all parasite species would also depend on the type of distribution the parasites exhibit. A higher sampling intensity would be required for parasites that exhibit an aggregated distribution rather than a regular or random (Poisson) distribution. Similarly, a more even distribution would require smaller sample intensities.

1.6 Parasite Community Structure

The ability to identify levels of host-specificity may also be aided by understanding the structure of parasite component communities. As mentioned above a predictable parasite community structure requires a lower sampling effort than an unpredictable structure. Predictable community structures among several host sample sites can be explained by several models. Two models, metapopulation dynamics (Levins, 1969) and core satellite theory (Hanski, 1982) are inter-related and are discussed below.

When a local extinction of a parasite species occurs, this species will be replaced by re-invasion from other populations within the metapopulation. Since metapopulations have a much longer time to extinction than local populations (Kennedy, 2001) metapopulation dynamics is seen as the key to a species' persistence in a locality. What needs to be determined is the size of the metapopulation, as there is a positive relationship between infra/component community similarity and connectivity and a negative relationship between infra/component community similarity and distance.

The core-satellite hypothesis explains two commonly observed patterns of species distribution. These patterns are a positive correlation between distribution and abundance and a bimodal distribution of species within a geographic area. Parasites species may be recognized as core (common) or satellite (rare). Core-satellite theory is usually applied at the infra-community level. However, it is sensitive to the spatial scale of study. Therefore I shall apply it to both the infra community and the component community level. The application of metapopulation theory and the identification of core/satellite species are addressed in Chapter 7.

1.6.1 Distribution Patterns of Parasites and Host

Helminth parasites are typically aggregated among host individuals (Shaw & Dobson, 1995). Consequently, most hosts harbour few or no parasites and only a few harbour a large number of parasites. Although aggregated distributions are typical, non-aggregated distributions may also occur (eg. nested community structure, Poulin, 1996; Rohde *et al.*, 1998).

Many factors can influence the extent of parasite aggregation. Among these, one I wish to mention is host schooling behaviour. Host aggregation behaviour has been identified as a factor in parasite community structure (Sasal *et al.*, 1999a). Their study examined intestinal digeneans in marine teleosts and a positive association of infection with gregariousness was reported. However no causal explanation was given and it is difficult to reason a cause and effect relationship in this case. For my ectoparasitic monogeneans, which tend to exhibit non-aggregated distributions (Section 7.6.1), a possible causal effect will be presented here. If fishes are randomly distributed within the water and little interaction occurs between them, then limitations are placed on a

parasites' ability to infect new hosts, increasing the probability of extinction within host infrapopulations. This could be one factor producing an aggregated structure in the parasite infracommunity. When hosts have a gregarious behaviour, the possibility of parasite transmission is increased to the point where a continuous recolonisation of parasites between hosts, could conceivably produce a non-aggregated parasite community. This is especially applicable to ectoparasites such as gill monogeneans which often have a limited motile larval stage. The type of distribution present in the component community is examined in Section 7.6.1.

1.6.2 Parasite Associations and Interspecific Competition

Competition between parasite species may explain high specificity and might also contribute to aggregation. Negative responses between parasite species are quite common in mixed infections of intestinal helminths and are often quite substantial, with infrapopulations of one species reduced by as much as 50% of that achieved when not sharing the host with another species (Dobson, 1985; Poulin, 1998). Extreme negative responses can lead to the exclusion of one species by the other. Typically, one helminth species causes severe reductions in the numbers of other species but is not affected itself by other species (Holland, 1984). A possible example of competitive exclusion by the monogenean parasites *Dactylogyrus extensus* over *D. anchoratus* has been reported (Ozer, 2002).

Although negative responses appear to be common in intestinal helminths (Poulin, 2001b), monogenean communities generally appear to show little evidence of intra- or inter-specific competition (Luque, 1996; Morand *et al.*, 2002; Simková *et al.*, 2001c). Competition can be avoided when co-occurring parasite species either do not interact,

because they are not abundant enough to exert mutual selective pressure, or because they differ in resource use and their fundamental niches do not overlap. A lack of niche overlap could be due to intraspecific interactions because of the need to find a mate (Rohde, 1993) thus causing conspecifics to aggregate, or it may be a consequence of the haptor architecture which limits the sites available for attachment (Rohde *et al.*, 1980).

Reproductive segregation among congeners parasitising the same host has been suggested as a mechanism to avoid competition (although see Rohde, 1979b). Morphological variation of attachment structure and copulatory apparatus supports this view (Morand *et al.*, 2002). If two congeneric parasites occurring on the same host species have very similar haptoral structures then segregation may occur by divergence of the copulatory apparatus structure while alternatively if copulatory structures are very similar then haptoral structure may show significant differences that forces species to attach at different sites. This has been shown to be very common among *Dactylogyrus* species (Gerasev, 1995). Differences in copulatory apparatus and haptoral morphology are examined in Chapter 4 and 5.

When positive associations among parasite species are present, the degree of host specificity may be reduced. Positive associations have been confirmed in several studies of monogeneans (El Hafidi *et al.*, 1998; Geets *et al.*, 1997; Lo & Morand, 2000; Rohde *et al.*, 1995; Simková *et al.*, 2000). This type of association could occur by facilitation processes, such as immunosuppression induced by one species benefiting other species. Thus, even a single key species might be able to create a suitable environment for several other species by its immunosuppressive capacity (Haukisalmi & Henttonen,

1998). I examine the presence of positive and negative host associations of parasites and parasite-parasite associations in Chapter 7.

1.6.3 Host Body Size and its Effect on Parasite Specificity

An association between host body size and host specificity has been identified where specialists are more common on larger fish species while generalists are more common on small species (Simková *et al.*, 2001a). Host body size has also shown a positive relationship with parasite abundance and richness of parasite species infecting a host (Guégan & Hugueny, 1994; Matejusová *et al.*, 2000; Poulin, 1995). Two explanations have been proposed (Guégan & Hugueny, 1994). Firstly, as gill area increases with fish size, there may be increased sampling of parasite larvae in the water. Secondly as fish become older, the length of time available for random parasite colonisation increases (Guégan & Hugueny, 1994). Under both these scenarios, the common parasite species are expected to be found on many fish while rare parasite species are restricted to a few fish, which have sampled parasites for the longest time or most intensively. A positive relationship between prevalence and mean per-host abundance is also expected (Wright, 1991). These aspects of parasite community structure and host size are examined in Chapter 7.

1.6.4 Specificity and the Identification of Host Hybridisation

Monogenean parasites have been used as tags for the identification of host species and host hybridisation (Cremonte & Sardella, 1997; Lambert & El-gharbi, 1995; Le Brun *et al.*, 1992; Mizelle *et al.*, 1943; Williams, 1964). On the basis of a multivariate analysis, the quantitative variations in some parasites show the possibility of their use as tags of different ecological conditions of the two geographic areas (Cremonte & Sardella,

1997). The use of monogenean parasites as tags could be applicable to identification of several host species and hybrids examined in this study. This is examined in Chapter 8.

1.7 The Subject of the Study

1.7.1 Host Fauna

The host group Atheriniformes comprises six families, 49 genera, and approximately 285 species (Dyer & Chernoff, 1996) and are found in marine and freshwater environments from tropical and temperate regions. In Australia and New Guinea, the three families Melanotaeniidae, Pseudomugilidae and Atherinidae represent Atheriniformes (see Allen *et al.*, 2002). They occur in a variety of habitats including streams of all sizes, lakes, pools, reservoirs, swamps and brackish waters.

Melanotaeniidae (rainbowfish) is a family of small freshwater fishes, generally confined to the northern Australian-New Guinea region. The family contains seven genera and approximately 69 species (Allen *et al.*, 2002; Allen & Renyaan, 1998; McGuigan, 2001). Australia has four genera: *Melanotaenia, Cairnsichthys, Iriatherina* and *Rhadinocentrus*, containing 18 species and sub-species: *Cairnsichthys*, *Iriatherina* and *Rhadinocentrus* are monotypic genera. Three other genera found in New Guinea are *Glossolepsis*, *Chilatherina* and *Pelangia,* none of which were sampled. The species found in Australia are *Melanotaenia splendida splendida* (Peters, 1876), *M. s. inornata* (Castelnau, 1875), *M. s. tatei* (Zietz, 1896), *M. australis* (Castelnau, 1875), *M. duboulayi* (Castelnau, 1878), *M. eachamensis* Allen & Cross, 1982, *M. exquisita* Allen, 1978, *M. fluviatilis* (Castelnau, 1878), *M. pygmae* Allen, 1978, *M. maccullochi* Ogilby, 1915, *M. gracilis* Allen, 1978, *M. nigrans* (Richardson, 1843), *M. trifasciata* (Rendahl, 1922), *M. utcheensis* McGuigan, 2001, *M. solata* Taylor, 1964, *Cairnsichthys*

rhombosomoides (Nichols & Raven, 1928), *Iriatherina werneri* Meinken, 1974 and *Rhadinocentrus ornatus* Regan, 1914. These species are found mostly in the tropical north and occur in a variety of habitats (Allen & Cross, 1982) and they range in maximum size from 50mm to 150mm in length. Their distributions are indicated in Figure 1.2.

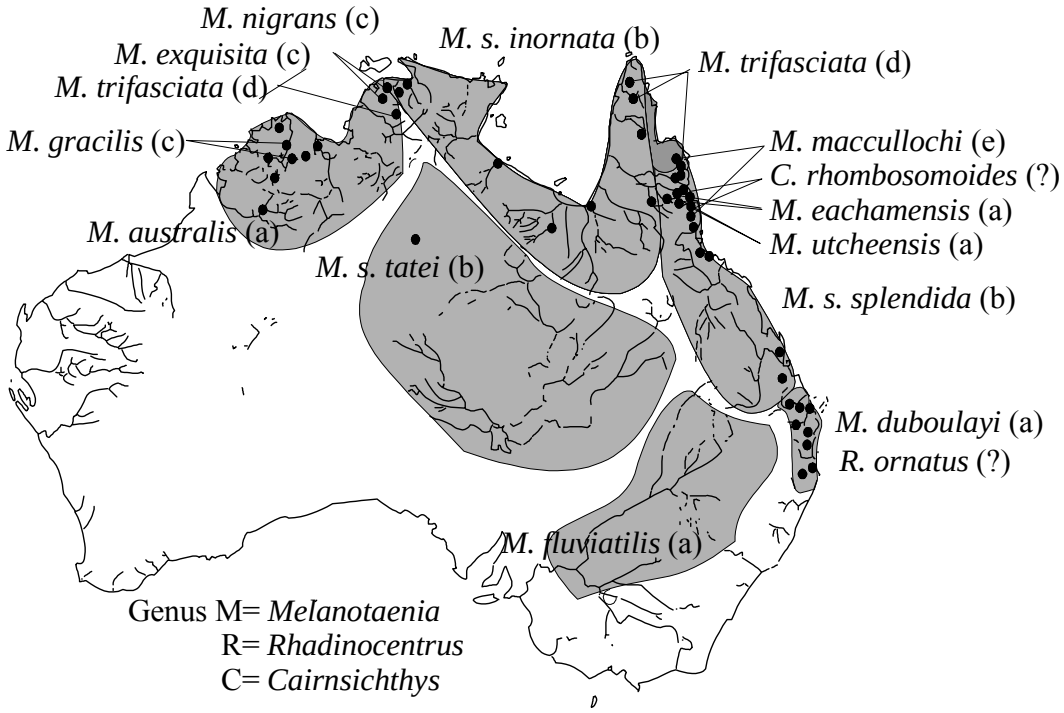


Figure 1.2. Distribution of melanotaeniids and sample sites in this study.
Host morphometric clades (a) '*australis*', (b) '*splendida*', (c) '*nigrans*', (d) '*goldiei*', (e) '*maccullochi*' (?) unknown.

Pseudomugilidae (blue eyes) comprises three genera: *Pseudomugil* with 15 species (6 in Australia) while *Scaturiginichthys* (Australia) and *Kiunga* (New Guinea) are monotypic genera (Allen *et al.*, 2002). Several *Pseudomugil* species also range into brackish or saltwater mangrove areas. Most species exist as isolated relict populations of limited distribution. The exception is *P. signifer* Kner, 1865, which is distributed along the entire length of the Australian east coast.

Atherinids (hardy heads) are small silvery fish, mainly confined to tropical and sub-tropical seas and estuaries throughout the world. The family comprises approximately 175 species belonging to 20 genera worldwide (Dyer & Chernoff, 1996). In Australia and New Guinea, two genera are present: *Atherinosoma* and *Craterocephalus*. *Craterocephalus* contains approximately 17 species.

The evolutionary history of the rainbowfish (Melanotaeniidae) remains obscure as there are no known fossil records. Allen and Cross (1982) proposed that melanotaeniids are closely related to the atherinids or silversides and probably evolved from a *Pseudomugil*-like ancestor, which was originally adapted to brackish estuarine conditions. Eventually rainbowfish evolved a purely freshwater life cycle, setting the stage for an explosive speciation in relatively recent times (Plio-Pleistocene, 5~1 Mya.) (Allen, 1980, 1989a, b, 1995; Allen & Cross, 1982; Merrick & Schmida, 1984). A dissenting view has suggested that craterocephalids and possibly melanotaeniids are much older, invading Australian freshwaters sometime between the mid-Cretaceous and Palaeocene (80~60 Mya) (Crowley, 1990; Unmack, 2001).

In recent years, systematics of the freshwater fish family, Melanotaeniidae, which is endemic to Australia and New Guinea, has been intensely studied using molecular techniques (Hurwood & Hughes, 2001; McGuigan *et al.*, 2000; McGuigan, 2001; Zhu *et al.*, 1994; Zhu *et al.*, 1998). Both molecular and morphological data have been used to elucidate the possible evolutionary relationships among *Melanotaenia* species. Within *Melanotaenia* a number of clades, defined on the basis of molecular studies, are recognised (McGuigan, 2000): "*splendida*", "*nigrans*", "*australis*" and "*goldiei* (trifasciata)" while "*maccullochi*" is separated from the "*splendida*" group and is

assigned its own group using morphological data (Schmida, 1997). A most notable pattern is the disjunct distribution of some clades (eg. clade a Figure 1.2). This could be due to large-scale extinction of intervening populations if each clade represents remnants of a formally widespread ancestral taxon (McGuigan, 2000).

The marine barrier, which is now the Arafura Sea, Gulf of Carpentaria, and Torres Strait, which separates Australia from New Guinea, is a recent development, having resulted from rising sea levels after the last major glacial period 6.5-8 Kya (Pleistocene) (Allen & Hoese, 1980). All of these seas are extremely shallow, with average depths ranging from about 15 to 60 metres. During much of the Pleistocene glacial period, this area formed a lowland alluvial plain dissected by numerous rivers and swamps producing a brackish or freshwater-brackish lake (Torgensen *et al.*, 1985; Torgensen *et al.*, 1988). The distribution range of several rainbowfish species occurring in both New Guinea and Australia provides evidence for this recent separation (Allen, 1980). This separation has also occurred during other geological periods (Torgensen *et al.*, 1985; Torgensen *et al.*, 1988). There is some belief that rainbowfishes originated in Western Australia and then spread eastward, north into New Guinea and southward down the northeast coast of Australia, differentiating into the various species we know today (Pusey *et al.*, 1997).

Many factors affect the distribution of rainbowfishes but one of the most important is biogeographical boundaries, principally the drainage division boundaries. Australia can be divided into six major drainage basins: Pilbara, Kimberley/western Northern Territory, Gulf of Carpentaria, Australian east coast, Murray Darling basin and the central Australian Lake Eyre basin (Figure 1.3). There is a pronounced difference

between the topography of Australia and that of New Guinea. New Guinea is divided physically by extensive mountain ranges that run from east to west with many peaks over 4000 metres forming an effective barrier. In New Guinea, the freshwater fish fauna can be clearly divided into two biogeographical regions. Freshwater bodies south of the central mountain range have fish fauna closely related to that of northern Australia. Rainbowfish inhabiting systems north of the mountain range are generally different from their southern cousins. Apart from the land barrier formed by the central mountain range, northern rivers are much younger than southern rivers. Because of its mountainous terrain and consequent abundance of isolated freshwater drainage systems, New Guinea is a particularly rich area for rainbowfish speciation harbouring more than 80% of the known rainbowfish species.

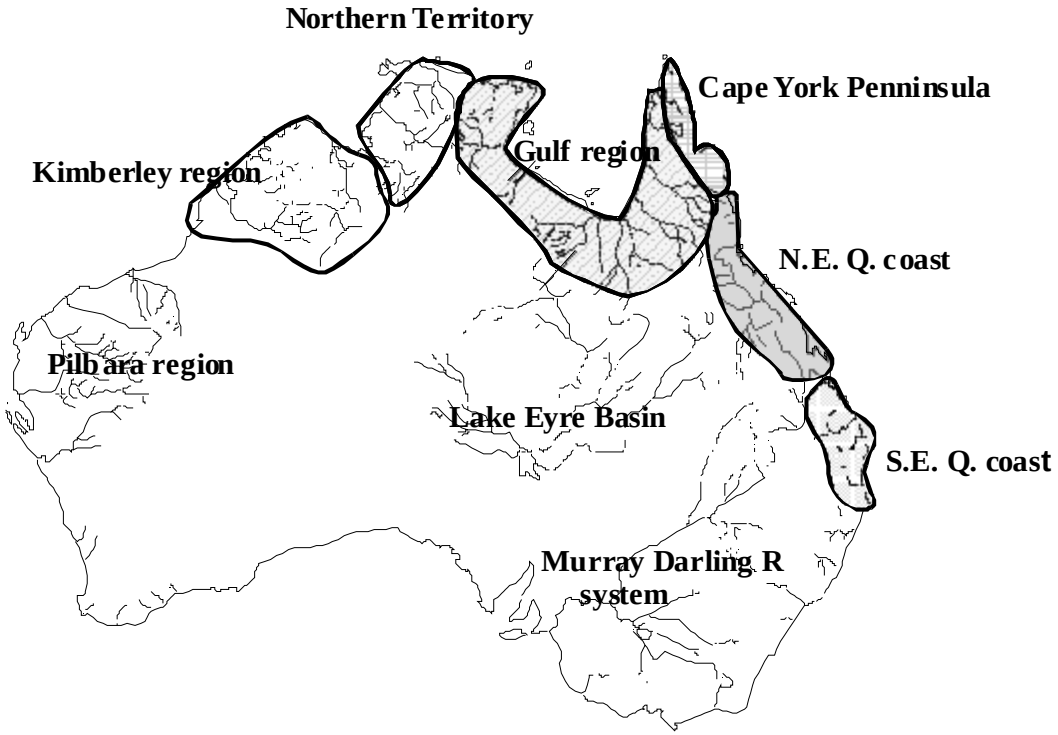


Figure 1.3. Major drainage regions identified in Australia.
Shaded regions represent those sampled.

1.7.2 Australian Monogenean Fauna

Monogeneans most often infect fish hosts, although they are known to infect other animals (eg. frog, squid, and hippopotamus (Bychowsky, 1957)). They have a direct life cycle and most commonly attach to the gills and fins of fish hosts. Certain species, however, do attach to other sites such as the nasal, buccal and intestinal tract. The posterior attachment organ, called the haptor, is usually furnished with a number of small hooks and a pair of usually larger sclerites called anchors.

The monogenean parasite fauna from Australian freshwater fishes has been little studied (Fletcher & Whittington, 1998) and the few publications available present only their taxonomy and descriptions. Although I consider these descriptions inadequate by modern standards, I have not re-examined them since none is the focus of this study.

The first monogeneans from Australian freshwater fishes were described by Johnston & Tiegs (1922) who recognised 13 species from six new genera. Since then a further nine publications have described species of Monogenea. To date 26 species have been described from 16 species of native freshwater fishes (Fletcher & Whittington, 1998). This represents roughly 5% of the approximately 300 species of freshwater fish known (Allen *et al.*, 2002). Three monogenean species per host species has been predicted for Australian freshwater fishes (Whittington, 1998). Therefore, approximately 900 species are yet to be described. Descriptions of monogenean parasites from atheriniform fishes from freshwater or from Australia are lacking although an unidentified species of *Ancyrocephalus* has been collected from *M. duboulayi* (see Pyecroft, 1994). Monogeneans from atheriniform fishes are also poorly known worldwide with only five species from four host species, all marine, being described. In the current study, I

examine a further 20 species of fish from Australian freshwaters, and report 19 ancyrocephaline species in 4 genera, all new to science.

An examination of monogenean species from the Melanotaeniidae, represents a unique opportunity for an integrated approach to studying several aspects of host-parasite association. In an attempt to give a broader picture of the possible evolutionary history of the host-parasite system, descriptions and patterns of association of monogenean parasites from several host species of two other atheriniform families, Pseudomugilidae and Atherinidae, are also included. This provides additional and comparative data on any phyletic associations and correlations identified, since all three families are closely related (belong to Atheriniformes).

1.8 Structure of the Thesis

Chapter 1

This chapter has presented ideas and models for the study of cospeciation between Australian teleost hosts and their monogenean parasites. Issues raised are:

1. Phyletic associations between hosts and parasites
2. Taxonomy of hosts and parasites
3. Parasite community structure
4. Parasite distribution
5. Parasite/host specificity
6. Cospeciation
7. Knowledge of host relationships
8. Paucity of knowledge of freshwater monogenea

Chapter 2

This chapter presents the methods used for:

1. Collection of hosts
2. Preparation of parasite specimens
3. Variables used for taxonomic descriptions and morphometric variation

Chapter 3

This chapter presents the statistical methods applied in the thesis and discusses issues of correct usage of statistics.

Chapter 4

This chapter discusses:

1. The merits of using reproductive and haptoral sclerite variation for defining genera and species of monogenean parasites
2. The uses of statistics in taxonomic descriptions and identification

Chapter 5

This chapter examines morphometric variation among parasite species on hosts from multiple locations.

Chapter 6

This chapter examines intraspecific variation of low host-specific parasites and its association with host species.

Chapter 7

This chapter examines:

1. Prevalence, abundance and intensity of parasites on hosts
2. Host specificity
3. Core-satellite species concepts
4. Parasite abundance and host habitat
5. Patterns of parasite and host species associations

Chapter 8

This chapter examines the use of parasite specificity and abundance for identifying host species and their hybridisation.

Chapter 9

This chapter examines:

1. If parasite interspecific morphometric variation is appropriate for inferring phylogenies
2. Cospeciation between hosts and parasites
3. Identifies cases of cospeciation, host switching, sympatric speciation and host addition
4. The use of parasite specificity for inferring introgression in host species
5. Presents a method using intraspecific variation of low-host-specific parasites for inferring delayed cospeciation

Chapter 10

This chapter forms a synthesis of ideas and presents possible outcomes of future research on Australian teleosts and their monogenean parasites.

CHAPTER 2 METHODS AND MATERIALS

2.1 Host Species Collection Sites

Twenty recognised species and sub-species of fishes from the Atherinidae, Melanotaeniidae and Pseudomugilidae were examined for monogenean gill parasites. These fish were collected from 51 sample sites (Table 2.1) distributed across tropical and sub-tropical Australia and resulted in 76 individual sample sets (Figure 2.1-2). A sample set is defined as all specimens of a single host species sampled from a single location. Where possible a minimum of ten specimens of each host species was examined. Identification of host species is according to Allen *et al* (2002) and McGuigan (2001). Specimens from Kangaroo Ck, North Queensland, were identified during collection as *M. s. splendida*. However this sample is here referred to as *M. sp.* (*Melanotaenia sp*.) because individuals possess a different colour pattern and sets of parasite species different from those seen in *M. s. splendida*. Melanotaeniids from the Atherton Tablelands have a complex taxonomy and identification of specimens is difficult. It is for this reason that, where possible, samples were gathered from sites previously examined using molecular techniques (McGuigan, 2001; Zhu *et al*. 1998). Individuals from sample sites were identified as *M. s. splendida, M. eachamensis* or '*M. eachutchee*' (possible hybrids between *M. s. splendida, M. eachamensis* and *M. utcheensis*).

Four collecting periods were used to gather specimens. Samples from North Queensland (Atherton Tablelands excepted), were collected during the months of September and October 1994 (this is the pre-wet season). The collection from S. E. Queensland occurred during December of the same year. The collection of samples from Central Australia, Kimberley region, Northern Territory and the western Gulf of Carpentaria was obtained during the pre-wet season period September-October 1995. Samples were

collected from the Atherton Tablelands during September 2000. Details of host species, sample sites, collection dates and sample size are given in Table 2.1, Figure 2.1-3.

Table 2.1. Sample locations, identity and date collected.

ID	Location	State	Longitude	Latitude	Date collected
1	Adcock G.	WA	125° 46.70' E	16° 55.55' S	29/9/1996
2	Amamoor Ck.	QLD	152° 40.26' E	26° 20.57' S	28/12/1994
3	Annan R.	QLD	145° 13.54' E	15° 44.20' S	20/10/1994
4	Barron R.	QLD	145° 25.59' E	16° 58.99' S	15/9/2000
5	Beerburrum Ck.	QLD	152° 57.52' E	26° 56.27' S	28/12/1994
6	Behana Ck.	QLD	145° 45.96' E	16° 40.42' S	9/10/1994
7	Bible Ck.	NSW	153° 32.88' E	28° 43.62' S	29/12/1994
8	Bluewater Ck.	QLD	146° 33.13' E	19° 10.57' S	10/8/1994
9	Brunswick R.	NSW	153° 26.20' E	28° 30.82' S	29/12/1994
10	Camp Ck.	WA	125° 50.52' E	14° 49.26' S	26/9/1996
11	Castaway Ck.	QLD	156° 06.36' E	26° 25.50' S	28/12/1994
12	Chinaman Ck.	QLD	145° 21.45' E	17° 23.80' S	15/9/2000
13	Comalie Ck.	NT	131° 06.75' E	13° 00.61' S	22/10/1996
14	Corduroy Ck.	QLD	146° 51.80' E	18° 05.37' S	7/10/1994
15	Daintree R.	QLD	145° 17.58' E	16° 11.79' S	12/10/1994
16	Dawn Ck.	WA	127° 39.45' E	15° 33.78' S	22/9/1996
17	Dirran Ck.	QLD	145° 36.16' E	17° 26.98' S	15/9/2000
18	Drysdale R.	WA	126° 23.22' E	15° 40.80' S	22/9/1996
19	Dulhunty R.	QLD	142° 30.24' E	11° 50.30' S	3/11/1994
20	Five Mile Ck.	QLD	146° 57.80' E	18° 07.37' S	7/10/1994
21	Granite Ck.	QLD	151° 39.90' E	24° 36.66' S	26/12/1994
22	Gregory R.	QLD	139° 14.45' E	18° 38.76' S	27/10/1996
23	Gwynne Ck.	QLD	145° 35.24' E	17° 18.66' S	15/9/2000
24	Howard Ck.	NT	131° 05.40' E	12° 27.67' S	20/10/1996
25	Ithica Ck.	QLD	145° 36.38' E	17° 24.26' S	15/9/2000
26	Kangaroo Ck.	QLD	145° 19.25' E	16° 08.50' S	13/10/1994
27	L. Kunnanurra	WA	128° 41.93' E	15° 47.50' S	10/10/1996
28	Lexilip Ck.	QLD	151° 11.80' E	24° 00.77' S	27/12/1994
29	Liverpool Ck.	QLD	145° 55.96' E	17° 43.42' S	8/10/1994
30	Manton Ck.	NT	131° 09.67' E	12° 52.81' S	22/10/1996
31	Mary R.	WA	127° 19.26' E	18° 32.43' S	9/10/1996
32	McIvor R.	QLD	145° 07.26' E	15° 07.16' S	21/10/1994
33	McIvor R. 1	QLD	145° 13.43' E	15° 06.71' S	22/10/1994
34	Moline Rock Pool	NT	132° 14.08' E	13° 35.19' S	24/10/1996
35	Nigger Ck.	QLD	145° 26.74' E	17° 25.24' S	15/9/2000
36	Norman R.	QLD	141° 08.06' E	17° 51.12' S	20/8/1994
37	Oscar Ck.	QLD	143° 11.88' E	13° 57.38' S	26/10/1994
38	Pentacoste R.	WA	127° 54.54' E	15° 49.59' S	19/9/1996
39	Roaring Meg Ck.	QLD	145° 19.35' E	16° 05.54' S	19/10/1994
40	Robinson R.	NT	137° 02.78' E	16° 28.25' S	26/10/1996
41	Ross R.	QLD	146° 45.60' E	19° 18.46' S	5/8/1994
42	Russ Ck.	WA	126° 41.96' E	16° 02.84' S	22/9/1996
43	Russell R.	QLD	145° 22.38' E	16° 28.06' S	14/10/1994
44	S. Mossman R.	QLD	152° 59.10' E	25° 59.90' S	27/12/1994
45	Seers Ck.	NT	134° 12.72' S	19° 36.44' S	27/10/1999
46	Tuan Ck.	QLD	152° 50.69' E	25° 41.46' S	27/12/1994
47	Tungamull Ck.	QLD	150° 41.19' E	23° 18.93' S	26/12/1994
48	Utchee Ck.	QLD	145° 56.20' E	17° 38.30' S	9/10/1994
49	Wenlock R.	QLD	142° 10.45' E	12° 23.18' S	3/11/1994
50	Wildman R.	NT	131° 57.23' E	12° 49.54' S	18/10/1996
51	Williams Ck.	QLD	145° 35.81' E	17° 23.55' S	15/9/2000

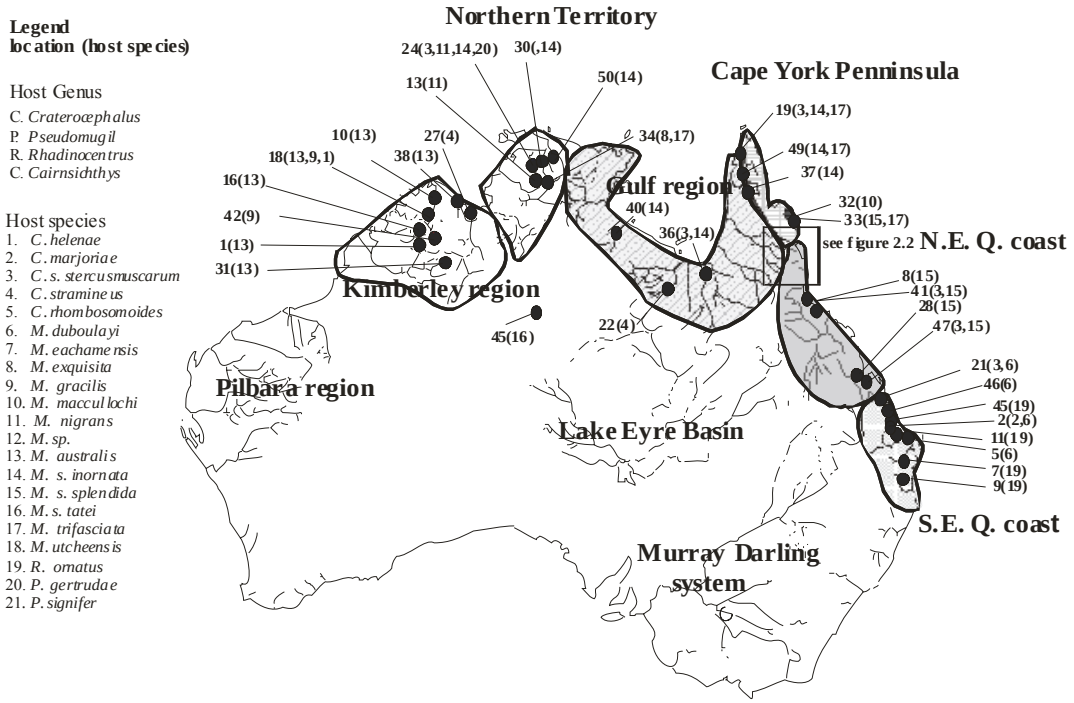


Figure 2.1. Collection sites for species examined.
Number preceding brackets is sample location.Outlined areas represent major geographic regions sampled (See Table 2.1 for location). ID# numbers in brackets refer to host species sampled.

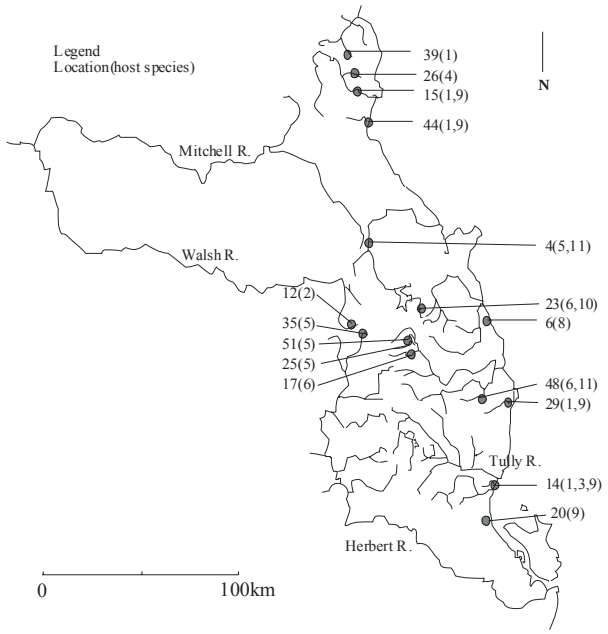


Figure 2.2. Collection sites within North Queensland for species of Melanotaeniidae, Pseudomugilidae and Atherinidae.
Number preceding brackets is sample location. ID# numbers in brackets refer to host species sampled.

Host species 1. *M. s. splendida*, 2. *M. s. splendida*, *M. maccullochi*, 4. *M. sp.* 5. "*M. eachutchee*", 6. *M. eachamensis*, 7. *M. utcheensis*, 8. *C. rhombosomoides*, 9. *P. signifer.* 10. *P. gertrudae*, 11. *C. s. stercusmuscarum*. For location identity, see Table 2.1.

Table 2.2. Host species, sample location and sample size.

Host species	Location	Sample size
"M. eachutchee"	Barron R.	11
"M. eachutchee"	Ithica Ck.	10
"M. eachutchee"	Williams Ck.	11
"M. eachutchee"	Nigger Ck.	21
C. helenae	Drysdale R.	20
C. marjoriae	Amamoor Ck.	13
C. rhombosomoides	Behana Ck.	7
C. rhombosomoides	Utchee Ck.	5
C. s. stercusmuscarum	Barron R.	13
C. s. stercusmuscarum	Dulhunty R.	3
C. s. stercusmuscarum	Granite Ck.	1
C. s. stercusmuscarum	Howard Ck.	3
C. s. stercusmuscarum	Norman R.	8
C. s. stercusmuscarum	Ross R.	3
C. s. stercusmuscarum	Tungamull Ck.	17
C. stramineus	Gregory R.	10
C. stramineus	L. Kunnanurra	20
M. australis	Adcock G.	5
M. australis	Camp Ck.	15
M. australis	Dawn Ck.	8
M. australis	Drysdale R.	11
M. australis	Mary R.	10
M. australis	Pentacoste R.	10
M. duboulayi	Amamoor Ck.	6
M. duboulayi	Beerburrum Ck.	10
M. duboulayi	Granite Ck.	15
M. duboulayi	Tuan Ck.	10
M. eachamensis	Dirran Ck.	19
M. eachamensis	Gwynne Ck.	10
M. exquisita	Moline Rock Pool	18
M. gracilis	Drysdale R.	18
M. gracilis	Russ Ck.	7
M. maccullochi	Corduroy Ck.	10
M. maccullochi	McIvor R. 1	6
M. nigrans	Comalie Ck.	2
M. nigrans	Howard Ck.	14
M. s. inornata	Chinaman Ck.	19
M. s. inornata	Dulhunty R.	5
M. s. inornata	Howard Ck.	2
M. s. inornata	Manton Ck.	10
M. s. inornata	Oscar Ck.	11
M. s. inornata	Robinson R.	4
M. s. inornata	Wenlock R.	13
M. s. inornata	Wildman R.	7
M. s. splendida	Annan R.	8
M. s. splendida	Bluewater Ck.	8
M. s. splendida	Corduroy Ck.	10
M. s. splendida	Daintree R.	6
M. s. splendida	Five Mile Ck.	1
M. s. splendida	Lexilip Ck.	4
M. s. splendida	Liverpool Ck.	13
M. s. splendida	McIvor R.	6
M. s. splendida	Roaring Meg Ck.	13
M. s. splendida	Ross R.	4
M. s. splendida	S. Mossman R.	6
M. s. splendida	Tungamull Ck.	10
M. sp.	Kangaroo Ck.	14
M. trifasciata	Dulhunty R.	6
M. trifasciata	McIvor R.	17
M. trifasciata	Moline Rock Pool	5
M. trifasciata	Wenlock R.	8
M. utcheensis	Utchee Ck.	20
P. gertrudae	Gwynne Ck.	10
P. gertrudae	Howard Ck.	17
P. signifer	Corduroy Ck.	10
P. signifer	Daintree R.	5
P. signifer	Liverpool Ck.	10
P. signifer	S. Mossman R.	5
R. ornatus	Bible Ck.	14

2.2 Parasite Preparation

2.2.1 Method 1

Host specimens, collected by a number of methods (6mm seine net, dip net), were killed by placing in iced water for thirty seconds. This kills the host but not the monogeneans. The gills were removed from the host, using fine needle forceps, and placed in a 50mm Petri-dish containing water from the collection site. Monogenean parasites were observed with a x40 dissecting microscope and removed from the gills using a fine needle. Live parasites were transferred to a 50mm x 25mm glass slide using a pipette; then a cover slip was placed on top. D.I.C. Nomarski optics at x400 and x1000 were used to examine live parasites to determine internal soft tissue morphology. As the slides dried, specimens became compressed, causing body fluids to be extruded. This produced a two dimensional imprint of the worm showing details of all measured morphometric characters and most of the morphological characters. The worms were subsequently preserved in glycerol-ammonium-picrate (Malmberg, 1970) by placing a drop at the edge of the cover glass and allowing capillary action to draw the preservative under the coverslip. Nail polish was used to seal the cover glass. This method was the only one that made visible the internal soft body structures i.e. the vas deferens, testis and vagina. This method has been criticised for its limited shelf life (< 5 years) (Ergens, 1969) where parasites turn brown or black and internal structure is no longer discernible. This was also observed in those parasites that did not exude their internal body fluids (after 3 years, pers. obs.). However, those that did exude their internal body fluids show clear, well-defined characters and no browning occurs after 10 years (pers. obs.). Ergens (1969) also recommended the exudation of the body fluids but goes on to use alcohol series and alternative mountant. This can be neglected. A

problem with my method is that external body measurements are exaggerated and cannot be used to compare body measurements from other slide preparation techniques.

2.2.2 Method 2

When live specimens could not be examined, gills were preserved in 5% formalin. Parasites were later extracted from the gills and mounted in Gray & Wess mountant (Humason, 1979) or glycerol-ammonium picrate. Parasites for permanent mounts were preserved in situ in 5% formalin, extracted from the gills, excess water was removed, and a small drop of Gray & Wess mountant was applied. A coverslip was then placed over the specimens and the mountant allowed to dry. This method was the most appropriate for the processing of large numbers. Most specimens could be measured after two weeks of curing. Few parasites are orientated on their side in slide preparations. However if preparations are checked while mountant is still liquid, they can be rolled on their side using a probe to manipulate the cover glass. This facilitates the examination of the three dimensional form of the haptor and its sclerotised parts. Although method one also shows some level of clarity, method two allows more time to study the details of the spatial orientation of sclerotised structures. Very few taxonomic descriptions report this aspect of the haptor. Slide preparations, due to increasing cover slip pressure as the mountant dries, often re-orientate and sometimes distort sclerites such as the dorsal and ventral anchors that in some genera are not normally directed along lateral planes. This can lead to a false interpretation of haptoral spatial structure. Method two is very convenient compared to alcohol dehydration methods and the use of Canada balsam mountant. Specimens are often lost or damaged when stains and alcohol series methods are applied.

2.2.3 Other Methods

A number of other methods were not very useful. Various stains were tried (eg. Gomori trichome stain, acid haematoxylin, triple stain). These however did not stain internal organs very well except the ovary. Alcohol series methods damage very small parasites by causing collapse of the soft body tissue so that features are not recognisable.

2.3 Measurements

Two types of data were collected in this research: measured continuous morphometric data and discrete (abundance and prevalence) data. Both data types were analysed using parametric methods; however each type of data requires a different set of analyses. Data were compiled in a database using Microsoft Access 2000 and analysed using the statistics package SPSS (Anon, 2001). The following parametric tests were used: Analysis of Variance (ANOVA), Multivariate Analysis of Variance (MANOVA), Linear Regression Analysis (LRA), Principal Component Analysis (PCA), Hierarchical Clustering Analysis (HCA), Discriminant Function Analysis (DFA) and Partial correlation analysis. Data that did not conform to a multivariate normal distribution and equal variance were transformed using $\ln(1 + x)$. Details of statistical methods and approach of analysis are given in Chapter 3 with modifications given in individual chapters.

CHAPTER 3 STATISTICS

3.1 Introduction

A multitude of statistical techniques has been developed for analysing taxonomic, ecological and evolutionary associations. Many of the techniques are used with little understanding by the individual researcher of the models behind them. This can lead to the publication of results that have been obtained using inappropriate statistical approaches (e.g. parametric tests when data are clearly not of a normal distribution). As quaintly stated by Mayr & Ashlock (1991) “The best way to understand the limits of numerical analysis is to understand the process fully”. It is for this reason I have given a detailed explanation of the statistical methods and how they are used here.

3.1.1 Data Considerations

Parametric tests require that the data conform to several assumptions: normal distribution of data, equal variance and equal sample size. Often however, one or more of the parametric criteria can be relaxed since many statistical methods are reasonably robust to departures from these criteria, without affecting α significance level. If departures are present, then the α level should be set to a more extreme value i.e. 0.01 instead of 0.05 (Stevens, 1999). It must be realised that when setting α at 0.01, significance levels identified would more closely approximate values for α set at 0.05. This technique of compensating for departures from the parametric requirements is only used in MANOVA tests and is not recommended as a general solution.

3.1.1.1 Normalisation

When examining morphometric variation, it is important to have data that are normally distributed. Two methods, the Kolmogorov-Smirnov test and Shapiro-Wilk test, can be used to test for normal distribution (Sokal & Rohlf, 1981). The Kolmogorov-Smirnov test is based on the largest absolute difference between the observed and the expected cumulative distributions. If the maximum deviation between cumulative data and the theoretical distribution is larger than the critical value from the table, for a given level of probability, normality must be rejected. Since the mean and the variance of the population are not specified but must be estimated from the sample, critical values given in standard Kolmogorov-Smirnov tables are consistently too large and lead to accepting as normal some distribution which are not (Legendre & Legendre, 1998; Legendre & Legendre, 1983). The Lilliefors test is a modification of the Kolmogorov-Smirnov test that tests for normality when means and variances are not known, but must be estimated from the data and is the preferred method (Lilliefors, 1967). Alternatively, frequency histograms and box plot graphs may be employed for visually identifying data that are not normally distributed (Stevens, 1999). Boxplots show the median, range and the first and third quartile percentage for data. However, visual methods suffer from a great deal of subjectivity since the deviation from the normal distribution is estimated qualitatively. If data are shown not to be of normal distribution using the above statistical tests, then an approximate normalisation can be obtained using some form of transformation. For approximate normalisation of continuous data, statisticians have identified certain transformations which are appropriate when standard deviation is functionally related to mean (Afifi & Azeni, 1979). If standard deviation is proportional to the mean, then a logarithmic transformation $\ln(x + 1)$ is used. The logarithmic

transform is also generally used when an examination of the data distribution shows a positive skew from normal.

Morphometric data for individual analyses were tested for normality. If data were not normal then an approximate normalisation was produced using the ln(x + 1) transformation.

3.1.1.2 Equal Variance

A common test of equal variance is the Levene test. This homogeneity of variance test is less dependent on the assumption of normality than most tests. For each case, it computes the absolute difference between the value of that case and its cell mean and performs a one-way analysis of variance on those differences. When unequal variance for variables occurs, the type I error rate is appreciably distorted only if the group sizes are sharply unequal (>1.5 to 1) (Stevens, 1999). If a significant difference in variances is present then statistical tests can be used that adjust for this unequal variance or a transformation can be applied. If the variance is proportional to the mean, the √ transformation will induce approximate equal variance (Afifi & Azeni, 1979). Many parametric tests are however robust enough to be little affected by unequal variance. In general if populations can be assumed to be symmetric, or at least similar in shape (eg. all positively skewed) and if the largest variance is less than 4 times the smallest variance then tests such as ANOVA are likely to be valid (Howell, 2002 pp340)

3.1.1.3 Equal Sample Size

To adjust for unequal sample sizes with multiple comparison tests, the harmonic mean should be used.

3.1.1.4 Standardisation

Data variables often have large differences in mean and range, or the data are measured in different scales. Standardisation is a method for making data dimensionless. This is often essential for mixed data (continuous, ordinal, nominal) since comparison of results when data are recorded in different scales is meaningless. The most common form of transformation is the z-score. This transformation reduces the variables to a mean of zero and variance to unity. Any form of data modification such as standardisation or transformation will tend to remove information. Standardisation will increase the emphasis on low data values while decreasing emphasis on high data values.

3.1.1.5 Outliers

An outlier is a sample of peculiar data composition that has low similarity to all other samples. Likewise a species is an outlier if it has low similarity to all other species. Many multivariate methods give unsatisfactory results if outliers are present in a data set, so for these methods it is important to be able to identify outliers and remove them from the data set prior to analysis. If the sample set contains peculiar samples unlike the others, it is best to remove these samples prior to data analysis, especially with ordination methods. Several methods can be used to identify outliers (e.g. hierarchical clustering, scatterplots, boxplots). The justification for omitting outliers is that their

relationship to other samples in the data set is not expressed by information in the data anyway.

3.1.1.6 Data Averaging

Averaging several samples together may form composite samples. Composite samples can be useful for two purposes; to summarise a large data set as a workable number of composite samples and to reduce noise by averaging together a number of replicate samples. Summarisation and noise reduction is often a valuable preliminary to subsequent detailed ordination and classification especially for a large data set. By averaging out the small differences among samples, the formation of composites tends to raise the level of abstraction, so that the broader features of the data are emphasized. For large data matrices, certain statistical techniques require some level of averaging (e.g. hierarchical clustering) in order to allow interpretation of results.

3.1.2 Descriptive Statistics

Most biological studies utilise descriptive statistics. Descriptives such as means, standard deviation and standard error are employed and are often misused or misconstrued (Anthony, 1999; Morrison, 2002). The use of mean and standard deviation or standard error requires a normal distribution of data. This is clearly not the case in many published result where reported standard deviations are greater than the mean. Many examples are available of this improper use of standard deviation (eg. Gutierrez, 2001; Gutierrez & Martorelli, 1999; Sasal *et al.*, 1999a; Simková *et al.*, 2000) and it is especially common for abundance data. If the data are not normally distributed then it is preferred that the median or mode is used and range or maximum and minimum values be used to describe the distribution of data.

3.1.3 Multivariate Analysis

Multivariate analysis (MANOVA) is an extension of the ANOVA method. MANOVA simultaneously tests for significant differences between means for multiple populations. Because there are multiple pairwise comparisons, α needs to be adjusted to account for this. The Bonferroni, Tukey's and Scheffé test are three commonly used methods that compensate for multiple comparisons. The Bonferroni test, based on Student's t statistic, adjusts the observed significance level for the fact that multiple comparisons are made. Sidak's t test also adjusts the significance level and provides tighter bounds than the Bonferroni test. Tukey's honestly-significant-difference (HSD) test uses the Studentised range statistic to make all pairwise comparisons between groups and sets the experiment-wise error rate to the error rate for the collection for all pairwise comparisons. Stevens (1999) considers the Scheffé test the best because the significance level is designed to allow for all possible linear combinations of group means to be tested, not just pairwise comparisons. However the result is that the Scheffé test is often more conservative than other tests, which means that a larger difference between means is required for significance. SNK, LSD, and Duncan's multiple range tests are commonly used multiple pairwise comparison methods but these tests do not control for overall α as claimed (Stevens, 1999).

When the variances are unequal, the α level again needs to be adjusted to a more stringent significant level. When unequal variance is present in the data, tests such as the Tamhane's T2 (conservative pairwise comparisons test based on a t test), Dunnett's T3 (pairwise comparison test based on the Studentised maximum modulus), Games-Howell pairwise comparison test (sometimes liberal), or Dunnett's C (pairwise

comparison test based on the Studentised range) can be used. For unequal sample sizes a test is also available, the Waller-Duncan t test and Tukey's HSD uses a Bayesian approach. These range tests use the harmonic mean of the sample size when the sample sizes are unequal. I used post-hoc multivariate test to identify significant differences between morphometric variable of parasite species.

3.1.4 Measures of Association

3.1.4.1 Covariance and Correlation

Covariance and correlation are two measures of the linear association between variables. Covariance is an unstandardised measure of the dependence between two variables around their means, whereas correlation is a standardised measure of the dependence between two variables and therefore dimensionless. The Pearson correlation coefficient r, is a measure of linear association between two variables and values range between -1 (a perfect negative relationship) and +1 (a perfect positive relationship). A value of 0 indicates no linear relationship.

The value calculated for the correlation coefficient needs to be interpreted with care. A value of r=0.1 accounts for 1% of the variance between two variables while for example a value of r=0.5 accounts for 25% of explained variance. To give a better description of the size of the explained variance, the squared coefficient r^2 is often used and is called the coefficient of determination. This value gives the exact amount of variance accounted for by the correlation coefficient. When using averaged data, it must be understood that a correlation based on averaged data is usually higher than if we had data for individuals.

For ordination methods (eg. Principle Component Analysis) analyses can be performed on either the variance-covariance matrix or a correlation matrix and a decision must be made as to which one is used. Pimental (1979) has suggested the following guidelines. When the data matrix is composed of data drawn from different kinds of measurements (e.g. length, counts), then a correlation matrix is probably preferred, while for data composed of the same kind of measurements (e.g. length only), a decision must be made to determine which of the two types of data matrix should be used. If the variance-covariance matrix is used, then absolute changes in morphology can be studied. If the correlation matrix is used, then changes relative to standardised data can be interpreted. Frequently both analyses are worthwhile. In this study I used both types of matrices and the results were essentially the same. Covariance matrices retain more of the original data structure since correlation matrices are standardised thus removing factors of scale. Data do not need to be standardised (z-score) if the correlation matrix is used in analyses. As the data analysed in this study are not of mixed types (metric and scale), covariance matrix analyses were used.

3.1.4.2 Similarity and Distance Measures

If multivariate analysis recognizes significant differences between population sample means then data can be converted into a matrix of associations. The association matrix can then be analysed further with analyses such as PCA, DFA, HCA and Cladistics. Distance coefficients, as well as similarities, measure the association between objects (Sokal & Rohlf, 1995). Distance coefficients are a series of measures that have the common characteristic of reaching their maximum value for two objects that are entirely different, and having a value of 0 for two identical descriptors (Legendre &

Legendre, 1983). The majority of distance coefficients are metric, which contrasts with similarity measures, which are never metric.

Many similarity measures are available in the statistics package SPSS. Similarity measures were first developed for binary data and are commonly used to assess association of taxa and regions. Data can be analysed via Q-mode, which measures the relationship between objects (eg. regions, hosts) based on descriptors (parasites) while R-mode measures the relationship between descriptors based on objects. For parasite presence/absence data each similarity measure uses zeros slightly differently and because of these slight differences the output may not truly represent answers to questions hypothesized for the data. A favourable characteristic of similarity coefficients is their ability to exclude zeros, otherwise regions with low species richness would be grouped on the basis of shared absences rather than presences (Legendre & Legendre, 1983). It is for this reason that an understanding of the weighting given to double and single zeros is needed since interpretation of results differs according to the coefficient used. Legendre & Legendre (1998) and Anderberg (1973) provide reviews and classification of similarity coefficients. Binary similarity measures can be obtained using the Russell and Rao measure. This is a binary version of the inner (dot) product where equal weight is given to matches and non-matches. Dice index excludes joint absences from consideration, and matches are weighted double. This is also known as the Czekanowski or Sorensen measure. Jaccard's (Coefficient of community or percent similarity coefficient) is an index in which joint absences are excluded from consideration. Equal weight is given to matches and non-matches. Ochiai index is the binary form of the cosine similarity measure. It has a range of 0 to 1. These measures have been used extensively in ecology and biogeography (eg. Gutierrez & Martorelli,

1999; Unmack, 2001). Several similarity measures were used in analyses (see Section 3.1.7)

Distance measures are typically used for continuous data. Two of the most commonly used measures City Block and Euclidean distance belong to a general class of distance functions called Minkowski metrics. Minkowski metrics measure the pth root of the sum of the absolute differences to the pth power between the values for the items. Squared Euclidean distances use the sum of the squared differences between the values for the items. It is also known as taxonomic distance and this measure is invariant when rotated (eg. Ordination methods), but is inappropriate for measuring evolutionary distances between taxa (Mayr & Ashlock, 1991). City Block, also known as Manhattan distance, measures the sum of the absolute differences between the values of the item. This measure differs from the Euclidean distance in that it neither squares the character difference nor takes the square root. The Manhattan distance is not invariant when rotated and is thus inappropriate for ordination (Mayr & Ashlock, 1991). It is appropriate for measuring evolutionary distance between taxa, since it measures character state differences as steps, therefore this measure is used in numerical cladistics (Mayr & Ashlock, 1991). Mahalanobis distance is a measure of how much a case's values on the independent variables differ from the average of all cases. For a single independent variable, it is simply the square of the standardized value of the independent variable. A large Mahalanobis distance identifies a case as having extreme values on one or more of the independent variables. Distance measures for counts can be obtained using Chi-square measure. This measure is based on the chi-square test of equality for two sets of frequencies. It should be remembered that converting a data

matrix into distances always entails a loss of information (Steel *et al.*, 1988). These distance measures were used in several analyses (see Sections 3.1.6.2 and 3.1.7).

3.1.5 Linear Regression Analysis

Standard linear regression analysis involves minimizing the sum of squared differences between a response (dependent) variable and a weighted combination of predictor (independent) variables (Pollard, 1977). The estimated coefficients reflect how changes in the predictors affect the response. The response is assumed to be numerical, in the sense that changes in the level of the response are equivalent throughout the range of the response. In linear regression analysis, the relationship between one variable, called the dependent variable, and several other variables called independent variables is examined. The independent variables are related to the dependent variable by a function called the regression function, which involves a set of unknown parameters. The strength of the linear relationship between the two variables is measured by the simple correlation coefficient, while the strength of the linear relationship between one variable and a set of variables is measured by the multiple correlation coefficients. Another measure of association, the partial correlation coefficient, measures the linear association between two variables after the removal of the linear effect of a set of other variables. The correlation coefficient R is the slope of the least-squares regression line when we measure both x and y in standard units. R measures only the strength of linear association. The square of the correlation R is the fraction of the variance of one variable that is explained by least-squares regression on the other variable. A regression line is a mathematical model for the overall pattern of a linear association between an explanatory variable and a response variable. I used linear regression analysis to examine the association between the following factors: morphometric variables of the

haptoral sclerites and host length, haptoral sclerite variation between parasite species, changes in population structure between parasite species.

3.1.6 Ordination Methods

Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) are used as a means of data reduction. This is achieved by reducing a large number of character variables into a much smaller number of new variables (eigenvectors) called principal components and discriminant functions respectively and are often graphically represented as two- or three-dimensional scatter plots. These methods also identify which characters have the highest correlation for each new variable. The two statistical methods, however, use different approaches. Principal Component Analysis extracts new variables based solely on the correlation within and between variances of characters without any *a priori* grouping of the character variants. Canonical Discriminant Analysis uses an *a posteriori* approach to produce a set of new variables that best maximises differences between the predetermined groups, using correlation within and between characters variances. Character variables are also identified which best reflect these *a priori* groups. However, the characters chosen change depending on *a priori* sample grouping. This method is able to identify morphological characters that define the *a priori* groups. This is not possible using PCA if clusters are not clearly defined. The advantage of DFA over PCA in taxonomy is that when species have been identified, using morphometric variables, measured unknown specimens and especially cryptic species, can be identified using cross-validation. Discriminant Function Analysis identifies sets of characters that are not included in the final analysis at the various levels of investigation. These character sets indicate redundancy of information in the data and are not necessary in the analysis. The sets of characters, however, change at the

various levels of analysis limiting *a priori* selection of characters for removal. Taxonomists often use alternative methods such as K-cluster or hierarchical cluster analysis for the same purpose (Anderberg, 1973). However, these methods do not identify the most useful characters for the separation of clusters but rely on *a priori* selection, usually obtained by multivariate analysis.

3.1.6.1 Canonical Discriminant Function Analysis (DFA)

Discriminant Function Analysis is a form of data transformation that considers variables to be correlated and is designed to present contrasting, as opposed to independent, patterns of variation. Unlike PCA, DFA identifies the extent to which given variables permit known groups to be distinguished and a statistical test for significant difference between group centroids is also used (Wilks λ) for discriminant analysis. Thus, DFA is more useful in identifying potentially useful morphometric characters in morphologically distinct species. Correlation of explained variance is accounted most in the first three discriminant functions and the last few usually produce a non-significant difference in the centroid means of clusters. A positive eigenvalue indicates a size relationship while a negative eigenvalue indicates a shape change. Discriminant analysis can also be utilised for classifying unknown objects using variance from known objects. Classification is also used to confirm the identification of an object. This confirmation of objects uses a process called cross-validation.

A step-wise selection criterion can be applied to the analysis. The stepwise method removes uninformative characters from analysis based on the significance levels set. Two methods are commonly used for setting selection of variables, the F statistic or the

significance of correlation. For my study the probability of F to enter was set at P=0.01 and for F to remove P=0.1.

3.1.6.2 Principle Component Analysis

Principal component analysis (PCA) is a method of data reduction designed to clarify the relationships between two or more characters and to divide the total variance of all characters into a limited number of uncorrelated new variables. These are termed "principal components". The first component summarises more of the variability than any other variable. The second variable summarises more of the variability not summarised by the first and uncorrelated with the first and so on. Because the new variables are not correlated, they may be interpreted independently. Thus, the total variation of a population may be broken into components, each of which may say something about the size, shape, or other quantitative aspect of the members of the population. PCA ordination scores are derived from the data matrix alone and it is thus an especially objective method where the analyst gives no *a priori* weighting. A further advantage is the simultaneous production of species and sample ordination scores in one integrated analysis.

Rotation of eigenvalues is often used to concentrate as much of the explained variation of a single variable into a single eigenvector. This helps to define the components in reference to a particular set of variables. One method is varimax rotation, which I applied to the data.

3.1.7 Cluster Analysis

The purpose of cluster analysis is to find groups in a data cloud of sampling units in the absence of any *a priori* information about which point belongs to which cluster. Clusters may be described as continuous regions of space containing a relatively high density of points, separated from other regions by regions containing relatively low-density points. Unlike ordination methods, (e.g. PCA, DFA), cluster analysis techniques operate on an un-partitioned data matrix to find, or impose, structure in the data cloud. No single clustering method can be used definitively to represent associations of similarity in the data and there have been many discussions on the relative merits of the various methods and their appropriate use on particular types of data (Jardine & Sibson, 1971). Because each method uses a slightly different approach and algorithm to produce the phenograms, several standard methods of analysis should be used and compared. These are described below.

3.1.7.1 Hierarchical Agglomerative Methods

Most of the hierarchical methods are agglomerative. Unlike the optimisation or partition methods, most agglomeration methods can use a broad range of similarity or difference measures. The various methods differ mainly in the detail of the fusion rule (i.e. two clusters should be fused if the distance between them has reached a certain threshold). The following are in general use.

3.1.7.1.1 Group Average Linkage (UPGMA)

This method is a compromise between single and complete linkage and identifies clusters by using the average of all the similarities between pairs of cases. It has the problem of being affected by different cluster sizes. In group average linkage, one of the

clusters might be smaller than the other. After fusion, the larger cluster determines the distance to the third cluster. That is, the number of cases in each cluster in the sample is determining the location of the cluster. This method can be divided into within-group and between-group and use the within-group and between-group variance respectively. It is a commonly used method for producing phylogenetic trees (Felsenstein, 1995).

3.1.7.1.2 Single Linkage (Nearest Neighbour)

This method identifies clusters based on isolation, i.e. how far they are apart at their nearest points. If there are any intermediate points, then the groups will be fused and any traces of their separate identities are lost. This is called chaining and leads to characteristic and uninformative phenograms. The resulting phenogram will look like a staircase as single cases are split off one at a time from a larger group. Its strength is that if clusters are well separated in the data, then single linkage can handle groups of different sizes and shapes that other methods often cannot recover. The algorithm is in essence the same as minimum spanning tree. In general, this method tends to exaggerate the similarity between groups and may thus suggest groups that are not realistic. This method is also commonly used for producing phylogenetic trees (Felsenstein, 1995).

3.1.7.1.3 Complete Linkage (Furthest Neighbour)

This method can be considered opposite to single linkage. It identifies clusters based on how far they are apart at their furthest points. The consequence is that the resulting clusters are compact and spherical. It is sensitive to even a single change in the rank order of the distances in the similarity matrix (Seber, 1984), and does not cope well with outliers (Milligan, 1981). In Monte Carlo simulations, this method mostly performed better than single linkage, though not as well as Ward's or group average (Milligan,

1981). Complete linkage is a conservative method in showing the more cohesive clusters but may miss others that are less clearly defined.

3.1.7.1.4 Centroid Clustering (UPGMC)

This method averages the cases of each group to find a group centre point, and then takes the similarity between the group centroids. It suffers from the occurrence of reversals. When two clusters are combined during the clustering process, it can happen that the level of similarity at which the combination takes place is higher than previously, instead of always being lower, as in other clusters (Legendre & Legendre, 1983).

3.1.7.1.5 Median Clustering (Weighted Centroid Clustering) (WPGMC)

When sample groups of varying sizes are used in clustering analysis, the position of the centroids may be biased toward the over represented groups, which can distort the clustering. This problem is corrected by giving equal weight to both clusters on the verge of fusing. This method suffers from the same bias as does average linkage in being affected by cluster size.

3.1.7.1.6 Ward's Method

This method is the hierarchical version of the trace optimisation method. At each fusion, it attempts to minimise the increase in total sum of squared distances within clusters. This is equivalent to minimising the sum of squared within-cluster deviation from the centroid. Since at any one stage it can only fuse those clusters already in existence, it is not allowed to reallocate points (i.e. it can only be stepwise optimal). Ward's method tends to form clusters containing equal numbers of observations, regardless of the true

number. Of all the cluster methods, average linkage or Ward's method appear to perform best in practice (Dunn & Everitt, 1982).

Since not all methods are necessarily appropriate for all types of datasets, an initial assessment of the various hierarchical cluster techniques was done. To determine which methods were most useful for analyses, I used samples of parasite species as a test of how well they group specimens. Group average (between), complete linkage and Ward's method were most applicable to the data analysed and were thus used in this thesis.

3.1.8 Abundance and Prevalence Data

Abundance and prevalence data are used extensively in this thesis to analyse host-parasite and parasite-parasite associations. To examine abundance data, it must be recognised that biological processes responsible for the abundances of species are often of a stochastic nature (Legendre & Legendre, 1983). This can lead to an enormous range of values. Thus when analysing this type of data, only the few dominant species, rather than the entire species composition, control the results of many multivariate analyses unless ln transformations (or similar) are applied in order to put the species on a more equitable footing (Gauch, 1982). Similarly, if the species vary greatly in their mean or variance, they may be standardised (z-score transformation). Species standardisation, however, may give too much emphasis to minor species.

If a data matrix is composed of two or more blocks of samples, with very few species occurring in more than one block, the matrix is said to be disjunctive. In disjunctive sample sets, samples have mostly nonzero similarities within blocks but mostly zero

similarities between blocks. Disjunction is the common term for large groups of singular samples, whereas outlier is the common term for one singular sample (or a few similar but singular samples) (Legendre & Legendre, 1983). Likewise, if the sample set is disjunctive, having several subsets of related samples that have little or nothing in common, it is best to separate the samples into several data subsets suitable for further analysis. Disjunction may be detected by a preliminary ordination using reciprocal averaging to produce an arranged data matrix. This also reveals outliers. Reciprocal averaging involves the standardisation by sample totals and by species totals and contributes to the effectiveness of this ordination method (Legendre & Legendre, 1983). Reciprocal averaging applies this standardisation internally. In comparison to algorithms without internal standardisation, there is less need to standardise prior to analysis and there is less difference in results caused by prior standardisation. Legendre *et al*. (1983) recommended that outliers be deleted and disjunctive sample sets be separated, rare species be omitted sample totals be standardized and abundance values be expressed logarithmically. These recommendations pertain to editing the data matrix as a whole.

General experience indicates a preference for abundance values with an intermediate range of 0-10 (Maarel, 1979a in Gauch, 1982). This range allows both quantitative and qualitative information to be expressed without either dominating the other. Compression of data to meet this range can be accomplished by using a logarithmic transformation. This type of transformation is also used to generate an approximate normal distribution of variables. The general recommendations to use a species abundance range of approximately 0 to 10 has exceptions if the raw data have a range not vastly greater than recommended above (e.g. 0-300), then multivariate analysis of

the raw data may be fine without the need to apply a transformation (Gauch, 1982). The choice of dissimilarity measure also has a similar effect.

Since species abundances are generally quantitative, it might seem appropriate to first look at parametric measures of independence when searching for species associations. If the abundance data are first normalised (e.g. ln(1 + x)), a covariance measure or a Pearson's linear correlation, may seem appropriate. Covariance or Pearson's correlations use the zeros as if they were another quantitative value, but normalising data does not change the basic problem (Legendre & Legendre, 1983). Many zeros without any interpretable significance appreciably distort the dispersion ellipse of the samples on both species axes compared, which biases the measure. Sample sets that contain all zeros for a variable also have the problem of zero variance, which can nullify the use of several parametric tests. Squared Euclidean distance measures are also often used. There are two problems with Euclidean distances. Because this distance measure uses squared values of original data, this measure emphasizes dominant species. In addition, Euclidean distances should not be used when examining species abundance because of double zero problems (Legendre & Legendre, 1983). When the data matrix consists of mean abundances of parasite species infecting host species, the parametric criteria of normality and equal variance would usually not be met. A non-parametric dissimilarity measure can be obtained by using a Chi-square measure. This measure is based on the Chi-square test of equality for two sets of frequencies.

Associations have to group species that are usually part of each other's biological environment. Covariance or correlation coefficients give only the linear correlation between the variations in the abundances of two species. Therefore, if two species are

always present together, but do not have their variations of abundances in a linear relationship, a Pearson's correlation coefficient will not detect the relationship. This limit of the covariance and correlation coefficients calls for an operational definition of association, and stresses the fact that low correlation values do not have any significance and do not show that two species are unrelated. This is a major drawback in the search for associations when using quantitative counts. In order to minimise this effect, Gauch (1982) has suggested several options:

1. Using presence/absence binary data (prevalence)
2. Eliminate all zeros from the comparisons by declaring the zeros as missing data
3. Eliminate the less frequent species (rare) from the study

Prevalence data may also be used where biological species associations can be defined based on the co-occurrence of species, rather than from the correlation between their fluctuations in abundances. Therefore, to examine species associations one can transform abundance data matrix to a presence/absence data matrix and use the similarity measures discussed in Section 3.1.4.2.

Rare is a relative term but typical criteria include species occurring in less than about 5% of the samples or in fewer than about 5 of 20 samples (Legendre & Legendre, 1998). There are several justifications for excluding rare species (Gauch, 1982). The occurrences of rare species are usually more a matter of chance than an indication of ecological or evolutionary conditions. Most multivariate techniques are little affected by rare species since they carry such a small percentage of the overall information or variance, hence rare species can be removed. Some multivariate techniques (e.g. ordination techniques) perceive rare species as outliers, thus obscuring the analysis of

the data set as a whole. Information on rare species may however be valuable for purposes (foreseen or not) other than multivariate analysis and therefore a careful decision should be made. When analysing species prevalence, there is also the problem of what to do with absences since biological communities are generally made up of a small number of dominant species and a large number of rare species.

Usually one wishes to base a search for biological associations on all the species of a given community, but in most cases the raw data contains many zeros. Consequently, the measure of dependence between species, upon which the identification of biological associations will be established, will be based on many pairs of zeros, for which there is no biological interpretation. It also follows that parasite species, which are host specific, also produce a similar effect, as does the examination of host associations based on parasite species where a number of host are only infected by mono host-specific parasite species. The absence of a rare species in a given sample constitutes a stochastic phenomenon, which does not necessarily indicate that the environment where it comes from is unfavourable.

3.2 Summary of How Methods were Used

Two types of data, morphometric and meristic, were analysed and each require slightly different approaches.

3.2.1 Morphometric Data

For the morphometric data in the taxonomic sections of Chapter 4, 5 and 6, probability values (P) represent exact probabilities. Examination of sclerite variables used MANOVA, PCA, DFA and HCA. Descriptive statistics were obtained for the soft body

and the haptoral sclerite variables. Means and range are expressed for soft body measurements using Method 2 (Section 2.2.2) and means and standard deviation are given for sclerite variables. Initial examination of the data for normality and homoscedasticity, graphically using frequency distributions and statistically using the Kolmogorov-Smirnov test and Levene's test (with α=0.01) respectively, indicated a need for transformation of several variables among several species. It was decided to natural log-transform and z-score standardise all variables to approximate multivariate normality and homoscedasticity when PCA and DFA analysis were used. A MANOVA test was used to detect differences in variable means between species. Only congeneric species were examined together. Raw data were used for MANOVA. Thus tests were considered significant at the experiment-wise error rate α=0.01. A post-hoc analysis (MANOVA) for pair-wise comparisons and range used the Scheffé test. Tukey HSD was also conducted and results compared with the Scheffé test as unequal group sizes were present in the data.

For PCA the covariance coefficient matrix was used and initially all characters were entered in analyses but subsequently removed from the final set of analyses based on low communality (< 0.7). Data were examined at two levels. Firstly, all species together; secondly, each genus was examined separately. Visual examinations of scatter plots assessed if separation of genera and species occurred.

Discriminant Function Analysis was used to identify morphometric sclerite characters having highest correlation with the genera and species recognised by morphology. The analysis used the stepwise approach and the within-group covariance distance matrix (Mahalanobis distances). Probability of F to enter and F to remove were set at ($P<0.01$)

and ($P<0.05$) respectively. Significant differences between group centroid means were assessed using Wilks λ ($P=0.01$). A cross-validation (leave-one-out classification) assessed the validity of the genera and species, and groups were weighted by sample size. Analyses were conducted at two levels. Firstly, examination of all species together; secondly, each genus was examined separately. Scatter plots were examined to determine if separation of parasite species or genera occurred using the first three functions. Discriminant analysis was also used to group samples at other levels. This included separation of cases into species-defined groups, correlation of association at the genus level and assessment of whether these groupings reflect the copulatory apparatus morphology groupings at these various levels. In Chapter 5, I extended the use of these statistical techniques to assess parasite species validity using variation on different host species.

In Chapter 6 I used DFA cross-validation to examine intra-species variation and its association with host species. Two parasite species that showed low host-specificity were used. For each parasite species, cases were grouped as host species and a cross-validation was used to assess the strength of intraspecific variation associated with hosts. Linear regression analysis was used to identify associations of sclerite variation between parasite species found on the same host individual and for correlation with host length. Data for this were log-transformed. To test for significant correlation between variables, the α level was set at $P= 0.01$.

In Chapter 6 I used cluster analyses to represent the morphometric associations between the parasite species and also host-associated intra-specific variation of two low host-specific species and compared resultant trees with the host phylogenetic tree. This

required the use of variable means for the parasite species or the eigenvectors produced from DFA. Manhattan and Squared Euclidean distance measures were used for the SPSS clustering methods group average (between), complete linkage and Ward's method. Manhattan distances measures were also used for analyses in the statistical package PHYLIP (Felsenstein, 1995). Distance matrices (see above) of the parasite species associations were examined using Neighbour-joining and UPGMA. Trees were either rooted or unrooted. For the rooted tree, parasites from *C. s. stercusmuscarum* were used as the outgroup. Cospeciation between hosts and parasites was analysed using TREEMAP (Page, 1995).

3.2.2 Meristic Data

Measures of prevalence, abundance and intensity of parasite infections on hosts are dealt with in Chapter 7. To measure associations between parasite species a Pearson's correlation was used and data were log-transformed. The problem of double absences was recognised (produced –ve associations) and a second analysis was done with host-specific species removed. Parasite species that showed linear associations were further analysed using linear regression analysis. Cluster analysis was used to display associations between parasite species. Mean intensities of parasite species on host species were log-transformed and I used the Chi-squared distance matrix as a measure of association. A presence/absence binary matrix was also used for comparison using Jaccard's and Ochiai index of similarity.

CHAPTER 4 TAXONOMY OF MONOGENEAN PARASITES

4.1 Introduction

Knowledge of monogeneans from Australian freshwater fishes is very sparse. To date 26 species of monogenean have been described from 16 species of native freshwater fishes (Fletcher & Whittington, 1998) that is roughly 5% of the approximately 300 species of freshwater fish known (Allen *et al.*, 2002) (see Section 1.7.2.). In the current study I examine a further 19 species of fish from Australian freshwaters, and report four new genera of Monogenea in the Dactylogyridae: *Longidigitis* gen. nov., *Recurvatus* gen. nov., *Iliocirrus* gen. nov. and *Helicirrus* gen. nov.and 19 new species. The genera are defined by copulatory apparatus morphology while species are defined by haptoral sclerite morphology. A new species of gyrodactylid is also reported.

In Chapter 1 I introduced the two criteria used by taxonomists to define ancyrocephaline genera: copulatory apparatus morphology and haptoral sclerite morphology. Other criteria such as position of vagina, gonads and other reproductive are also usually included. These approaches are compared and congruence of taxonomic groupings is examined. Species are recognised here by morphological as well as morphometric variation of haptoral sclerites. Anchors, bars and hooks, contained in the haptoral region, are most often the only sclerotised parts of the parasites measured in detail and reported in the literature. The number of individual character measurements may vary from five to over 25, depending on the genus and the researcher. Although details of these measurements are often required for species identification, more often than not the number of parasites measured is fewer than 10. This precludes the use of statistical analyses.

4.2 Methods

A requirement of any new parasite taxonomic description is the designation of a type host and location. In selecting type localities, preference was given to sample sites from my original study area of Queensland and to those sample sets that contained the greatest range of parasite species and intensity. Only parasites from type localities were used for statistical analyses and descriptions (see Appendix A, for species description details). Morphometric variables were modified from those defined by Klassen (1991) and measured with an eyepiece graticule. Five soft body and 24 sclerotised variables of the haptoral apparatus were measured (Figure 4.1). Where possible, a minimum of 20 specimens of each parasite species was measured. Hook numbering follows that of Mizelle (1936) (Figure 4.2). The haptoral terminology follows that of Gussev (1973) and male copulatory apparatus terminology follows Klassen (1991). Counts of the number of coils found at the distal end of the copulatory apparatus shaft of *Helicirrus* spp. are approximate due to the problems of resolution. Direction of coils will be determined as clockwise or counterclockwise according to Kritsky *et al.* (1985). The coil direction is determined by viewing the copulum ventrally. If the copulum shaft is directed in a clockwise direction from the base to the ventral lip of the shaft, the rings are defined to have a clockwise direction, and conversely so, counterclockwise.

4.2.1 Statistical Analyses

The statistical methods, Multivariate Analysis of Variance, Principal Component Analysis and Discriminant Function Analysis are used to assess congruence of genera and species initially distinguished by morphology. Details of the statistical analyses are given in Section 3.2.

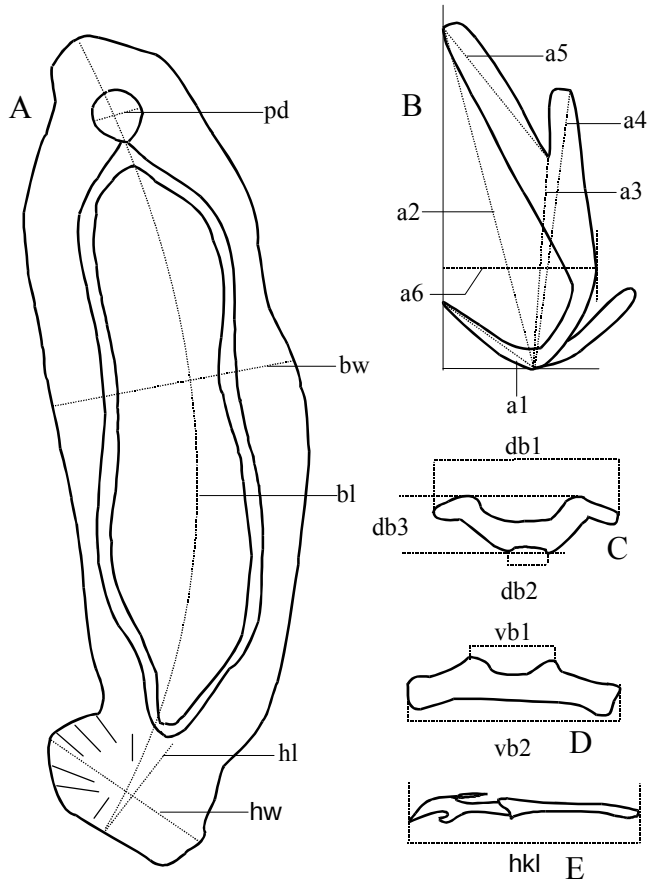


Figure 4.1. Parasite measurements.
A. Body measurements: bl body length; bw, body width; hl, haptor length; hw, haptor width; pd, pharynx dia.. B. Anchor measurements a1-a6; C. Dorsal bar measurements db1-3; D. Ventral bar, vb1-2; E. Hook length, hkl 1-7.

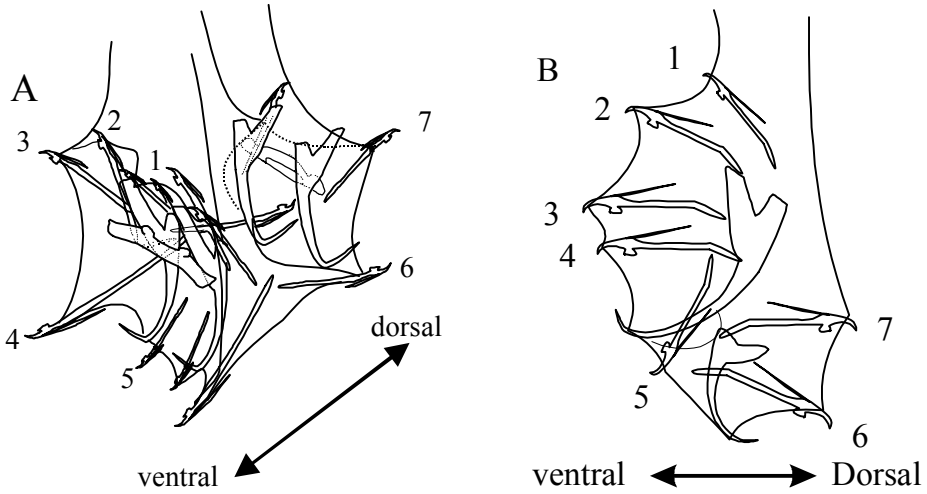


Figure 4.2. A. Haptoral sclerite relationships and orientation for *Longidigitis*, *Iliocirrus*, *Recurvatus*, and *Helicirrus* species *H. marjoriaea*, *H. mcivori* and *H. gertrudaea*.
1-7, hook numbering. B. For *Helicirrus* species *H. megaloanchor*, *H. splendidae* and *H. maccullochii*.

4.3 Results

Descriptions of all new species are in Appendix A. The following species are recognized: *Longidigitis auripontiformis*, *L. robustus*, *Iliocirrus iliocirrus*, *Helicirrus splendidae* and *H. megaloanchor* from *Melanotaenia splendida splendida* (Peters, 1876), *L. gracilis* from *M. utcheensis* McGuigan, 2001, *I. mazlini* from *M. eachamensis*, *L. maccullochii* and *H. maccullochii* from *M. maccullochi* Ogilby, 1915, *I. trifasciatae*, *H. mcivori* and *L. hopevalensis* from *M. trifasciata* (Rendahl, 1922), *I. ornatusi* from *Rhadinocentrus ornatus* Regan, 1914, *L. utcheei* from *Cairnsichthys rhombosomoides* (Nichols & Raven, 1928), *Recurvatus chelatus* and *I. rossi* from *Craterocephalus stercusmuscarum stercusmuscarum* (Günther, 1867), *H. marjoriaea* from *Craterocephalus marjoriae* Whitley, 1948, *R. signiferi* from *Pseudomugil signifer* Kner, 1865 and *H. gertrudaea* from *Pseudomugil gertrudae* Weber, 1911 are described.

4.3.1 Remarks on Morphological Characters

4.3.1.1 Copulatory Apparatus Structure.

Four copulatory apparatus types are identified (Figure 4.3).

Type I. A type I copulatory apparatus identifies species of *Recurvatus* and consists of a ball-shaped base with the shaft emanating from the right-anterior side. The shaft consists of a thin tube forming a counter-clockwise curving single loop (ventral view), which encircles the base (see Section 4.2). The distal end of the shaft terminates anterior to and to the right of the base. The accessory piece is attached to the anteromedial end of the base, dextral to the copulum shaft.

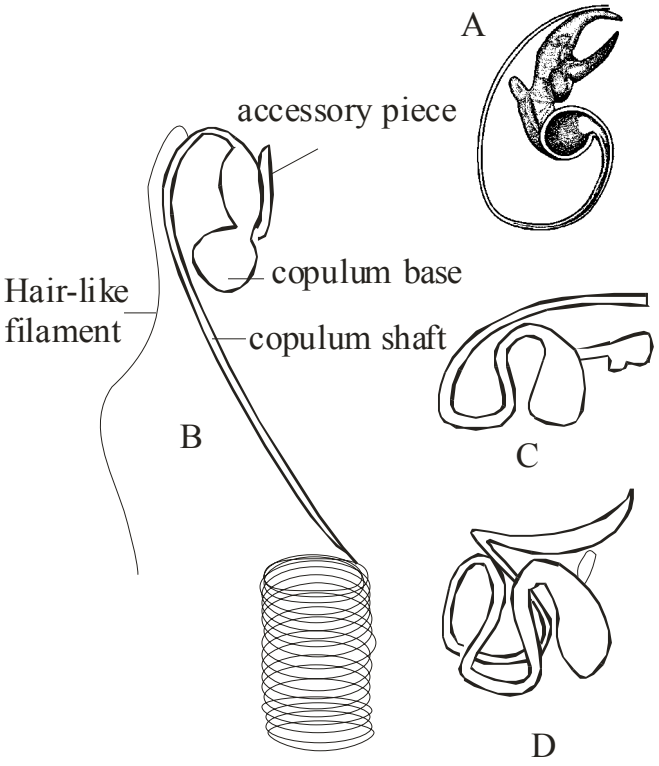


Figure 4.3. Copulatory apparatus types.
A. type I "*Recurvatus*", B. type II "*Helicirrus*", C. type III "*Longidigitis*", D. type IV "*Iliocirrus*" all dorsal view.

Type II. A type II copulatory apparatus identifies species of *Helicirrus* and consists of a single teardrop-shaped base with the shaft emanating from the anteromedial end. The shaft projects anteriorly initially then sharply reverses direction to continue left and posteriorly to the base, ending in a helix anterior to gonads. A thin spike-shaped accessory piece projects anteriorly from the right anterior end of the copulum base and to the right of the shaft. A long thin filament arises from near the anterior end of the shaft. This filament was not observed in live specimens or most permanent mounts but was easily discernable when the internal fluids of the parasite were extruded in preserved material using Malmberg's glycerol-ammonium-picrate. This was also the case for the vas deferens.

Type III. A type III copulatory apparatus identifies species of *Longidigitis* and consists of a bulbate base with the shaft emanating from the anterior end. The shaft consists of a tube with a single sinistral loop with distal end anterodextral to the base. The accessory piece is sclerotised; thumb shaped and arises from the anterosinistral side of the shaft.

Type IV. A type IV copulatory apparatus identifies species of *Iliocirrus* and consists of a bulbate base with the shaft arising from the anterior end. The shaft consists of a tube with two sinistral loops. The first loop is posterosinistral and the second loop anterosinistral to base. The copulum shaft distal end is anterior and to the right of the base. The accessory piece is sclerotised; thumb shaped and emanates from the right-anterior side of the copulum.

HAPTORAL STRUCTURE. The haptoral sclerite morphology can be discussed in three parts, anchors, bars and hooks. In the new genera described here, variations of these characters do not clearly reflect generic groupings.

Anchors have a large variation in size and shape although two patterns are recognized. Pattern one has dorsal anchors slightly smaller than ventral, while pattern two has dorsal anchors much smaller than ventral. Pattern one is seen in all species of *Recurvatus* and *Longidigitis* but also occurs in several species of *Helicirrus*, *H. mcivori*, *H. gertrudaea* and *H. marjoriaea*. Pattern two occurs in three species of *Helicirrus*, *H. splendidae*, *H. megaloanchor* and *H. maccullochii* and all species of *Iliocirrus* and is associated with the reduced hook pattern (see below).

The dorsal bar has three morphological variants. The simple arch with the anteromedial membrane present occurs in *L. auripontiformis*, *L. hopevalensis* and *L. robustus.* The second variant of the dorsal bar possesses a posteromedial notch. This notch is present in *L. gracilis* and all species of *Iliocirrus.* The third variant is the highly reduced dorsal bar which is observed as a thin filament and occurs in *H. splendidae, H. megaloanchor* and *H. maccullochii.* All species of *Recurvatus* and the species *L. maccullochii, L. utcheei, H. mcivori, H. marjoriaea* and *H. gertrudaea* have a well-developed arched dorsal bar and lacking both the medial notch and anteromedial membrane.

Hook lengths show two character patterns that are spread across members of more than one genus. In the first pattern, hooks are of reduced form; all smaller than ventral anchors with the dorsal hooks H6 always the longest. This pattern is found in the species *H. splendidae*, *H. megaloanchor* and *H. maccullochii* and is associated with highly reduced dorsal anchors and bar. The second pattern, occurring in members of all four genera, consists of elongated and widely differing lengths though generally increasing from H1 to H4. Ventral hook H4 is always the longest and always longer than the ventral anchors.

Several patterns of associated hook lengths are identified in the elongated form. Size differences between H2 and H7 have three patterns. H2 is longer than H7 in *I. ornatusi*, *I. mazlini*, *I. trifasciatae*, *L. auripontiformis*, *L. utcheei*, and *H. marjoriaea*. H2 equals H7 in *R. chelatus*, *R. signiferi*, *I. rossi*, *I. iliocirrus*, *L. gracilis*, *L. maccullochii*, *L. robustus* and *H. mcivori*. *Helicirrus gertrudae* has H2 shorter than H7. Generally, H3 is noticeably longer than H6, however in *I. trifasciatae* and *L. utcheei* H3 and H6 are of

similar size. Most species have hook H3 longer than H2, but this is reversed for *R. signiferi*.

4.3.2 Remarks on Morphometric Variation

Mean and range of measured soft body characters, and mean and standard deviation of haptoral sclerite measurements are presented in Appendix B1-4. Soft body measurements show large variation and are influenced by preparation method. Slide preparations using Method 2 (formalin-preserved parasites) had much smaller soft body dimensions than those prepared by the Method 1 (live-parasite technique) (pers. obs.). This is an important point when considering comparisons with species from other studies. However, several species show obvious differences in body width. The species *H. splendidae, H. maccullochii, I. mazlini* and *I. ornatusi* have greater body widths, relative to body length, than other species examined. *Iliocirrus mazlini* and *I. ornatusi* are easily distinguished from other species of the genus based on this observation.

4.3.2.1 Multivariate Analysis

A significant difference (Scheffé test between-subjects MANOVA) between character means for all sclerite variables was identified in species of *Longidigitis* and *Helicirrus* ($P<0.01$). A significant difference ($P<0.01$) between means for species of *Recurvatus* was observed for all variables except DA5 and DA2, ($P>0.01$) and DA1, VA6 and VA2 ($P>0.05$). For *Iliocirrus* species a significant difference ($P<0.01$) between means was observed for all variables except DA1 ($P<0.05$) and VA6 ($P>0.05$).

A MANOVA using the Scheffé test for pair-wise comparisons ($P<0.01$) of species within each genus showed the maximum number of significantly different variable means between any two species was 21 of the possible 24 variables measured, while the minimum was seven. The maximum difference of 21 occurred between the species *L. gracilis* and *L. hopevalensis* (Table 4.1) and *H. splendidae* and *H. gertrudaea* (Table 4.2). The minimum number of seven significant differences ($P<0.001$) was between *L. hopevalensis* and *L. auripontiformis* (Table 4.1) and between *H. gertrudaea* and *H. marjoriaea* (Table 4.2). For species of *Iliocirrus*, the maximum was 17 between *I. iliocirrus* and *I. mazlini* and between *I. rossi* and *I. ornatusi* (Table 4.3). The minimum for species of *Iliocirrus* was eight between *I. trifasciatae* and *I. rossi* (Table 4.3). For the species of *Recurvatus*, the difference was 18. A MANOVA using Tukey's HSD test for pair-wise comparisons of species within each genus showed the maximum number of significantly different variable means ($P<0.01$) between any two species was 22, while the minimum was 13 between *H. mcivori* and *H. gertrudaea* (Table 4.2). The maximum number of 22 occurred between the following pairs of species: *H. maccullochii* and *H. marjoriaea, H. megaloanchor* and *H. gertrudaea, H. gertrudaea* and *H. splendidae, H. marjoriaea* and *H. splendidae, H. maccullochii* and *H. mcivori, L. gracilis* and *L. hopevalensis* and *L. utcheei, L. auripontiformis* and *L. gracilis* and *L. maccullochii, I. rossi* and *I. ornatusi*.

Table 4.1. Number of statistically significant morphometric character differences between species of *Longidigitis*.

SPECIES	MINIMUM DIFFERENCE	MAXIMUM DIFFERENCE
L. auripontiformis	*L. hopevalensis* (7) (18)* *L. robustus* (13) (19)* *L. utcheei* (14) (19)*	*L. gracilis* (19) (22)* *L. maccullochii* (20) (22)*
L. robustus	*L. maccullochii* (11) (20)* *L. hopevalensis* (14) (16)*	*L. gracilis* (17) (21)* *L. utcheei* (15) (18)*
L. maccullochii	*L. utcheei* (16) (19)* *L. gracilis* (17) (20)*	*L. hopevalensis* (20) (21)* *L. auripontiformis* (20) (22)*
L. utcheei	*L. hopevalensis* (14) (20)* *L. robustus* (14) (17)*	*L. gracilis* (19) (22)*
L. hopevalensis	*L. auripontiformis* (7) (18)*	*L. gracilis* (21) (22)*
L. gracilis	*L. maccullochii* (17) (20)*	*L. hopevalensis* (21) (22)*

( ) Scheffé pairwise comparisons (α= 0.01).
( )* Tukey's HSD test (α= 0.01).

Table 4.2. Number of statistically significant morphometric character differences between species of *Helicirrus*.

SPECIES	MINIMUM DIFFERENCE	MAXIMUM DIFFERENCE
H. splendidae	*H. maccullochii* (10) (15)* *H. megaloanchor* (13) (14)* *H. mcivori* (13) (18)*	*H. marjoriaea* (20) (22)* *H. gertrudaea* (17) (20)*
H. maccullochii	*H. splendidae* (10) (15)* *H. megaloanchor* (15) (16)*	*H. marjoriaea* (18) (20)* *H. gertrudaea* (16) (20)* *H. mcivori* (20) ((22)*
H. megaloanchor	*H. gertrudaea* (18) (22)*	*H. marjoriaea* (18) ((20)* *H. mcivori* (19) (21)*
H. mcivori	*H. gertrudaea* (7) (13)* *H. marjoriaea* (13) (21)*	*H. megaloanchor* (19) (21)*
H. gertrudaea	*H. marjoriaea* (11) (17)*	*H. megaloanchor* (18) (22)*
H. marjoriaea	*H. gertrudaea* (11) (17)*	*H. splendidae* (21) (22)*

( )Scheffé pairwise comparisons (α= 0.01)
( )* Tukey's HSD test (α= 0.01).

Table 4.3. Number of statistically significant morphometric character differences between species of *Iliocirrus*.

SPECIES	MINIMUM DIFFERENCE	MAXIMUM DIFFERENCE
I. trifasciatae	*I. rossi* (8) (16)* *I. mazlini* (9) (17)*	*I. iliocirrus* (14) (17)* *I. ornatusi* (12) (19)*
I. iliocirrus	*I. ornatusi* (12) (19)* *I. rossi* (14) (16)*	*I. mazlini* (17) (21)*
I. rossi	*I. mazlini* (11) (16)*	*I. ornatusi* (17) (22)*
I. ornatusi	*I. trifasciatae* (12) (19)* *I. iliocirrus* (12) (19)*	*I. mazlini* (15) (20)*
I. mazlini	*L. trifasciatae* (9) (17)*	*I. iliocirrus* (17) (21)*

( ) Scheffé pairwise comparisons (α= 0.01).
( )* Tukey's HSD test (α= 0.01).

4.3.2.2 Principal Component Analysis

Initially all analyses were run to examine communalities between variables and functions. All variables with communalities <0.7 were removed and a final analysis was done. A summary of explained variance and morphological characters associated with principle components is presented in Table 4.4 while detailed results of analyses are given in Appendix B5-9. For all species analysed together, PCA extracted four components, accounting for 84.4 % of the explained variance, after the removal of characters H1, and H5. The components PC1 accounted for 48.6%, PC2 for 18.23%, PC3 for 9.0% and PC4 accounted for 7.3%. Specimens did not cluster into their respective genera (Figure 4.4). Two clusters were present on the first two components. One cluster (bottom left) represented *H. splendidae*, *H. maccullochii* and *H. megaloanchor* and identify the reduced-hook haptoral type. The main cluster, which included species from all four genera, is characterised by the adult hook haptoral type.

Table 4.4. Summary table of explained variance and morphological characters associated with principle components.

	PC1	PC2	PC3
All species	48.6% DA1-6 DB 3 VA 1	18.23% H2-4, 6, 7, DB1, 2	9% VA2- 6 VB1
Recurvatus spp.	39.8% H2-4, 6, 7 DA3, 4 VA1, VB1	22.4% VA2, 4, 5 DA2	16.4% DA1 VB2
Helicirrus spp.	50.8% H2-4, 6, 7 DA1-6 DB1, 3 VA1	29.4% VA2-6 VB1, 2	5.6%
Longidigitis spp.	51.3% DA1-6 H2, 7 VA2-5	14.5% H3, 4, 6	10.7% VB1, 2
Iliocirrus spp.	37.5% VA2-5 H2, 3, 7 DA2-4	22.2% H4, 6 VB1	12.5% VB2 DA5

% explained variance.

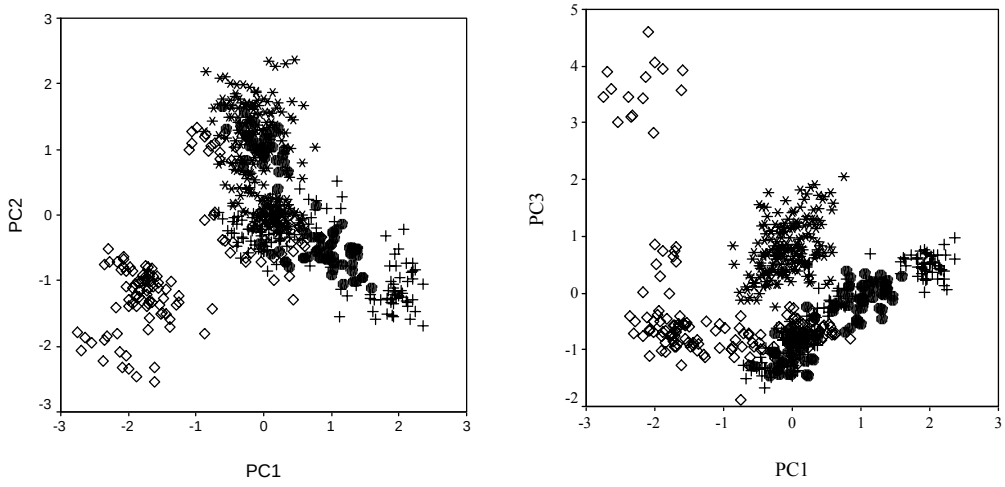


Figure 4.4. Genus-level scatter plot of first three axes of principal component analysis. Species of ●*Recurvatus*, +*Longidigitis*, ∗*Iliocirrus,* ◇*Helicirrus*.

Dorsal anchor and hook characters dominated the first two components respectively while ventral anchor and bar characters dominated PC3. Characters of PC1 and PC3 separated the *Iliocirrus* genus from the main cluster.

When species of *Helicirrus*, *Recurvatus*, *Longidigitis* and *Iliocirrus* were analysed separately, clusters were generally well defined. Four components were identified for *Recurvatus,* after the removal of eight characters: DA-6, VA3, VA6, DB1-3, H1 and H5. The components accounted for 85.8% of the explained variance. PC1 accounted for 39.8%, PC2 for 22.4%, PC3 for 16.4% and PC4 for 7.2%. Species were clearly separated on the first component (Figure 4.5) which was dominated by hooks H2-4 and H6-7 and dorsal anchor characters DA3-4.

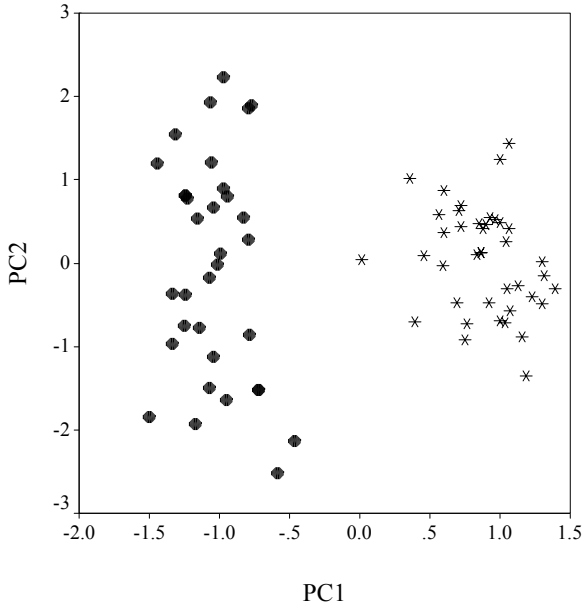


Figure 4.5. Scatter plot of first two axes of principal component analysis for species of *Recurvatus*
∗*R. signiferi*, ●*R. chelatus*.

Analysis of the *Helicirrus* species identified three components, after the removal of three characters: DB2, H1 and H5. The first two components clearly separated *H. megaloanchor, H. splendidae* and *H. maccullochii* (Figure 4.6A) while the combination of PC1 and PC3 clearly separated *H. marjoriaea*, *H. gertrudaea* and *H. mcivori* (Figure 4.6B). Dorsal anchor, ventral anchor, and H6-7 and VB2 had highest correlation with

the first three components respectively and accounted for 85.8% of the explained variance.

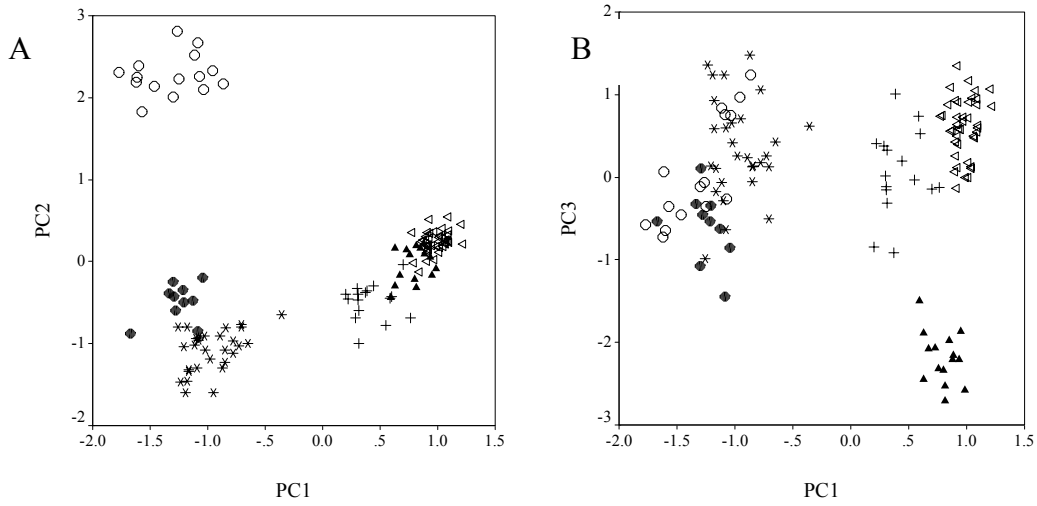


Figure 4.6. Scatter plot of first three axes of principal component analysis for species of *Helicirrus*.
A. PC1 and PC2. B. PC1 and PC3. ✳ *H. splendidae*, ○ *H. megaloanchor*, + *H. mcivori*, ◁ *H. marjoriaea*, ● *H. maccullochii*, ▲ *H. gertrudaea*.

Four components were identified for the *Longidigitis* species after the removal of six variables: VA1, VA6, DB2-3, H1 and H5. The four components accounted for 83.3% of the explained variance. PC1 accounted for 51.3%, PC2, for 14.5%, PC3 for 10.7% and PC4 for 6.8%. The first two components separated *L. maccullochii*, *L. robustus*, *L. auripontiformis* and *L. gracilis* from each other, with *L. utcheei* slightly overlapping *L. gracilis* and *L. hopevalensis* partially overlapping *L. auripontiformis* (Figure 4.7A). The combination of PC1 and PC3 clearly separated *L. gracilis*, *L. utcheei* and *L. maccullochii* from each other while *L. auripontiformis, L. maccullochii* and *L. hopevalensis* overlapped and *L. utcheei* and *L. robustus* overlapped (Figure 4.7B). *Longidigitis auripontiformis* and *L. hopevalensis* clearly separated on PC4 (not shown). Dorsal and ventral anchor characters dominated component PC1 while hook

measurements dominated PC2. Ventral bar characters dominated PC3 and dorsal bar character DB1 dominated PC4.

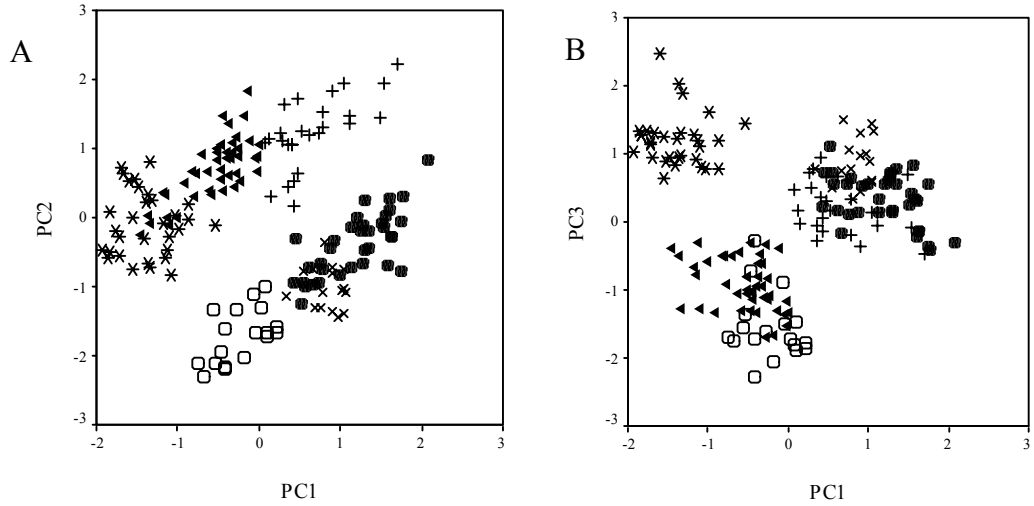


Figure 4.7. Scatterplots of first three axes of principal component analysis for species of *Longidigitis*.
A. PC1 and PC2. B. PC1 and PC3. +*L. maccullochii*, ◂*L. utcheei*, ×*L. hopevalensis*, ✳*L. gracilis*, ○*L. robustus*, ●*L. auripontiformis*.

Four components were identified for *Iliocirrus* species after the removal of nine variables: DA1, DA6, VA1, VA6, DB1-3, H1 and H5. The four components accounted for 79.3% of the explained variance. The first two components separated *I. mazlini*, *I. rossi* and *I. iliocirrus* from each other, while *I. ornatusi* overlapped *I. mazlini* and *I. trifasciatae* and slightly overlapped *I. rossi* and *I. iliocirrus* (Figure 4.8A). The combination of PC1 and PC3 clearly separated *I. mazlini* from *I. ornatusi* while *I. trifasciatae* slightly overlapped *I. rossi* and *I. iliocirrus* (Figure 4.8B). A combination of dorsal and ventral anchor and hook characters dominated the first component, while hook characters dominated the second component. Ventral bar character VB2 and dorsal anchor character DA5 correlated highest with PC3.

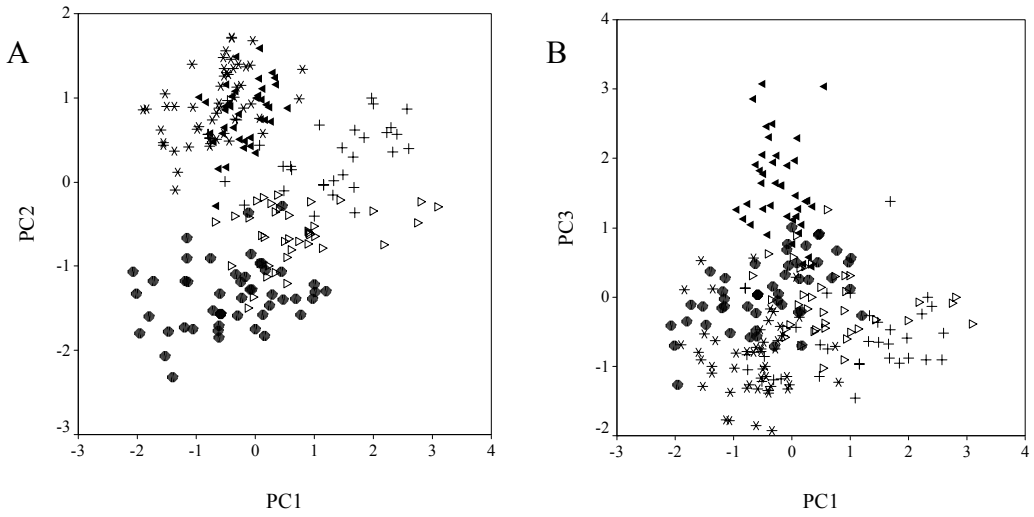


Figure 4.8. Scatterplots of first three axes of principal component analysis for species of *Iliocirrus*.
A. PC1 and PC2. B. PC1 and PC3. ▷ *I. trifasciatae*, + *I. rossi*, ∗ *I. ornatusi*, ◂ *I. mazlini*, ● *I. iliocirrus*.

Table 4.5. Summary table of explained variance and morphometric characters associated with discriminant functions.

	F1	F2	F3
Genus level	76.1%	18.9%	5.0%
	DB2	VB1, 2	H2-4, 6, 7
	VA2	DA3, 4	DA1, 2, 6
		VA3, 4, 6	VA1, 5
		H5	DB1, 3
All species	54.8%	22.8%	5.5%
	DB2	H4	VA2-4
		DA2	DA3, 4
Recurvatus spp.	H1, 2, 7		
	VB1		
Helicirrus spp.	74.8%	17.8%	4.0%
	H4	VA1-3, 6	DA1, 2, 6
	DB1, 3	VB1,	VB2
		H5	H6
			VA5
Longidigitis spp.	48.5%	28.9%	11.8%
	DA1-3, 5, 6	H4	H1, 3, 6, 7
	DB3	VA2-4	
Iliocirrus spp.	58.4%	18.5%	14.5%
	H3, 4, 6	VB2	VA1-4
	VA6	H5	DB1, 2
		DA5, 6	DA1-4

% explained variance.

4.3.2.3 Discriminant Function Analysis

A summary of explained variance and morphological characters associated with discriminant functions is presented in Table 4.5 while detailed results of analyses are given in Appendix B10-15. Stepwise analysis of all specimens, with parasite species grouped into genera, identified three characters DB3, DA5 and H1 not fitting the selection criteria of F to enter ($P<0.05$) and F to remove ($P<0.1$). These characters were removed from the final analysis. The first function (F1) accounted for 76.1%, F2 accounted for 18.9% and F3 accounted for 5.0% of total variance. *Helicirrus, Longidigitis* and *Iliocirrus* were clearly separated using F1 characters (Figure 4.9A). Characters of F1 and F2 do not separate *Recurvatus* and *Helicirrus. Recurvatus* clearly separated from the other three genera using F3 characters; however, *Helicirrus, Iliocirrus* and *Longidigitis* do not separate from each other (Figure 4.9B). A significant difference between group centroid means was identified for all functions (Wilks' λ, $P<0.001$). The characters, VA2 and DB2 had the highest correlation with F1, while DA3, VB1-2, VA4, VA6 and DA3-4 had the highest correlation with F2 and F3 had highest correlation with hook characters H2-4, H7, dorsal anchor characters DA1-2, DA6 and ventral anchor characters VA1 and VA5. The functions were not dominated by a set of variables from any one morphological character (i.e. anchors, bars or hooks), but a mixture of variables from each. All eigenvalues were positive except DA3-4 indicating a size difference in the characters between genera.

Analysis of *Recurvatus* identified 19 variables that did not fit the selection criteria of F to enter ($P<0.05$) and F to remove ($P<0.001$). Most of these rejected variables were anchor and bar measurements. The remaining five variables, hook characters H1, H2,

H7, and VB1 VA4 clearly separated *R. signiferi* and *R. chelatus* (not shown). The low number of species in the analysis can explain the low number of characters used.

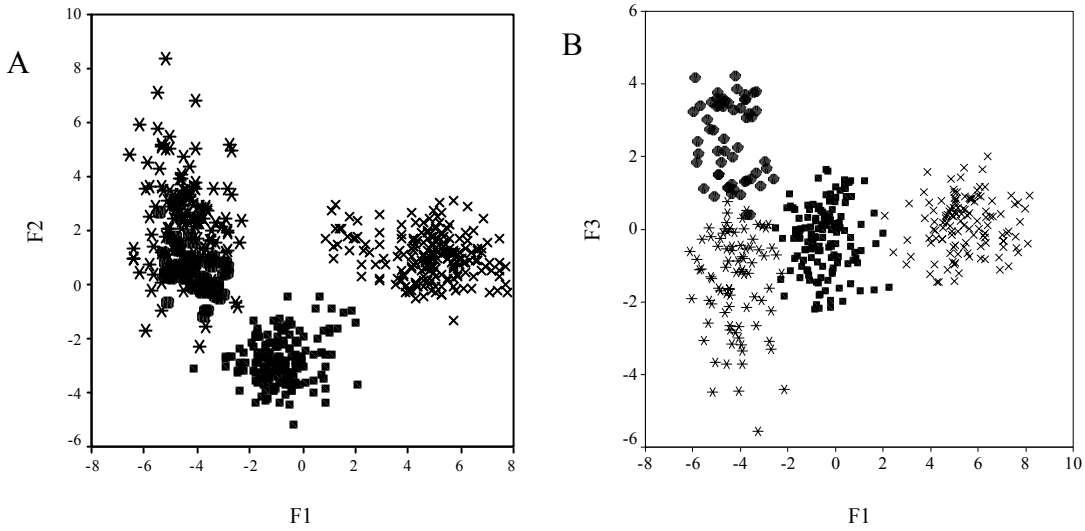


Figure 4.9. Scatterplot genus-level grouping using first three axes of discriminant analysis. Specimens entered as genera. A. F1 and F2. B. F1 and F3. ×*Iliocirrus*, ■*Longidigitis*, ✳*Helicirrus*, ●*Recurvatus*.

Analysis of species of *Helicirrus* identified four variables DA2, DA6, H1, and H5 not fitting the selection criteria of F to enter ($P<0.05$) and F to remove ($P<0.001$). The first three functions accounted for 96.6% of the total explained variance. Three distinct clusters were evident for the combined functions F1 and F2. *Helicirrus megaloanchor* separated from all other species, *H. splendidae* and *H. maccullochii* from all other species and *H. mcivori*, *H gertrudaea* and *H. marjoriaea* from all other species (Figure 4.10A). Characters of F1 and F2 do not clearly separate *H. splendidae* from *H. maccullochii* or *H. mcivori*, *H. gertrudaea* and *H. marjoriaea* from each other. Characters of F3 clearly separate *H. splendidae* from *H. maccullochii*, and *H. mcivori*, *H. marjoriaea* and *H. gertrudaea* from each other (Figure 4.10B). Dorsal bar characters

DB1, DB3 and H4 have highest correlation with F1, F2 correlates highest with ventral anchor characters and F3 correlates highest with characters VA5, VB2, H6 and DA1.

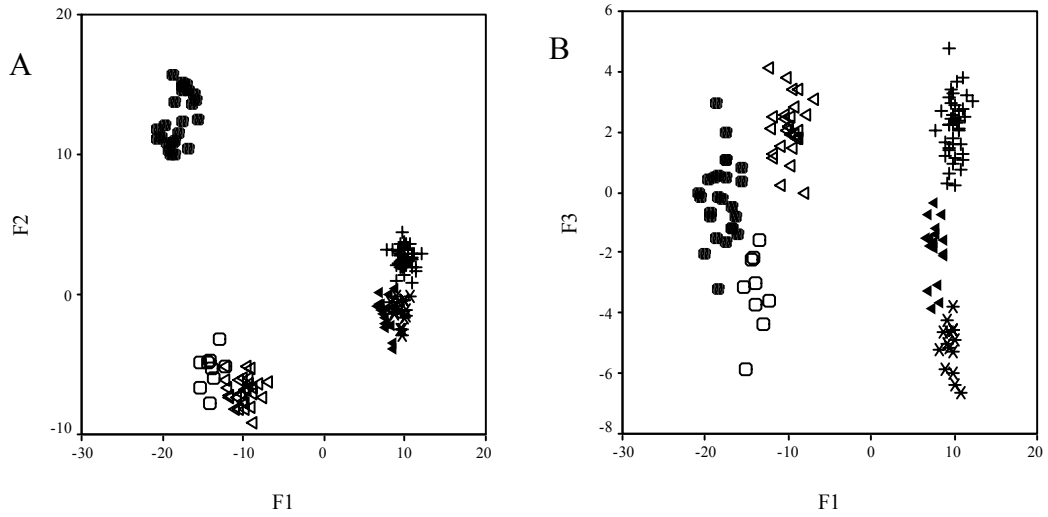


Figure 4.10. Scatterplots of first three axes of discriminant functions for species of *Helicirrus*.
A. F1 and F2. B. F1 and F3. ◁ *H. splendidae*, ● *H. megaloanchor*, ◀ *H. mcivori*, + *H. marjoriaea*, ○ *H. maccullochii*, ✳ *H. gertrudaea*.

Analysis of species of *Longidigitis* identified the three characters H1, VA5-6 as not fitting the selection criteria of F to enter ($P<0.05$) and F to remove ($P<0.001$). The first three functions account for 89.2% of the total explained variance. Characters of F1 clearly separated *L. utcheei*, *L. maccullochii* and *L. gracilis* from *L. auripontiformis*, *L. robustus* and *L. hopevalensis* while F2 characters clearly separated *L. auripontiformis*, *L. hopevalensis* and *L. robustus* from each other (Figure 4.11A). Functions F1 and F2 characters do not clearly separated the species *L. gracilis*, *L. maccullochii* or *L. utcheei* from each other. Function F3 characters clearly separated *L. gracilis* from *L. utcheei* and *L. maccullochii* (Figure 4.11B), while *L. maccullochii* and *L. utcheei* clearly separated using F4 characters (not shown). Dorsal anchor characters dominate F1, ventral anchor characters dominate F2, hook characters dominate F3, and ventral bar characters dominate F4.

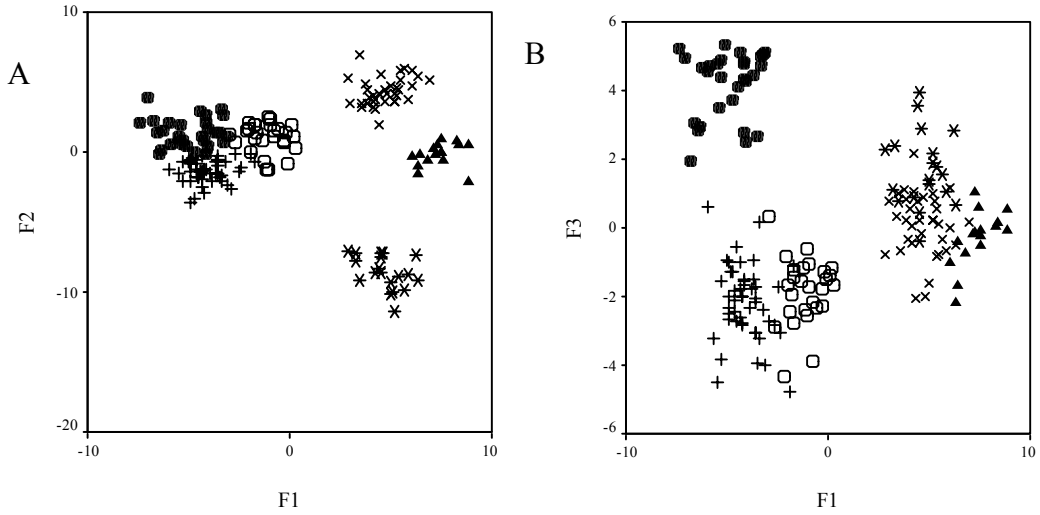


Figure 4.11. Scatter plots of first three axes of discriminant functions for species of *Longidigitis*.
A. F1 and F2. B. F1 and F3. ■ *L. utcheei*, + *L. maccullochii*, ▲ *L hopevalensis*, ● *L. gracilis*, ∗ *L. robustus*, × *L. auripontiformis*.

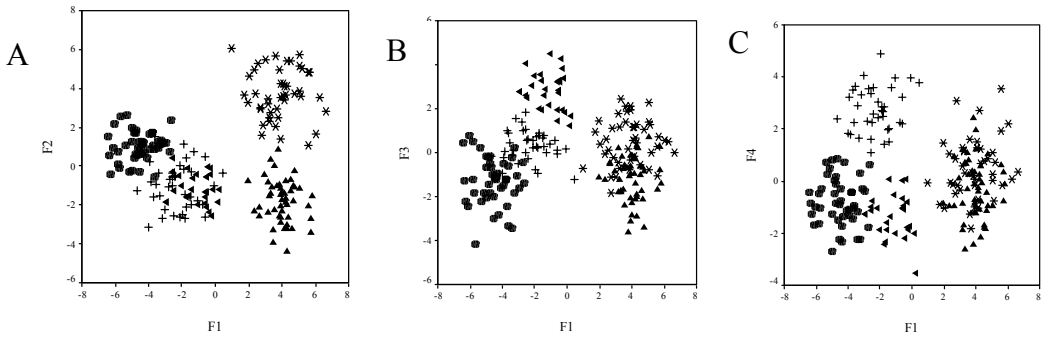


Figure 4.12. Scatter plot of first four axes of discriminant functions for species of *Iliocirrus*.
A. F1 and F2. B. F1 and F3. C. F1 and F4. + *I. trifasciatae*, ◂ *I. rossi*, ▲ *I. ornatusi*, ∗ *I. mazlini*, ■ *I. iliocirrus*.

Analysis of *Iliocirrus* species identified 12 variables that did not fit the selection criteria of F to enter ($P<0.05$) and F to remove ($P<0.001$). The first three functions account for 91.4% of total explained variance. *Iliocirrus ornatusi*, *I. mazlini* and *I. iliocirrus* clearly separated from each other using F1 and F2 (Figure 4.12A). *Iliocirrus rossi* and *I. trifasciatae* cluster together with partial overlapping of *I. iliocirrus* (Figure 4.12A). Function F3 characters separate *I. rossi* from *I. iliocirrus* and *I. trifasciatae* (Figure

4.12B) while F4 characters separate *I. trifasciatae* from *I. ross*i and *I. iliocirrus* (Figure 4.12C). Hook characters dominate F1; characters VB2, DA5 and H5 dominate F2. Dorsal and ventral bar characters are correlated highest with F3 and characters H1-2 and DB3 correlate highest with F4.

Table 4.6. Cross-validation of genus level grouping.
Specimens entered as genus groups. 97.1% of cross-validated grouped cases correctly classified.

		Predicted Group Membership				Total
	Genus	*Recurvatus*	*Helicirrus*	*Longidigitis*	*Iliocirrus*	591
Count	*Recurvatus*	66	8	0	0	74
	Helicirrus	5	125	3	0	134
	Longidigitis	0	0	174	0	174
	Iliocirrus	0	0	0	209	209
%	*Recurvatus*	89.2	10.8	.0	.0	100.0
	Helicirrus	3.7	93.3	2.2	.0	100.0
	Longidigitis	.0	.0	100.0	.0	100.0
	Iliocirrus	.0	.0	.0	100.0	100.0

4.3.2.4 Cross Validation

Using the within-group covariance matrix and probability weighted for sample size, with parasites entered as genera, cross-validation analysis correctly classified 97.1% of the 591 measured specimens (Table 4.6). For *Recurvatus*, 10.8% of specimens were mis-classified as *Helicirrus*, while for *Helicirrus* specimens 3.7% were classified as *Recurvatus* and 2.2% were classified as *Longidigitis*. All specimens of *Longidigitis* and *Iliocirrus* were classified correctly. This high level of correctly classified specimens indicates that combinations of haptoral morphometric variables can identify the genera that were defined by copulatory apparatus morphology. When all parasites were analysed together and entered as species, 99.5% of specimens were classified into their correct species. Specimens mis-classified were not identified as other species within

their own genus but across genera. Two specimens (13.3%) of *H. gertrudaea* were classified as *R. signiferi* while one specimen of *H. mcivori* was classified as *L. gracilis.*

4.4 Discussion

Both haptoral and copulatory apparatus structure are recognised as important in defining the genera and there is congruence between the two approaches. Parasite species were grouped into genera based on copulatory apparatus morphology. Discriminant function analysis and PCA clearly identified a number of clusters, recognised *a posteriori* as the parasite genera and species described using morphology. Thus, the genera and species were validated using only morphometric haptoral characters. Reproductive morphology appears to be the most natural taxonomic criterion, rather than haptoral morphology, for grouping the species assigned to *Helicirrus*, *Recurvatus, Iliocirrus* and *Longidigitis*. Haptoral sclerite morphology groupings can also reflect generic groupings based on copulatory apparatus structure. However, no single or set of morphological characters is unique to each genus. Separation of each genus is by a set of broadly recognised morphometric variations between characters defined by DFA.

The amount of variation in haptoral morphology differed between genera. Species of *Helicirrus* have the greatest haptoral sclerite variation while species of *Iliocirrus* have the least. For species of *Iliocirrus*, *I. trifasciatae* is least discernable from others of the genus, especially *I. rossi* and *I. iliocirrus*, based on haptoral sclerite variation; however, *Iliocirrus trifasciatae* is the most distinct species of *Iliocirrus*, based on copulatory apparatus morphology. Species of *Longidigitis* could be separated into two groups *L. gracilis*, *L. maccullochii* and *L. utcheei* on the one hand and *L. auripontiformis*, *L. hopevalensis* and *L. robustus* on the other based on haptoral sclerite variation. Members

of the "*gracilis*" group show consistent similarities in haptoral and copulatory apparatus structures and clustered together in both PCA and DFA analyses. *Longidigitis auripontiformis* and *L. hopevalensis* have similar haptoral sclerite morphologies but they are easily distinguished by the differences in copulatory apparatus morphology.

Two haptoral morphologies were identified in species of *Helicirrus*. Well-developed dorsal anchors, dorsal bar and elongated hooks are present in *H. mcivori*, *H. gertrudaea* and *H. marjoriaea*. Highly reduced dorsal anchors, dorsal bar and hooks are found in *H. splendidae*, *H. maccullochii* and *H. megaloanchor*. This may be associated with the attachment site on the host. All species of *Longidigitis, Iliocirrus* and *Recurvatus* attach to the secondary gill filaments while *H. splendidae* and *H. maccullochii* most often attach small spines located between the gill raker spines (pers. obs.). A similar reduction can be also found in gyrodactylids. It is suggested that this modification is related to their attachment to a different, more simplified, part of the gill structure. The well-developed haptoral sclerite structures of species of *Longidigitis*, *Iliocirrus* and *Recurvatus* and of *H. mcivori*, *H. gertrudaea* and *H. marjoriaea* may be an adaptation to the complexity of the soft gill tissue requiring more developed ventral and dorsal sclerites. *Helicirrus megaloanchor* has reduced hooks and highly reduced dorsal bar and anchors; however this species attaches to the gill filaments and not between the gill raker spines. This species also has extremely large ventral anchors, which may be a compensatory mechanism for attachment to the soft gill tissues. Reduced and elongated hook patterns and their corresponding orientation of anchors and hooks may also indicate a major branching in the evolution of species in this genus.

Multivariate analysis identified significant differences in character means between species and the species descriptions use these differences. An example is the length relationships among hooks among species. However, different MANOVA methods often identify a varying number of significant differences. This was shown with the comparison of the Scheffé and Tukey's HSD tests ($P< 0.001$). For example, for *H. marjoriaea* and *H. mcivori*, 11 significantly different characters were identified using the Scheffé test while 17 were identified using the Tukey's HSD test. This indicates that the Scheffé test is much more conservative in identifying significant character differences than the Tukey's HSD test. The true number of differences lies somewhere between these two extremes for $\alpha = 0.05$-0.01.

A much better understanding is needed of the diversity of morphological and morphometric variation of the haptoral structures and possible correlations with the genera. The same understanding is also needed for the reproductive system. The species of *Iliocirrus* may represent variation within a single species. They show little easily identifiable copulatory apparatus or haptoral morphological variation and are difficult to recognize visually, but were easily identified using morphometric analysis of haptoral sclerite variability. However, samples of these cryptic species from additional hosts and sample sites are needed to confirm the taxonomy (see Chapter 5). This lack of morphological variation also has implications for cladistic studies, which traditionally use morphological characters. Methodologies have been developed for analysing morphometric variation in a cladistic manner (Archie, 1985; Baum, 1988). However, cladistic studies have never been attempted on haptoral variation of monogenean taxa (e.g. Gyrodactylidae, although it is a well-studied group).

Comparison by me of the monogenean parasites described previously from other atherinid species identified extremely elongated hooks in *Atherinicus cornutus*, *Ancyrocephalus atherinae, Ancyrocephalus littoralis* and *Diversohamulus tricuspidatus*. However, a gonadal bar is present in *A. cornutus*, which is absent in species of *Longidigitis, Iliocirrus*, *Recurvatus* and *Helicirrus* described here as well as all other species infesting atheriniform fishes. *Diversohamulus tricuspidatus* has a haptoral sclerite complex similar to *H. megaloanchor* with similar increase in length of hooks, large ventral anchors and bar and highly reduced dorsal bar and anchors. However, the former species (as well as *A. atherinae* and *A. littoralis*) differs from the four new genera in the copulatory apparatus structure. The copulatory apparatus of *D. tricuspidatus* does not resemble any of the four types described earlier but consists of a straight tubiform copulum shaft and accessory piece with a few distal ramifications and is attached to the proximal surface of the copulum base. The copulatory structure of *A. cornutus* has similarities to the Type III copulatory apparatus found in species of *Longidigitis* consisting of a single recurved loop. However, the copulum shaft is directed anteriorly not dextrally and accessory piece is different. The vaginal opening in *A. cornutus* and *D. tricuspidatus* is ventral and dextral in *A. atherinae* and *A. littoralis* while it is sinistral in species of *Longidigitis* and *Iliocirrus*.

KEY TO MONOGENEAN PARASITE GENERA OF AUSTRALIAN ATHERINIFORM FISHES

1. Copulum shaft coiled at distal end posterior to copulum base……..*Helicirrus*

2. Copulum shaft single counter-clockwise coil (ventral view) encircling copulum base……..……...…..*Recurvatus*

3. Copulum shaft comprises a single sinistral recurved loop….....*Longidigitis*

4. Copulum shaft comprises two sinistral recurved loops……..….....*Iliocirrus*

4.5 KEY TO AUSTRALIAN SPECIES OF HELICIRRUS

1a. Hooks reduced, H4 shorter than ventral anchor……..…..…….……...2

1b. Hooks elongated form, H4 always longer than ventral anchor…….....…….....4

2a. Copulum shaft proximally expanded to form a chamber..……..……...3

2b. Copulum shaft not expanded……..…....*H. splendidae*

3a. Ventral anchor extremely large, dorsal anchor highly reduced…....*H. megaloanchor*

3b. Ventral anchor slightly larger than dorsal anchor……....….*H. maccullochii*

4a. Copulum shaft not expanded proximally…..…….....……...…5

4b. Copulum shaft expanded proximally…….……..……..….*H. mcivori*

5a. Copulum shaft highly coiled at distal end (30 coils)……..…..…*H. marjoriaea*

5b. Copulum shaft with few coils (<10)…….…....*H. gertrudaea*

4.6 KEY TO AUSTRALIAN SPECIES OF RECURVATUS

1a. Accessory piece large, claw shaped……..…..…...*R. chelatus*

1b. Accessory piece not as above…….……..…..*R. signiferi*

4.7 KEY TO AUSTRALIAN SPECIES OF LONGIDIGITIS

1a. Notch present on posterior medial edge of dorsal bar….....2

1b. Notch absent from posterior medial edge of dorsal bar….....4

2a. Anchors roots thickened…….....*L. robustus*

2b. Anchor roots not thickened…….…….……...3

3a. Dorsal bar ear-shaped, with membrane infill on anterior edge.....*L. hopevalensis*

3b. Dorsal bar not ear-shaped, without infill on anterior edge……..…..…..*L. gracilis*

4a. Dorsal bar ear-shaped, with membrane infill on anterior edge ….*L. auripontiformis*

4b. Dorsal bar not ear-shaped, without infill on anterior edge……5

5a. Hooks H3 and H6 similar length…….……...…….……...*L. utcheei*

5b. Hooks H3 longer than H6……..…….....*L. maccullochii*

4.8 KEY TO AUSTRALIAN SPECIES OF ILIOCIRRUS

1a. Hooks H2 and H6 similar in length…….…….……..……...*I. mazlini*

1b. Hooks H2 much smaller in length than H6……...…..…….…….2

2a. Ventral bar with deep V-shaped notch on posterior medial edge……....*I. ornatusi*

2b. Ventral bar without deep V-shaped notch on posterior medial edge……....…..…3

3a. Hooks H3 and H6 similar size……..……..……...…....4

3b. Hooks H3 longer than H6….....*I. rossi*

4a. Copulum shaft thin, recurved loops reduced……*I. trifasciatae*

4b. Copulum shaft thick, recurved loops well-developed…….....*I. iliocirrus*

CHAPTER 5 MORPHOMETRIC VARIATION OF PARASITE SPECIES AMONG LOCALITIES AND HOSTS

5.1 Introduction

Studies of host-parasite specificity require confidence in the taxonomy of the species studied. For the host species studied in this thesis, confidence in their identification can only be confirmed by congruence between morphological and genetic variation. Unfortunately, this aspect of the host taxonomy was not examined. However, published details of species descriptions and distributions (see Chapter 2) were available which provided much confidence in the identification of hosts. In Chapter 4, an analysis of the association between parasite morphometric variation and diagnoses of genera and descriptions of species was presented with parasite specimens from a limited set of sample sites being used. However, the genus and species taxonomy needs further investigation to confirm validity. In species-groups that show little morphometric variation, confidence in distinguishing features becomes increasingly important. To validate the taxonomy of the parasite genera and species, variation in samples from multiple host species and collection sites were examined using multivariate statistics such as principle component and discriminant analysis. These methods have rarely, if ever been used by other workers in taxonomic studies. Species of *Iliocirrus* were recognised by morphometric variation of the measured haptor variables and it was suggested in Section 4.4 that the species identified may only be variations of a single species. The validity of the species of *Iliocirrus* is examined further in Section 5.2.1.

5.2 Methods

5.2.1 Confirmation of Genera and Species using Morphometric Variation

Principle component analysis and scatterplot examination and DFA cross-validation classification and scatterplot examination were used to confirm species identity according to morphology, while morphometric variation and association with genera used DFA cross-validation and scatterplot examination. These methods are described in Section 3.2.1 and Chapter 4. Parasite specimens (n=2130) were measured to obtain means, range and standard deviation for sclerite variables. Data were ln transformed and z-score standardised to approximate multivariate normality and to reduce colinearity between variables. Discriminant function analyses were conducted using Mahalanobis distances and the step-wise method (F to enter= 0.05, F to remove = 0.1). Cases were classified, using cross-validation, by grouping specimens either into parasite genera or into species. Hierarchical cluster analysis was also used to identify genus and species groups and species variable means were used. The analysis applied three hierarchical cluster methods, average linkage (between-group), complete linkage (furthest neighbour) and Ward's method. The distance measures used were either squared Euclidean or city block (Manhattan).

5.3 Results

5.3.1 Genus and Species Confirmation

5.3.1.1 Descriptive

The means, range and standard deviation of sclerite characters for the parasite species described in Chapter 4 are presented in Appendices C1-C4. These represent pooled samples from multiple hosts and locations (Appendices C5-C35).

5.3.1.2 Principal Component Analysis

A summary of explained variance and morphological characters associated with principle components is presented in Table 5.1. Initial analyses were run to examine communalities between variable and functions. All variables with communalities <0.3 were removed and a final analysis was undertaken. Because of the large size of the database, genera were not analysed all together. Species of *Helicirrus* and *Recurvatus* were analysed together as were species of *Longidigitis* and *Iliocirrus*.

For the analysis of *Longidigitis* and *Iliocirrus*, the species were clearly separated into the two genera on the first two components (Figure 5.1A) which were dominated by hook, ventral anchor and bar, and dorsal anchor characters respectively. Separation between *L. gracilis*, *L. auripontiformis*, *L. utcheei* and *L. maccullochii* was also evident. However, *Longidigitis robustus* and *L. hopevalensis* were not well separated from the *L. auripontiformis* cloud while separation of *Iliocirrus* species was not evident from the scatterplot. When a varimax rotation was applied, the species of *Iliocirrus* are better separated, however the separation of species of *Longidigitis* is reduced (Figure 5.1B). This lack of separation of species within each of the genera prompted a further examination and each genus was examined separately.

For species of *Longidigitis* dorsal and ventral anchor characters dominated PC1, hooks dominated PC2 and VB2 dominated PC3, accounting for 79.2% of explained variance. Species were only partially separated (Figure 5.2A) on the first two components, however components PC1 and PC3 more clearly separate each species (Figure 5.2B).

Analysis for species of *Iliocirrus* shows they are not clearly separated on the first three components (Figure 5.3), which accounted for 55.6% of the explained variance, although *I. mazlini, I. ornatusi* and *I. rossi* clearly separated from the *I. iliocirrus* cloud. Hook characters, which dominate PC1 loosely separate *I. mazlini, I. ornatusi* and *I. rossi*, while ventral anchor characters that dominated PC2 clearly separate *I mazlini* from all other species and dorsal anchor characters, which dominated PC3 loosely separate *I. ornatusi* and *I. mazlini*.

Table 5.1. Summary table of explained variance and morphological characters associated with principle components and discriminant functions.

	PC1	PC2	PC3	F1	F2	F3
All genera				67.5% H3, 5-6	28.35% DA1-6	4.2% DB1, H2, 4, 7
All *Helicirrus/Recurvatus* spp.	67.8% DA1-6, H2-4, DB1, 3	18.1% VA1-6, VB1-2	2.9% H2, 6-7	73.6% H4, DA2	17.0% VA1-3, 5-6	5.7% H2, 7
Helicirrus/Recurvatus spp. (elongated hooks)	37.7% VA2-4 DB1, 3 VB1-2	22.0% DA1-6	10.2% H2-7			
Iliocirrus/Longidigitis spp.	42.6% H2-4, 6-7 VA2-4, 6 VB1-2 DB1, 3	29.2% DA1-6 DB2 VA1, 5	4.3% H1, 5	73.7% H3	12.3% VA2-6 DA1-4 H1	6.7% H4, 6-7
Iliocirrus spp.	39.5% H2-4, 6-7, DB1, 3	11.5% VA1-6, H1, 5, VB1-2	6.9% DA1-6	68.3% H3-4, H6- 7, DB3	14.5% VB2, DA2, 4, 5	9.8% VA1, DB1, VB1, H5
Longidigitis spp.	67.2% DA1-6, VA1-6, DB3, VB1	7.1% H1-7, DB1	4.9% VB2	76.3% DA1-6, VA2, 4-5, DB3	13.7% VB2, H4-5	5.4% VA6, H7
I. iliocirrus/host				45.3% H1, 3, 5-6 VA2-6 DA2, 5-6 VB1, DB1	16.5% DA2 VA6	10.5% DB3
L. auripontiformis/host				40.6% VA2-5 DA2-4 H1- 3, 5	25.0% DA1, 6	9.1% VA1, DB3 VB2

% explained variance.

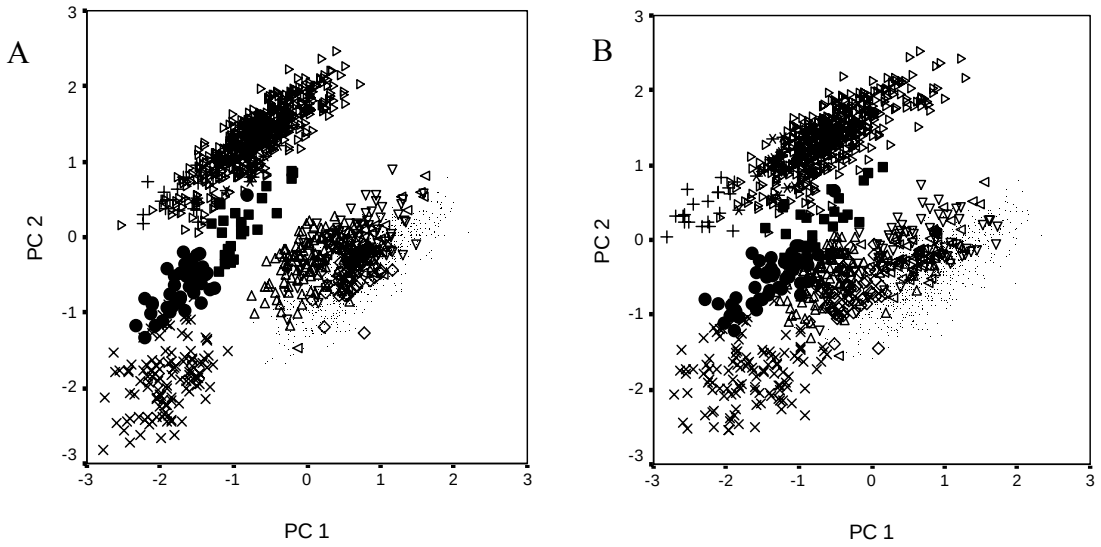


Figure 5.1. Scatterplot for species of *Longidigitis* and *Iliocirrus* using principal component analysis.
A. no rotation applied. B. Varimax rotation applied. · *I. iliocirrus*, ◇ *I. mazlini*, △ *I. ornatusi*, ▽ *I. rossi*, ◁ *I. trifasciatae*, ▷ *L. auripontiformis*, + *L. robustus*, × *L. gracilis*, ∗ *L. hopevalensis*, ● *L. maccullochii*, ■ *L. utcheei*.

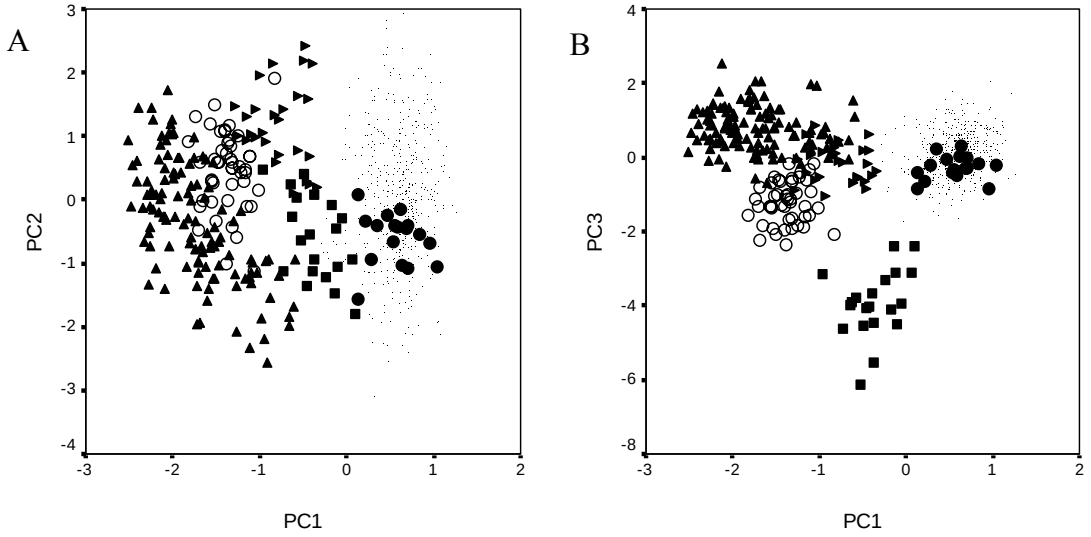


Figure 5.2. Separation of species of *Longidigitis* using PCA with varimax rotation applied.
A. PC1/PC2, B. PC1/PC3. ○ *L. maccullochii*, ● *L. hopevalensis*, · *L. auripontiformis*, ▲ *L. gracilis*, ▶ *L. utcheei*, ■ *L. robustus*.

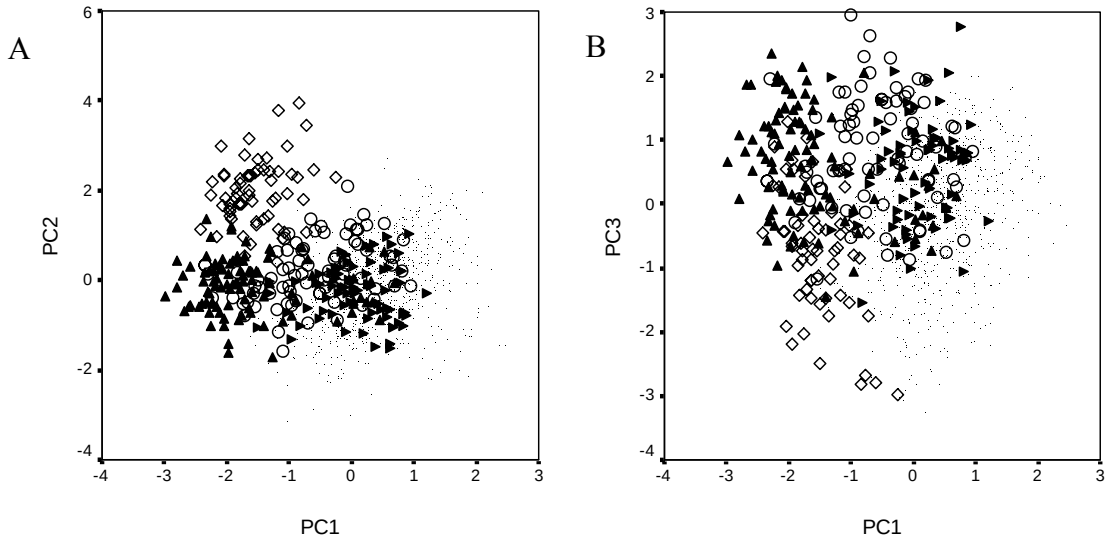


Figure 5.3. Separation of species of *Iliocirrus* using PCA with varimax rotation applied. A. PC1/PC2, B. PC1/PC3. ○ *I. rossi*, ◇ *I. mazlini*, · *I. iliocirrus*, ▲ *I. ornatusi*, ▶ *I. trifasciatae*.

Specimens of *Helicirrus* and *Recurvatus* species were examined in two stages. When all species were analysed, *H. megaloanchor* clearly separated from all other species which formed a single elongated cluster (Figure 5.4A) on the first two components which were dominated by hook and dorsal anchor, and ventral anchor characters respectively. The three species of *Helicirrus* possessing reduced hooks clearly separated from the species of *Recurvatus* and *Helicirrus* possessing elongated hooks (Figure 5.4B). The three components accounted for 88.8% of the explained variation.

The analysis of species of *Recurvatus* and species of *Helicirrus* with elongated hooks only, clearly separates each species on the first three components (Figure 5.5). It is interesting to note that species of *Helicirrus* with elongated hooks do not form separate clusters from species of *Recurvatus*. Instead each cluster represents species from the same host genus (e.g. *H. marjoriaea/R. chelatus* from *Craterocephalus*) (Figure

5.5B).Ventral and bar, dorsal anchor, and hooks dominated the components respectively, accounting for 69.9% of the total explained variation.

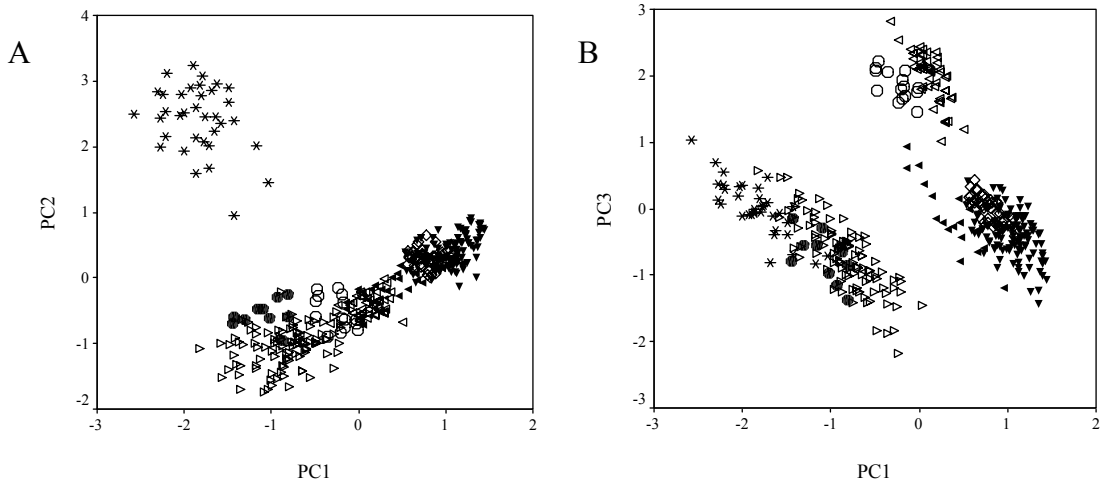


Figure 5.4. Separation of species of *Helicirrus* and *Recurvatus* using PCA. All species included.
A. PC1/PC2, B. PC1/PC3. ✳ *H. megaloanchor*, ▷ *H. splendidae*, ○ *H. gertrudaea*, ◇ *H. marjoriaea*, ◀ *H. mcivori*, ● *H. maccullochii*, ◁ *R. signiferi*, ▼ *R. chelatus*.

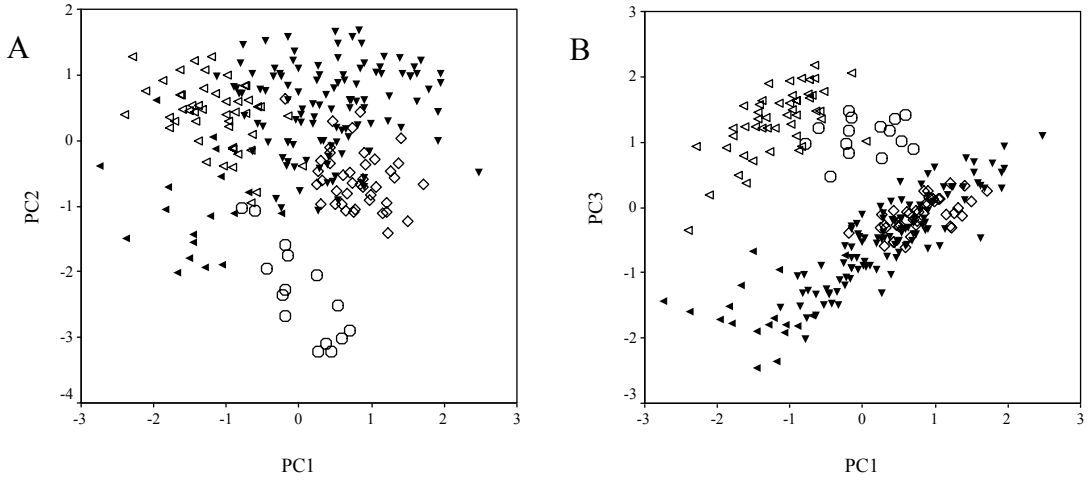


Figure 5.5.Separation of species of *Helicirrus* and *Recurvatus* using PCA. Species with elongated hook form only.
A. PC1/PC2, B. PC1/PC3. ○ *H. gertrudaea*, ◇ *H. marjoriaea*, ◀ *H. mcivori*, ◁ *R. signiferi*, ▼ *R. chelatus*.

5.3.1.3 Discriminant Function Analysis

A summary of explained variance and morphological characters associated with discriminant functions is presented in Table 5.1. Analysis of the combined genera *Longidigitis*, *Recurvatus*, *Helicirrus* and *Iliocirrus* identified a significant difference

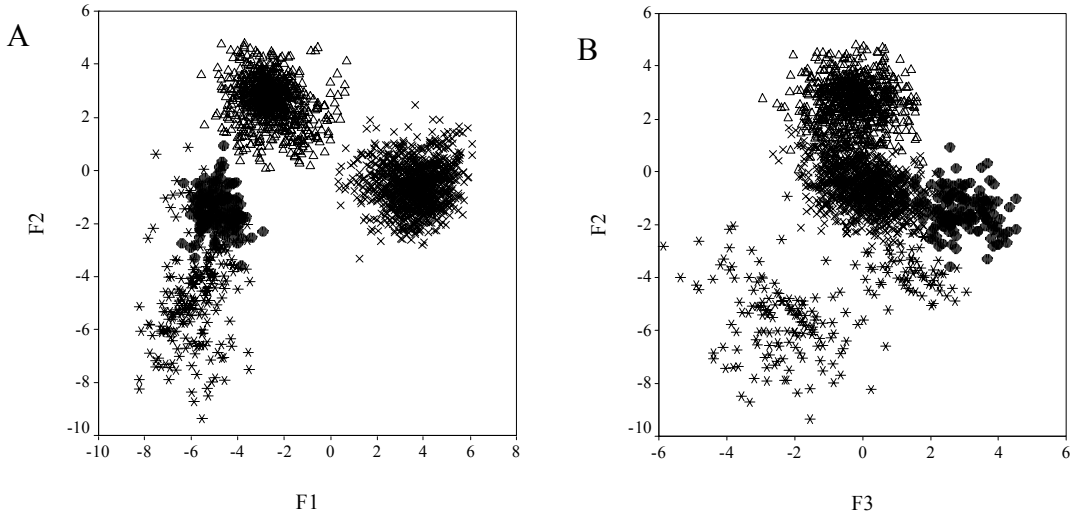


Figure 5.6. Separation of monogenean genera using DFA.
A. F1 and F2, B. F2 and F3. ✳ *Helicirrus*, ● *Recurvatus*, ▲ *Longidigitis* and × *Iliocirrus.*

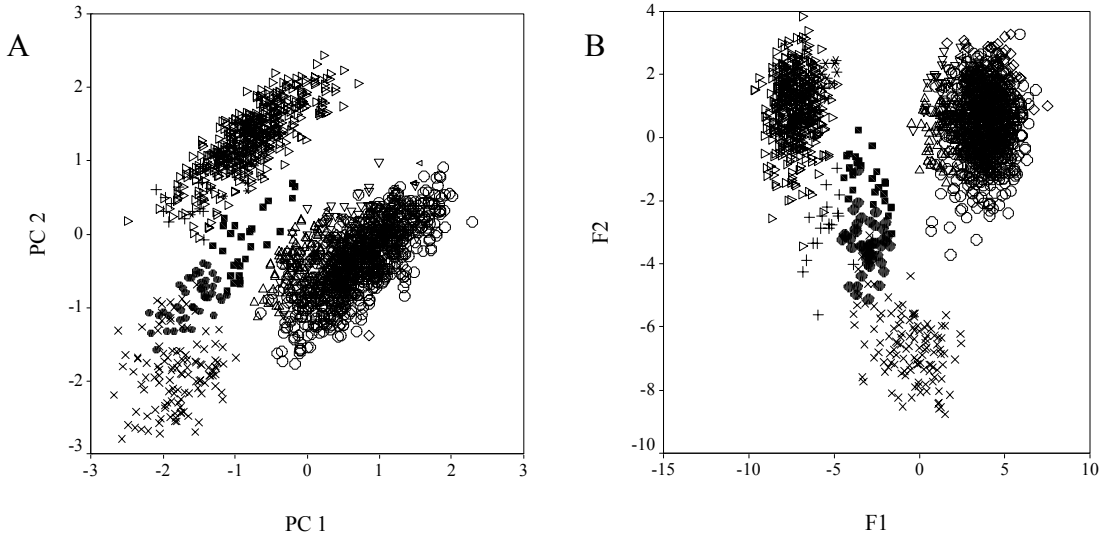


Figure 5.7. Scatterplot for parasite species of *Longidigitis* and *Iliocirrus*. A. PCA. B. DFA.
○ *I. iliocirrus*, ◇ *I. mazlini*, △ *I. ornatusi*, ▽ *I. rossi*, ◁ I. trifasciatae, ▷ *L. auripontiformis*, + *L. robustus*, × *L. gracilis*, ✳ *L. hopevalensis*, ● *L. maccullochii*, ■ *L. utcheei*.

between centroid means for all functions (Wilk's $\lambda = 0.609$, $Chi^2_{21} = 1115$, $P < 0.001$ for last function). Genera were clearly separated using the first three functions (Figure 5.6) which were dominated by hook (3, 5, 6) and ventral anchor, dorsal anchor, dorsal bar and hook (2, 7, 4) variables respectively and accounted for 99.2% of the total explained variance. It can be seen that for species of *Helicirrus* (Figure 5.6B), two clusters form. One cluster represents specimens with reduced hooks while the second, which groups with all other genera, represents specimens with elongated hooks.

Species separation was examined by analysing genera as two groups: *Longidigitis/Iliocirrus* and *Helicirrus/Recurvatus*. Analysis of the species of *Longidigitis* and *Iliocirrus* as a group identified a significant difference between species group centroid means (Wilk's $\lambda = 0.905$, $Chi^2_{14} = 90.1$ $P<0.001$ for last function). Species of *Iliocirrus* and *Longidigitis* clearly clustered into their respective genera (Figure 5.7B). Hook (H3), dorsal and ventral anchor, and hook variables dominated the three components respectively, accounting for 92.7% of the total explained variance. The scatterplots (Figure 5.7) of DFA and PCA appear very similar in pattern showing a degree of linearity in the shape of the species clouds, particularly for *I. iliocirrus* and *L. auripontiformis.* However, there is a rotation of the species clouds.

A further analysis of *Iliocirrus* was undertaken and a significant difference between group centroid means (Wilk's $\lambda = 0.706$, $Chi^2_{19} = 368.8$, $P<0.001$ for last function) was identified. The *Iliocirrus mazlini* cluster clearly separated from all other *Iliocirrus* species on the first two functions (Figure 5.8). Two species, *I. rossi* and *I. ornatusi,* show some overlap but are clearly separated from *I. iliocirrus* and *I. trifasciatae* for functions F1 and F2 which was dominated by hook and dorsal anchor characters

respectively. *Iliocirrus trifasciatae* does not separate from *I. iliocirrus* on the first two functions but is separated by F3 which was dominated by bar characters (Figure 5.8B).

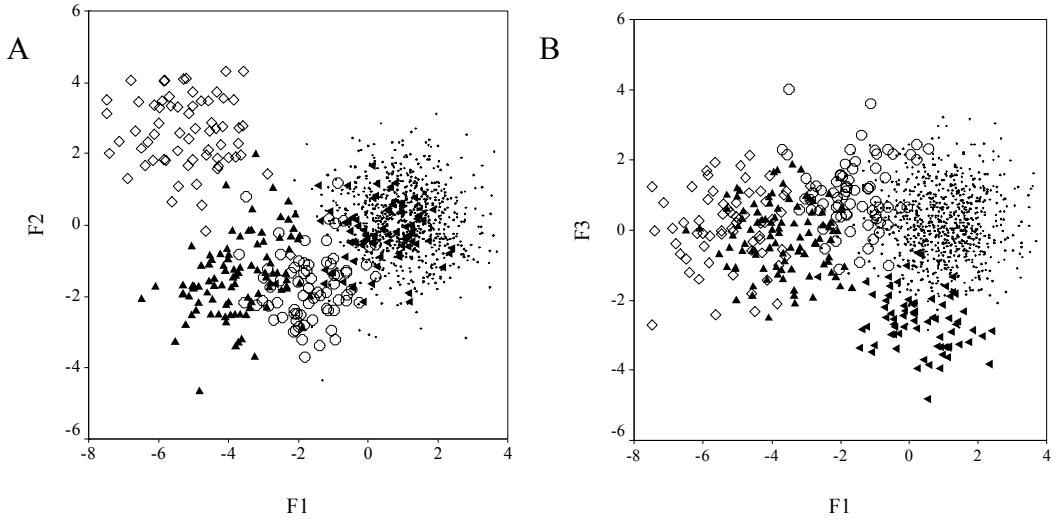


Figure 5.8. Separation of species of *Iliocirrus* using DFA.
A. F1 and F2, B. F1 and F3. · *I. iliocirrus*, ◇ *I. mazlini*, ◂ *I. trifasciatae*, ▲ *I. ornatusi*, ○ *I. rossi*.

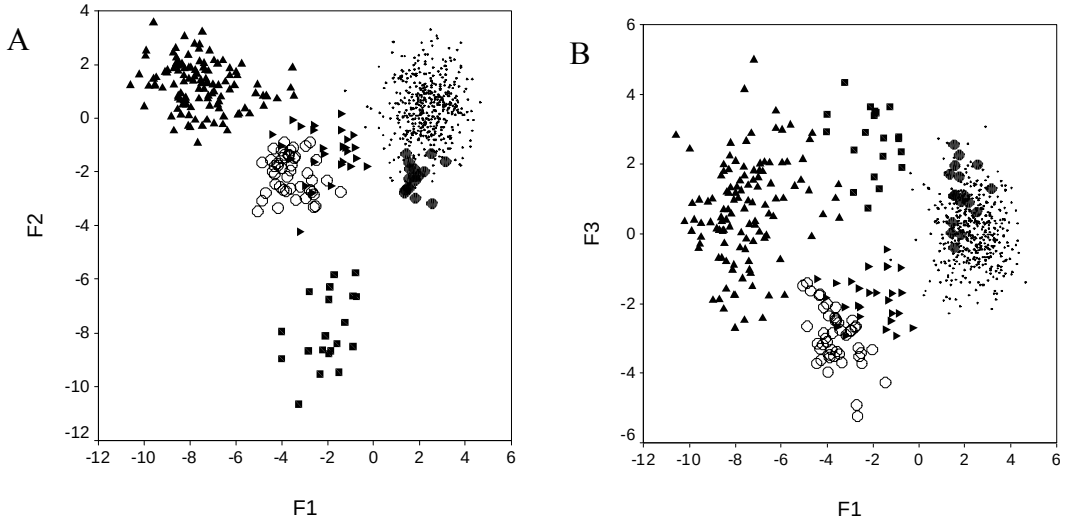


Figure 5.9. Separation of species of *Longidigitis* using DFA.
A. F1 and F2. B. F1 and F3. ○ *L. maccullochii*, ● *L. hopevalensis*, · *L. auripontiformis*, ▲ *L. gracilis*, ▸ *L. utcheei*, ■ *L. robustus*.

The analysis of species of *Longidigitis* only, identified a significant difference between group centroid means (Wilk's $\lambda = 0.747$, $Chi^2_{18} = 201.9$, $P<0.001$ for last function). *Longidigitis robustus* and *L. gracilis* were clearly separated from all other species on the first two functions while *L. maccullochii* and *L. utcheei* show some overlap, as do *L. auripontiformis* and *L. hopevalensis* (Figure 5.9). Dorsal and ventral anchor, VB2 and H4-5, and VA6 and H7 dominated the three functions respectively, accounting for 95.4% of the total explained variance.

5.3.1.4 Cross-Validated Classification

The first analysis examined parasite morphometric variation and its association at the genus level. Cross-validation of the 2130 specimens representing *Helicirrus*, *Longidigitis*, *Iliocirrus* and *Recurvatus* correctly classified 98% of original cases and 97.7% of cross-validated cases. All specimens of *Recurvatus*, *Longidigitis* and *Iliocirrus* were correctly classified. Approximately twenty-two percent (47 specimens) of specimens of *Helicirrus* were mis-classified as belonging to *Recurvatus* using cross-validation (Table 5.2). These mis-classifications represent specimens possessing elongated hooks. A second analysis, with specimens of *Helicirrus* possessing the reduced hook form removed, mis-classified 5.3% (4 specimens) of these (Table 5.3). This confirms the association between haptoral sclerite and copulum morphology identified in Chapter 4. Classification of the association between sclerite variability and parasite species was examined using 2130 specimens and included all species of *Longidigitis*, *Recurvatus*, *Iliocirrus* and *Helicirrus* (DB2 omitted from analysis) correctly classified 98.0% of original cases into their correct species while cross-validation correctly classified 97.4% of cases (Table 5.4). All specimens of species of *Longidigitis* and *Recurvatus* were correctly classified while all species of *Iliocirrus* had

some mis-classification of specimens. *Iliocirrus trifasciatae* had the lowest correct cross-validation with 77.8% while *I. iliocirrus* was the highest with 97.7% of cross-validated cases classified correctly.

Table 5.2. Cross-validation of specimens of *Recurvatus*, *Longidigitis*, *Iliocirrus* and *Helicirrus* with elongated and reduced hooks.
98% of original grouped cases were correctly classified. 97.7% of cross-validated grouped cases correctly classified. The within-group covariance matrix was used.

	Predicted group membership				
P. genus	1	2	3	4	Sample size
1. *Recurvatus*	**100**	.0	.0	.0	147
2. *Helicirrus*	22.2	**77.8**	.0	.0	216
3. *Longidigitis*	.0	.0	**100**	.0	706
4. *Iliocirrus*	.0	.0	.0	**100**	1061

Table 5.3. Cross-validation of specimens of *Recurvatus*, *Longidigitis* and *Iliocirrus* and *Helicirrus* with elongated hooks only.
99.7% of original grouped cases were correctly classified. 99.7% of cross-validated grouped cases correctly classified. The within-group covariance matrix was used.

	Predicted group membership				
P. genus	1	2	3	4	Sample size
1. *Recurvatus*	**98.6**	1.4	.0	.0	147
2. *Helicirrus*	5.3	**99.7**	.0	.0	76
3. *Longidigitis*	.0	.0	**100**	.0	706
4. *Iliocirrus*	.0	.0	.0	**100**	1101

A further analysis of species of *Iliocirrus* was performed because of the high number of mis-classifications. When only species of *Iliocirrus* species were analysed, 97.0% of the original cases were correctly classified while 95.7% of specimens were correctly classified using cross-validation (Table 5.5). This high level of correct classification confirms that the species of *Iliocirrus* recognised in chapter 4 are not variants of a single species. All specimens of *I. mazlini* and *I. ornatusi* were correctly classified while *I. trifasciatae* had the lowest at 80.6%. The lower correct classification of *I trifasciata*

Table 5.4. Cross-validation of species of *Longidigitis, Iliocirrus, Recurvatus* and *Helicirrus* with elongated hook form using all specimens.
98% of original grouped cases correctly classified and 97.4% of cross-validated grouped cases correctly classified. The within-group covariance matrix was used.

	Predicted group membership																
Parasite species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Sample size
1 *H. gertrudaea*	**100**	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	15
2 *H. marjoriaea*	.0	**100**	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	43
3 *H. mcivori*	.0	.0	**83.3**	.0	.0	**16.7**	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	18
4 *L. auripontiformis*	.0	.0	.0	**100**	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	478
5 *L. robustus*	.0	.0	.0	.0	**100**	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	20
6 *L. gracilis*	.0	.0	.0	.0	.0	**100**	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	118
7 *I. iliocirrus*	.0	.0	.0	.0	.0	**.5**	**97.7**	.0	.0	.0	**.9**	**.9**	.0	.0	.0	.0	789
8 *L. maccullochii*	.0	.0	.0	.0	.0	.0	.0	**100**	.0	.0	.0	.0	.0	.0	.0	.0	46
9 *I. mazlini*	.0	.0	.0	.0	.0	.0	.0	.0	**97.0**	**.5**	**1.5**	.0	.0	.0	.0	.0	65
10 *I. ornatusi*	.0	.0	.0	.0	.0	.0	**2.0**	.0	**2.0**	**93.9**	**2.0**	.0	.0	.0	.0	.0	99
11 *I. rossi*	.0	.0	.0	.0	.0	.0	**8.2**	.0	.0	**4.1**	**87.7**	.0	.0	.0	.0	.0	73
12 *I. trifasciatae*	.0	.0	.0	.0	.0	.0	**20.8**	.0	.0	**1.4**	.0	**77.8**	.0	.0	.0	.0	72
13 *L. utcheei*	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	**100**	.0	.0	.0	27
14 *R. chelatus*	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	**100**	.0	.0	91
15 *R. signiferi*	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	**100**	.0	49
16 *L. hopevalensis*	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	**100**	17

Table 5.5. Cross-validation of specimens into species of *Iliocirrus*.
97.0% of original cases correctly classified and 95.7% of cross-validated cases correctly classified. The within-group covariance matrix was used.

	Predicted group membership					
Host species	1	2	3	4	5	Sample size.
1. *I. iliocirrus*	**97.4**	.0	.0	1.2	1.4	785
2. *I. mazlini*	.0	**100**	.0	.0	.0	62
3. *I. ornatusi*	.0	.0	**100**	.0	.0	99
4. *I. rossi*	8.2	.0	.0	**91.8**	.0	73
5. *I. trifasciatae*	19.4	.0	.0	.0	**80.6**	72

compared to the other species of *Iliocirrus* may create issues of species identification. This however can be avoided if copulum structure is also examined. Although approximately 20% of specimens of *I. trifasciatae* were mis-classified as *I. iliocirrus*, these two species can be clearly identified since the former has a thin copulum shaft while the latter has a thick copulum shaft.

5.3.1.5 Hierarchical Cluster Analysis

Hierarchical cluster analysis was used to examine associations at several levels. The first analysis examined the morphometric associations between all described parasite species (Figure 5.10). To do this the species variable means were used. All dendrograms show species of *Iliocirrus* clustered together as did species of *Recurvatus* and species of *Helicirrus* possessing reduced hooks. The species of *Longidigitis* always formed a single large cluster however; the internal associations differ between cluster methods. Species of *Helicirrus* possessing elongated hooks did not group together but always grouped with parasite species from the same host genus.

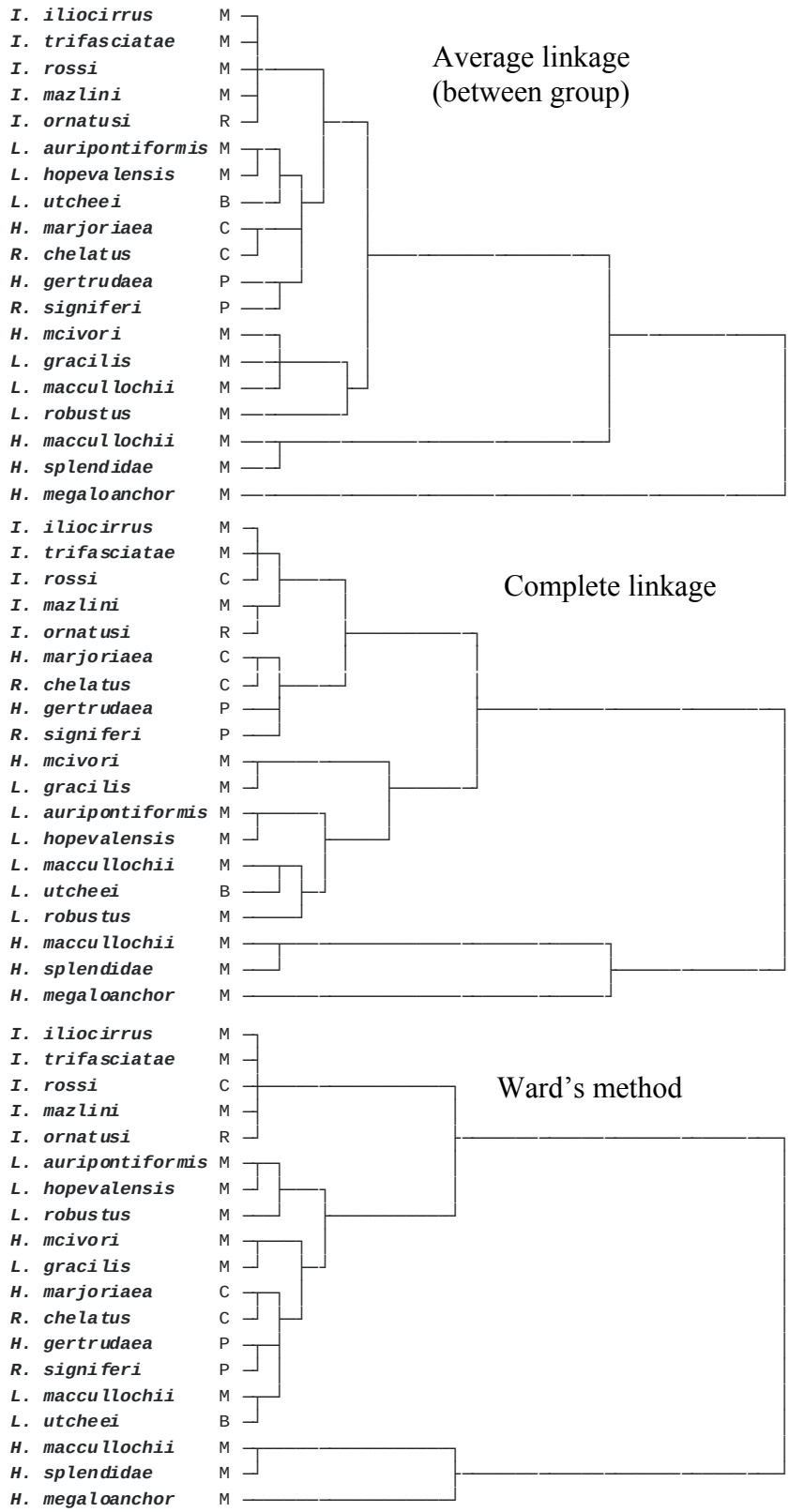


Figure 5.10. Hierarchical cluster analysis of monogenean species and association with host genera (Squared Euclidean distances used).
M. *Melanotaenia* (Melanotaeniidae), C. *Craterocephalus* (Atherinidae), P. *Pseudomugil* (Pseudomugilidae), B. *Cairnsichthys* (Melanotaeniidae), R. *Rhadinocentrus* (Melanotaeniidae).

5.4 Discussion

Within a species, there are genotypic variations that are expressed phenotypically in terms of morphometry. Morphometric variants are only obvious when many individuals of the species expressing the morphometric variation are measured. It is necessary to analyse these intraspecific variations as they have caused considerable confusion in taxonomy giving rise to validity issues. Morphometric differences are usually not sufficiently different to warrant recognition as valid species. In Chapter 1, I discussed the problem of incorrect identification of species when assessing host-parasite specificity. In this Chapter, I examined the validity of the parasite genera described in Chapter 4. Recognition of genera and species was confirmed using variation in the morphometric characters of haptoral sclerite morphology of species from multiple sample sites and host species. Cluster analysis and cross-validation methods of classification using DFA confirmed the association between copulum morphology, which was used to define the genera described in Chapter 4, and haptoral sclerite morphology. The results gave an extremely high level of congruence between the haptoral sclerite variation and the parasite genus (Section 5.3.3.1). The species identified in Chapter 4 were also validated, again with a high number of specimens being classified as belonging to their correct species. These results give confidence in the identification of taxa used in analyses.

Cluster analysis identified an interesting set of associations when grouping parasite species according to haptoral sclerite variability. Each of the three species of *Helicirrus* possessing elongated hooks clustered with parasite species from other genera. What is more interesting is that these parasite species grouped according to host genus infected.

These parasite species groups also add strength to the hypothesis that variation in haptoral sclerite architecture is associated with host attachment structure. This may indicate convergent evolution of haptoral structures through host association.

CHAPTER 6 INTRASPECIFIC VARIATIONS AND HOST ASSOCIATION

6.1 Introduction

Morphometric characters used to define species exhibit some variation. In ectoparasitic species, intra-specific variation could be caused by environmental factors such as temperature or variation could be the result of parasite-host interactions as suggested in Chapter 1. Environmental factors such as season or water temperature are known to cause morphometric variability in monogeneans. In species of *Gyrodactylus* (Denham & Long, 1999; Ergens, 1981; Ergens & Gelnar, 1985; Ferdig *et al.*, 1993; Mo, 1991), seasonal variation in size of the sclerites has been identified and an inverse relationship was identified. Similar results have been observed in ancyrocephaline monogeneans (Ferdig *et al.*, 1993). Relatively minor environmentally induced variability is seen in *Gyrdicotylus gallieni* (see Jackson & Tinsley, 1995). Parasites from cool temperate climates would be expected to show variation related to temperature as is common for many organisms that inhabit this region, however temperature is thought to have little effect on organisms inhabiting tropical regions (Lim, 1987). Although seasonal factors are important, they were not examined in this study for two reasons: samples were collected between September and December (limiting seasonal effects) and temperature appears not to be a major factor in tropical regions as noted above.

Host-associated sclerite variability has been recognized within a single monogenean species (Dmitrieva & Dimitrov, 2002; Geets *et al.*, 1999). *Gyrodactylus arcuatus*-like monogeneans collected from the gills of 3 goby species, *Pomatoschistus minutus*, *P. lozanoi* and *P. pictus*, were compared on a morphometric basis with *G. arcuatus* from

its type host, the three-spined stickleback, *Gasterosteus aculeatus* (see Geets *et al.*, 1999). Univariate statistics (ANOVA) were used to detect features that were useful in separating the gyrodactylids from the different host groups. Subsequent factor analysis and discriminant analysis, combining all variables, led to the separation of three distinct forms (possibly species) dependent on the host species harbouring them. This is possible through asexual reproduction, which may promote an accumulation of phenotypic distinctions between stocks within the same parasite species, or through morphometric changes in haptoral structures being directed by adaptation to variations of attachment structure in different host species. The latter cause of intra-specific variation is examined using the two low host-specific species (Chapter 7), *I. iliocirrus* and *L. auripontiformis*, and is the subject of this chapter. Size of haptoral sclerites may also be affected by host length with larger sclerites being required on larger hosts and should be observed in co-occurring parasite species.

6.2 Methods

The possibility of host-induced haptoral sclerite variability was examined using *I. iliocirrus* and *L. auripontiformis*. The strength of the intra-specific variation and parasite-host associations was examined using discriminant analysis and cross-validation classification. Firstly, I used individual parasite specimens as the case unit and secondly variable means for each parasite species at each host-site as the case unit.

Cluster analysis can be used to assess associations of morphometric variations and two algorithms were used. The eigenvalues for the group centroid means, obtained from the previous analysis of the two parasite species, were converted to Manhattan distances for the cluster analysis (UPGMA and complete linkage). The branches of the dendrograms

were labelled according to host infected. The host phylogeny, derived from mtDNA (Section 9.2) was then compared for similarity with parasite dendrograms. This latter method was employed since within-species variation generally showed little major differentiation and any host associated sclerite variation may need to be concentrated into groups.

6.3 Results

6.3.1 Association between Intra-Specific Variation and Host Species Infected

6.3.1.1 Discriminant Function Analysis and Cross-Validated Classifications

Host-associated intra-specific variation of *I. iliocirrus* and *L. auripontiformis* was identified using DFA cross-validation. Classification of the 785 specimens of *I. iliocirrus* according to host infected, using the within-group covariance matrix, correctly classified 64.5% of original cases and 58.5% of cross-validated cases (Table 6.1). The least correct classifications were for specimens infecting *M. duboulayi* (38.5%) and *M. sp*. (30.8%) while the highest were for *M. australis* (72.2%) and *M. trifasciatae* (73.9%). Classification of specimens using the between-group covariance matrix correctly classified 71.6% of cases (Table 6.2). The highest correct classification (100%) was for specimens infecting *C. rhombosomoides* while the lowest was for specimens infecting *M. duboulayi* (59.4%). This would appear to indicate the presence of a strong association between haptoral sclerite variation and host infected.

Table 6.1. Cross-validation of specimens of *I. iliocirrus* according to host infected, using the within-group covariance matrix
64.5% of original grouped cases correctly classified and 58.5% of cross-validated grouped cases correctly classified.

	Predicted Group Membership											
Host sp.	1	2	3	4	5	6	7	8	9	10	11	Sample size.
1. *M. exquisita*	**41.7**	.0	16.7	16.7	4.2	.0	20.8	.0	.0	.0	.0	24
2. *M. gracilis*	.0	**50.0**	.0	21.7	.0	.0	17.4	6.5	.0	4.3	.0	46
3. *M. nigrans*	2.8	2.8	**52.8**	16.7	2.8	5.6	13.9	2.8	.0	.0	.0	36
4. *M. australis*	3.2	7.1	4.8	**72.2**	.8	.8	10.3	.0	.8	.0	.0	124
5. *M. duboulayi*	.0	4.3	.0	.0	**39.1**	2.9	14.5	27.5	.0	2.9	8.7	68
6. *M. sp.*	7.7	.0	.0	11.5	.0	**30.8**	38.5	11.5	.0	.0	.0	26
7. *M. s. splendida*	.6	1.1	1.7	9.1	2.3	1.1	**52.3**	27.3	.6	1.1	2.8	175
8. *C. rhombosomoides*	.0	.5	1.6	1.1	4.9	1.1	17.0	**68.7**	.0	2.7	2.2	9
9. *M. s inornata*	.0	11.1	.0	.0	.0	.0	33.3	.0	**55.6**	.0	.0	180
10. *M. trifasciata*	.0	2.2	2.2	10.9	.0	.0	4.3	4.3	.0	**73.9**	2.2	46
11. "*M. eachutchee*"	.0	2.0	.0	.0	9.8	3.9	15.7	27.5	.0	.0	**41.2**	51

Table 6.2. Classification of specimens of *I. iliocirrus* according to host infected, using between-group covariance matrix.
71.6% of original grouped cases correctly classified.

	Predicted Group Membership											
Host sp.	1	2	3	4	5	6	7	8	9	10	11	Sample size.
1. *M. exquisita*	**83.3**	.0	4.2	.0	.0	.0	8.3	.0	.0	.0	4.2	24
2. *M. gracilis*	.0	**71.7**	2.2	13.0	.0	.0	4.3	4.3	2.2	2.2	.0	46
3. *M. nigrans*	.0	2.8	**75.0**	8.3	.0	5.6	2.8	5.6	.0	.0	.0	36
4. *M. australis*	.8	3.2	1.6	**82.5**	.8	.0	11.1	.0	.0	.0	.0	124
5. *M. duboulayi*	.0	2.9	.0	.0	**59.4**	1.4	10.1	17.4	4.3	.0	4.3	68
6. *M. sp.*	7.7	3.8	.0	.0	.0	**69.2**	15.4	3.8	.0	.0	.0	26
7. *M. s. splendida*	1.1	1.7	.6	3.4	2.8	2.3	**66.5**	16.5	1.7	1.7	1.7	175
8. *C. rhombosomoides*	.0	.0	1.6	1.1	3.3	2.2	10.4	**75.3**	2.2	1.6	2.2	9
9. *M. s inornata*	.0	.0	.0	.0	.0	.0	.0	.0	**100.0**	.0	.0	180
10. *M. trifasciata*	.0	2.2	.0	6.5	.0	.0	2.2	4.3	.0	**82.6**	2.2	46
10. "*M. eachutchee"*	.0	.0	.0	2.0	7.8	2.0	7.8	15.7	2.0	.0	**62.7**	51

The classification of specimens of *L. auripontiformis* according to host infected correctly classified 67.2% of original cases and 60.0% of cross-validated cases using the within-group covariance matrix (Table 6.3). The lowest percentage of correctly classified specimens was for specimens infecting "*M. eachutchee"* (18.2%) and *M. trifasciata* (26.7%) while the highest was for specimens infecting *M. s. inornata* (76.5%). When the between-group covariance matrix was used, 75.3% of specimens were correctly classified according to host infected (Table 6.4). The least correctly classified group was for those infecting *M. s. splendida* (56.7%) while the highest was for *M. sp*. (90.5%). The majority had more than 75% of specimens correctly classified

according to host infected. Again, as with specimens of *I. iliocirrus*, there appears to be a strong association between haptoral sclerite variation and host species infected.

Table 6.3. Classification using cross-validation of specimens of *L. auripontiformis* according to infected host, using the within-group covariance matrix.
67.2% of original grouped cases correctly classified and 60.0% of cross-validated grouped cases correctly classified.

	Predicted Group Membership										
Host sp	1	2	3	4	5	6	7	8	9	10	Sample size
1. *M. exquisita*	**56.3**	.0	.0	6.3	.0	.0	6.3	31.3	.0	.0	16
2. *M. gracilis*	8.3	**58.3**	.0	8.3	.0	.0	.0	25.0	.0	.0	12
3. *M. nigrans*	.0	.0	**55.6**	18.5	7.4	.0	11.1	.0	3.7	3.7	27
4. *M. australis*	6.4	.0	12.8	**57.4**	4.3	6.4	2.1	6.4	4.3	.0	47
5. *M. duboulayi*	.0	1.4	.0	2.8	**63.9**	1.4	19.4	11.1	.0	.0	72
6. *M. sp.*	.0	.0	.0	.0	9.5	**71.4**	9.5	9.5	.0	.0	21
7. *M. s. splendida*	.0	1.0	.0	2.9	9.6	3.8	**43.3**	37.5	1.0	1.0	104
8. *M. s. inornata*	.7	.0	.0	.7	2.0	.7	18.3	**76.5**	1.3	.0	153
9. *M. trifasciata*	6.7	.0	13.3	.0	6.7	.0	13.3	33.3	**26.7**	.0	15
10. "*M. eachutchee"*	.0	.0	.0	.0	.0	.0	36.4	45.5	.0	**18.2**	11

Table 6.4. Classification of specimens of *L. auripontiformis* according to host infected, using the between-group covariance matrix.
75.3% of original grouped cases correctly classified.

	Predicted Group Membership										
Host sp.	1	2	3	4	5	6	7	8	9	10	Sample size
1. *M. exquisita*	**81.3**	.0	.0	.0	.0	.0	6.3	12.5	.0	.0	16
2. *M. gracilis*	.0	**83.3**	.0	8.3	.0	.0	.0	8.3	.0	.0	12
3. *M. nigrans*	.0	.0	**85.2**	7.4	3.7	.0	.0	.0	3.7	.0	27
4. *M. australis*	2.1	.0	8.5	**78.7**	2.1	2.1	4.3	2.1	.0	.0	47
5. *M. duboulayi*	.0	.0	.0	1.4	**76.4**	.0	15.3	5.6	.0	1.4	72
6. *M. sp.*	4.8	.0	.0	.0	.0	**90.5**	.0	4.8	.0	.0	21
7. *M. s. splendida*	1.0	1.0	1.0	2.9	7.7	.0	**56.7**	28.8	.0	1.0	104
8. *M. s. inornata*	1.3	.0	.0	.7	2.0	.7	13.1	**81.0**	.7	.7	153
9. *M. trifasciata*	.0	.0	.0	.0	.0	.0	6.7	20.0	**73.3**	.0	15
10. "*M. eachutchee"*	.0	.0	.0	.0	.0	.0	.0	18.2	.0	**81.8**	11

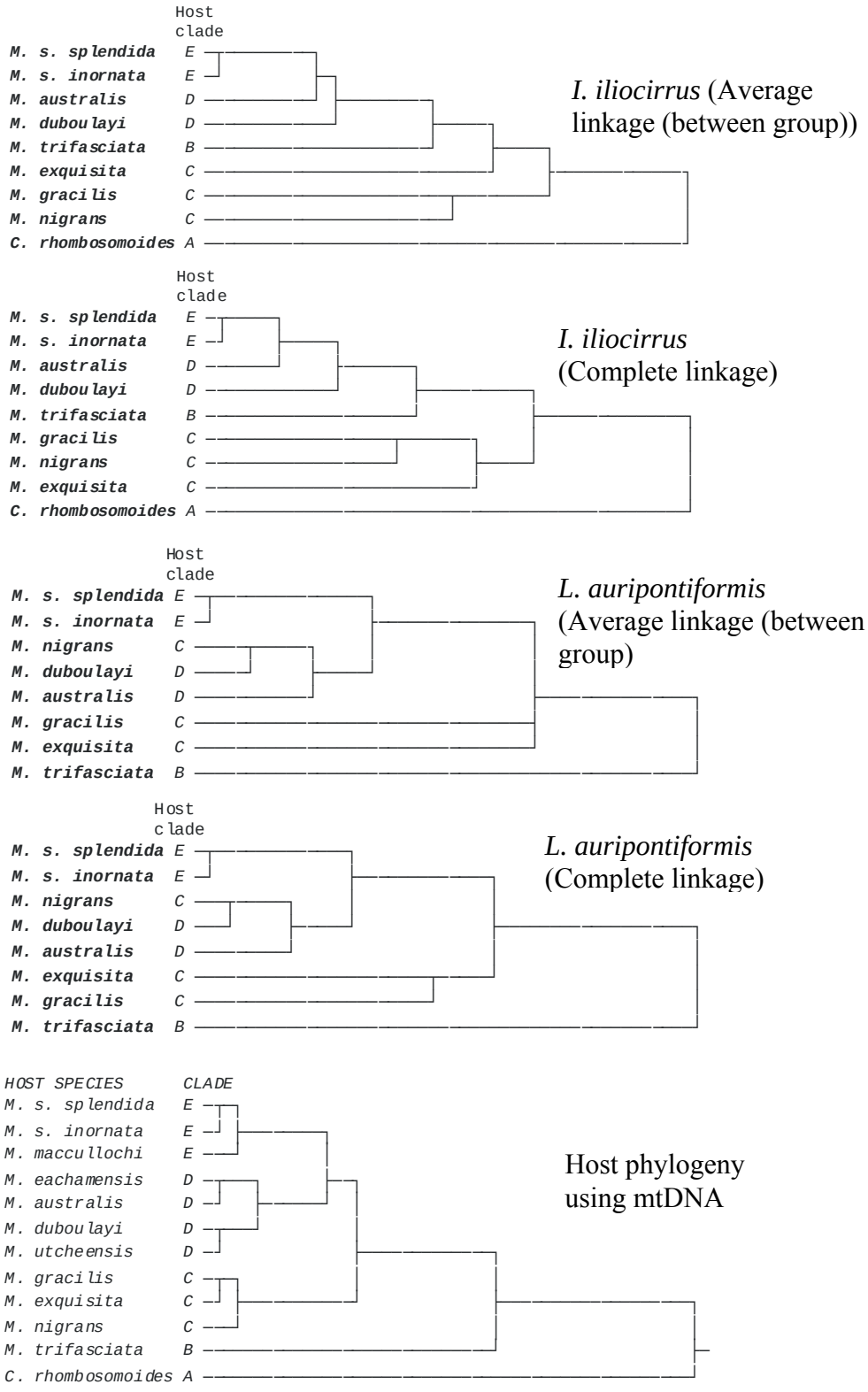


Figure 6.1. Cluster analysis (using Manhattan distances) of intraspecific variation for *I. iliocirrus* and *L. auripontiformis*.
Group centroid means of discriminant analysis were used as measures Specimens clustered according to host species infected. Host clades based on mtDNA. A. "*rhombosomoides*", B. "*goldiei (trifasciata)*", C. "*nigrans*", D. "*australis*", E. "*splendida*".

6.3.1.2 Hierarchical Cluster Analysis

Analysis of the associations of parasite intraspecific variation and host infected almost mirrors that of the host phylogeny (Figure 6.1). In the analysis of *I. iliocirrus* and *L. auripontiformis* variation associated with *M. trifasciata* and *M. nigrans*, respectively, did not cluster in the positions inferred by the host phylogeny.

6.3.2 Correlation between Host Length and Parasite Intra-Specific Sclerite Variation

The strength of the association between sclerite variation and host length was examined for all parasite species using Pearson's correlation. All variables were significantly correlated with host length ($P < 0.05$) (Table 6.5). However for most species, few variables were correlated (<50%). *Iliocirrus iliocirrus* and *L. auripontiformis* had a high number of correlations (>80%). For *I. iliocirrus* the four variables DA1, VA1, DB2, VB2 were not significantly correlated with host length ($P>0.05$), while for *L. auripontiformis* the two variables VA1 and VB2 were not significant ($P>0.05$). These two species were examined further to assess the strength of correlation at the host species level.

A significant correlation ($P<0.05$) between sclerite variables for *I. iliocirrus* and length of individual host species was identified (Table 6.6). The two species *M. s. inornata* and "*M. eachutchee*" had the highest number of correlations with the former having twenty correlated variables and the latter having seventeen while eleven variables were correlated with host length of *M. s. splendida*. The other host species had few sclerite variables correlated with host length. Most significant correlations were weak having r-values less than 0.5. A similar pattern was observed for *L. auripontiformis* where a

Table 6.5. Spearman correlations for sclerite variables of each parasite species and host length.

	H. gerrardiana	*H. macracanthus*	*H. marjoriae*	*H. melvani*	*H. megaloanchor*	*H. spinulatum*	*L. lineatus*	*L. mclati*	*L. ornatus*	*L. cosi*	*L. triflectus*	*L. surifomiformis*	*L. gracilis*	*L. boguraiensis*	*L. macroclavicle*	*L. robustus*	*L. wicheri*	*R. ...*	*R. ...*
#Parasite	15	10	41	11	45	78	107	87	99	17	31	403	92	17	41	18	27	42	39
#Host	10	5	8	4	33	43	164	31	43	7	13	133	48	10	9	13	4	11	8
DA1	-.156	.466	.293	-.173	-.611	**-.431	.044	.009	-.098	-.023	**.332	*.133	.186	-.110	.133	.006	**-.388	.091	.334
DA2	-.277	-.146	.337	-.381	-.115	-.098	**.439	.309	.134	**.737	**.402	**.307	**.380	.111	**.313	-.332	**-.333	**.433	.163
DA3	-.387	-.119	-.100	-.385	-.148	-.083	**.379	-.008	.185	.466	.310	**.310	**.341	-.147	*.318	-.314	-.113	.303	-.172
DA4	-.418	-.350	.301	*-.193	.119	.037	**.409	-.033	.133	.471	.339	**.301	**.317	-.039	**.400	-.392	**-.466	**.431	-.393
DA5	-.401	.039	**.433	.211	-.131	**.375	**.383	.139	*-.348	.383	.191	**.370	.083	-.001	.333	-.318	*-.431	**.433	.007
DA6	-.341	.137	**.434	*-.130	-.346	.011	**.379	.301	**-.301	.378	*.413	**.319	**.481	-.333	.183	-.311	-.317	**.408	-.141
VA1	-.132	-.351	-.039	*-.194	.303	.019	**.013	.041	-.119	.407	.329	-.013	**.313	.079	-.137	-.119	*-.413	.139	-.348
VA2	-.333	.319	-.139	.333	-.117	-.010	**.333	.149	*.334	**.139	.376	**.474	-.012	.311	**.383	.033	**-.182	.304	.176
VA3	*-.377	-.033	.086	-.152	-.133	-.160	**.343	.113	**.377	.449	*.398	**.403	.044	.375	*.333	-.193	*-.433	*.390	.143
VA4	-.433	-.331	.140	-.183	-.131	-.100	**.347	**.301	.089	**.833	.147	**.413	.071	.094	*.334	-.113	**-.493	.381	-.034
VA5	.318	-.131	*.343	.348	.317	-.071	**.331	.083	.073	*.304	.131	**.403	.088	-.101	.143	-.033	**-.476	.034	-.014
VA6	.087	.030	.091	-.113	.143	-.041	**.303	-.133	-.018	.347	.334	**.383	*.308	.339	-.001	-.386	-.031	.134	-.183
DB1	-.307	*.161	**.413	.171	.193	.333	**.394	*.048	.034	*.339	.319	**.373	-.006	.073	**.410	-.318	.319	.198	-.114
DB2							**.037	.033	-.181	-.191	.198		-.019						
DB3	.306	.043	.193	-.331	.171	-.319	**.313	.080	-.097	.373	-.183	**.197	.047	.341	.033	.103	*-.441	**.449	.183
VB1	-.163	.000	.113	.338	-.143	*.377	**.319	-.038	.013	*.101	.014	**.438	.303	.071	*-.314	-.307	.373	.393	-.337
VB2	.331	.113	**.419	-.171	-.169	-.003	.031	-.304	.137	.388	**-.418	.032	.018	.431	-.118	.493	.333	.108	-.149
H1	-.417	.040	.378	-.333	.388	.139	**.393	.037	-.093	.343	.333	**.399	-.076	-.334	-.010	-.114	**-.317	-.333	*.391
H2	.033	.030	-.373	.376	.331	.197	**.343	.033	.086	**.713	.313	**.484	-.010	.147	**.131	-.383	.191	.113	.003
H3	-.178	-.308	.333	.337	-.131	*.339	**.339	-.033	.084	*.380	.131	**.459	**-.441	.081	**.338	-.391	-.338	**.377	-.173
H4	.331	.151	**.433	-.131	.133	.171	**.310	.018	.133	.433	.301	**.473	**-.438	.071	.373	-.316	-.301	**.410	.149
H5	.038	.057	**.318	-.110	-.138	**-.374	**.310	.073	*-.311	.133	*.413	**.374	**.311	.003	.030	-.073	.033	*.011	*.398
H6	-.457	.117	.398	**.883	-.683	**.133	**.403	-.018	.089	.103	.116	**.317	**-.380	-.083	**.439	.046	.103	.343	-.031
H7	-.103	.161	-.080	.131	.130	.191	**.173	.137	.033	*.334	.313	**.437	-.131	-.037	**.313	-.018	.198	.334	*.014

Sample size.
* Correlation is significant at the .05 level (2-tailed).
** Correlation is significant at the .01 level (2-tailed).

Table 6.6. Correlations between haptoral variables of *I. iliocirrus* and length of individual host species.

	C. rhombosomoides	*M. australis*	*M. duboulayi*	*"M. eachutchee#*	*M. exquisita*	*M. gracilis*	*M. nigrans*	*M s. inornata*	*M. sp.*	*M. s. splendida*	*M. trifasciata*
#Parasite	10	101	71	64	22	46	36	143	26	79	5
#Host	3	26	25	15	7	14	11	28	7	23	2
DA1	.312	-.159	*-.376	150	-.026	.250	.115	.017	-.174	-.184	-.327
DA2	.292	-.176	-.025	**.465	*.448	-.067	.256	**.558	.268	**.277	**.993
DA3	.564	-.184	-.195	.117	*.487	-.171	-.184	**.472	.048	**.389	**.968
DA4	.500	-.195	-.140	**.365	*.507	-.253	.081	**.644	.150	**.472	**.990
DA5	-.218	-.053	-.129	**.478	*.532	.034	*-.347	**.548	-.057	-.060	.840
DA6	.046	*-.246	-.194	*.316	.191	-.116	-.123	**.304	-.084	.109	.662
VA1	-.253	.030	.098	*.297	.192	.290	-.159	*-.210	.081	-.116	-.408
VA2	.565	-.124	-.140	**.401	-.177	.202	*.364	**.475	.194	*.290	**.963
VA3	-.028	-.119	-.061	**.367	-.019	.189	*.423	**.457	.006	**.316	.840
VA4	.367	-.183	-.092	**.543	.080	.100	.237	**.532	.350	**.318	*.904
VA5	.335	.036	-.040	**.481	-.170	.039	**-.573	**.400	*.396	.137	.444
VA6	.283	-.030	.212	**.439	.077	-.078	**-.438	*.177	.096	.075	.102
DB1	.289	**-.270	-.154	**.404	.363	-.239	**.457	**.541	.290	.153	.811
DB2	-.195	*.216	**-.359	**.328	-.100	-.121	**.583	-.098	-.141	.082	.612
DB3	-.371	.156	.212	-.049	-.332	.139	-.059	.161	*.479	.042	-.408
VB1	.174	-.065	.139	.136	.396	-.096	.042	**.486	**.532	-.043	.185
VB2	-.454	.053	.084	-.035	*-.425	.015	*-.369	.067	.383	-.059	-.764
H1	.169	.065	-.048	.133	.185	.065	-.301	**.281	.262	.178	.881
H2	-.070	.010	.009	**.423	.351	-.132	-.277	**.483	*.393	**.313	**.955
H3	.506	*-.220	-.175	**.447	*.481	.125	.193	**.535	**.657	**.321	.843
H4	-.181	*-.234	.025	**.395	-.059	-.076	-.115	**.477	*.466	*.268	*.890
H5	.077	.040	**-.479	.216	.329	.178	-.269	.046	.285	-.174	-.645
H6	.444	-.187	.109	**.445	-.163	-.179	-.168	**.582	*.488	**.294	.645
H7	.454	-.093	.028	**.554	*.525	-.098	.012	**.534	.349	**.318	.807

Sample size.
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

significant correlation (P<0.05) between sclerite variables and length of host species was identified (Table 6.7). The two species *M. s. inornata* and *M. trifasciata* had the highest number of correlations with the former having twenty correlated variables and the latter having seventeen. The other host species had few or no sclerite variables correlated with host length.

Since a high number of significant correlations between sclerite variables of *L. auripontiformis* and *I. Iliocirrus* and host length were identified for *M. s. inornata* and

"*M. eachutchee*", a further analysis was conducted to see if correlations were present at the host-site level (Table 6.8). For *I. iliocirrus* the correlation with host length of "*M. eachutchee*" was significant (P<0.05) for 13 variables at the Barron R. site and five variables at the Ithica Ck. site. For *M. s. inornata* 16 variables were correlated with host length from the Wenlock R. site, while few sclerite variables were correlated with host length at the three other sites. Correlations between variables of *L. auripontiformis* and host length identified 5 significant results for the Chinaman Ck. site, while other sites had few or no variables correlated with host length.

Table 6.7. Correlations between haptoral variables of *L. auripontiformis* and length of individual host species.

	M. australis	*M. duboulayi*	*"M. eachutchee"*	*M. exquisita*	*M. gracilis*	*M. nigrans*	*M. s. inornata*	*M. sp*	*M. s. splendida*	*M. trifasciata*
#Parasite	21	75	19	22	12	27	150	21	45	8
#Host	13	25	7	9	6	11	45	12	19	4
DA1	**.552	-.187	.131	-.355	-.192	**-.533	.036	.316	-.194	.502
DA2	.286	.075	*.541	.237	.368	.119	**.630	.036	-.086	.696
DA3	.240	.171	.221	.087	.535	.130	**.583	.123	.111	*.806
DA4	.244	.063	.469	-.015	.540	-.069	**.629	.119	-.046	*.807
DA5	*.456	-.002	.410	-.148	.148	*-.462	**.421	.201	*-.371	-.367
DA6	.238	-.112	.416	-.224	.267	-.059	**.276	.032	.070	*.836
VA1	.170	-.051	-.122	-.010	.158	-.155	-.119	.200	*-.375	**.878
VA2	.335	.153	.338	-.045	.303	-.130	**.587	-.041	.063	**.939
VA3	-.241	.083	.293	-.322	-.013	-.017	**.371	.270	.226	.574
VA4	-.014	.017	.435	-.244	-.071	-.120	**.558	.088	.149	**.903
VA5	*.458	.082	.288	.392	.437	-.162	**.487	-.030	-.138	**.847
VA6	-.115	.184	-.148	-.008	.193	-.110	**.223	.303	.175	.404
DB1	-.397	-.105	.346	.208	-.291	-.201	**.444	-.244	-.022	**.900
DB3	.066	.073	.304	**.793	-.245	.028	**.289	-.133	-.226	.181
VB1	.323	-.107	.342	.071	.358	.302	**.614	*.435	*-.313	*.794
VB2	.254	-.030	.426	-.266	.235	.319	*.172	.039	**-.486	.000
H1	*.651	.004	.210	.144	**-.716	*-.474	**.216	.115	-.264	**.870
H2	.255	-.037	.322	**.614	**.726	-.296	**.613	-.021	-.207	*.729
H3	**.712	.166	.426	.068	.327	.192	**.628	-.344	*-.345	**.864
H4	.117	.120	*.554	.199	.291	.120	**.626	-.159	-.203	*.827
H5	**.701	-.204	.473	.261	-.456	**-.734	.027	.121	.121	**.853
H6	.002	.048	.157	**-.559	.203	.152	**.550	.187	-.227	**.916
H7	**.649	.179	.301	.075	.324	-.161	**.583	-.222	-.204	*.758

Sample size.
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 6.8. Spearman correlations between sclerite variables of *I. iliocirrus* and *L. auripontiformis* and host length of "*M. eachutchee*" and *M. s. inornata* at different sample locations.

Parasite	*I. iliocirrus*						*L. auripontiformis*					
Host	*"M. eachutchee"*		*M. s. inornata*									
Location	Barron R	Ithica Ck.	Manton Ck.	Oscar Ck.	Wenlock R.	Wildman R.	Chinaman Ck.	Manton Ck.	Oscar Ck.	Robinson R.	Wenlock R.	Wildman R.
#Parasite	28	36	20	20	44	43	39	20	23	14	14	38
#Host	6	9	3	5	10	6	15	5	7	3	8	7
DA1	-.028	.110	-.320	*.542	-.064	-.073	.194	.264	-.161	.078	-.132	.014
DA2	.329	*.352	.082	.025	**.469	-.094	.304	*.529	.113	.318	.243	.137
DA3	*.427	.087	.269	-.156	*.314	*.486	**.461	*.463	**.680	.332	-.046	-.004
DA4	*.527	.214	-.052	*-.516	**.570	*.561	**.434	.407	**.533	.306	.082	.144
DA5	**.648	.124	-.236	.337	**.606	-.037	-.130	.114	-.005	-.158	.230	-.072
DA6	*.436	.155	-.385	.427	.273	-.014	.151	-.071	-.237	-.081	.140	-.229
VA1	.068	*.395	-.026	-.392	.007	*-.365	.201	*.775	.022	-.079	-.336	-.254
VA2	.116	.171	-.059	*-.534	**.616	.164	.279	.389	.202	.054	.196	.038
VA3	.142	.250	-.412	.102	*.322	-.042	.276	.298	.384	-.022	-.336	.162
VA4	.172	**.452	-.254	-.340	**.488	.050	*.376	.017	.319	-.052	-.183	.060
VA5	.296	.231	-.321	-.308	**.559	.269	.181	.169	-.031	-.093	.376	.139
VA6	.359	*.352	-.329	-.170	.177	-.026	-.148	.181	.191	-.059	-.024	**.534
DB1	**.545	*-.379	-.090	.188	.193	.188	.090	-.103	.252	.194	.070	.117
DB2	.313	-.019	-.380	-.212	-.175	-.083						
DB3	.169	-.052	.072	.066	*.335	-.118	.301	-.225	-.071	.075	.412	.091
VB1	-.021	-.105	.013	-.318	.275	.243	.304	-.174	.176	-.084	.323	-.127
VB2	*.423	.101	-.149	-.298	.292	.084	-.106	-.100	-.027	-.085	-.088	-.161
H1	**.510	-.010	-.281	.151	**.417	-.022	-.093	-.069	.006	.063	-.311	.070
H2	*.400	-.005	-.192	.286	**.550	*.350	.225	.085	.381	.271	.117	.000
H3	**.504	.257	.161	.107	**.499	.266	-.046	.226	.109	.089	*.514	.003
H4	**.535	.162	-.297	.123	*.364	.087	**.486	-.225	-.094	.041	.231	.021
H5	*.453	.108	-.248	-.236	*.486	-.013	.214	.008	.302	.110	-.100	.226
H6	**.415	.132	-.287	*.446	**.661	-.063	.152	-.337	.139	.354	.192	-.122
H7	**.613	.316	.018	.212	*.346	*.441	*.339	.133	.060	-.054	.198	.093

Sample size.
* Correlation is significant at the .05 level (2-tailed).
** Correlation is significant at the .01 level (2-tailed).

For a final examination of the effect of host length, a classification of *L. auripontiformis* and *I. iliocirrus* at the host species level was undertaken. For the classification of *I. iliocirrus*, host length was most strongly associated with F2 (not shown) while for *L. auripontiformis* specimens and their host species association, host length was most strongly associated with the function 3 (not shown). The use of host length as a variable improved the percentage of correctly classified specimens of *I. iliocirrus* from 64.5 to 70.3% and 58.5 to 62.5% using cross validation and the within group matrix. For *L. auripontiformis*, correctly classified specimens improved from 60.0 to 69.1% and from 67.4 to 79.2% using cross validation and the within-group matrix.

6.4 Discussion

Intraspecific variations have been given little attention in the literature although their presence has been noted by various workers (Ergens, 1976; Gussev, 1976; Gussev & Kulemina, 1971). The occurrence of this phenomenon has been recognised in taxonomic papers where taxonomic ranges have been given. Species with wide ranges probably have several morphometric forms. This phenomenon has been considered as polymorphism (Ergens, 1981, 1991; Gussev, 1976): the dominant morphometric variant has been termed as the typical form or morph while the others are atypical forms.

Morphometric variations can occur in some monogenean species found on the same host individual, as well as on different host individuals from the same and different locations. The factors causing differences in a monogenean species could be inherrent genetic variability, host or micro-environment variability, and macro-environmental differences.

Different monogenean species respond differently to these interactions, some affected by macro-environmental factors, others being more affected by host variation and others apparently unaffected by both environmental circumstances. Discriminant analyses of parasites indicate that variants can be separated but at the same time, these variants are shown to be morphologically similar based on the scatterplot results.

In summary, intraspecific morphometric variants occur in monogenean species capable of infecting a range of host species (micro-environmental differences); infecting host species possessing a wide geographical range or occurring in widely differing macro-

environmental; infecting different individuals of a host species which is physiologically or genetically heterogeneous (resulting in host induced differences). The existence of morphometric variants suggests that taxonomists should be more cautious in proposing new species based solely on morphometric differences.

Intra-specific morphometric variation of the sclerites was examined for an association with host species infected and two species, *L. auripontiformis* and *I. iliocirrus*, were used. A classification of specimens of each species revealed a strong association with host species infected. For the cluster analysis, intra specific variation in both species shows a strong association with host species clades (Figure 6.1). The strength of this association may indicate that haptoral architecture variation is influenced by host species differences in gill architecture, which may represent host phylogenetic signal. The potential for the use of this phylogenetic signal to identify evolutionary associations between the host species is examined further in Chapter 9.

The relationship between host length and sclerite variability identified several significant correlations although these correlations are considered weak. Overall, host length showed little consistent correlation with sclerite variability and it is for this reason that a correction was not applied to the cluster, principle and discriminant analyses. To reduce the effect of host length, it is advised that future research should try to collect host specimens of similar size. It must be noted that this retriction would cause a loss in information on intraspecific variation, thus increasing the problem in taxonomy of host induced differences.

CHAPTER 7 HOST-PARASITE ASSOCIATIONS AND SPECIFICITY

7.1 Introduction

In this Chapter I, introduce a new method for assessing host specificity. Previous methods have been restrictive in their definitions and are thus limited in their use for studying host-parasite phylogenetic relationships. Four levels of host specificity are defined with mono-host-specificity being the most restrictive and low-host-specificity the least restrictive. Since Australian freshwater congeneric host species rarely occur in the same locality (Unmack, 2001) (also see Chapter 10), these levels of host-specificity and parasite/parasite associations may indicate phylogenetic relationships among host species, assuming that host switching has not occurred, or may indicate ecological associations if host addition has occurred. The association of host specificity and host phylogeny is examined further in Chapter 9.

The measure of host specificity can be affected by sampling effort and the structure of parasite distributions. The latter can be examined at three levels. At the lowest, the infrapopulation, the individual host specimen is the sample being examined. The next level, the component population, includes all specimens of a single parasite species on all specimens from a single host species in a sample site, while the metapopulation includes all specimens of a parasite species from a single host species from all sites. Patterns of parasite distribution may be similar at all levels of scale and therefore predictable or may have vastly different patterns. Two questions can be asked. For example, what are the patterns present and are the patterns the same at all levels examined and therefore predictable? This is the thrust of this chapter.

7.2 Generalist or Specialist

Parasites have usually been divided into two classes, generalists and specialists, and are based on their host specificity. Generalist parasites are those species that infect more than one host species while specialists are those species which infect only one species of host (Poulin, 1992; Sasal *et al.*, 1999b). An alternative definition has been presented (Euzet & Combes, 1980). Three classes of specificity: strict or oioxenic, when a parasite species can only live in a single host species; close or stenoxenic when a parasite species parasitises different, phylogenetically related species; and broad or euryxenic when the parasite is found in numerous hosts whose similarity is more ecological than phylogenetic.

Specialist parasites are usually termed host-specific, however all parasites are host-specific but the degree varies. Thus parasites known to infect only one host species should be referred to as mono-host-specific. No distinction has previously been made between generalist parasites which occur on two or three host species and those that occur on more than, for example, ten phylogenetically related host species. Hence, for example, parasites that infect two to five phylogenetically closely related host species may be referred to as highly-host-specific and parasite species that infect more than five host species may be referred to as having low host-specificity. This separation between high and low specificity is rather arbitrary and would not be appropriate for comparisons of specificity between parasites of phylogenetically distant taxa. A more appropriate method could use a percentage criterion, since specificity is used to identify different levels of host association (usually comparing parasite species of the same genus). Thus, parasite species that infect more than 66% of the known host species

infected by congeners are referred to as having low-host-specificity. Those infecting between 66% and 33% show medium host specificity while parasites infecting less than 33% are referred to as highly-host-specific. This also gives a quantitative value to the level of host-specificity. Generalists should be regarded as those species that infect multiple phylogenetically disparate taxa. This scheme is examined below and I will use these levels when referring to host-specificity.

7.3 Core and Satellite Species

A number of hypotheses have been proposed to explain community patterns and processes. One such hypothesis, the core-satellite species concept (Hanski, 1982; Hanski & Gilpin, 1991; Hartvigsen & Halvorsen, 1993, 1994), presents predictions about the mechanisms that influence the distribution of a species within a region.

If there is stochastic variation in the rate of colonisation or extinction (or both) of habitat patches within the region and if the probability of extinction within a patch declines as population increases, then each species within a community will tend towards one of two opposite states. Some species will colonise most patches and be present in high numbers within a patch. These regionally common and locally abundant species are termed core species (Hanski, 1982). Other species will tend to colonise few patches and, where found, are in low numbers. These regionally uncommon and locally rare species are termed satellite species. Core species have higher dispersal ability and hence greater distribution (Nee *et al.*, 1991). Therefore, core species should be well dispersed within niche space while satellite species should be restricted.

The core-satellite species concept was expanded on by Bush & Holmes (1986) who represented the regional dispersion of parasites by prevalence and local abundance by intensity. They explicitly tested for a positive distribution-abundance correlation. Also examined were the modes of distribution before assigning parasite species to core or satellite species status, and evaluation of the linear niche relationships of the two groups. The original model (Hanski, 1982) only predicted a bimodal distribution however a third category called secondary species has also been identified (Bush & Holmes, 1986). The use of core and satellite species as simply synonyms of high prevalence or low prevalence respectively has been applied and discouragement of this has been recommended (Nee *et al.*, 1991). Typically, a species has been classed as a core species if it occurs on more than 70% of specimens examined. Bush *et al.* (1997) recommend that the use of core and satellite species be restricted to situations in which the predictions or assumptions of the core-satellite hypothesis are being tested and that the full set of criteria used to assign species within each study be stated explicitly.

7.3.1 Metapopulations

Metapopulation theory has been proposed as an alternative approach to island biogeography model (MacArthur & Wilson, 1967) for studying the spatial patterns in helminth communities of fish (Hartvigsen & Halvorsen, 1994). Hanski & Gilpin (1991) defined a metapopulation as a system of local populations connected by dispersing individuals. A second definition identifies a metapopulation as all individuals of one parasite species in one population of one host species (Simberloff & Moore, 1997). The latter definition is very applicable to monogeneans since this definition may be appropriate for mono-host-specific parasites with a direct life cycle (Kennedy, 2001).

Applications of metapopulation theory usually assume that local populations are not connected but are separated by unsuitable habitats (Hanski & Gilpin, 1991). However, a lack of connectivity is not necessary for the application of metapopulation theory (Kennedy, 2001). He reasoned that a river and its tributaries should be viewed as metapopulations and locations in the rivers and tributaries as local populations. A consequence is that the predicted levels of similarity may be higher than normal between communities given their proximity and connectivity (Poulin & Morand, 1999). Kennedy (2001) found that metapopulation theory did not satisfactorily explain changes in helminth community structure in his study, i.e. similarity between communities did not reflect connectivity of localities within stream.

7.4 Parasite-Host Interactions

Host body size has often shown a positive relationship with parasite abundance and richness of parasite species infecting a host (Barse, 1998; Chubb, 1979; Guégan & Hugueny, 1994; Kim *et al.*, 2001; Matejusová *et al.*, 2000; Morand *et al.*, 1999). Two explanations have been proposed for ectoparasites (Guégan & Hugueny, 1994). The effect of passive sampling of parasite species with increased gill surface areas: increased body size implies a larger gill surface area, which gives rise to a greater random occurrence of parasite richness in fish. The effect of passive sampling of parasite species and fish age has also been suggested. As fish become older, the length of time a host has available for random parasite colonisation increases (Guégan & Hugueny, 1994). Thus under the passive sampling hypothesis, the common parasite species are expected to be found on many fish while rare parasites are restricted to a few fish which have sampled parasites for the longest time. A positive relationship between occurrence and mean/host abundance is also expected (Wright, 1991). Therefore, when

sampling hosts for parasites larger host specimens should be examined. This increases the chance of detecting all the parasite species that infect a particular host species and hence better ensuring that the levels of host specificity are correctly identified.

7.4.1 Host Habitat Relationships

The habitat type may have an effect on parasite species diversity, prevalence and abundance. Although temperature appears to have little effect, rainfall or waterflow does affect parasite abundances, dispersal and invasion (Lim, 1987), although too high a rainfall or waterflow may be disadvantageous to larval colonisation and establishment (Barker & Cone, 2000). A study looking at eels identified water velocity as a causal factor in variations of parasite abundance (Barker & Cone, 2000). Freshwater habitats can be divided into several types from small fast flowing streams, large slow flowing streams to small, almost dry ponds. These stream types are often of a seasonal nature.

7.5 Methods

The gills of 21 host species (see Table 2.2) consisting of 719 fish specimens from 75 host/location samples were examined for monogenean parasites. The following quantifying variables were measured: prevalence, abundance, maximum intensity, overall abundance and overall mean abundance of each species. Host length was also examined to ascertain if it affected the parasite parameters abundance, prevalence and species diversity.

7.6 Results

7.6.1 Prevalence

Of 719 fish specimens, 85.7% (620) were parasitised by monogeneans and 8,587 gill parasites were removed. Three host species *Craterocephalus helenae* from the Drysdale R., *Craterocephalus stramineus* from L. Kunnanurra and the Gregory R, and *Melanotaenia s. tatei* from Tennant Ck. were uninfected (Table 7.1). Also no monogeneans were found on *P. gertrudae* from Gwynne Ck. These host-location samples were removed from all further analyses (70 specimens). Of the remaining 649 host specimens, 95.5% were infected while for the 70 remaining sample sets, 82.3% (56) had an overall prevalence of 100%. For the 12 sample sites with a prevalence of less than 100%, only *P. gertrudae* (58.8%) from Howard Ck. had a prevalence of less than 83%.

Overall prevalence of infection for each parasite species on host species (19) is *I. iliocirrus* 63.2%, *I. mazlini* 15.8% *I. rossi* 5.3%, *I. ornatusi* 5.3%, *I. trifasciatae* 5.3%, *L. auripontiformis* 57.9%, *L. gracilis* 42.1%, *L. maccullochii* 5.3%, *L. utcheei* 5.3%, *L. robustus* 36.8%, *H. splendidae* 68.4%, *H. megaloanchor* 42.1%, *H. mcivori* 5.3%, *H. maccullochii* 5.3%, and *H. marjoriaea* 5.3%. Three parasite species *I. iliocirrus*, *L. auripontiformis* and *H. splendidae* had a prevalence greater than 50%. Three species *H. megaloanchor, L. gracilis* and *L. robustus* had a prevalence of approximately 40%. All other species had a prevalence of approximately 5%. These prevalences are affected by sample size, thus they only give a rough understanding of prevalence patterns. However, they do give indications of which parasites may be mono, high or low-host-specific and which are core, secondary and satellite species (see Section 7.6.4 and 7.6.5.).

Table 7.1. Host species, sample location, State, number of fish sampled, host length, range, mean parasite abundance, maximum intensity, prevalence.

Host sp	*Location*	*State*	*No.*	*Avg. t L*	*Host range*	*Mean Abun.*	*Ma. ints*	*Prev. %*
C. helenae	Drysdale R.	WA	20	66.9	60-77	0	0	0.0
C. marjoriae	Amamoor Ck.	QLD	13	46.6	37-52	21.5	28	100
C. rhombosomoides	Behana Ck.	QLD	7	28	23-33	2.9	14	85.7
C. rhombosomoides	Utchee Ck.	QLD	5	32.5	31-34	8.7	14	100
C. s. stercusmuscarum	Barron R.	QLD	13	57.6	37-68	13.4	63	100
C. s. stercusmuscarum	Dulhunty R.	QLD	3	60.7	58-62	1	2	100
C. s. stercusmuscarum	Granite Ck.	QLD	1	52	52	5	10	100
C. s. stercusmuscarum	Howard Ck.	NT	3			13.5	61	100
C. s. stercusmuscarum	Norman R.	QLD	8			10.8	38	100
C. s. stercusmuscarum	Ross R.	QLD	3			16.8	36	100
C. s. stercusmuscarum	Tungamull Ck.	QLD	12			12.2	45	100
C. stramineus	Gregory R.	QLD	10	31.8	27-41	0	0	0.0
C. stramineus	L. Kunnanurra	WA	20	31.4	27-38	0	0	0.0
M. australis	Adcock G.	WA	5	44.3	36-53	2.2	8	100
M. australis	Camp Ck.	WA	15	40.8	34-49	4.0	37	100
M. australis	Dawn Ck.	WA	8	46.3	39-54	4.7	27	100
M. australis	Drysdale R.	WA	11	33.6	32-37	3.2	25	100
M. australis	Mary R.	WA	10	45.5	38-51	6.4	60	100
M. australis	Pentacoste R.	WA	10			18.6	117	100
M. duboulayi	Amamoor Ck.	QLD	6	42.2	36-53	2.3	15	83.3
M. duboulayi	Beerburrum Ck.	QLD	10	42.6	38-47	2.1	14	90.0
M. duboulayi	Granite Ck.	QLD	15	50.5	43-55	3.7	42	100
M. duboulayi	Tuan Ck.	QLD	10	55.3	40-68	5.4	33	100
M. eachamensis	Dirran Ck.	QLD	19	45.2	38-55	3.6	25	100
M. eachamensis	Gwynne Ck.	QLD	10	41.7	35-57	2.6	14	90.0
M. eachamensis	Nigger Ck.	QLD	20	43.4	37-51	1.7	10	90.0
M. eachutchee	Barron R.	QLD	11	47.4	39-60	5.8	39	100
M. eachutchee	Ithica Ck.	QLD	10	51.1	46-62	7.1	33	100
M. eachutchee	Williams Ck.	QLD	10	45.5	37-57	3.5	13	100
M. exquisita	Moline Rock Pool	NT	17	42.1	34-47	4.4	29	100
M. gracilis	Drysdale R.	WA	18	46.0	38-57	3.1	23	86.9
M. gracilis	Russ Ck.	WA	7	52.8	42-61	9.2	28	100
M. maccullochi	Corduroy Ck.	QLD	9	36.5	26-46	4.9	14	100
M. maccullochi	McIvor R. 1	QLD	6	31.5	25-41	6.1	22	100
M. nigrans	Comalie Ck.	NT	2	41	40-42	6.3	27	100
M. nigrans	Howard Ck.	NT	14	33.4	28-43	3.2	13	92.8
M. s. inornata	Chinaman Ck.	QLD	19	47.2	38-59	5.5	39	100
M. s. inornata	Dulhunty R.	QLD	5			5.3	17	100
M. s. inornata	Howard Ck.	NT	2			9.6	32	100
M. s. inornata	Manton Ck.	NT	10	60.5	53-68	21.6	93	100
M. s. inornata	Oscar Ck.	QLD	11	46.7	43-57	9.9	50	100
M. s. inornata	Robinson R.	NT	4	72.2	65-83	4.8	27	100
M. s. inornata	Wenlock R.	QLD	13	40.6	29-46	4.9	26	100
M. s inornata.	Wildman R.	NT	7	49.8	40-56	15.8	122	100
M. s. sp	Kangaroo Ck.	QLD	14	53.5	36-67	5.9	40	100
M. s. splendida	Annan R.	QLD	8	48.3	34-66	11.3	47	100
M. s. splendida	Bluewater Ck.	QLD	6			6.2	27	100
M. s. splendida	Corduroy Ck.	QLD	10	34.3	26-49	2.6	26	90.0
M. s. splendida	Daintree R.	QLD	6	49	47-53			100
M. s. splendida	Five Mile Ck.	QLD	1	55	55	7.3	22	100
M. s. splendida	Lexilip Ck.	QLD	2			10	25	100
M. s. splendida	Liverpool Ck.	QLD	13			7.8	64	100
M. s. splendida	McIvor R.	QLD	5	57.1	48-68	8.1	46	100
M. s. splendida	Roaring Meg Ck.	QLD	13	60.9	56-69	5.4	30	100
M. s. splendida	Ross R.	QLD	4			5.4	23	100
M. s. splendida	S. Mossman R.	QLD	6			5.1	23	100
M. s. splendida	Tungamull Ck.	QLD	9	47	47	3.7	16	88.8
M. s. splendida	Walkaman Res. St.	QLD	10	42.5	38-55	0.1	1	10.0
M. s. tatei	Tennant Ck.	NT	3	28	27-29	0	0	0.00
M. trifasciata	Dulhunty R.	QLD	6			8.1	51	100
M. trifasciata	McIvor R.	QLD	15	56.5	45-78	10.2	52	100
M. trifasciata	Moline Rock Pool	NT	5	48	42-75	1.5	4	100
M. trifasciata	Wenlock R.	QLD	8	35	35-35	2.3	16	100
M. utcheensis	Utchee Ck.	QLD	20	52.2	42-62	3	18	100
P. gertrudae	Gwynne Ck.	QLD	10	25.5	24-27	0	0	0.0
P. gertrudae	Howard Ck.	NT	17	19.6	15-35	1.4	4	58.8
P. signifer	Corduroy Ck.	QLD	10	28.9	27-31	1.3	4	90.0
P. signifer	Daintree R.	QLD	5	41.2	37-47	33	48	100
P. signifer	Liverpool Ck.	QLD	10			10.6	20	100
P. signifer	Russell R.	QLD	5			2	5	60.0
P. signifer	S. Mossman R.	QLD	5	44.2	42-46	20.4	40	100
R. ornatus	Bible Ck.	NSW	14	32.4	27-45	4.1	10	100
R. ornatus	Brunswick R.	NSW	11	40.1	35-43	5	13	100

Table 7.2. Host species and % prevalence of each parasite species

Host species	No. examined	*R. chelatus*	*R. signifer*	*H. gertrudaea*	*H. marjoriaea*	*H. mcivori*	*H. sp.*	*H. maccullochii*	*H. splendidae*	*H. megaloanchor*	*L. kupevalensis*	*L. maccullochi*	*L. utcheei*	*L. gracilis*	*L. robustus*	*L. auripontiformis*	*L. iliocirrus*	*I. maclini*	*I. trifasciatae*	*I. ornatusi*	*I. rossi*	*A. dirrani*	*Gyrodactylus sp.*
C. stramineus	30																						
C. kalonae	20																						
C. marjoriae	13				100																		
C. s.	40	92.5					4.7														85.0		
P. signifer	35		88.6																				
P. gertrudae	17			64.7																			
R. ornatus	59								6.3											94.9		1.7	
C.	12												57.2				41.6						
M. trifasciata	34					44.1			14.7		32.4					26.5	52.9		76.5				
M. maccullochi	15							33.3	20.0	26.7		100											
M. exquisita	17								29.4					23.5		82.4	100						
M. gracilis	25								12.0					36.0	4.0	24.0	88.0						
M. nigrans	16													56.2		62.5	93.7						
M. sp.	14								64.3	21.4				21.4		100.0	100						
M. s. splendida	83								32.5	27.7					8.4	74.1	84.3					1.2	
M. s. inornata	71								39.4	35.2					12.7	93.0	80.3						
M. australis	59								64.4	8.5				67.8	1.7	74.6	93.2						
M. eachamensis	50								42.0	4.0				38.0		2.0	4.0	86.0				16.0	2.0
M. utcheensis	20								84.0					80.0				80.0					
"*M. eachutchee*"	32								31.2	18.7					12.5	31.2	71.9	43.7					
M. duboulayi	41								14.6	19.5				12.0	12.2	92.7	90.2						

Prevalence of infections varies greatly on those hosts that are known to have a particular parasite species (Table 7.2). *Iliocirrus iliocirrus* had a prevalence greater than 70% for nine of the twelve (75%) host species it is known to infect. *Longidigitis auripontiformis* had a prevalence greater than 70% for six of the eleven host species and greater than 20% for ten of the eleven host species it is known to infect. *Helicirrus splendidae* had a prevalence > 70% for one of the thirteen host species and a prevalence of 20% or more for nine of the thirteen species it is known to infect. Prevalences for *H. megaloanchor* were less than 40% for all nine hosts, however only two had a prevalence less than 10% for species it is known to infect. *Longidigitis gracilis* had a prevalence greater than 20% for seven of the eight host species and a prevalence greater than 80% for *M. utcheensis*. *Longidigitis robustus* had a prevalence less than 16% for host species they are known to infect. Seven of the twelve remaining parasites had a prevalence greater than 70% for hosts they are known to infect.

Histograms were also used to examine the number of parasite species infecting specimens of each host species (Figure 7.1). From the graphs it can be clearly seen that multiple parasite species infections are the most common. *M. australis* specimens had up to five parasites species with the most common number being four and the presence of a single parasite species was least common. For the host species *M. s. splendida, M. s. inornata, M duboulayi, M. sp*. and *M. utcheensis,* three parasite species were most commonly present. Of the host species known to be infected with multiple parasite species only *R. ornatus* was most commonly infected with a single parasite species (not shown).

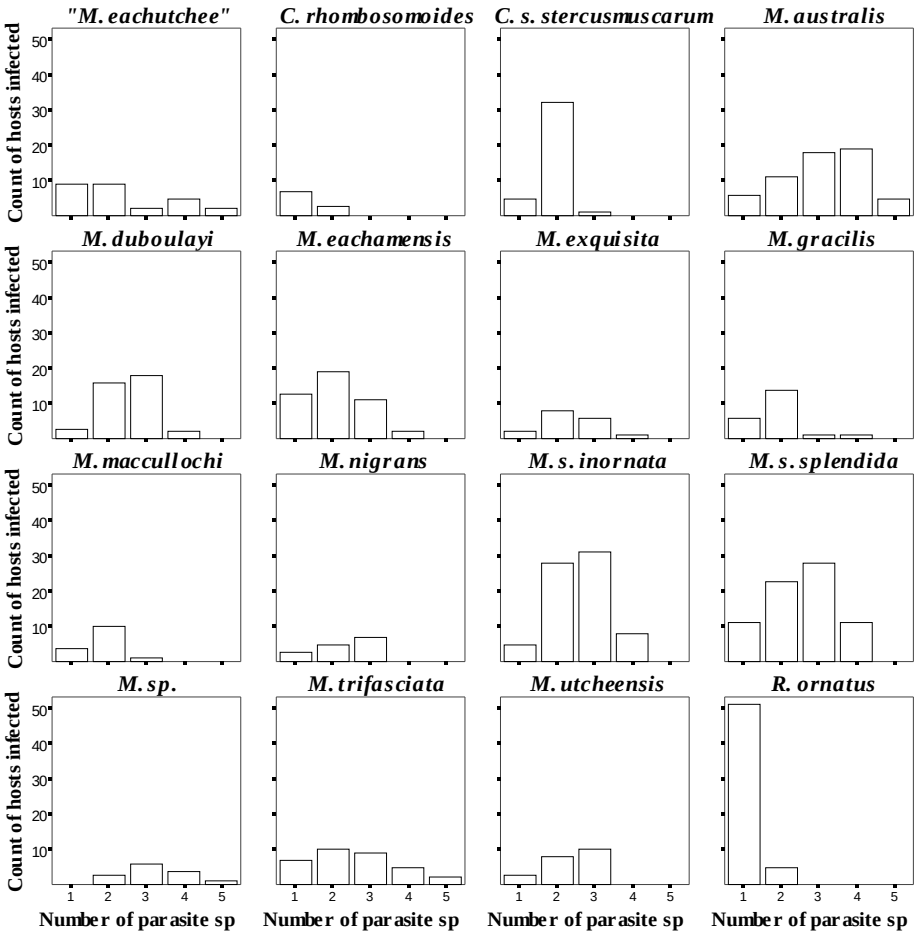


Figure 7.1. Number of parasite species present and percentage of host specimens infected for each host species. All locations included.

7.6.2 Abundance

The overall mean abundance was 13.17 monogenean parasites per host specimen (649 specimens). Four host sample sets had a mean abundance greater than 20 (Table 7.1). The highest sample host-site mean abundance of 33 occurred on *P. signifer* from the Daintree R. while *M. s. inornata* from Manton Ck, *C. marjoriae* from Amamoor Ck. and *P. signifer* from the S. Mossman R, had mean abundances of 21.6, 21.5 and 20.4 respectively. *Helicirrus* species were found in low numbers (usually less than one specimen per host). Exceptions were *H. marjoriaea* found on *C. marjoriae* and *H. splendidae* found on *M. utcheensis* (Table 7.3). *Iliocirrus iliocirrus* was the most

Table 7.3. Mean abundance of parasite species on host species examined.

Host species	Sample no.	*R. chelatus*	*R. signifer*	*H. gertrudeae*	*H. marjoriae*	*H. mawsoni*	*H. sp.*	*H. maccullochii*	*H. splendidae*	*H. megalancanchor*	*L. hopevalensis*	*L. maccullochii*	*L. ukhei*	*L. gracilis*	*L. robustus*	*L. aripontiformis*	*L. filocirrus*	*L. waelini*	*L. trifasciatae*	*L. ornatusi*	*L. rossi*
C. stramineus	2																				
C. helenae	1																				
C. marjoriae	1				19.8																
C. s.	6	14.3					0.05														7.15
P. signifer	4		7.97																		
P. gertrudae	2			0.85																	
R. ornatus	4								0.19											3.76	
C.	2												4.92				1.83				
M. trifasciata	4					1.24			0.24	0.03	0.68					0.62	2.71		11.3		
M. maccullochi	3							0.57	0.27	0.47		7.40									
M. exquisita	1								0.29					0.35		2.88	6.88				
M. gracilis	2								0.16					0.96	0.08	1.48	6.80				
M. nigrans	2													1.13		1.81	5.25				
M. australis	6								1.71	0.59				2.64		6.19	11.1				
M. eackamensis	3								0.70	0.07				0.70			0.11	4.19			
M. utchensis	2								3.26					2.00				1.37			
M. duhoulayi	4								0.15	0.27				0.12	0.46	2.66	5.05	0.02			
M. sp.	1								0.86	1.00				0.29	0.07	4.07	12.3		0.07		
M. s. splendida									0.69	0.53					0.09	2.99	9.58				
M. s. inornata	6								2.05	1.25					0.17	6.67	11.5				
"M. eachutchae"	3								0.67	0.06					0.22	0.76	4.47	1.38			

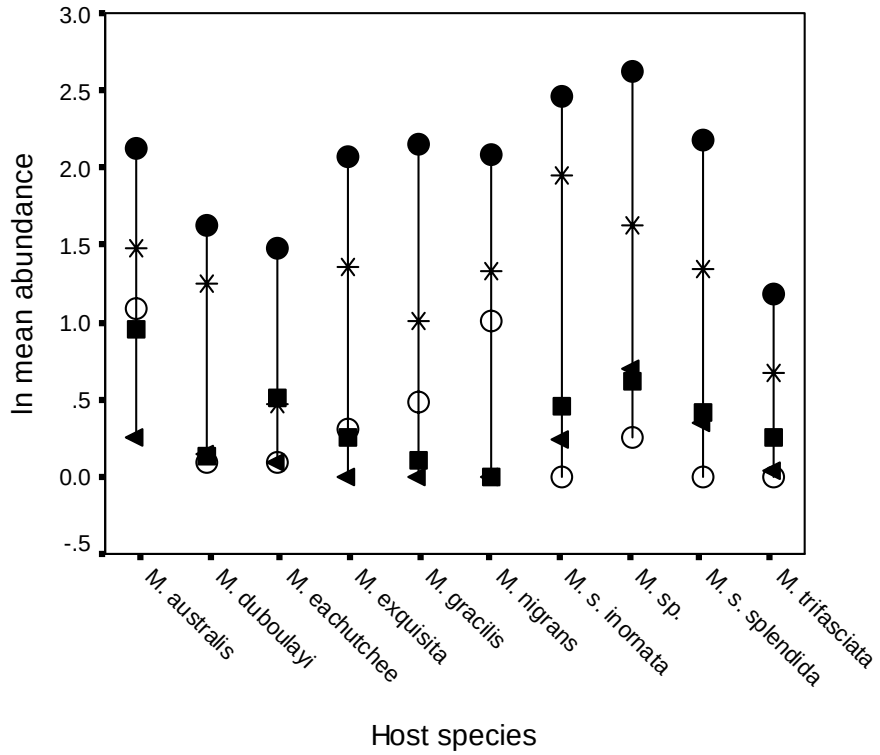


Figure 7.2. ln mean abundance of the five most common parasite species on host species. ●. *I. iliocirrus*, ✳ *L. auripontiformis*, ■ *H. splendidae*, ◂ *H. megaloanchor*, ○ *L. gracilis*.

common species of the genus with a mean abundance >5 for most host species (75%) infected while *L. auripontiformis* was the most common species for *Longidigiti*s. A comparison of the relative abundances of the five most common species on each host species indicates that *I. iliocirrus* was always the most common while *L. auripontiformis* was the second most common (Figure 7.2). There also appears to be a hierarchical structure of parasite species abundances and this is examined below.

7.6.3 Intensity

The mean intensity for the 620 infected host specimens was 13.85 parasites/fish. A maximum intensity of infection of 122 occurred on a host specimen of *M. s. inornata* from Wildman R. while mean intensity for this sample site was 15.8. A maximum intensity of infection of 117 occurred on a specimen of *M. australis* from the Pentacoste

R. with the mean for the site being 18.6. Four host-site sample sets had a mean intensity greater than 20 and values are the same as for mean abundances (see Section 7.6.2).

Mean and maximum intensity of infection for each parasite species is shown in Table 7.4. The four highest mean intensities of infection are for the mono-host-specific parasites (see Section 7.6.4) *H. marjoriaea* (21.54), *R. chelatus* (15.86), *I. trifasciatae* (13.96) and *R. signiferi* (12.74), while for the low-host-specific parasites, *I. iliocirrus* had the highest mean intensity of infection. The highest maximum intensity for a single parasite species on a single host specimen was for *I. iliocirrus* (72). Four other parasite species had maximum intensities greater than 45: *R. chelatus, R. signiferi, I. trifasciatae* and *L. auripontiformis* (Table 7.4).

Table 7.4. Maximum and mean parasite species intensity on host specimens.

Parasite sp	Max. intensity	Mean intensity
H. gertrudaea	4	2.27
H. maccullochii	4	2
H. mcivori	6	2.33
H. megaloanchor	10	2.74
H. splendidae	16	2.61
H. marjoriaea	28	21.54
L. hopevalensis	5	2.18
L. robustus	7	2.18
L. utcheei	14	5.62
L. gracilis	17	2.86
L. maccullochii	18	8.53
L. auripontiformis	50	5.36
I. ornatusi	10	3.96
I. mazlini	14	3.17
I. rossi	28	8.85
I. iliocirrus	72	10.52
I. trifasciatae	49	13.96
R. chelatus	47	15.86
R. signiferi	48	12.74

Intensity and host length was examined further to see if there was an association between these variables and the number of parasite species present. Only those host species known to be infected with more than two species were included in the analysis. Variables host length and parasite intensity were ln transformed and z-score standardised before analysis. LRA identified a significant association between the parasite intensity and the number of species present ($P<0.001$, $R^2=0.365$). This indicated that the probability of a multiple parasite species infection on a host increased with parasite intensity. Host length was included in a second analysis. However this variable did not produce a significant increase in the regression coefficient ($P=0.229$, $R^2 =0.0016$). A significant difference in ln mean intensity and number of species infections was identified (ANOVA $F_{1, 427} =233.4$, $P<0.0001$). A multiple pairwise comparison using Tamhane post-hoc multiple range test identified a significant difference in ln mean intensity for infracommunities containing 1, 2, 3 and 4 species (Figure 7.3). No significant difference in ln mean intensity was observed between infracommunities having 4 or 5 species (Figure 7.3).

Positive associations were observed between several pairs of parasite species (Appendix TABLE E4). The highest positive correlation between any two parasite species was between *R. chelatus* and *I. rossi* (R 0.91, P< 0.01) which occur on *C. s. stercusmuscarum*. Other species that show a significant association are *I. iliocirrus* and *L. auripontiformis*, *I. iliocirrus* and *L. gracilis*, *L. auripontiformis* and *L. gracilis*. For parasite mean abundance/sample location, a positive significant association was identified between the following species: *L. auripontiformis* and *I. iliocirrus* ($R^2= 0.73$, $P<0.01$), *L. auripontiformis* and *L. gracilis* ($R^2= 0.40$, $P<0.01$), *I. iliocirrus* and *L. gracilis* ($R^2= 0.13$, $P<0.01$) (Figure 7.4).

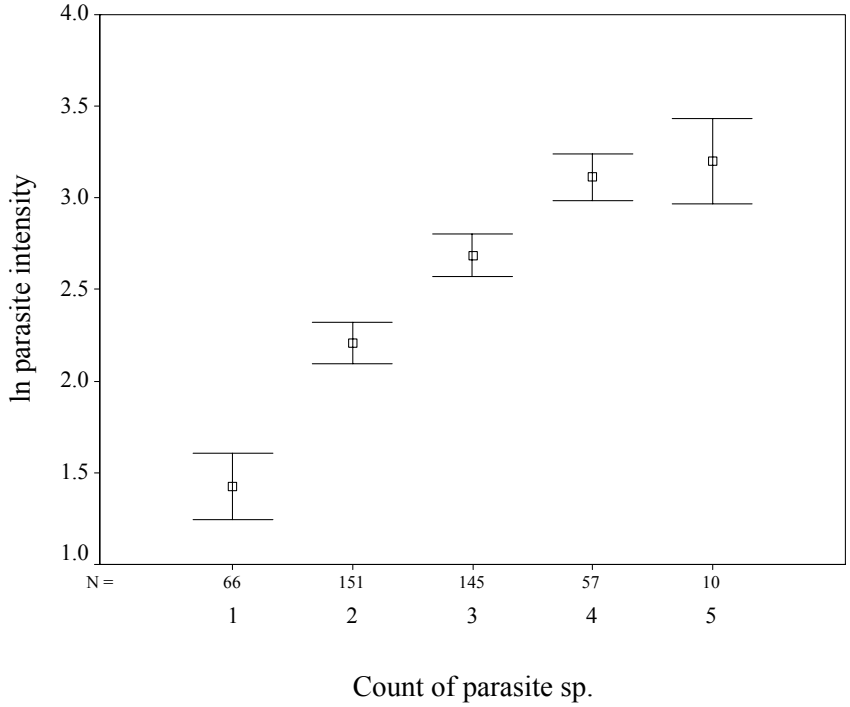


Figure 7.3. Parasite intensity of infection and number of species occurring on host specimens.
Error bars represent mean 95% C.I.

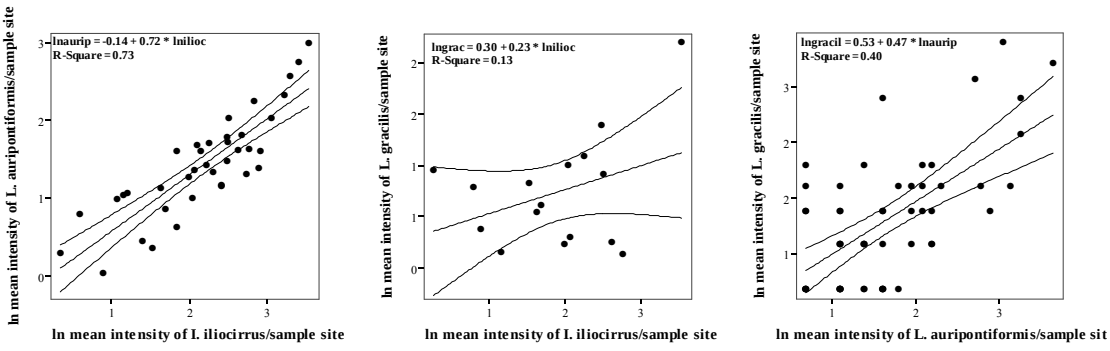


Figure 7.4. Association of mean intensity/sample host-site between parasite species infections. Data was ln transformed.

Table 7.5. Number of host species infected by each parasite species.
Number of host species infected.
*Level of host specificity 1. mono; 2. high; 3. medium: 4. low.

Parasite sp.	N#	Status*
H. gertrudaea	1	1
H. maccullochii	1	1
H. marjoriaea	1	1
H. mcivori	1	1
H. megaloanchor	8	3
H. splendidae	13	4
I. iliocirrus	12	4
I. mazlini	3	2
I. ornatusi	1	1
I. rossi	1	1
I. trifasciatae	2	2
L. auripontiformis	11	4
L. robustus	6	3
L. gracilis	8	3
L. hopevalensis	1	1
L. maccullochii	1	1
L. utcheei	1	1
R. chelatus	1	1
R. signiferi	1	1

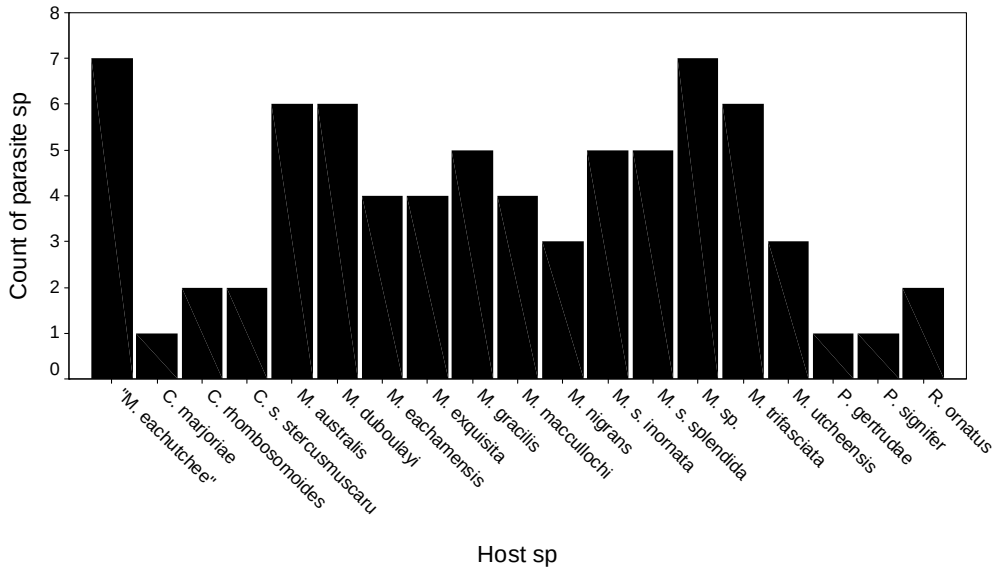


Figure 7.5. Host species and the number of known parasite species infecting them.

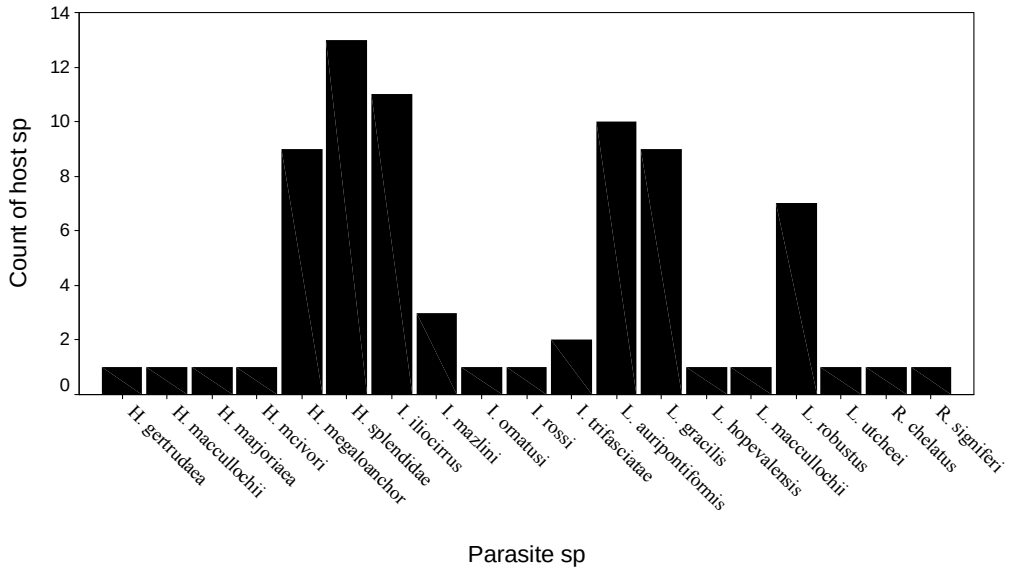


Figure 7.6. Parasite species and number of host species they infected.

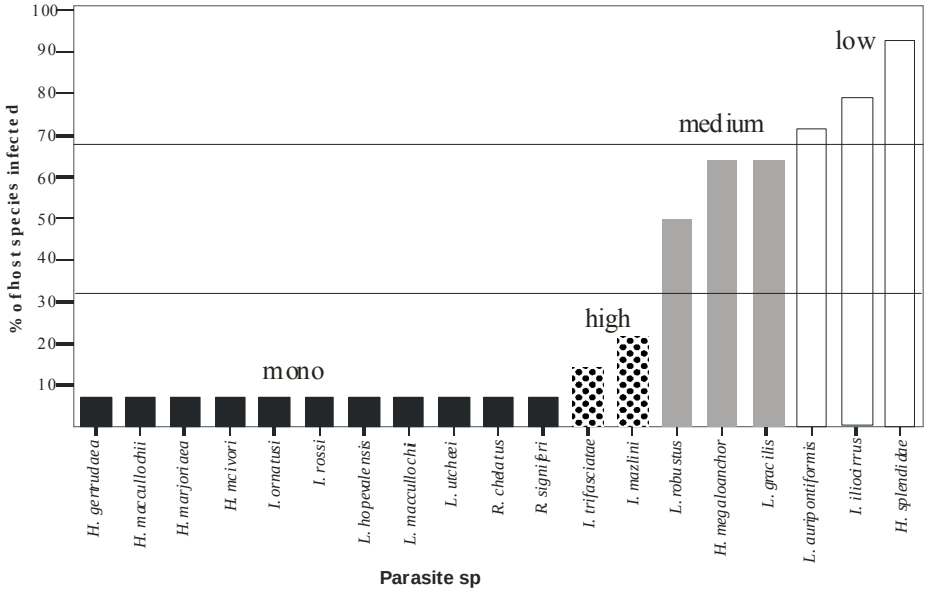


Figure 7.7. Parasite species infecting Melanotaeniidae host species and their host specificity classification.

7.6.4 Host Specificity

The majority of host species (>75%) were infected by three or more parasite species (Figure 7.5). When the generalist /specialist approach to host specificity is used, eleven species (68.4%) are recognized as specialists i.e. infect only one host species (Figure 7.6). All species infecting atherinids and pseudomugilids are specialists. This high

specificity may be related to the low number of species examined from these families. For this reason specificity was examined separately for melanotaeniids. Using the modified groupings I proposed, species recognised as mono, high, medium or low host-specific are presented in Table 7.5. These levels of host specificity were determined from frequency of host species infected (Figure 7.7). Fourteen monogenean species are known to infect melanotaeniid hosts.

Looking at host specificity within each parasite genus four of the six described species of *Helicirrus* are mono-host-specific: *H. mcivori* from *M. trifasciata, H. marjoriaea* from *M. marjoriae, H. maccullochii* from *M. maccullochi* and *H. gertrudaea* from *P. gertrudae. Helicirrus splendidae* and *H. megaloanchor* show low and medium host specificity respectively. Both species of *Recurvatus* were mono-host-specific: *R. chelatus* from *C. s. stercusmuscarum* and *R. signiferi* from *P. signifer*.

Two of the five species of *Iliocirrus* are mono-host-specific: *I. ornatusi* from *R. ornatus* and *I. rossi* from *C. s. stercusmuscarum.* Three species infected multiple host species. *Iliocirrus iliocirrus* occurs on the same host species as *L. auripontiformis* and also occurs on *C. rhombosomoides* and is designated as low-host-specific for the Melanotaeniidae. *Iliocirrus mazlini* occurs on the two species *M. eachamensis* and *M. utcheensis* and is identified as highly host-specific as is *I. trifasciatae* from *M. trifasciata.*

Three of the five species of *Longidigitis* identified are mono-host-specific: *L. maccullochi* from *M. maccullochi, L. utcheei* from *C. rhombosomoides* and *L. hopevalensis* from *M. trifasciata.* Several parasite species occurred on multiple host

species. *Longidigitis gracilis* is identified as medium host-specific for species in Melanotaeniidae; *L. auripontiformis* is identified as showing low host-specificity.

Longidigitis robustus was very rare; however it did occur on more than one host species: *M. s. splendida*, *M. s. inornata*, *M. gracilis*, *M. duboulayi* and *M. sp*. This species was also present on specimens signified as *M. "eachutchee*".

A high level of host-specificity is identified at the parasite genus level. Species of *Longidigitis* are specific to the Melanotaeniidae while species of *Recurvatus* occur only on atherinids and pseudomugilids. Species of *Helicirrus* with the elongated hooks occur on species from all families examined: Atherinidae, Melanotaeniidae and Pseudomugilidae. Species of *Helicirrus* with reduced hooks occur on melanotaeniids. Species of *Iliocirrus* are specific to the melanotaeniids except *I. rossi,* which occurs on *C. s. stercusmuscarum*.

7.6.5 Core-Satellite species

To identify core, secondary and satellite parasite species, frequency histograms representing the number of specimens of each parasite species on each host species at each sample site and all sites were examined (not shown). The findings are summarised in Table 7.6. *Pseudomugil signifer*, *P. gertrudae* and *C. marjoriae* were infected only with a single monogenean species and hence these monogeneans are not recognised as core species since a minimum of a two-parasite species infection in a host species is required under the strict definition. However prevalence of species infecting these hosts were generally >70%

Three patterns of distribution are identified: unimodal, bimodal and trimodal distribution. The designation of core, secondary and satellite species is dependent on the host species infected. *Melanotaenia s. splendida* and *M. australis* were used to illustrate core, secondary and satellite parasite species since these had the greatest number of samples. When host specimens were grouped as a single sample the pattern identified for *M. australis* shows a clear bimodal distribution, which identifies as core species *I. iliocirrus*, *L. auripontiformis, L. gracilis, H. splendidae* and as satellite species *H. megaloanchor* and *L. robustus* (Figure 7.8). *Melanotaenia s. splendida* shows a trimodal distribution (Figure 7.8) identifying as core species *I. iliocirrus*, *L. auripontiformis*, as satellite species *L. robustus* and as secondary species *H. splendidae* and *H. megaloanchor*.

A bimodal distribution identifying core and satellite species is seen in the following host species: *C. s. stercusmuscarum, M. australis, M. duboulayi, M. exquisita, M. maccullochi, M. nigrans, M. utcheensis* and *R. ornatus*. A trimodal distribution identifying core, satellite and secondary species is reflected in *M. eachamensis, M. s. inornata, M. s. splendida, M. sp.* and *"M. eachutchee"*. One species, *C. rhombosomoides* had only core species and a unimodal distribution.

Iliocirrus iliocirrus and *L. auripontiformis* are recognised as core species for the following: *M. s. splendida, M. s. inornata, M. duboulayi, M. australis, M. exquisita* and *M. sp*. Two core species, *I. rossi* and *R. chelatus*, are recognised for *C. s. stercusmuscarum* and *I. iliocirrus* and *L. utcheei* for *C. rhombosomoides. Melanotaenia nigrans* and *M. gracilis* only had *I. iliocirrus* identified as a core species. Other host species with only one core species are *M. maccullochii*, *M. trifasciata, R. ornatus* and

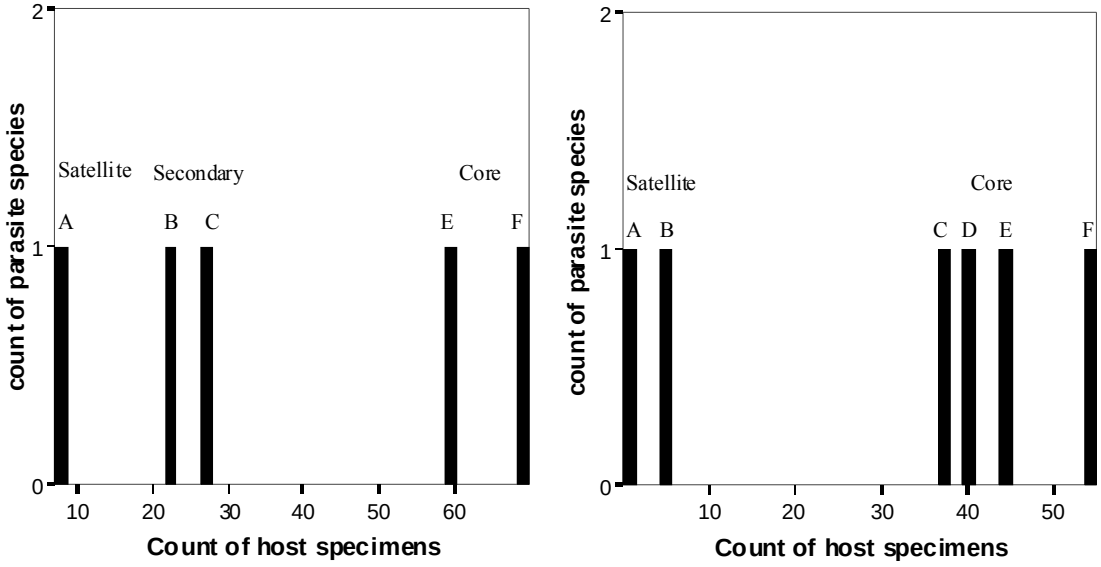
Table 7.6. Designation of core, secondary and satellite parasite species when all host specimens are included

Host species	No. examined	*R. chelatus*	*R. signifieri*	*H. gertrudaea*	*H. marjoriaea*	*H. mcivori*	*H. sp.*	*H. maccullochii*	*H. splendidae*	*H. megaloanchor*	*L. hopevalensis*	*L. maccullochii*	*L. utcheei*	*L. gracilis*	*L. robustus*	*L. auripontiformis*	*I. iliocirrus*	*I. mazlini*	*I. trifasciatae*	*I. ornatusii*	*I. rossi*
C. stramineus	30																				
C. helenae	20																				
C. marjoriae	13				core																
C. s.	40	core					sat														core
P. signifer	35		core																		
P. gertrudae	17			sec																	
R. ornatus	59								sat											core	
C.	12												core				core				
M. trifasciata	34					sec			sat		sat					sec	sec		core		
M. exquisita	17								sat					sec		core	core				
M. gracilis	25								sat					sec	sat	sat	core				
M. nigrans	16													sec		sec	core				
M. sp.	14								sec	sat				sec		core	core				
M. s. splendida	83								sec	sat					sat	core	core				
M. s. inornata	71								sec	sec					sat	core	core				
M. maccullochi	15							sec	sat	sat		core									
M. australis	59								sec	sat				sec	sat	core	core				
M. eachamensis	50								sec	sat				sec		sat	sat	core			
M. utcheensis	20								core					core				core			
"M. eachutchee"	32								sat	sat					sat	sec	core	sec			
M. duboulayi	41								sat	sat				sat	sat	core	core				

M. eachamensis. Three species *I. mazlini, L. gracilis* and *H. splendidae* are recognised as core species for *M. utcheensis*. Both *M. s. splendida* and *M. s. inornata* have *H. megaloanchor* and *H. splendidae* as secondary species while *M. sp.* has *H. splendidae* as a secondary species. *Longidigitis robustus* was recognised as a satellite species on the six host species; *M. s. splendida*, *M. s. inornata, M. duboulayi, M. australis, M. sp. M. gracilis* and the host "*M. eachutchee*".

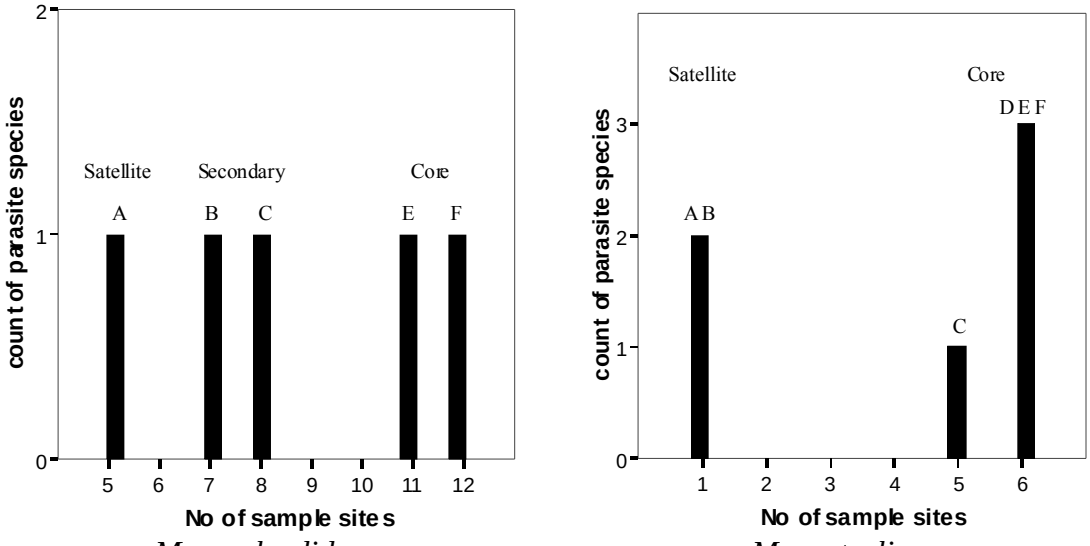
Core and satellite species identified above included all specimens of a host species as a single population. Frequency histograms were also used to see if the patterns identified above are identical for parasite species prevalences at sample sites (i.e. is the same distribution pattern repeated at each sample site?). Three host species are used as examples. The distribution of the parasites species on *M. australis* and *M. s. splendida* shows the pattern is repeated for sample sites (Figure 7.9). This indicates that the same sets of core, secondary and satellite species are present at each sample site for these two host species. This repeated pattern was generally universal for all host species. The host species *M. s. inornata* showed a variation from this generality (Figure 7.10) where the normally most common species *I. iliocirrus* was relegated to satellite species status. This also changed the status of other species where *H. splendidae* and *H. megaloanchor* became core species.

When observed/expected presence of parasite species at sample locations is used to categorise core, secondary and satellite species generally the same status is shown as for abundance. The exception was *H. mcivori*, which was always identified as a satellite species but was found at 75% of sample sites indicating core status (Table 7.7).



M. s. splendida *M. australis*

Figure 7.8. Frequency distribution of infections on specimens of *M. s. splendida* and *M. australis* showing core, secondary and satellite parasite species.
Specimens from all sites included. Bars represent parasite species. A. *L. robustus,* B. *H. megaloanchor,* C. *H. splendidae*, D. *L. gracilis,* E. *L. auripontiformis*, F. *I. iliocirrus.* Total specimens examined for *M. s. splendida* (72), *M. australis* (58).



M. s. splendida *M. australis*

Figure 7.9. Frequency distribution of infection for sample sites of *M. s. splendida* and *M. australis* showing core, secondary and satellite parasite species.
Bars represent parasite species. A. *L. robustus*, B. *H. megaloanchor*, C. *H. splendidae*, D. *L. gracilis*, E. *L. auripontiformis*, F. *I. Iliocirrus*. Total number of sample sites examined *M. s. splendida* (12), *M. australis* (6)

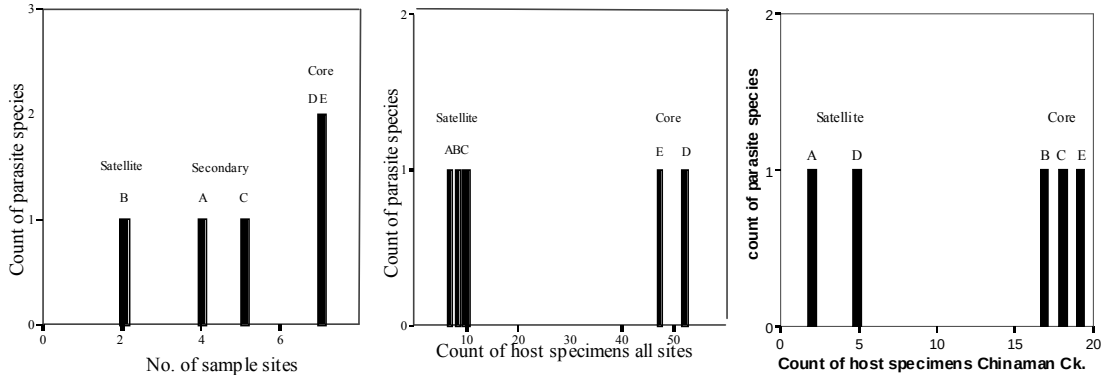


Figure 7.10. Frequency distribution of parasite species infection on *M. s. inornata* showing core, secondary and satellite parasite species.
Bars represent parasite species. A. *L. robustus,* B. *H. megaloanchor,* C. *H. splendidae,* D. *I. iliocirrus,* E. *L. auripontiformis.* Total sample sites (7), host specimens all sites (71), host specimens Chinaman Ck (19).

Table 7.7. Parasite species and the observed percentage of host sample sites collected.

Species	Locations found	Locations exp.	Percent found	status
H. gertrudaea	1	1	100.00	core
H. maccullochii	1	2	50.00	secondary
H. marjoriaea	1	1	100.00	core
H. mcivori	3	4	75.00	satellite
H. megaloanchor	15	34	45.45	satellite
H. splendidae	28	45	63.64	secondary
I. iliocirrus	34	41	82.93	core
I. mazlini	7	7	100.00	core
I. ornatusi	4	4	100.00	core
I. rossi	6	6	100.00	core
I. trifasciata	3	4	75.00	core
L. auripontiformis	31	40	77.50	core
L. gracilis	16	19	84.21	core
L. maccullochii	2	2	100.00	core
L. robustus	13	32	40.63	satellite
L. utcheei	2	2	100.00	core
R. chelatus	6	6	100.00	core
R. signiferi	5	5	100.00	core

7.6.6 The Effect of Host Length

Means and range of host length for sample sites are presented in Table 7.1. It must be noted that several host sample sites have missing data for host length and range due to lost data. Correlation between host length and parasite abundance was examined at two

levels. A significant positive correlation (R^2=0.17, P<0.01) was detected when all host specimens and parasites specimens were analysed together. However, when this correlation was examined in detail (i.e. host species were examined separately), only three host species have positive significant correlations *M. australis* (R^2=0 25, P<0.001), *M duboulayi* (R^2=0.16, P<0.05) and *P. signifer* (R^2=0.66, P<0.05) (Appendix FIGURE D1). Each of these three hosts was examined at the individual sample site level and no significant correlation (P>0.05) between host length and abundance was observed (Appendix FIGURE D2) indicating that associations between host length and parasite abundance is highly variable. The correlation between parasite species abundance and host length was also examined for those sample sites that, for statistical reasons, had twenty or more host samples. Three host sample sites were tested and no significant correlation was observed: *M. s. inornata* from Chinaman Ck. (R^2=-0.03, P>0.05), *M. utcheensis* from Utchee Ck (R^2=0.00, P>0.05) and *M. eachamensis* from Dirran Ck (R^2=0.01, P.0.05) (Appendix FIGURE D3). Since prevalence was generally 100% for host-site samples the results for intensity and host length were similar to abundance and host length. The host-site sample of *M. s. inornata* from Chinaman Ck was also examined for correlation between abundance of each parasite species and host length and no significant correlation was detected (P>0.05) (Appendix FIGURE D4).

7.6.7 Parasite abundance and Habitat Prevalence

The mean abundance of parasites from 620 host specimens was 13.87 parasites/fish. This abundance was not consistent across all sample sites. Sample sites were divided into five categories and ranked by stream flow and size.

1. Small fast-flowing stream with little plant life or organic detritus present
2. Medium fast-flowing stream, some aquatic plant life and detritus present

3. Large slow-flowing stream with plentiful aquatic plants and detritus

4. Large non-flowing pond (billabong) with large amount of aquatic plant and detritus present

5. Small almost stagnant pond with detritus and little aquatic plant life usually almost dried pond

Parasite abundance and intensity show a positive linear relationship, increasing from category one type stream to category five, with the latter having a three fold larger number per host specimen compared to the former. A significant difference was detected between habitat types for mean parasite load/host specimen (ANOVA $F=71.2$, $P<0.001$). Habitats in category one had significantly lower mean parasite intensities ($P<0.001$) than those in category two. There was no significant difference in parasite intensity between categories two, three and four ($P>0.05$). A significantly higher intensity of parasite load is present in habitat category five than for habitat categories one, two, three, and four. Examining the marginal mean, shows an increasing intensity of parasite load from habitat type one to type five (Figure 7.11A). With a correction for host length, using partial correlation, a general trend of increasing parasite load was still present although the association is not as strong (Figure 7.11B).

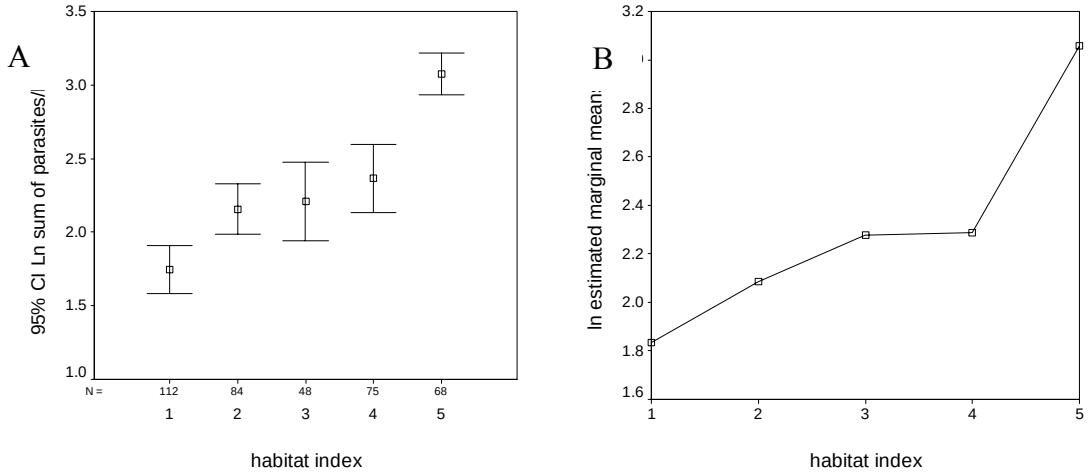


Figure 7.11. Parasite abundance and habitat type for species of *Melanotaenia*. A. means 95% C.I. Effect of host length not removed, B. Marginal means with correlation of host length removed.

7.7 Patterns of Parasite Associations and Host Species Associations

Patterns of associations among parasite species have been known to reflect phylogenetic associations of their hosts (Chapter 1.2.1). Host associations were examined by using presence/absence of parasite species. This was accomplished by HRA using a Jaccards coefficient matrix and the dice method for the binary data. Results of pattern of association were the same for both methods. A comparison of the dendrogram with the host phylogeny shows a number of similarities (Figure 7.12). Firstly, there is a strong congruence in the relative positions of host clades using parasite presence/absence and the host phylogeny; secondly, host species (parasite presence/absence) generally clustered in their correct designated phylogenetic clade. The two monotypic genera *Cairnsichthys* and *Rhadinocentrus* were infected with mono-host-specific parasites.

Melanotaenia species were generally identified by the presence of three low-host-specific species (*H. splendida*, *L. auripontiformis* and/or *I. iliocirrus*) (Figure 7.12A). Mono-host-specific parasites separated the "*goldiei*" (*trifasciata*) clades from the other

species. The disjunct clades "*australis*" and "*nigrans*" were infected with *L. gracilis* while absence of this species grouped host species as the clade "*splendida*" (Figure 7.13). The "*australis*" and "*nigrans*" clades are separated by the presence of *H. megaloanchor* on the former; however, this division is not very strong.

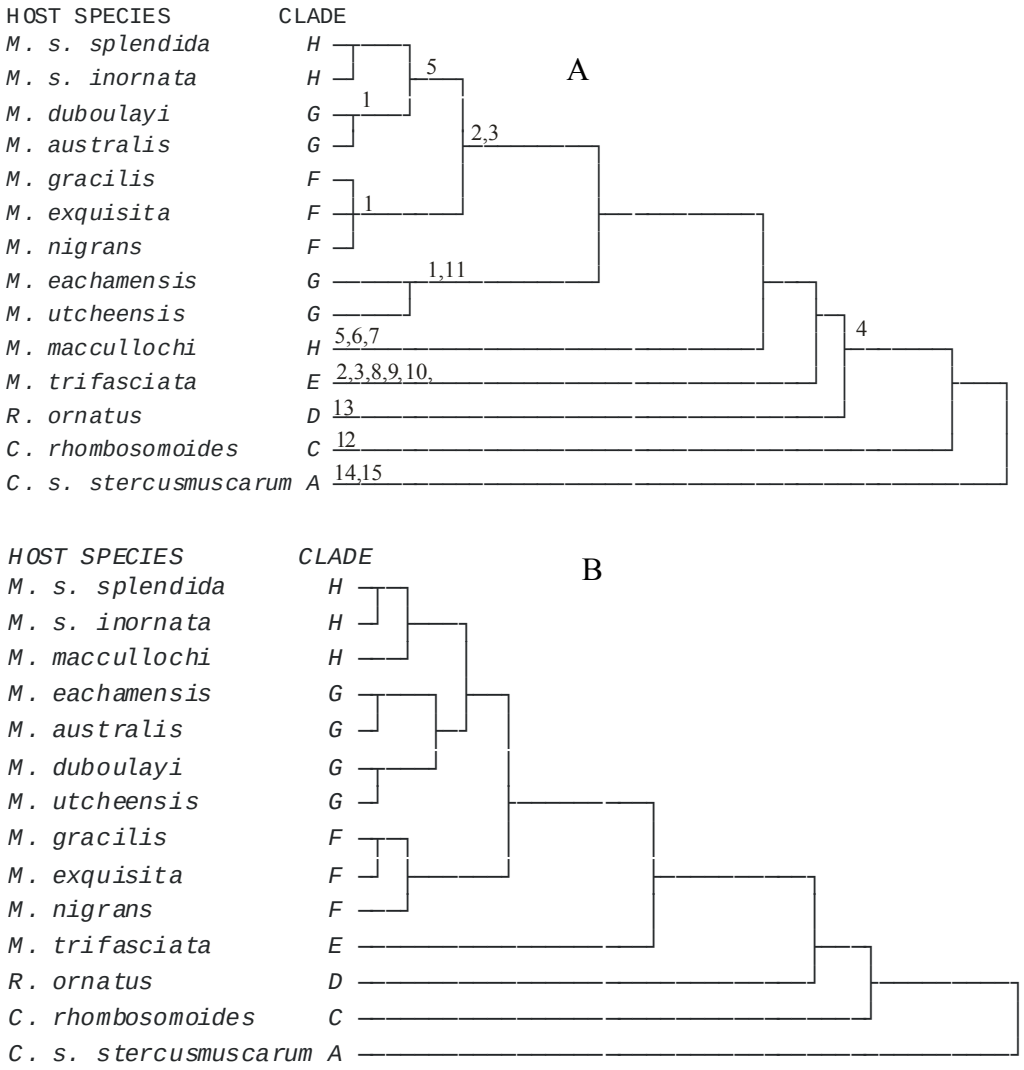


Figure 7.12. A. Associations between host species using parasite species presence/absence. B. Host mtDNA phylogeny.
Parasite species 1. *L. gracilis*, 2. *L. auripontiformis*, 3. *I. iliocirrus*, 4. *H. splendidae*, 5. *H. megaloanchor*, 6. *L. maccullochii*, 7. *H. maccullochii*, 8. *L. hopevalensis*, 9. *I. trifasciatae*, 10. *H. mcivori*, 11. *I. mazlini*, 12. *L. utcheei*, 13. *I. ornatusi*, 14. *I. rossi*, 15. *R. chelatus*. Host clades. A. "*Craterocephalus*", B. "*Pseudomugil*", C. "*ornatus*", D. "*rhombosomoides*" E. "*goldiei* (*trifasciata*)", F. "*nigrans*", G. "*australis*", H. "*splendida*".

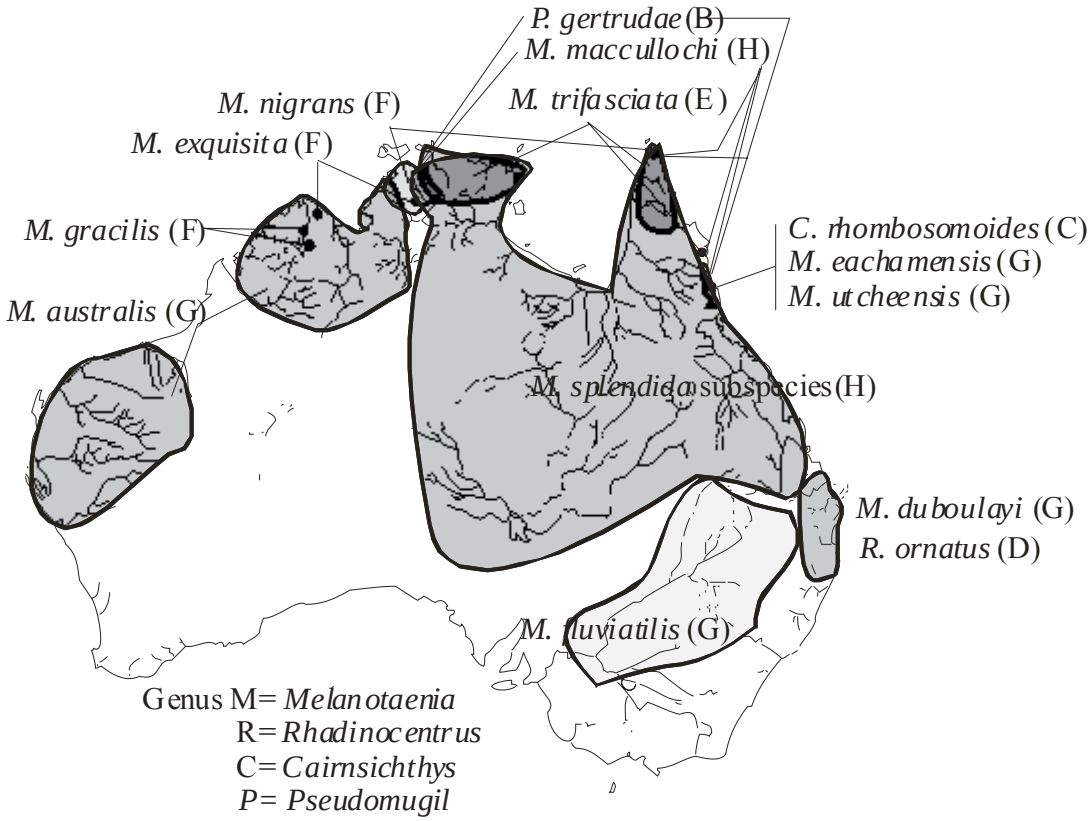


Figure 7.13. Host species distributions showing their disjunctions.
Letter after species name indicates mtDNA species clades; B. "*Pseudomugil*", C. "*rhombosomoides*", D. "*ornatus*", E. "*goldiei*" (*trifasciata*), F. "*nigrans*", G. "*australis*"; H. "*splendida*".

Two exceptions were present: *M. maccullochi* did not cluster in "*splendida*" clade and *M. eachamensis* and *M. utcheensis* did not cluster in "*australis*" clade. These exceptions are the result of the hosts having parasite species not found on other hosts. *Melanotaenia maccullochi* has two mono-host-specific parasites, *L. maccullochi* and *H. maccullochi*, while *M. eachamensis* and *M. utcheensis* have *I. mazlini* present. *Longidigitis auripontiformis* and *I. iliocirrus* are also absent from these species while they occur on all other melanotaenids.

7.8 Discussion

Host specificity is measured by examining the prevalence of parasite species on host species, and this measure is often affected by location sample size (Cribb *et al.*, 1994). However location sample size seems to have little influence in this study. Almost all host specimens were infected with parasites and most had several species, but several anomalies were identified. Four atherinid species were examined; however two species *C. helenae* and *C. stramineus* had no parasites despite the fact that twenty specimens of each of these host species were examined. *Melanotaenia australis* and *M. gracilis* were also collected from the same location as *C. helenae*, yet all were infected with up to four parasite species. Both *C. stramineus* and *C. helenae* are very restricted in their distribution and possible reasons for the absence of monogeneans may be that these species have evolved from a small founder population that was not infected ("missing the boat" hypothesis proposed by Paterson & Gray (1997), or the parasites become extinct. Alternatively, these two species may have developed immune systems that have prevented infection by monogeneans.

Another host, *P. gertrudae*, from Gwynne Ck. was not infected by monogeneans although *M. eachamensis* from the same site had three parasite species. *Pseudomugil gertrudae* is known to be infected by monogeneans (*H. gertrudaea*) elsewhere. However, the absence of monogeneans from Gwynne Ck. can be explained. This fish species has not been recorded from any other region on the Atherton Tablelands and is outside its usual known distribution (usually coastal flood plains). It has been suggested that this population originated from a release of specimens from the aquarium trade and hence the original stock may not have carried monogeneans (Buckley, pers.com).

The absence of monogenean parasites on *M. s. tatei* from Tennant Ck. may be attributed to the small sample size (2) and the small mean host length. It is expected that this host species does have a monogenean parasite fauna and based on the infections of the two other subspecies, *L. auripontiformis*, *I. iliocirrus*, *H. splendidae* and *H. megaloanchor* are likely to be present. Further sampling is needed to confirm this.

A hierarchy of relative prevalence and intensity of parasite species infections on host species was identified. The most common species is *I. iliocirrus*, followed in descending order by *L. auripontiformis*, *L. gracilis*, *H. splendidae*, *H. megaloanchor* and *L. robustus*. The dominance of *I. iliocirrus* in terms of intensity of infection over all other parasite species may indicate competition between monogenean parasite species, a higher fecundity rate or may represent a key species in immunosuppression.

Parasite associations may be statistical artifacts, or be real due to interactions among the parasite species on the host or due to common features of parasite and host biology. Competition between parasite species is often cited as a cause of differential intensities (but see Rohde, 1979a). Often the dominance of one species can cause the exclusion of another. Alternatively, a key species that suppresses the immune response, may allow further infection by other species. Four parasite species were examined for linear associations in mean sample site intensity. *Iliocirrus iliocirrus* and *L. auripontiformis* had the strongest positive association. A similar association was detected between *L. gracilis* and *L. auripontiformis*, which had a slightly lower R^2 value. The association between *I. iliocirrus* and *L. gracilis* was weakest of these three. These associations would appear to indicate that once *I. iliocirrus* infects a host the host immune system is compromised.

Iliocirrus iliocirrus was almost universally the most common species on melanotaeniids; however, some host sample sites did have an alternative dominant species. The Chinaman Ck. sample of *M. s. inornata* was dominated by *H. splendidae* and *L. auripontiformis* and these two species occurred at a higher level of intensity than found at any other location. The third species present at this site, *H. megaloanchor*, was also present at a much greater intensity than at other sites. This increased level of intensities in the absence of *I. iliocirrus* appears to confirm a competitive interaction between species. This competition may be niche-associated for the species *I. iliocirrus*, *L. auripontiformis*, *L. gracilis* and *H. megaloanchor* since these four species attach to the gill filaments. This does not appear to be the case for *H. splendidae* as this species is only found on the gill raker.

Host sample size can have a major impact on assessments of parasite distribution on hosts. *Helicirrus* species were mostly prevalent in low numbers (< 1/host) but were widespread. If the number of sample locations had been restricted, these parasite species may have been missed or may be classified as incidental infections. *Longidigitis robustus* provides an example of this problem. Specimens of this species were found on a number of host species and up to four per host specimen were observed. However, it was more common to find a single specimen for every ten hosts examined. If this species was considered a spurious infection then it would not be expected to appear on several host species across a wide geographic distribution, thus theseassociations are considered a valid. Because of the rarity of this speciees the complete set of associations remains ambiguous , therefore it is suggested that this species is not a reliable example of the true host associations.

When a host contains a mono-host-specific parasite, the relative intensity and prevalence of other parasite species change. The mono-host-specific parasite has the greatest prevalence and intensity compared to the other species from the same genus. This is shown for *M. trifasciata* where *I. trifasciata* is more prevalent than the normally dominant *I. iliocirrus*.

In his hypothesis of core and satellite species Hanski (1982) explicitly stated that it should only be tested with sets of species, which may establish populations at the same sites. In the context of the expanded model I have proposed (see Section 7.3), these locations are the host species. Therefore, different populations of the same host species have the potential to exhibit the same set of core and satellite species as was found in this study. The hypothesis also predicts a bimodal distribution of species within each habitat (host) and this was shown to be the case in several host species. The presence of a trimodal distribution and secondary species, however, was not predicted by Hanski (1982) although it was later noted by Bush & Holmes (1986).

Can satellite species become core species or vice versa? My study shows this to be a possibility. For example, *I. iliocirrus* is normally a core species on *M. s. inornata* while *H. megaloanchor* and *H. splendidae* are recognised as secondary species. However, for the Chinaman Ck. location, these ranks are reversed. These fluctuations are possibly due to stochastic variance or possibly sampling heterogeneity. With this variation in mind, core and satellite species designations should be recognised at two levels, the infrapopulation level and the component population level of the host species.

In a study by Kennedy (2001), predictions relating to core and satellite species were met. However, his data did not completely support the view that species abundances in one patch were paralleled in other patches. He also found the application of the concept of core and satellite species to the parasite species identified on eels was difficult since the methods advocated by Hanski (1981, 1982) distinguishing these categories was not readily applicable to species poor communities.

Characterising the pattern of parasite distribution at the infracommunity level is important in assessing the sampling effort needed to identify the degree of host specificity present. The variables prevalence, abundance and intensity are commonly used for this (see Sections 7.6.1-3).

Prevalence of infection was observed to be very high (95.5%) (Section 7.6). This may be due to the schooling behaviour of the fish and is partially explained by metapopulation theory. Metapopulation dynamics is seen as the key to a species' persistence in a locality as metapopulations have a much longer time to extinction than local populations (Kennedy, 2001). There is a positive relationship between patch similarity and connectivity and a negative relationship between patches and distance. Local extinction of parasite species will be followed by re-invasion from other populations within the metapopulation. The probability of population extinction increases when the population decreases (Schaffer & Samson, 1985) and species reaching high densities have less chance of extinction and are thus able to colonise a wider range of hosts.

Guégan & Hugueny (1994) have suggested that monogenean infrapopulations are very different from free-living populations on true islands by having life cycles with a "high" immigration rate between host individuals. Thus, gill parasite infrapopulations can collectively be considered as a parasite metapopulation. Therefore, extinction rates in monogenean communities must be considered in a metapopulation context, in which extinction in an individual host is compensated by immigration of parasites from other host individuals. If hosts have a strong schooling behaviour, then the possibility of parasite extinction is reduced. Thus, schooling behaviour would produce a more homogeneous distribution of parasite species, a pattern that is present in the hosts examined in this thesis. This is also confirmed with habitat type where highly concentrated hosts had the highest parasite intensities, while hosts from small fast flowing streams, which would tend to disperse the hosts and swimming parasite larvae, had a low parasite load. A non-aggregated parasite distribution should also be present in aquaculture systems where there is a close physical association between hosts. A study on variations in a population of the monogenean *Ancyrocephalus mogurndae* Gussev, 1955 on gills of cage-cultured mandarin fish, *Siniperca chuatsi* (Basilewsky) (see Nie, 1996) identified prevalence as high (75-100 %) throughout the study period, and this prevalence did not vary significantly between months. Other studies have also found high levels of prevalence in aquaculture systems (Nielsen & Buchmann, 2001).

Parasite associations on hosts show a strong congruence with the host phylogeny. Two incongruences, *M. maccullochi* and *M. utcheensis/M. eachamensis*, may reflect cases of host addition, host switching or an anomaly in the host mtDNA phylogeny. These issues are examined further in Chapter 9.

CHAPTER 8 IDENTIFICATION OF HOST SPECIES USING PARASITE SPECIES AS INDICATORS

8.1 Introduction

Three melanotaeniid species, *M. splendida*, *M. eachamensis* and *M. utcheensis*, are found on the Atherton Tablelands and their taxonomic differentiation using visual means is difficult. DNA has been used to identify interspecific differences (McGuigan, 2001; Zhu *et al.*, 1998) and some level of hybridisation has been detected among them. The use of monogenean parasite tags is assessed for the identification of the three different fish species and their hybrids.

The Lake Eacham rainbowfish (*M. eachamensis*) from Lake Eacham on the Atherton Tablelands in North Queensland was described in 1982 and has since been examined extensively using morphology and genetic characters (McGuigan, 2001; Pusey *et al.*, 1997; Zhu *et al.*, 1998). In the early 1980s, *M. eachamensis* was placed on the endangered species list and in the late 1980s was declared extinct in the wild (Wager, 1993; Wager & Jackson, 1993). This presumption was later found to be incorrect as two other localities, Lake Euramo and Dirran Ck, were found to contain this species (Allen, 1989b, 1995). This prompted a study on morphometric variation in the species and concluded that the Lake Eacham rainbowfish is widespread across the Atherton Tablelands (Pusey *et al.*, 1997).

Studies using mtDNA have identified *M. eachamensis* as being more closely related to the Western Australian fish *M. australis* than to the local *M. s. splendida* (see Zhu *et al.*, 1994). Their study also identified a variant genotype, which was later recognised as belonging to a new species *M. utcheensis* McGuigan (2001). A second DNA study,

(Zhu *et al.*, 1998) examined the distribution of *M. eachamensis* more intensively and supported the view of widespread distribution, previously recognized by Pusey *et al* (see above). However, clarity of the distinction of *M. eachamensis* from *M. s. splendida*, using mtDNA, was confused by the admixture of several "*eachamensis*" and "*splendida*" variant genotypes in a number of sample sites. Some of these "*eachamensis*" variants were later recognized as *M. utcheensis* (see McGuigan, 2001).

With the description of *M. utcheensis*, a reanalysis and further sampling has defined the distribution of this species (McGuigan & Moritz unpub., see McGuigan, 2001). Their study found several pure *M. utcheensis* populations, as well as admixtures of "*eachamensis-utcheensis*", and "*utcheensis-splendida*" mtDNA lineages in other localities. Whether the admixtures of mtDNA lineages are due to the occurrence of sympatry between the species, or to either current or historical hybridisation is still unclear (McGuigan, 2001). The problem is how to recognise pure *M. eachamensis*, *M. utcheensis* and *M. s. splendida* populations and distinguish them from mixed populations or hybrids of these species?

Hybrids are commonly thought to be intermediate between parents. However numerical taxonomic studies show that they are not usually exactly intermediate in the sense of the line in phenetic hyperspace that joins the two parents (Sneath & Sokal, 1973, p 373) but that they appear to one side. Such a displacement indicates some genes from one parent are dominant and others are recessive, or the result of overdominance. Several studies have shown that parasite species can be used to identify hybrids and mixed populations (Lambert & El-gharbi, 1995; Williams, 1964).

Seven species of parasite, *H. splendidae*, *H. megaloanchor*, *I. mazlini*, *I. iliocirrus*, *L. auripontiformis*, *L. gracilis* and *L. robustus* are known to infect the host species examined in this chapter (see Chapter 7).

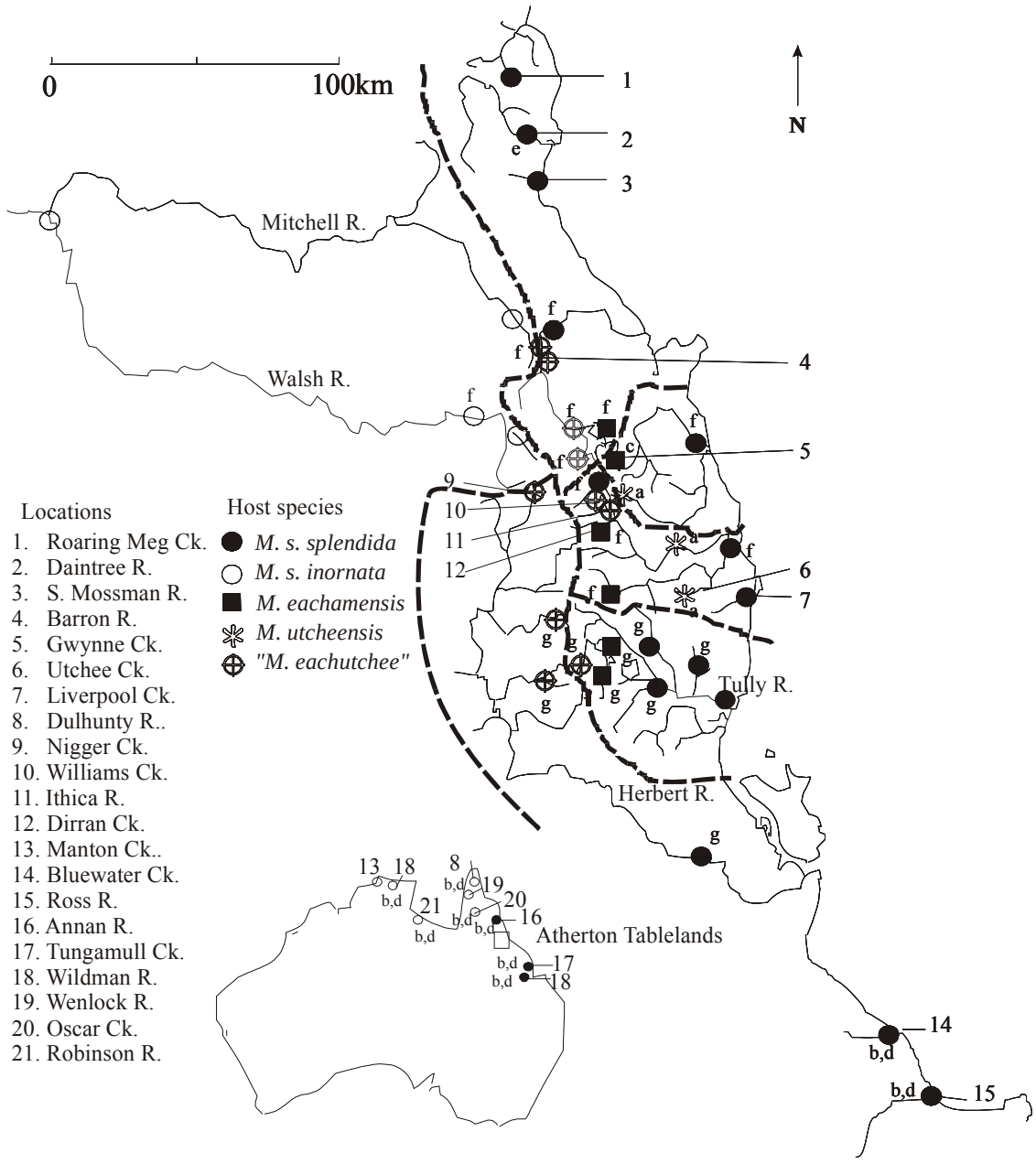


Figure 8.1. Sample sites and presumed identities of hosts at each.
Detailed map represents the Atherton Tablelands and associated regions. Numbers represent sites sampled in this study. References for species identification: McGuigan, 2001[a], Allen & Cross, 1982[b], Allen, 1989b[c], Allen *et al.*, 2002[d], Zhu *et al.*, 1994[e], Zhu *et al.*, 1998[f], Pusey *et al.*, 1997[g].

8.2 Methods

Sample sites for the three species included, where possible, those examined by previous investigators (Figure 8.1.). Using information from the published literature, sample sites were identified as containing pure *M. s. splendida, M. eachamensis*, *M. utcheensis* or admixed "*M. eachutchee*" lineages. Host specimens were examined for parasites and the number of each species was recorded (Table 8.1). Three approaches to the analysis of specificity were assessed for their usefulness in detecting host species clusters: 1. Principal component analysis of counts of parasites on individual host specimens and mean counts of each parasite species/location, 2. Hierarchical cluster analysis of mean counts of parasite species/host/location using single linkage (squared Euclidean distances), 3. Hierarchical cluster analysis of presence/absence of parasite species/location. Data were ln transformed.

8.3 Results

Analysis using PCA and counts of parasite species on host individuals produced two components. *Melanotaenia s. splendida* and *M. s. inornata* were grouped together for ease of identifying clusters. *Melanotaenia utcheensis* and *M. eachamensis* showed a small overlap of their clusters but they were clearly separated from the *M. splendida* cluster (Figure 8.2.). The samples of "*M. eachutchee*" formed a cluster partially overlapping the other three. These clusters showed the greatest separation on PC1 which was dominated by *I. mazlini*, *I. iliocirrus*, *L. auripontiformis* and *L. gracilis*. The second component, which was dominated by *H. splendidae* and *H. megaloanchor*, nearly separated *M. eachamensis* from *M. utcheensis*. When the mean abundance of parasite species at different sample sites was analysed, all four species clusters were clearly

Table 8.1. Parasite species abundance on species of the *Melanotaenia*.
For location see Table 2.1.

		H. splendidae			*H. megaloanchor*			*I. iliocirrus*			*L. auriportiformis*			*L. gracilis*			*I. mazlini*		
Host sp.	**loc**	**Mean**	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**
M. eachamensis	17	.67	0	2	.00	0	0	.00	0	0	.00	0	0	.17	0	2	5.22	0	14
	23	.75	0	2	.00	0	0	.38	0	3	.00	0	0	1.87	0	4	3.25	0	10
M. utcheensis	48	3.20	0	12	.00	0	0	.00	0	0	.00	0	0	2.00	0	11	1.35	0	4
M. s. splendida	3	.63	0	1	.00	0	0	24.38	11	34	9.13	3	21	.00	0	0	.00	0	0
	8	2.67	0	10	1.50	0	5	11.33	6	21	4.67	1	9	.00	0	0	.00	0	0
	20	2.00	2	2	.00	0	0	17.00	17	17	3.00	3	3	.00	0	0	.00	0	0
	29	.31	0	2	.92	0	4	14.38	0	35	2.69	0	5	.00	0	0	.00	0	0
	39	1.23	0	5	.23	0	1	10.15	4	24	2.23	1	5	.00	0	0	.00	0	0
	41	2.00	1	3	.00	0	0	10.75	5	18	4.50	3	6	.00	0	0	.00	0	0
	44	.33	0	1	1.83	0	7	7.17	5	10	4.33	0	11	.00	0	0	.00	0	0
	47	.00	0	0	.67	0	2	5.22	0	12	2.78	0	9	.00	0	0	.00	0	0
M. s. inornata	19	.20	0	1	.00	0	0	8.20	5	11	3.20	1	6	.00	0	0	.00	0	0
	30	.20	0	2	.00	0	0	29.30	13	57	14.50	2	36	.00	0	0	.00	0	0
	37	.36	0	2	.45	0	4	20.36	5	43	6.64	2	15	.00	0	0	.00	0	0
	40	1.00	0	3	.00	0	0	7.00	2	16	5.33	3	7	.00	0	0	.00	0	0
	49	.38	0	2	.38	0	2	9.00	1	19	2.54	0	7	.00	0	0	.15	0	1
	50	.00	0	0	.00	0	0	26.29	4	72	12.00	4	40	.00	0	0	.14	0	1
"M. eachatchee"	4	.67	0	3	.44	0	2	13.89	6	24	4.33	2	8	.00	0	0	2.56	0	8
	25	.00	0	0	.00	0	0	10.63	1	32	.00	0	0	.00	0	0	.75	0	2
	35	.41	0	2	.00	0	0	1.18	0	7	.06	0	1	.59	0	2	.82	0	2
	51	1.86	0	3	.00	0	0	.00	0	0	.00	0	0	.00	0	0	3.14	0	11

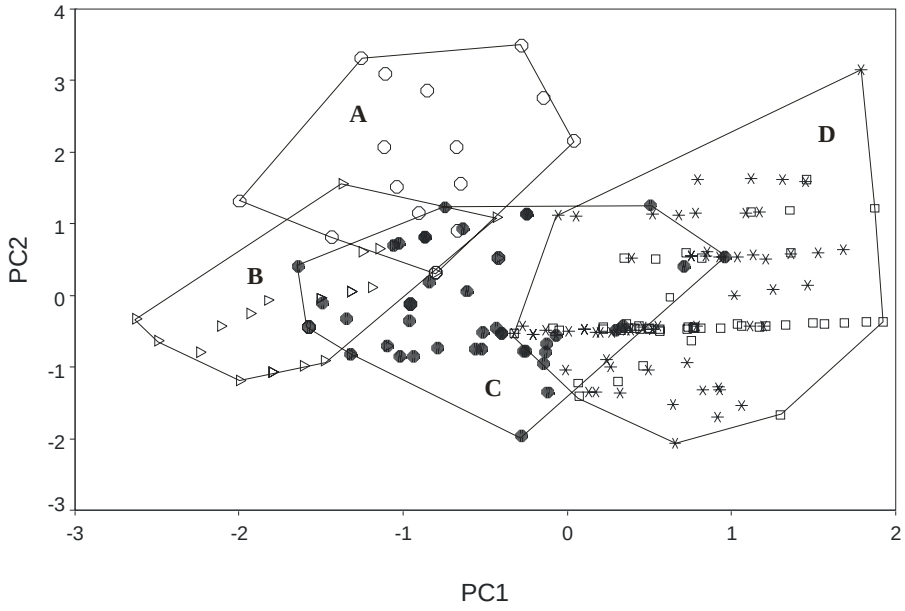


Figure 8.2. Principal component analysis separation of host species using parasite species abundance on host specimens.
Lines represent host species boundaries. ○ (A) *M. utcheensis*; ▷ (B) *M. eachamensis*; ● (C) *"M. eachutchee"*, ∗ (D) *M. s. splendida*, □ (D) *M. s. inornata*

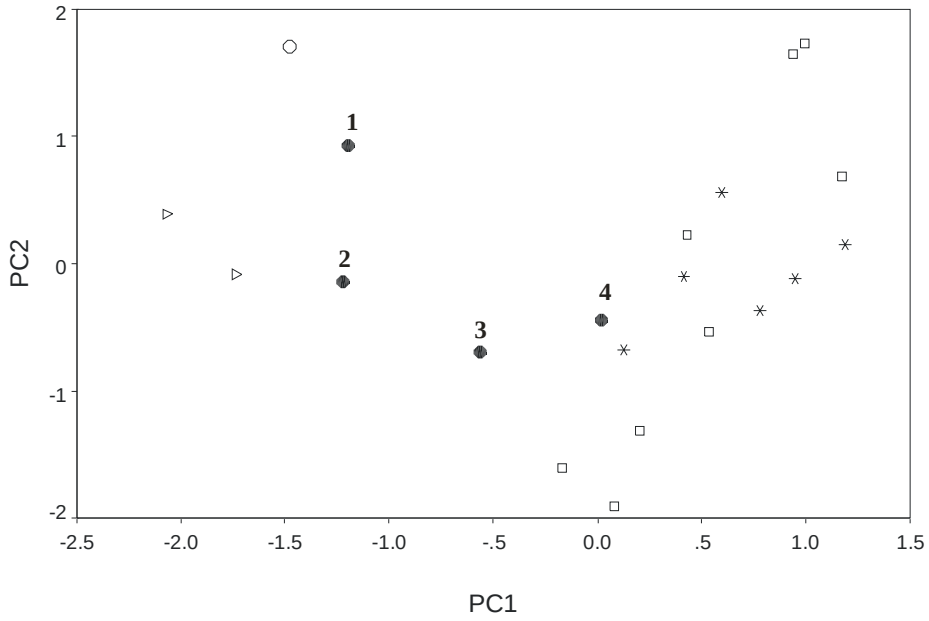


Figure 8.3. Principal component analysis separation of host species using average parasite species abundance for each sample site.
∗ *M. s. splendida*; □ *M. s. inornata*; ▷*M. eachamensis*; ○ *M. utcheensis*; ● *"M. eachutchee"*. Sample site 1. Williams Ck.; 2. Nigger Ck., 3. Ithica Ck., 4. Barron R.

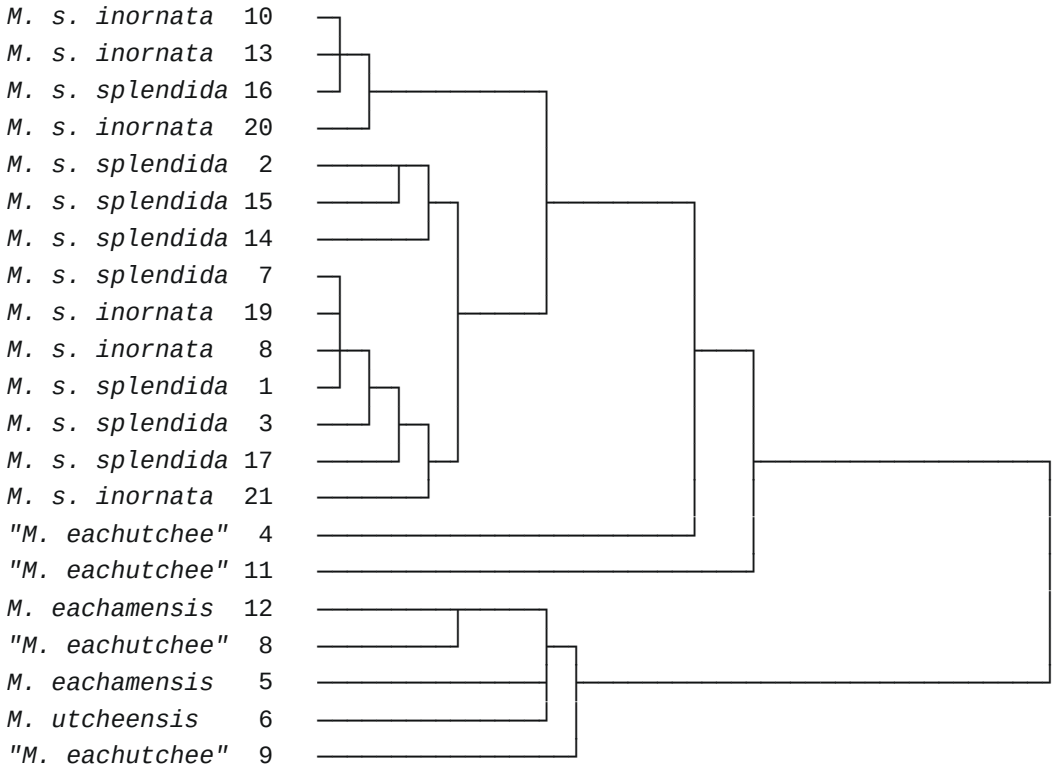


Figure 8.4. Hierarchical cluster analysis (single linkage) of host species using parasite species mean abundance/sample site.
Numbers represent locations (see Figure 8.1.)

separated (Figure 8.3). The *"M. eachutchee"* sample from Barron R. clustered with *M. splendida*. The other three *"M. eachutchee"* samples clustered between the *M. splendida*, the *M. eachamensis* and *M. utcheensis* clusters. The two components were again dominated by the same parasite species as the previous analysis.

Hierarchical cluster analysis of the parasite species distributions on hosts using mean abundance/sample site identified two distinct clusters, one grouped all samples of *M. s. splendida* and *M. s. inornata*, while the second grouped *M. eachamensis* and *M. utcheensis* together (Figure 8.4). The positions of the "*M. eachutchee*" samples were distributed among these two clusters. The Williams Ck. and Nigger Ck. population grouped within the *M. eachamensis/M. utcheensis* cluster while the Barron R. and Ithica

R. populations of "*M. eachutchee*" loosely grouped with the *M. splendida* cluster. The analysis of parasite presence/absence data produced the two clusters identified above however all *"M. eachutchee"* samples except the Barron R. population grouped with the *M. eachamensis/M. utcheensis* cluster (not shown).

To identify the nature of the differences in parasite species among the host species, the mean abundances of parasites from combined samples of host species were examined (Figure 8.5). Using prevalence and abundance of parasite species as a criterion for identifying host species, the following associations can be recognised. Pure *M. s. splendida* and *M. s. inornata* are generally infected with the four parasite species *L. auripontiformis, I. iliocirrus, H. splendidae* and *H. megaloanchor*. They are never infested with *I. mazlini* or *L. gracilis.* Pure *M. eachamensis* and *M. utcheensis* hosts are associated with *I. mazlini, H. splendidae* and *L. gracilis*. For the "*M. eachutchee*" populations all sample populations had *I. mazlini* present.

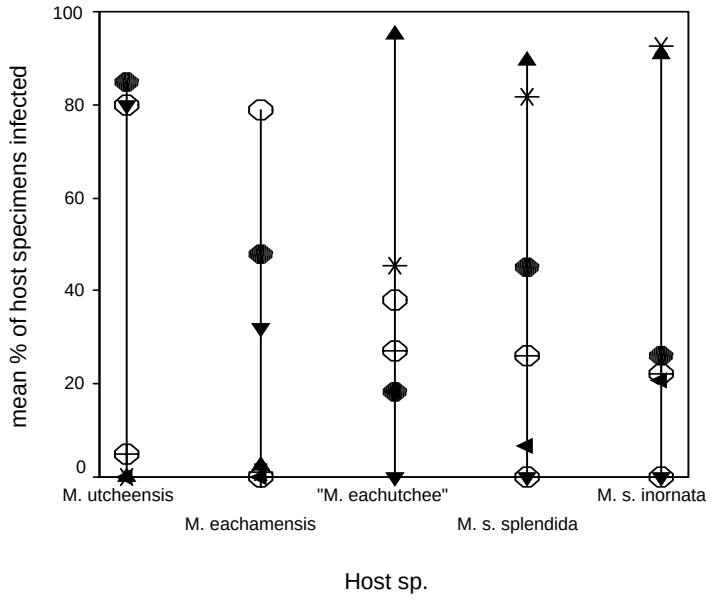


Figure 8.5. Parasite species mean % of specimens infected for each host species. ⊖ *H. megaloanchor*; ● *H. splendidae*; ▲ *I. iliocirrus*; ○ *I. mazlini*; ✳ *L. auripontiformis*; ◄ *L. robustus*; ▼ *L. gracilis*.

8.4 Discussion

The comparison of parasite species prevalence and abundance of infection on host species has been used in other studies as a possible method for the identification of host species and hybrids (see introduction to this chapter). Using this method, *M. eachamensis* and *M. utcheensis* were clearly separated from *M. s. splendida* and *M. s. inornata* by the presence of *L. gracilis* and the absence of *I. iliocirrus* on the former two hosts and the absence of *L. gracilis* and *I. mazlini* and the presence of *L. auripontiformis* and *I. iliocirrus* on the latter two. There is, however, a need for a greater sampling of *M. utcheensis* since only a single sample site was examined. Further sampling may confirm that the dominance of *H. splendidae* and *L. gracilis* can be used to distinguish *M. utcheensis* from *M. eachamensis*, which was dominated by *I. mazlini* and *H. splendidae*. If this method of host species identification is confirmed, then it represents a method complementary to DNA studies.

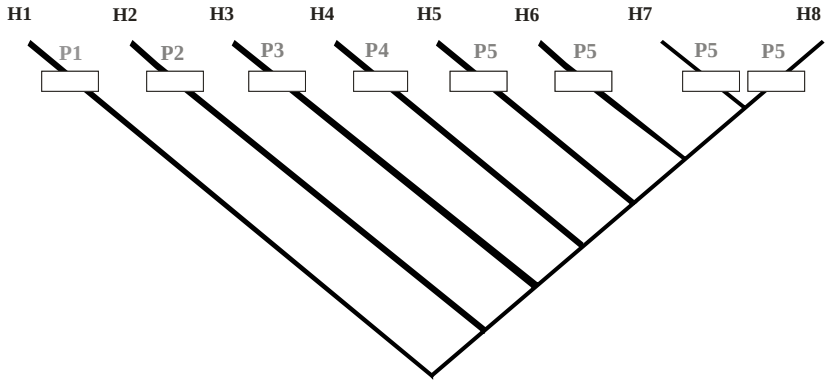
A major difficulty of this study was confirmation of host species and admixed populations from the resampled sites. Host specimens show localised colour patterns related to stream locality and no guarantee can be given that multiple host species are not present at the same site. Colour descriptions were not given for fish used in published studies, either molecular or morphometric. I am assuming admixed populations are absent. A re-examination of the distribution of *M. eachamensis*, *M. utcheensis* and *M. s. splendida* is required with a three-way integrated approach being applied. Host species samples need to be examined using molecular, morphological and parasitological techniques, together with detailed colour descriptions.

CHAPTER 9 DOES MORPHOMETRIC VARIATION REFLECT PARASITE PHYLOGENY?

9.1 Introduction

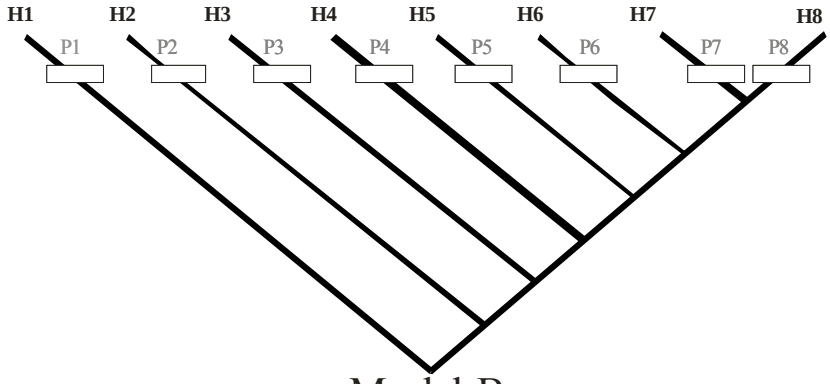
Congruence of the association between species of hosts and species of parasites has often been attributed to synchronous cospeciation. This term describes those cases in which host speciation and parasite speciation are approximately contemporaneous and is represent by Model B (Figure 9.1) (i.e. cospeciation requires only that parasite speciation events occur sometime between consecutive host speciation events) (Hafner & Nadler, 1990). Alternatively, delayed cospeciation might occur whereby speciation in the parasite may lag behind that of the host (Manter, 1955) and is represent by Model A (Figure 9.1). In such circumstances, basal host lineages should have more associations attributable to cospeciation than derived host lineages (i.e. basal lineages have mono-host-specific parasite species while derived lineages have parasites of low host specificity). Speciation in the host may lag behind that of the parasite (Brooks & McLennan, 1993; Hafner & Nadler, 1988). This would produce a pattern where multiple congeneric species of parasite occur on a single host species (Model C, Figure 9.1). In this chapter, I examine whether synchronous or delayed cospeciation best explains the host-parasite associations.

Models of host-parasite cospeciation, and computer programs exploring coevolution, usually assume a one-host one-parasite association. However, this is usually not the case in real systems. Monogenean parasites are said to be highly host-specific hence, they should be good models for examining coevolution. Coevolution between host and parasite species is uncommon (Morand *et al.*, 2002) although examples have been



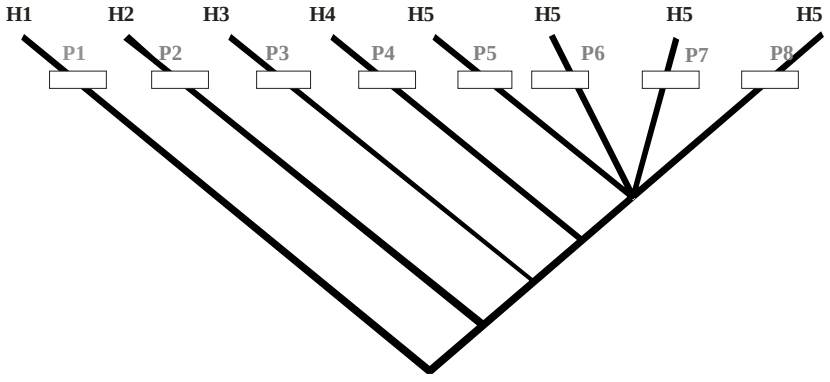
Model A

Parasite speciation time event later than host



Model B

Parasite and host speciation time event synchronous



Model C

Parasite speciation time event earlier than host

Figure 9.1. Models of cospeciation.
Model A: parasite speciation lags host speciation. Model B: parasite speciation is synchronous with host speciation. Model C: host speciation lags parasite speciation.

inferred (Beverley-Burton, 1995; Boeger, 1988; Boeger & Kritsky, 1997; Carney & Dick, 2000; Choudhury & Dick, 2001; Desdevises *et al.*, 2000; Garfias & de Leon, 1998; Kritsky *et al.*, 1997; Mollaret *et al.*, 2000; Van Every & Kritsky, 1992). Several factors have been offered as explanations for this incongruence. Among these are host switching, duplications (sympatric speciation) and sorting events (extinctions).

Incongruence in host/parasite phylogenies can also be due to infections of multiple host species by a single parasite species (widespread taxon). Jackson (1999) explained the occurrence of widespread taxa by:

a) Host switching or addition onto related or unrelated host species in sympatry

b) As a result of the parasite not speciating in the same order as the host does (asynchronous speciation)

c) Parasites speciate in the same order as their hosts but with a time lag between the two events (i.e. hosts speciate before parasites)

This last scenario I call delayed or retarded synchronous cospeciation.

Studies of cospeciation usually involve comparisons of host and parasite phylogenies. Phylogenies are usually inferred from DNA or morphological data, however continuous morphometric data can also be utilised for inferring a phylogeny (Donoghue & Sanderson, 1992; Rae, 1998; Theile, 1993) despite arguments to the contrary (Pimentel & Riggins, 1987). These forms of data are converted to a binary data matrix. However binary data are sensitive to the algorithm used for producing the similarity matrix as discussed in Chapter 3.

Cladistic methods infer a phylogeny from synapomorphic character states. Continuous morphometric data coded as a distance matrix are recognised as phenetic and hence are not usually considered appropriate for inferring a phylogeny since synapomorphies cannot be identified. However, phylogenies inferred from DNA sequences do not identify synapomorphic character states either. Therefore objections to the use of continuous data, utilising distance matrix coding, for inferring a phylogeny appear to be misdirected. Therefore, I regard the trees produced using morphometric variation as a phylogenetic hypothesis.

To test for the presence of cospeciation, a multitude of methods are available (eg. Adams, 1972; Huelsenbeck *et al.*, 1997; Johnson *et al.*, 2001; Siddall, 1996). Presently there are several working methodologies (Brooks, 1981; Charleston, 1998) with associated software packages (Charleston & Page, 2002; Maddison & Maddison, 1992; Page, 1995; Ronquist, 2000). Typically, these search for congruence between independently derived host and parasite phylogenies using one of two different approaches. Reconciliation methods maximise cospeciation events and may modify or eliminate input data to maximise fit of single parasite clades to a null hypothesis of cospeciation. In contrast, Brooks Parsimony Analysis (BPA) is designed to assess cospeciation among multiple parasite clades (Brooks, 1981) using parasite phylogenetic trees recoded as additive binary characters (through inclusive or-ing) and minimising the *ad hoc* assumptions, duplications, sorting events and host switching. From the association tree a host phylogeny is inferred. The pattern of congruence for both methods is tested for a significant departure from randomness.

Errors in phylogeny inference can occur at three different levels: species sampling, character sampling and selection of tree construction algorithm. Tree construction algorithms may make assumptions that are not realistic models of evolution, thus they may not reflect the true history of evolution (Carpenter, 1996). Four programs, TREEMAP 1 (Page, 1995), TREEMAP 2 (Charleston, 1998), COMPONENT 2 (Page, 1993) and MACLADE (implementing BPA) (Maddison & Maddison, 1992) are commonly used to analyse host-parasite phylogenies and much discussion has been published regarding which is the best (Dowling, 2002; Dowling *et al.*, 2003; Page, 1996; Page & Charleston, 2002; Siddall & Perkins, 2003). All these programs have limitations due to their underlying models. The single most common issue is whether the program can adequately accommodate host switching. COMPONENT 2 does not allow host switching at all, TREEMAP 1 allows host switching but does not deal with cases appropriately (it excludes sets of switches which are weakly incompatible) (Charleston, 1998). Brooks parsimony analysis cannot adequately deal with host switching either (Page, 1994). Furthermore, none of the programs accommodates host addition or delayed cospeciation as an option. Charleston (1998) developed the jungle algorithm, implemented in TREEMAP 2, which applies costs (weights) to evolutionary events (cospeciation, host switching, duplications, and sorting events). Charleston (1998), however, has noted that weighting event costs plays a pivotal role in which solution will be optimal, but estimation of appropriate weights is difficult, especially in the case of host switching (Huelsenbeck *et al.*, 1997). In addition, TREEMAP 2 has the problem of requiring each host species to have an associated parasite species; hence, some sorting events are automatically removed before analysis, which reduces the true cost. TREEMAP 1 and 2 have the added problem of not being able to deal with widespread taxa due to host switching without speciation. A solution was proposed by

Siddall & Perkins (2003). This is accomplished by giving a parasite species a different designation each time it inhabits a different host, effectively turning one parasite into multiple sister taxa. This fix effectively turns a host-switch without speciation (one event) into a host switch with speciation (two events), which TreeMap can handle Brooks *et al.* (2004). If, widespread species are due to host speciation without parasite speciation (Brooks, 1979, 1981, 1990; Hennig, 1966; Wiley, 1988), the fix created by Siddall and Perkins then leads to erroneous reconstructions, whereas BPA continues to accurately reflect the pattern Brooks *et al.* (2004). A solution to this problem is proposed in Section 9.6 (use of intraspecific variation of parasites associated with infected hosts).

With the issues of program limitations acknowledged, TREEMAP 1 (Page, 1995) was chosen with a set of *a posteriori* assumptions applied by me. These assumptions are addressed in the methods section.

9.2 Methods

Means of morphometric sclerite variables (Appendix TABLE C1-4) were used to produce the parasite phylogeny (Figure 9.2). The means were z-score standardised. Species of *Helicirrus* with elongated hooks and species of *Recurvatus* do not naturally group with congeners (Section 5.3.1.5). Therefore, a variable that assigned species to genera was included. This was given a weight of ten. A tree topology was produced using the neighbour-joining method in PHYLIP (Felsenstein, 1995). This was derived from a distance matrix using Manhattan distances. Tree topologies are known to be affected by input order of taxa; hence, the "jumble" option was used. As the large number of hosts infected by some parasite species (widespread taxa) confounded

interpretations of reconciled trees, separate analyses were done for members of *Helicirrus* and *Longidigitis,* while *Recurvatus* and *Iliocirrus* species were combined. However, a combined genus analysis is also included.

A single complete phylogeny of all host species examined is not yet available; therefore, the host tree was compiled from three sources. For the family level associations, I used the cladistic analysis of Dyer & Chernoff (1996). For *Melanotaenia* species, the cytochrome b mtDNA phylogeny (McGuigan *et al.*, 2000; Zhu *et al.*, 1994) was used instead of the combined D-loop cytochrome b and $tRNA^{pro}$ control region data phylogeny (has trichotomy of "*nigrans*", "*splendida*" and "*australis*" clades) as TREEMAP 1 requires a completely resolved binary tree. *Cairnsichthys rhombosomoides* and *Rhadinocentrus ornatus* were placed basally to the *Melanotaenia* species because preliminary cytochrome b mtDNA results (Unmack, 2002; Unmack, pers. com.) and morphology (Allen & Cross, 1982) indicate this. Allen & Cross (1982) consider Melanotaeniidae is derived from a pseudomugil-like ancestor: hence, species of *Pseudomugil* were placed basally to the melanotaeniids. Previous phylogenetic analyses have used an atherinid host species as the outgroup (*Craterocephalus stercusmuscarum fulvus*) and hence a similar outgroup, which includes *M. marjoriae*, is used in the analyses. The proposed host phylogeny and parasite phylogeny for each genus (Figure 9.2) was compared using TREEMAP 1.

The host/parasite associations are represented as a tanglegram. A reconciled tree was produced in TREEMAP 1 using the exact search method and the most parsimonious trees were examined. Because of the limiting assumptions of the software model, these

trees were inspected *a posteriori* for alternative, potentially more parsimonious, explanations of cospeciation in the host parasite association pattern (i.e. the aim was to find an explanation that incurred the least costs). These explanations are presented below. The cost of the alternative explanations was assessed and, if cheaper, the alternatives were presented as changes in the host/parasite reconciled tree. Costs were recognised as the *ad hoc* assumptions (duplications, host switches, host additions and sorting events) needed to explain the number of cospeciations identified. To test if the number of cospeciations was significantly different from random, 10,000 random trees were generated using the "proportional to distinguishable" method. This was repeated five times and the average taken.

Intraspecific variation was examined at the host-site-level and haptoral sclerite variable means were used (Appendix Tables A11-23). For the analysis of *I. iliocirrus*, *I. rossi* and *I. trifasciata* were included as outgroup taxa. For the analysis of *L. auripontiformis*, *L. hopevalensis* from *M. trifasciata* was used as the outgroup.

A discriminant function analysis was used to concentrate the variation associated with each host species using host species as the discriminant grouping. The eigenvectors were then used to produce a distance matrix using Manhattan distances. The matrix was then analysed using UPGMA in PHYLIP to produce the association tree. This tree was then compared with the host phylogeny.

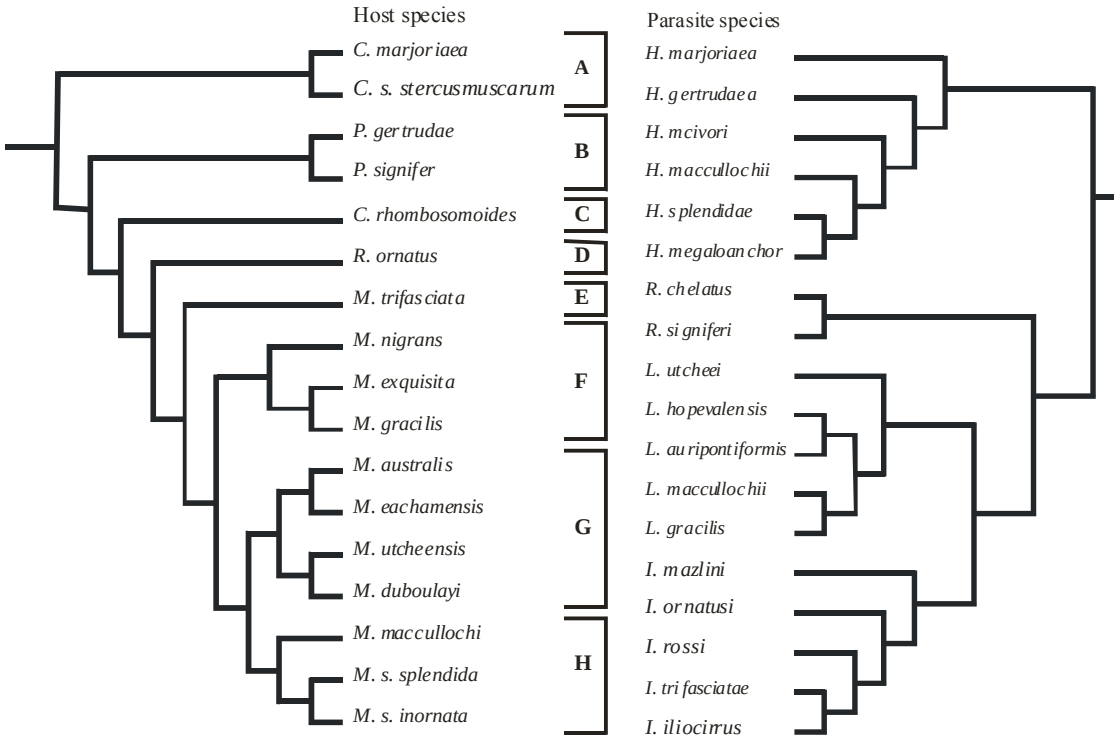


Figure 9.2. Inferred host and parasite phylogeny.
Bold type host species clades identified. A. "*Craterocephalus*", B. "*Pseudomugil*", C. "*rhombosomoides*", D. "*ornatus*" E. "*goldiei* (*trifasciata*)", F. "*nigrans*", G. "*australis*", H. "*splendida*". Family level associations adapted from Dyer (1996). Clades A-D adapted from Allen & Cross (1982), Unmack (2002); (Unmack pers.com). E-H adapted from McGuigan (2000), Zhu, *et al* (1994). Parasite phylogenies inferred using morphometric variation of parasite species for *Iliocirrus, Recurvatus, Longidigitis* and *Helicirrus*.

9.2.1 Exclusion of Parasite or Host Species from Analysis

Sometimes it may be necessary to remove samples from analyses when the distribution of parasite or host species does not unambiguously reveal their correct associations. Small sample size or rarity of a host or parasite species can present erroneous patterns (false negatives) thereby disguising the full extent of the association. Alternatively, false positive results may occur where host species are sympatric, enabling the occasional cross contamination of parasite species. In addition, there may be doubt regarding the taxonomic affinity of the parasite or host with its congeners.

9.2.2 Polarisation of Morphological Changes in Parasite Haptor

Character polarisation is used to place a direction to changes in characters thereby producing an evolutionary sequence of events. Polarisation, however, can place unvalidated restrictions on the possibilities of the associations, which may reduce the chance of identifying the true host/parasite association. Often character polarisation can force evolutionary associations to conform to some preconceived notion. If a strong enough phylogenetic signal is present in the characters, then polarisation should not be necessary. Occasionally a species may be placed incorrectly in the inferred phylogeny. This can occur if an excess of characters not related to the polarisation swamp the data matrix or if character weighting is not applied. For haptoral morphology, a change in host attachment site may be followed by a morphological change in the haptoral structure. *Helicirrus megaloanchor*, as noted in Chapter 4, has greatly enlarged ventral anchors. I proposed that this species evolved from a *Helicirrus splendidae*-like ancestor because of an adaptation to a new attachment site, namely moving from the gill rakers to the soft gill filaments. This scenario is presented in the phylogeny of the *Helicirrus* species (Figure 9.2). The alternative scenario, where *H. megaloanchor* is basal to *H. maccullochii* and *H. splendidae*, is used as an initial assumption in the analyses. These alternative possibilities are tested by examining the pattern of the parasite/host association and *ad hoc* costs.

9.2.3 Host Switching and Addition

Switching and addition of parasite species on new hosts can occur when hosts are, or become, sympatric. Due to a close phylogenetic relationship between the hosts, barriers to host switching or addition may be incomplete. A parasite may thus be able to

establish on a more basally derived host. TREEMAP 1 will interpret this new association as the progenitor of this parasite, thus misinterpreting the time of the true speciation event. Such host additions cannot be identified with software for phylogeny inference (Waegele, 1999). To discover cases of possible host addition, the parasite-host association is removed from the analysis but is still counted as a cost. If a more parsimonious reconciled tree results, then host addition is considered as a possible *ad hoc* assumption.

9.2.4 Missing Taxa

Incongruence between host and parasite associations can occur because of missing parasite taxa. This can be due to extinctions of a host-parasite association as well as inadequate sampling effort. One way in which such associations can be inferred is by examining host-parasite associations known to be present at higher and lower nodes in the tree. If a derived host species obtains a parasite by host addition, the cost of sorting events is reduced in those sister host species.

9.3 Results

The *a posteriori* assumptions used are presented in Table 9.1 and validation of these is given in the associated sections for the parasite genera. A summary of the analyses indicating the number of *ad hoc* assumptions (i.e. duplications, host switchings, additions and sorting events) needed to produce the cospeciations identified, and the probability of obtaining this number from random associations, is presented in Table 9.2.

9.3.1 Association 1: *Iliocirrus/Recurvatus* Species

For the unmodified host/parasite associations (Figure 9.3), the TREEMAP 1 model produced one most-parsimonious reconciled tree. Two cospeciation events were identified with 16 sorting events, three host switches and one duplication (Figure 9.4), which resulted in a total cost of 20 *ad hoc* assumptions. The probability of obtaining this number of cospeciation events was not significantly different (p=0.2421) from random chance. The reconciled trees were examined visually, the following *a posteriori* assumptions being implemented, and costs determined on the trees by reanalysis.

Table 9.1. *A posteriori ad hoc* assumptions applied to analyses

Analysis of unmodified host phylogeny
Assumptions for *Iliocirrus/Recurvatus* analysis
A1. Most host additions reflect delayed cospeciation.
A2. Host addition of *I. iliocirrus* on *C. rhombosomoides* A3. *Iliocirrus mazlini* missing on *C. rhombosomoides* A4. Host addition of *I. mazlini* on *M. utcheensis* and *M. eachamensis*
Assumptions for *Longidigitis* analysis
B1. Most host additions reflect delayed cospeciation
B2. Rare species *L. robustus* removed
Assumptions for *Helicirrus* analysis
C1. Most host additions reflect delayed cospeciation
C2. Host addition of *H. splendidae* on *R. ornatus* C3. Host addition of *H. splendidae* on *I. trifasciata*
Modified host phylogeny with *M. maccullochi* placed basal to *"nigrans"* clade
Assumptions for *Helicirrus* analysis
D1. Most host additions reflect delayed cospeciation
D2. Host addition of *H. splendidae* on *R. ornatus* D3. Host addition of *H. splendidae* on *M. trifasciata* D4. Host addition of *H. splendidae* on *M. maccullochi* D5. Host addition of *H. megaloanchor* on *M. maccullochi* D6. *Helicirrus maccullochii* basal to *H. megaloanchor*
Assumptions for *Longidigitis* analysis
E1. Rare species *L. robustus* removed

Table 9.2. Costs associated with reconciled trees for each parasite genus and host phylogeny.
For modified assumptions, see figures and related sections.

Unmodified Host phylogeny								
Genus	Cosp.	Dupl	Host switch	Host addition.	Sort	Total cost	Prob.	Fig
Iliocirrus/Recurvatus output from TREEMAP 1.	2	1	3	0	16	20	0.1242	Figure 9.4
Iliocirrus/Recurvatus using additional assumption A1, A2	2	2	2	1	12	17	0.1242	not shown
Iliocirrus/Recurvatus using additional assumptions A1, A3	2	1	3	0	15	19	0.1242	not shown
Iliocirrus/Recurvatus using additional assumption assumptions A1, A2, A3	2	2	2	1	12	17	0.1242	not shown
Iliocirrus/Recurvatus using additional assumptions A1, A2, A3, A4	3	1	2	3	5	11	0.0375	Figure 9.4
Longidigitis using additional assumptions B1, B2.	1	3	0	0	10	13	0.1885	Figure 9.6
Helicirrus output from TREEMAP 1	2	3	0	0	16	19	0. 0243	Figure 9.8
Helicirrus using additional assumptions C1, C2	2	3	0	1	14	18	0. 0243	Figure 9.9
Helicirrus using additional assumptions C1, C2, C3	3	2	0	2	11	15	<0.0001	Figure 9.9
Separate analyses combined using most parsimonious assumptions	7	6	2	5	26	39	<0.0001	Figure 9.10
Modified Host Phylogeny								
Genus	Cosp.	Dupl	Host switch	Host addition	Sort	Total cost	Prob.	Fig
Longidigitis output from TREEMAP 1.	2	2	0	0	7	9	0.0017	Figure 9.11
Helicirrus output from TREEMAP 1.	2	3	0	0	14	17	0.0243	Figure 9.12
Helicirrus using additional assumptions D1, D2.	2	3	0	1	12	16	0.0243	not shown
Helicirrus using additional assumptions D1, 2, D3	3	2	0	2	9	13	<0.0001	not shown
Helicirrus using additional assumptions D1, D2, D3, D4	3	2	0	3	10	15	<0.0001	not shown
Helicirrus using additional assumptions D1, D2, D3, D6	3	2	0	2	9	13	<0.0001	not shown
Helicirrus using additional assumptions D1, D2, D3, D4 D6	3	2	0	4	10	16	<0.0001	not shown
Helicirrus using additional assumptions D1, D2, D3, D5	3	2	0	3	10	15	<0.0001	not shown
Helicirrus using all additional assumptions	4	1	0	4	8	13	<0.0001	Figure 9.12
Separate analyses combined. most parsimonious assumptions used	9	4	2	7	20	33	<0.0001	Figure 9.13

9.3.1.1 Validation of *a posteriori* Assumptions:

(A1). *Iliocirrus iliocirrus* distribution among hosts is treated as delayed cospeciation, not host addition or host switching: speciation events associated with the "*nigrans*" clade (see Figure 9.2). The alternative massive host switching or addition seems unlikely due to allopatric distribution of hosts.

(A2). The invasion of *M. s. splendida* into geographic regions occupied by *C. rhombosomoides* facilitated addition of *I. iliocirrus* to the latter host.

(A3). Missing ancestral association of *I. mazlini* with *C. rhombosomoides* can be explained by the small sample size of *C. rhombosomoides*, which raises the possibility of missed detection of association. *Cairnsichthys rhombosomoides* is morphologically very similar to *R. ornatus* and these species were once considered sisters, belonging to the same genus (Allen & Cross, 1982). Parasites *I. mazlini* and *I. ornatusi* are very similar in morphology and distinct from other congeners. The position of *I. mazlini* basal to *I. ornatusi* and *I. trifasciatae* in parasite tree mirrors basal position of *C. rhombosomoides* to *I. ornatus* and *M. trifasciata* in host phylogeny.

(A4). *Cairnsichthys rhombosomoides* is found in same area and sample sites as *M. utcheensis*, which allows the possibility of host additions to M. eachamensis, and *M. utcheensis*. The absence of *I. mazlini* from other host species within the clade of *M. utcheensis* and *M. eachamensis* and ancestral clades also adds strength to this assumption.

After the implementation of all assumptions, three cospeciation events were identified, *I. ornatusi* with *R. ornatus*, *I. mazlini* with *C. rhombosomoides* and *R. signifer* with *P. signifer* (Figure 9.4). This reduced the *ad hoc* costs to 11: two host switches (*I. rossi* and *R. chelatus* to *C. s. stercusmuscarum*); three host additions (*I. Iliocirrus* infected *C. rhombosomoides* and *I. mazlini* infected *M. utcheensis* and *M. eachamensis*); five sorting events (extinctions of *I. iliocirrus* on *M. eachamensis*, *M. utcheensis* and *M. maccullochi*, the apparent absence of *I. mazlini* from *C. rhombosomoides* and of a *Recurvatus species* on *P. gertrudae*). The probability of obtaining three cospeciations was significantly different from random (P=0.0375). The duplication event, *I. iliocirrus* and *I. trifasciata*, may also represent a host addition (*I. iliocirrus* on *M. trifasciata*). When this assumption was tested, a further cospeciation event was identified.

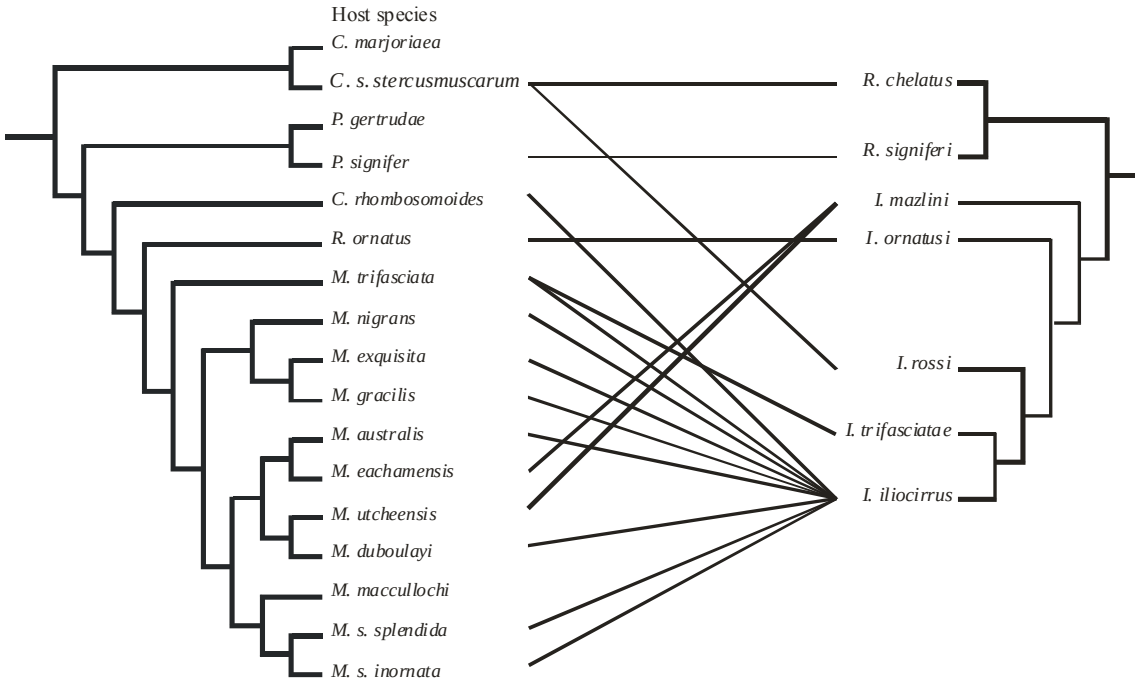


Figure 9.3. Tanglegram of associations between parasite species of *Iliocirrus* and *Recurvatus* and their hosts as initially produced by TREEMAP 1.

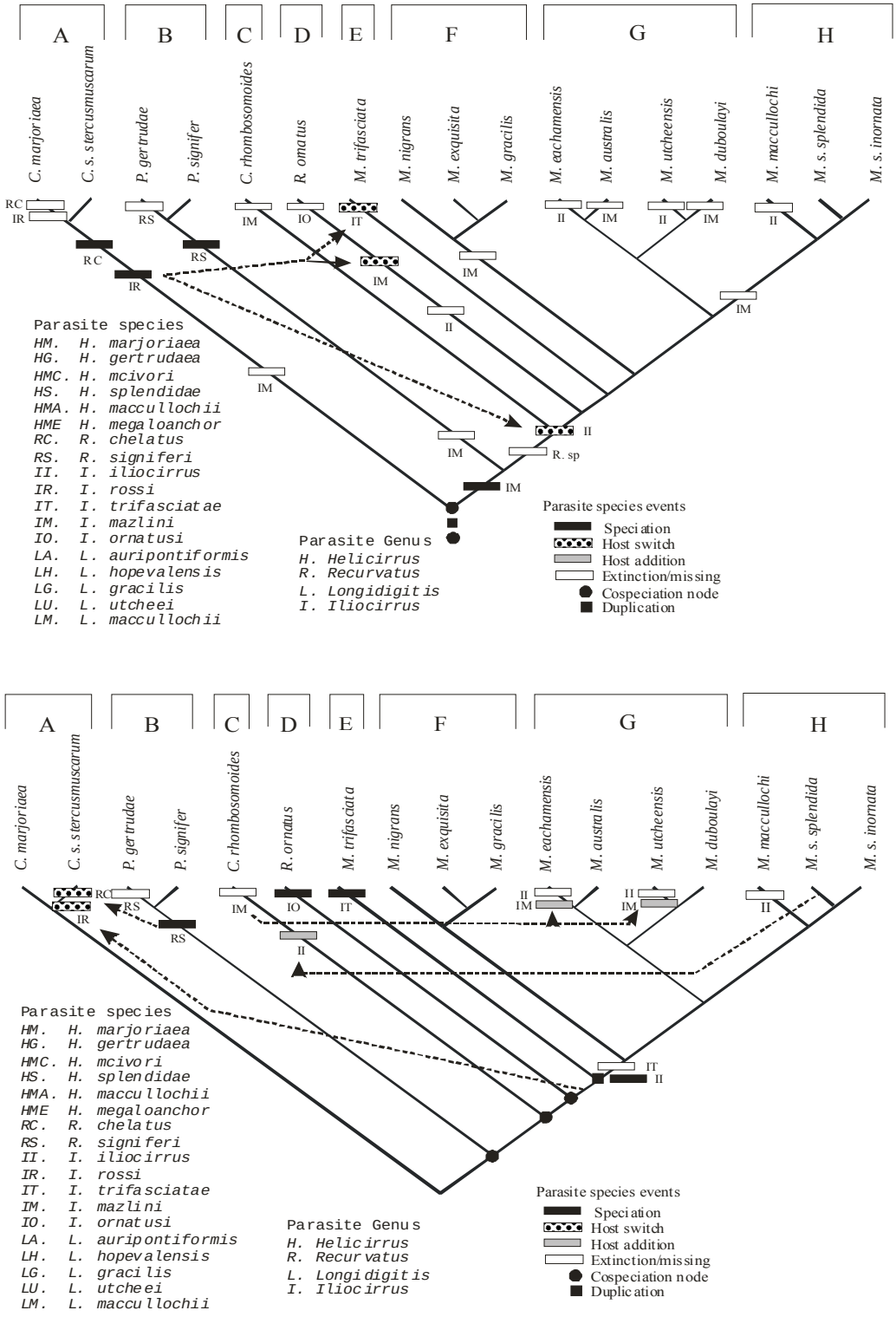


Figure 9.4. Reconciled trees of associations between *Iliocirrus* species and their hosts. Top. unmodified. Bottom. Absence of *Iliocirrus mazlini* on *C. rhombosomoides* is treated as a sampling error. *Iliocirrus iliocirrus* treated as an addition on *C. rhombosomoides*. I. mazlini treated as an addition on *M. utcheensis* and *M. eachamensis*. Costs are counted as host switches, host additions and duplication (sympatric speciation events). Dots at nodes represent cospeciation events. Arrows represent host additions and host switches.

9.3.2 Association 2: *Longidigitis* species.

The tanglegram of the unmodified associations is shown in Figure 9.5. *Longidigitis robustus* was removed prior to the initial analysis (see below for validation). The analysis produced one most-parsimonious tree and identified one cospeciation event between *Longidigitis utcheei* and *C. rhombosomoides* (Figure 9.6). The total cost of the associations was 13, representing three duplications (*L. maccullochi*, *L. gracilis* and *L. hopevalensis*) and 10 sorting events. The probability of obtaining a single cospeciation event was not significantly different from random (p=0.1885). The sympatric speciation of *L. auripontiformis* and *L. hopevalensis* on *M. trifasciata* may also be explained as a host addition (*L. auripontiformis* to *M. trifasciata*). When this assumption was analysed a further cospeciation event was identified.

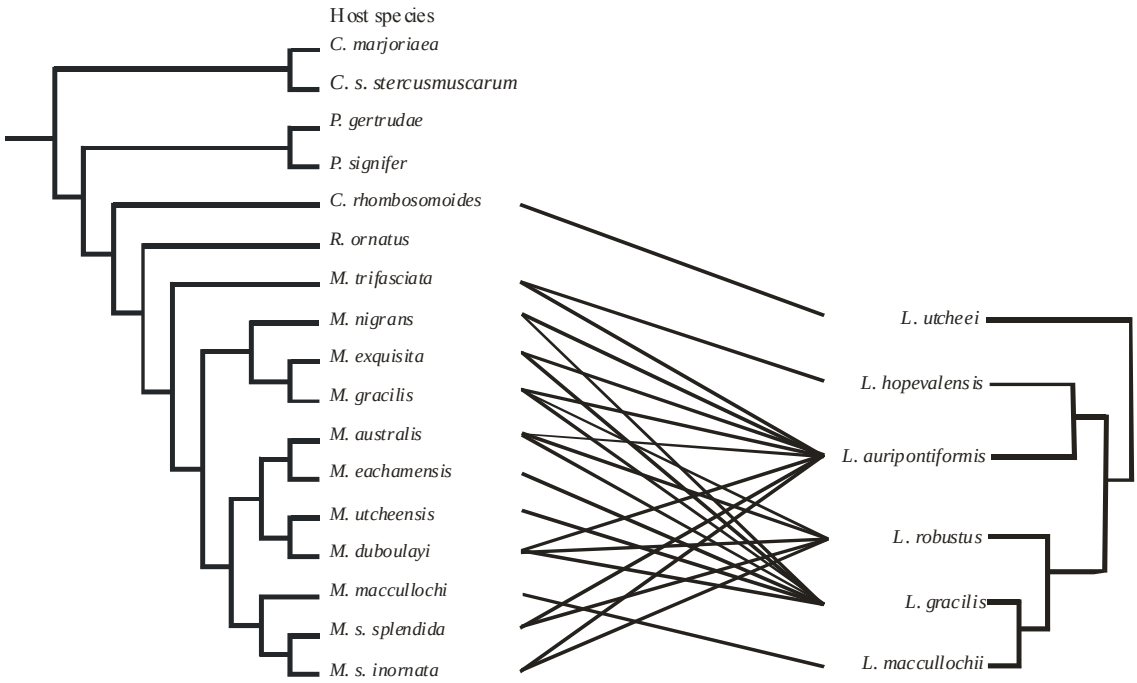


Figure 9.5. Tanglegram of host-parasite associations between *Longidigitis* species and their hosts.

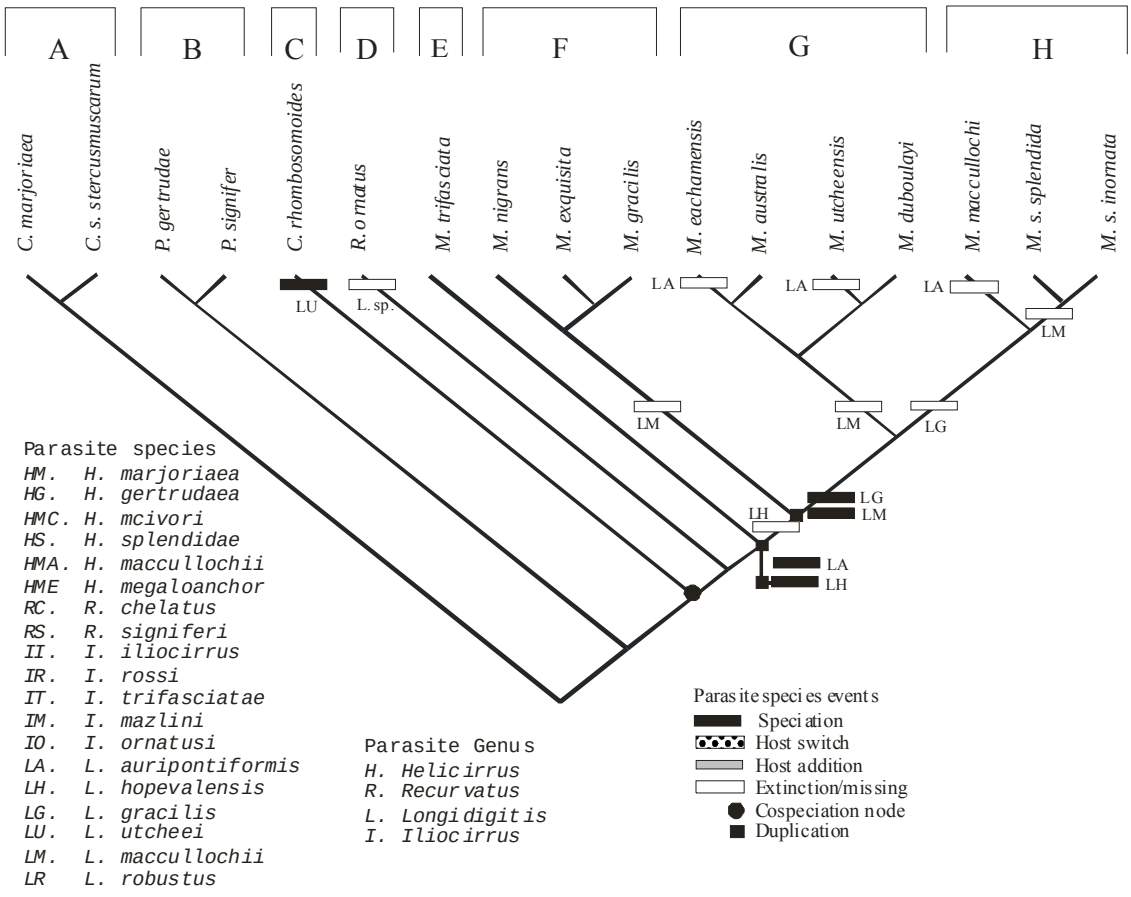


Figure 9.6. Reconciled trees of associations between *Longidigitis* species and their hosts. Longidigitis robustus removed from analysis. Costs are counted as host switches, host additions and duplication (sympatric speciation events). Dots at nodes represent cospeciation events. Arrows represent host additions and host switches.

9.3.2.1 Validation of modified assumptions

(B1). The distributions of *Longidigitis gracilis* and *L. auripontiformis* on most of their hosts do not represent host additions but are the result of delayed cospeciation.

(B2). Removal of rare species: *Longidigitis robustus* is very rare (see Chapter 6) and the chance of finding a consistent host association, especially when multiple infections are present is highly reduced. Secondly, as noted in Chapters 4 and 5, there is doubt regarding the correct taxonomic affinity of this species to other species within the

genus. This species is morphologically distinct from all its congeners. For these reasons, this species was removed from the analyses.

9.3.3 Association 3: *Helicirrus* species

The analysis of the unmodified associations (Figure 9.7) produced a single reconciled tree, which identified two cospeciation events, *H. marjoriaea* with *C. marjoriae* and *H. gertrudaea* with *P. gertrudae* (Figure 9.8). Three duplication events were also recognised where *H. megaloanchor*, *H. splendidae* and *H. maccullochii* speciated in sympatry with *H. mcivori* while 16 sorting events were identified producing an *ad hoc* cost of 19. The probability of two cospeciation events occurring was significantly different from random (P=0.0243). A set of *a posteriori* assumptions regarding the history of the associations is presented below.

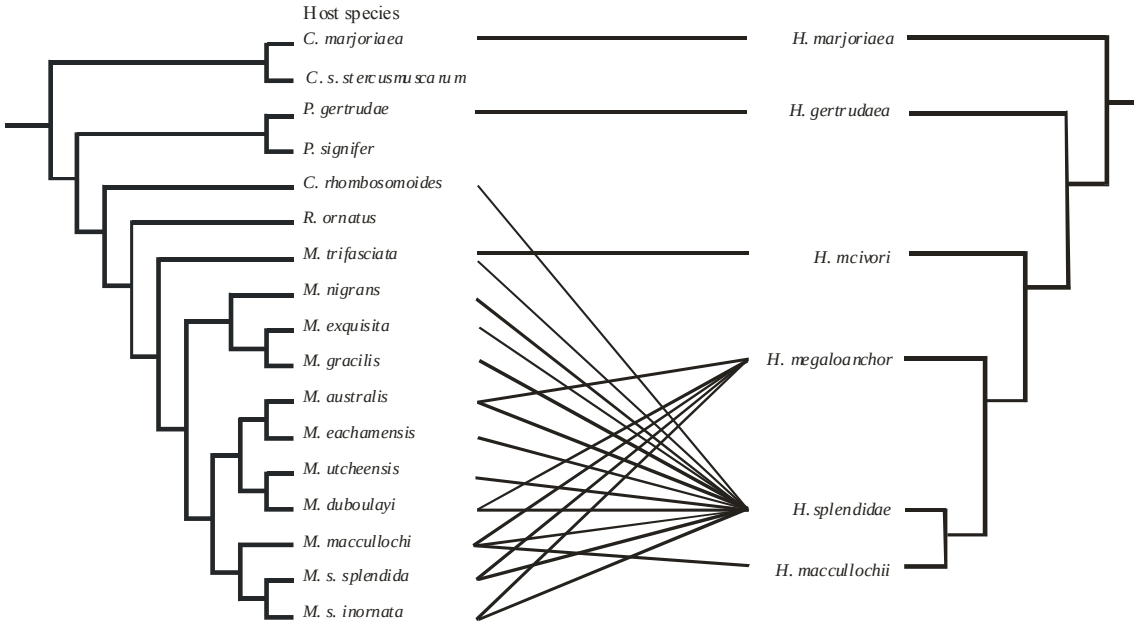


Figure 9.7. Tanglegram of unmodified associations between *Helicirrus* species and their associated hosts.

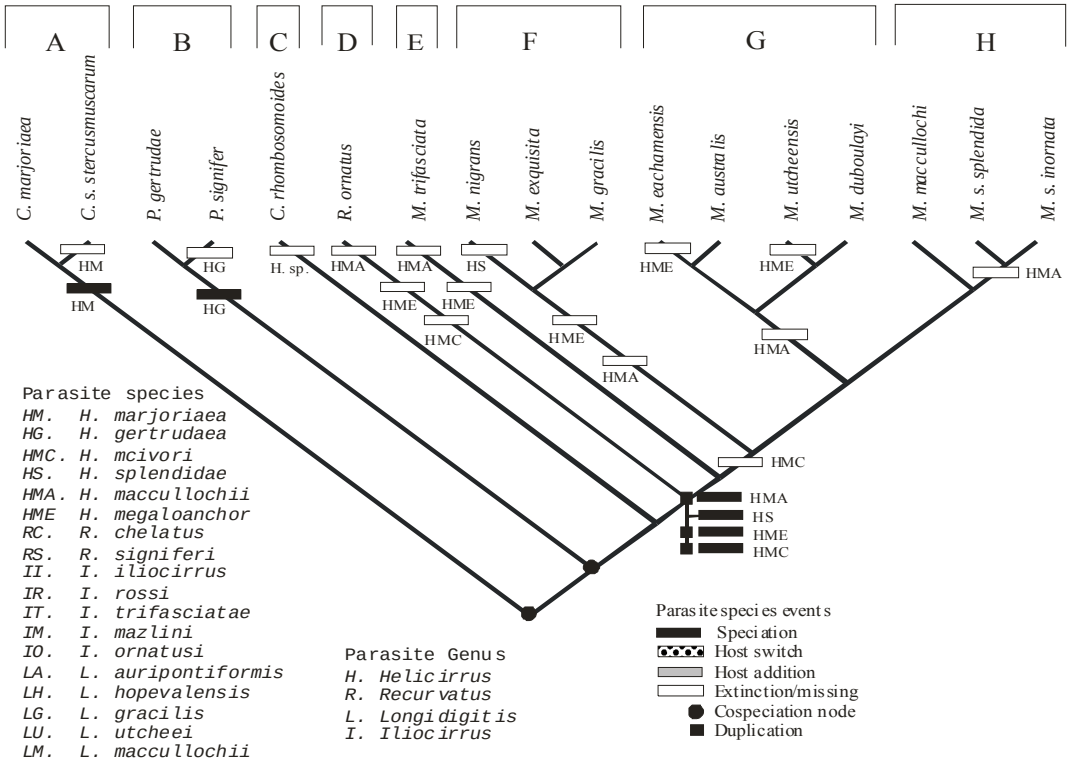


Figure 9.8. Unmodified reconciled tree of associations between *Helicirrus* species and their associated hosts.
Costs are counted as host switches, host additions and duplication (sympatric speciation events). Dots at nodes represent cospeciation events. Arrows represent host additions and host switches.

9.3.3.1 Validation of *a posteriori* assumptions.

(C1). The distributions of *H. megaloanchor* and *H. splendidae* on most of their hosts do not represent host additions but are the result of delayed cospeciation.

(C2). Host additions to species of ancestral lineages are allowed because of host geographic association. The invasion of *M. duboulayi* into the geographic region occupied by *R. ornatus* allowed host addition of *H. splendidae*.

(C3). The invasion of *M. s. splendida* into the geographic regions occupied by *M. trifasciata* allowed host addition of *H. splendidae*.

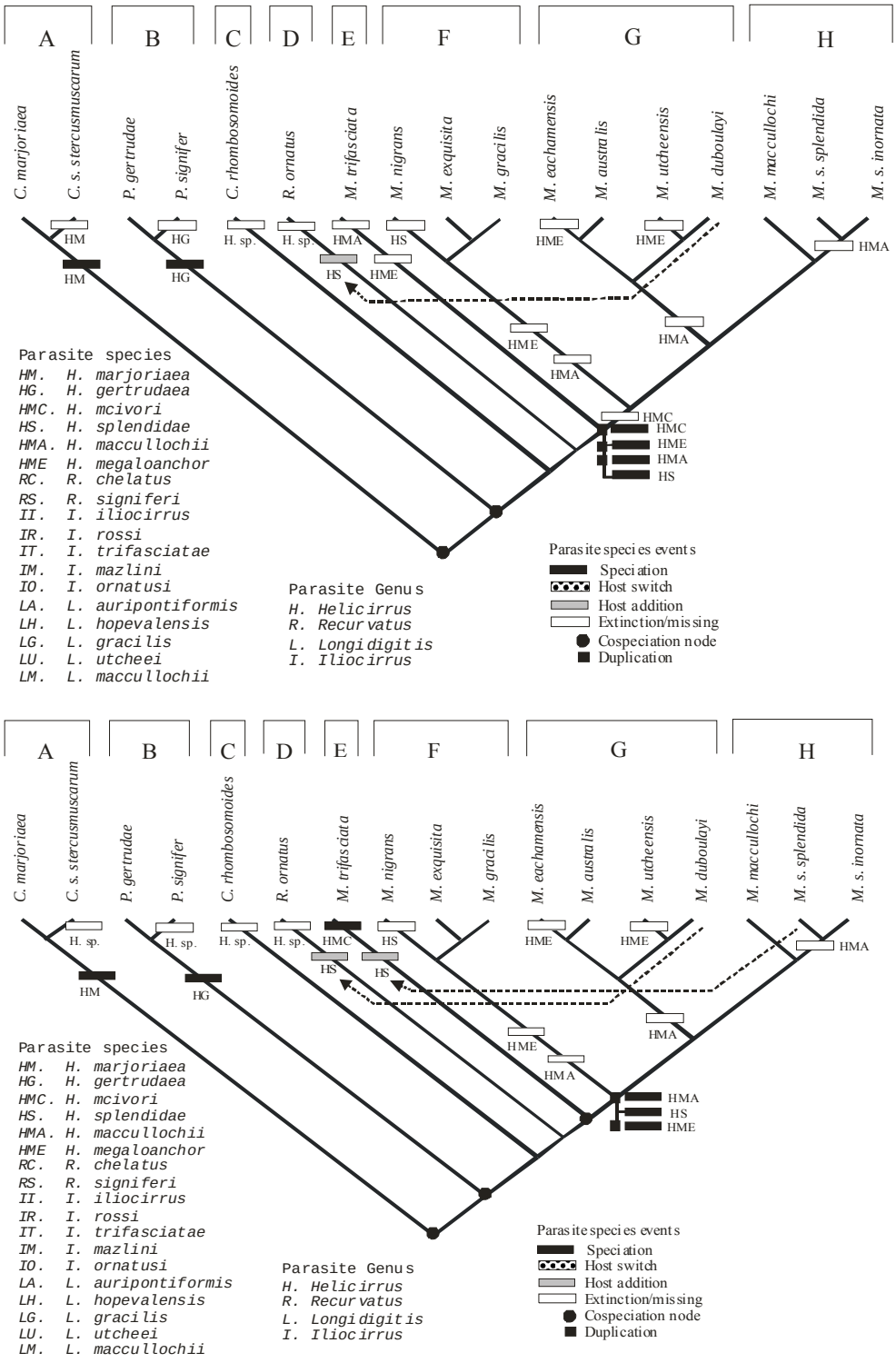


Figure 9.9. Modified reconciled tree of associations between *Helicirrus* species and their associated hosts.
Top. *Helicirrus splendidae* treated as a host addition on *R. ornatus*. Bottom. *Helicirrus splendidae* treated as a host addition on *R. ornatus* and *M. trifasciata*. Costs are counted as host switches, host additions and duplication (sympatric speciation events). Dots at nodes represent cospeciation events. Arrows represent host additions and host switches.

When *H. splendidae* was treated as an addition on *R. ornatus*, two cospeciation events were identified. *Helicirrus marjoriaea* cospeciated with *C. marjoriae* while *H. gertrudae* cospeciated with *P. gertrudae* (Figure 9.9). Three duplications were recognised with *H. mcivori* speciating in sympatry with *H. maccullochii, H. splendidae* and *H. megaloanchor*. Fourteen sorting events and one host addition were also present. The total *ad hoc* cost decreased from 19 to 18. When *H. splendidae* was also treated as an addition on *M. trifasciata*, three cospeciation events were identified (Figure 9.9). This reduced the sorting events to 11 and total *ad hoc* costs from 18 to 15. The associations of *H. splendidae* and *H. megaloanchor* with their hosts represent a possible case of delayed cospeciation with infections of nine and four hosts respectively

9.3.3.2 Combined associations

Combining the association trees of all parasite genera and host phylogeny shows the complexity of the host-parasite speciation events. This complexity is represented by two diagrams (Figure 9.10). Six duplications, two host switches, five host additions and 26 sorting events, totalling 39 *ad hoc* assumptions, were needed to account for the seven cospeciation events identified between hosts and parasites after applying *a posteriori* assumptions.

Cospeciation events were present for all host species basal to the speciation of the "*nigrans*" clade. Two major sympatric speciation events were identified. Firstly two sympatric speciation events (*L. hopevalensis* and *L. auripontiformis, I. iliocirrus* and *I. trifasciatae*) were associated with the speciation event that produced *M. trifasciata*. Secondly the "*nigrans*" clade is associated with three parasite sympatric speciation events; that of *L. gracilis* and *L. maccullochii*, and of *H. maccullochii* with *H.*

megaloanchor and *H. splendida*. Except for *L. maccullochii,* these species have widespread host distributions, being present on species of the "*nigrans*", "*australis*" and "*splendida*" clades. These widespread distributions, which may represent delayed cospeciation, are examined further in Section 9.6. Hosts of basal lineages have fewer parasite species than the more derived hosts. *Melanotaenia eachamensis* and *M. utcheensis* are associated with a number of sorting events (three) and a host addition. *Melanotaenia trifasciata* is associated with three host additions, which are assumed to be from the invasion of *M. s. splendida* and *M. s. inornata*. Delayed cospeciation events are represented by *I. iliocirrus*, *L. auripontiformis*, *H. splendidae* and *H. megaloanchor* and species of the associated host clades *"splendida", "nigrans"* and *"australis"*. *Melanotaenia maccullochi* shows a distinct difference of parasite associations from its sister species *M. s. splendida* and *M. s. inornata* and the two species clades just basal to it, being the only species having mono-host-specific parasite species (*L. maccullochii* and *H. maccullochii*) and also by the absence of the two widespread species *L. auripontiformis* and *I. iliocirrus*. The phylogenetic position of *M. maccullochi* is examined further in the following section.

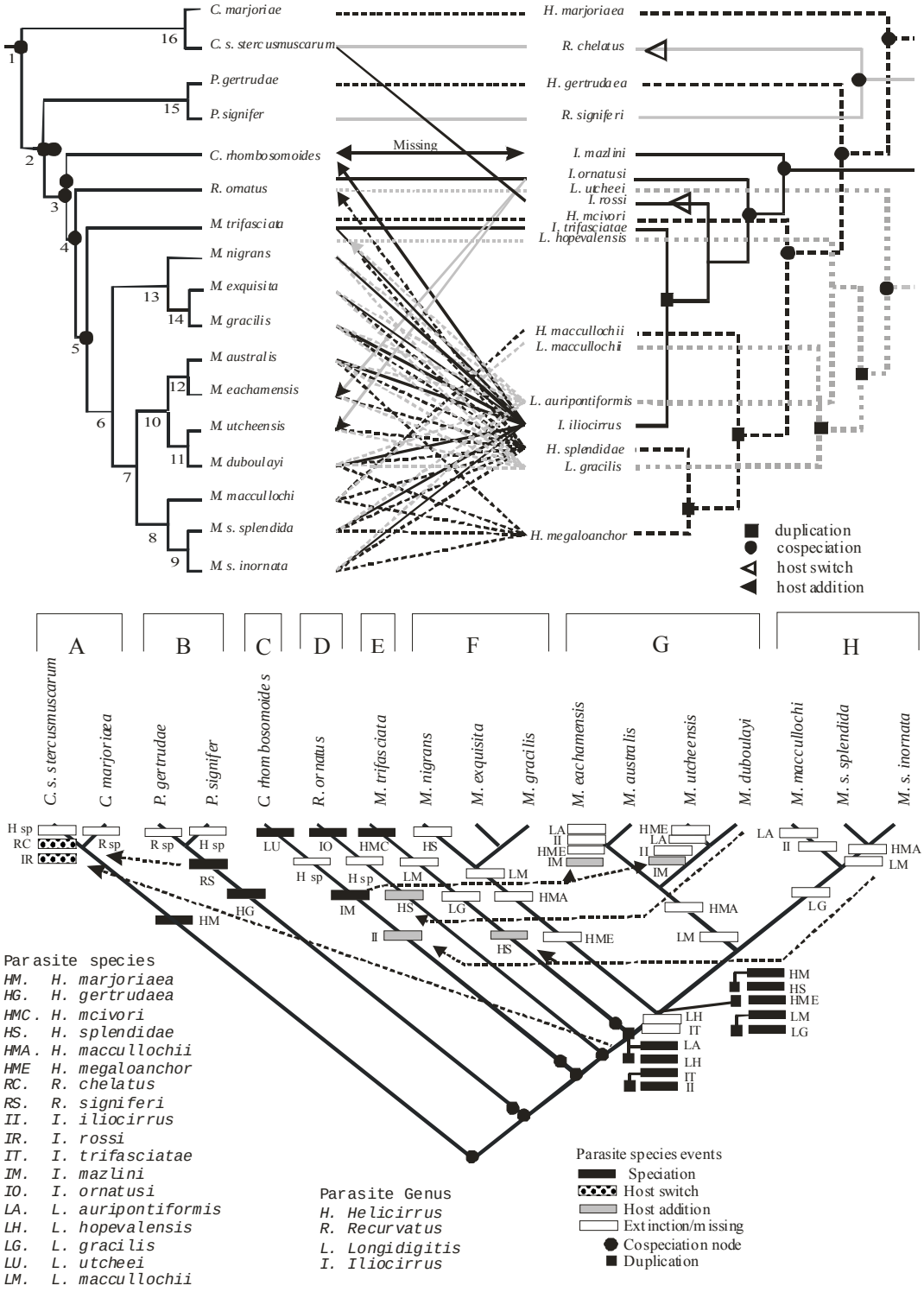


Figure 9.10. Combined reconstruction of host and parasite phylogenies and their associations after applying *a posteriori* assumptions.
Top. Tanglegram. Lines connecting hosts and parasites represent associations. Bottom. Reconciled tree. *Melanotaenia maccullochi* placed in "*splendida*" clade. Costs are counted as host switches, host additions and duplication (sympatric speciation events). Dots at nodes represent cospeciation events. Arrows represent host additions and host switches.

9.4 Anomalies of *M. maccullochi* in the phylogenetic tree

The parasite associations observed for *M. maccullochi* raises questions about the position of this species in the phylogenetic tree. As noted in the previous section this species has a set of unique parasite associations which appear to dis-associate it from its sister species ("*splendida*" clade) and the "*nigrans*" and "*australis*" clades. Doubt is also raised by morphological analysis (Schmida, 1997), which recognises this species as the sole species in a distinct clade ("*maccullochi*"). Furthermore, *M. maccullochi* has the same disjunct geographic distribution as species of older lineages, namely *P. gertrudae* and *M. trifasciata*. A further point to note is that hybridisation has been identified between rainbowfish species (McGuigan *et al.*, 2000, Unmack, pers. com.).

My hypothesis is that parasite associations can identify a more parsimonious reconciled tree, which indicates the "true" position of *M. maccullochi* in the host phylogeny. To test this, analyses were run in which *M. maccullochi* was positioned at different nodes along the host phylogeny. When *M. maccullochi* was placed between the "*goldiei*" (*trifasciata*) and "*nigrans*" clade, the most parsimonious reconciled tree was produced. For *Iliocirrus* species, there was no change in the number of cospeciations or *ad hoc* assumptions. For *Longidigitis* species, one additional cospeciation was recognised, with *ad hoc* assumptions of two duplications and seven sorting events (Figure 9.11). This reduced the total *ad hoc* assumptions from 13 to nine. For the unmodified analysis of *Helicirrus*, the two cospeciations identified were explained by three duplications, 14 sorting events and two host addition reducing the costs by two (Figure 9.12). When the *a posteriori* assumptions (excluding assumption 5) were analysed in various

combinations, there was no increase in the number of cospeciations, however total *ad hoc* cost were reduced from 15 to 13 (Table 9.2).

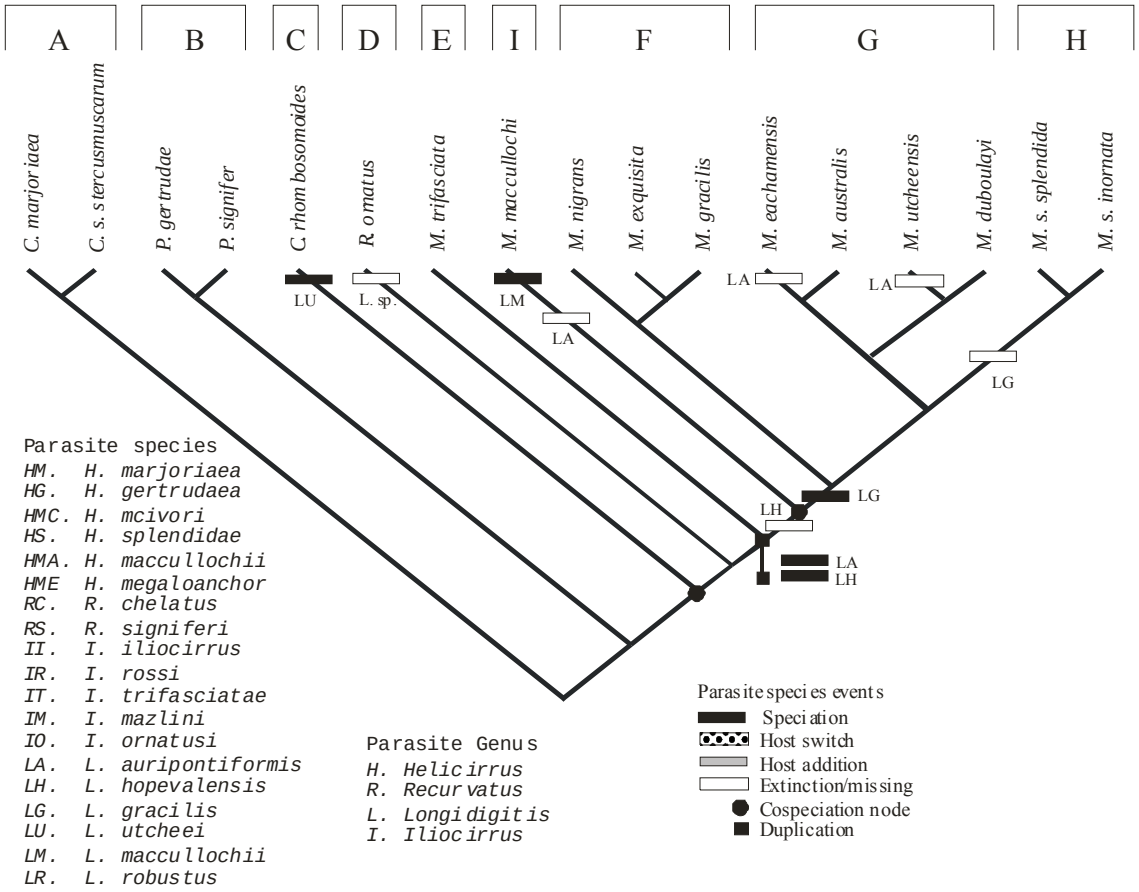


Figure 9.11. Reconciled tree of associations between *Longidigitis* species and their hosts with *M. maccullochi* lineage (I) placed basal to "*nigrans*" clade (F). *Longidigitis robustus* removed. Costs are counted as host switches, host additions and duplication (sympatric speciation events). Dots at nodes represent cospeciation events. Arrows represent host additions and host switches.

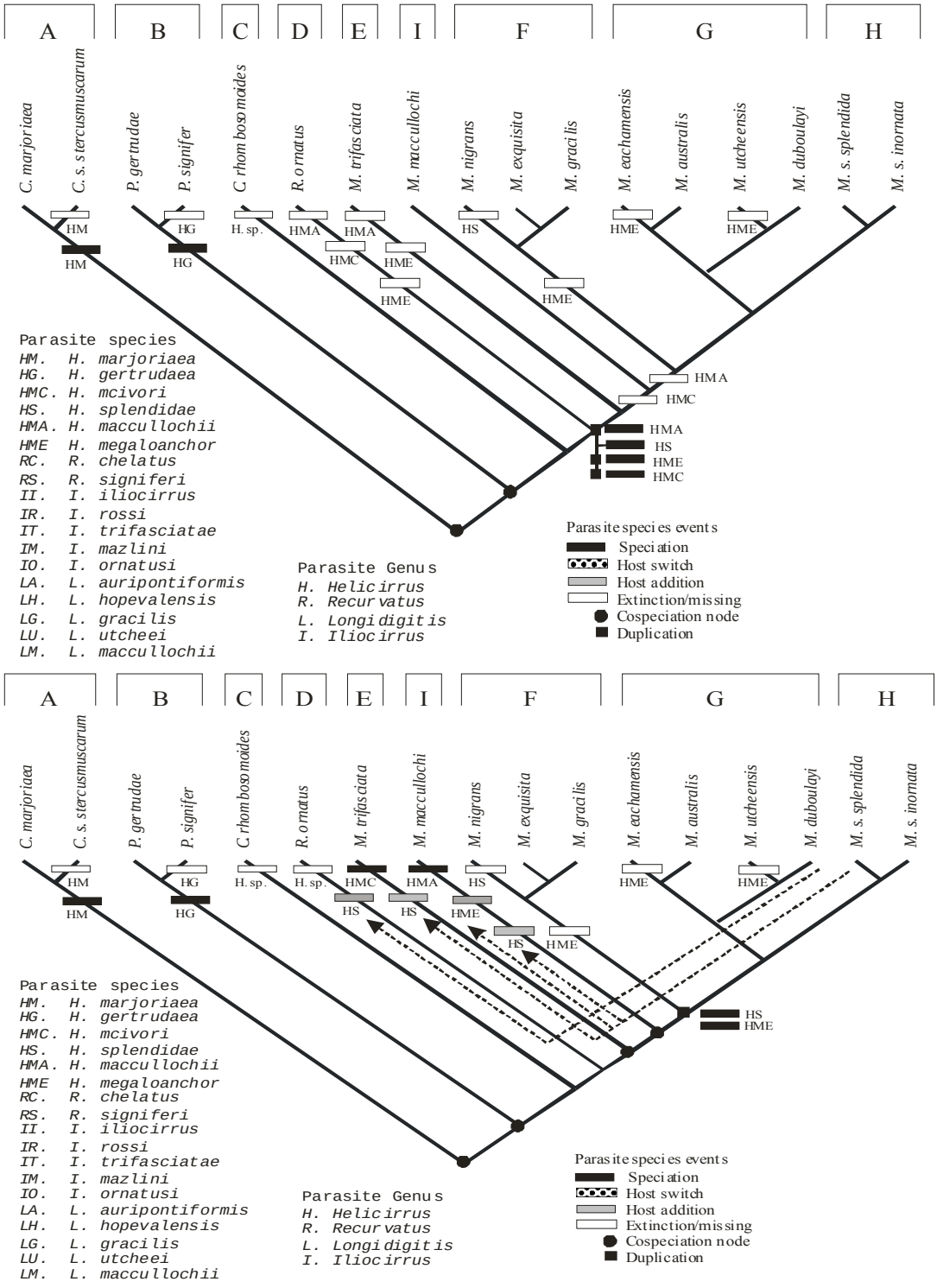


Figure 9.12. Reconciled tree of *Helicirrus* species and their associated hosts with *M. maccullochi* lineage treated as ancestral to "*nigrans*" (F) clade.
Top. Unmodified host-parasite associations. Bottom. *Helicirrus splendidae* treated as a host addition on *M. trifasciata*, *M. maccullochi* and *R. ornatus*. *Helicirrus megaloanchor* treated as a host addition on *M. maccullochi*. *Helicirrus maccullochii* lineage is treated as ancestral to *H. megaloanchor* lineage. Costs are counted as host switches, host additions and duplication (sympatric speciation events). Dots at nodes represent cospeciation events. Arrows represent host additions and host switches.

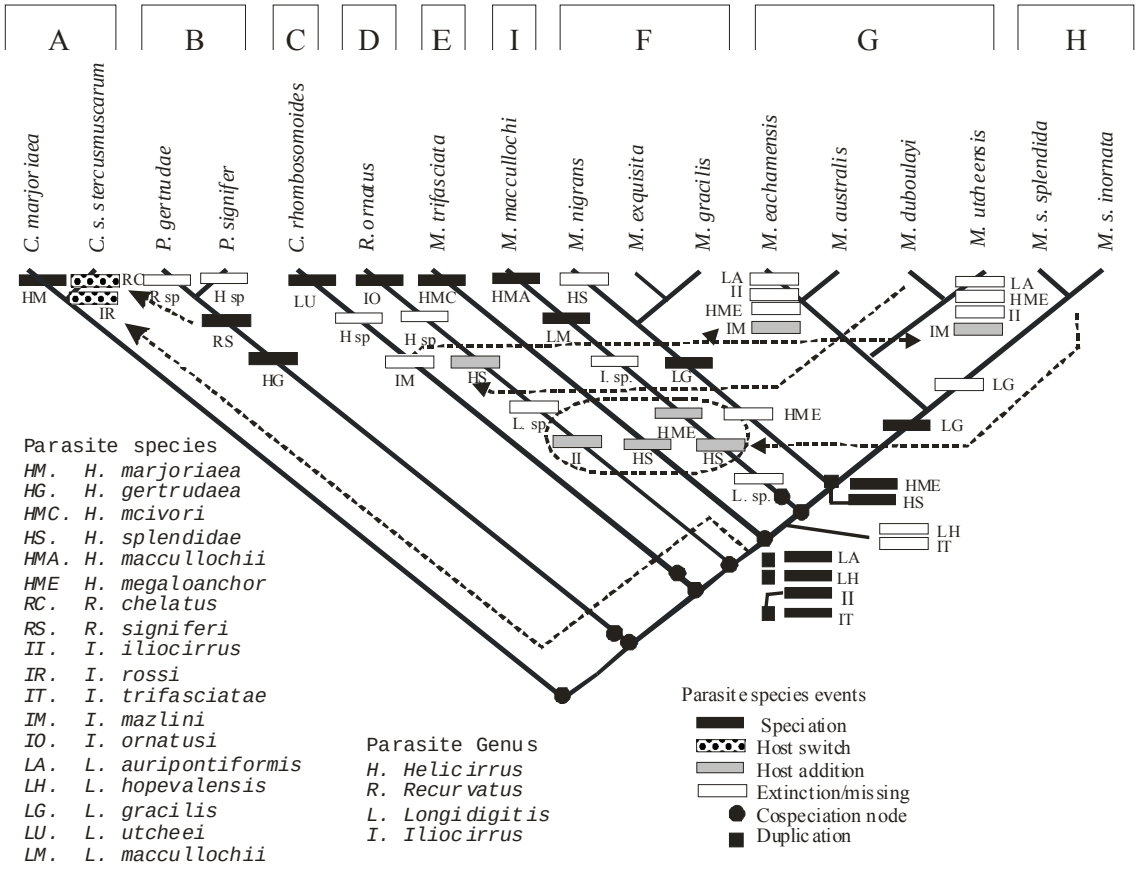


Figure 9.13. Reconstruction of host and parasite phylogenies and their associations. *Melanotaenia maccullochi* lineage placed basally to "*nigrans*" clade. Costs are counted as host switches, host additions and duplication (sympatric speciation events). Dots at nodes represent cospeciation events. Arrows represent host additions and host switches.

9.5 Ancestral or derived position of *H. megaloanchor*

The position of *H. megaloanchor* on the association tree is in doubt. As noted in Section 4.4, I proposed that this species is derived from a *H. splendidae-H. maccullochii*-like ancestor where a transfer from the gill arches to the gill filaments required enlarged anchors. The alternative proposal, *H. splendidae-H. macullochii* is derived from a *H. megaloanchor*-like ancestor, is compared. The resulting analysis using *a posteriori* assumptions (D1-D5) identified another cospeciation when *H. maccullochi* was placed basal to *H. megaloanchor* (Figure 9.12). When all assumptions were used, in the

combined analysis of all genera, two more cospeciations were suggested with an overall reduction of *ad hoc* costs of six (from 39 to 33) (Figure 9.13).

9.6 Intraspecific variation and the detection of delayed synchronous cospeciation

In the introduction, I suggested that multiple infections of many hosts in a single or several sister clades, by a single parasite species, may indicate delayed cospeciation rather than host addition. Five species *L. gracilis*, *I. iliocirrus*, *L. auripontiformis*, *H. splendidae* and *H. megaloanchor* were identified as candidates. If distributions of these species among hosts represent delayed cospeciation then some phylogenetic signal should be detectable as intraspecific variation among parasites associated with host clades or species. To test this hypothesis, I used the intraspecific morphometric variation of the two parasite species *L. auripontiformis* and *I. iliocirrus*. These two species occur in sufficient numbers to permit a micro-morphometric variation approach. A comparison between the parasite clades and host mtDNA phylogeny was conducted.

Trees inferred from intraspecific parasite morphometric variation associated with host species mirror each other and almost mirror the host phylogeny (Figure 9.14, 9.14). Six cospeciation events were recognised for intraspecific variation in *L. auripontiformis* and in *I. iliocirrus*. Both analyses identified a sympatric speciation (*L. hopevalensis* and *L. auripontiformis*, *I. trifasciatae* and *I. iliocirrus*) associated with the *M. trifasciata* lineage, and alternatively the host switch which was more parsimonious. Both analyses also identified a host switch from *M. australis* to the "*nigrans*" clade. The inference of this is that intraspecific variation of the haptoral structure does show phylogenetic signal.

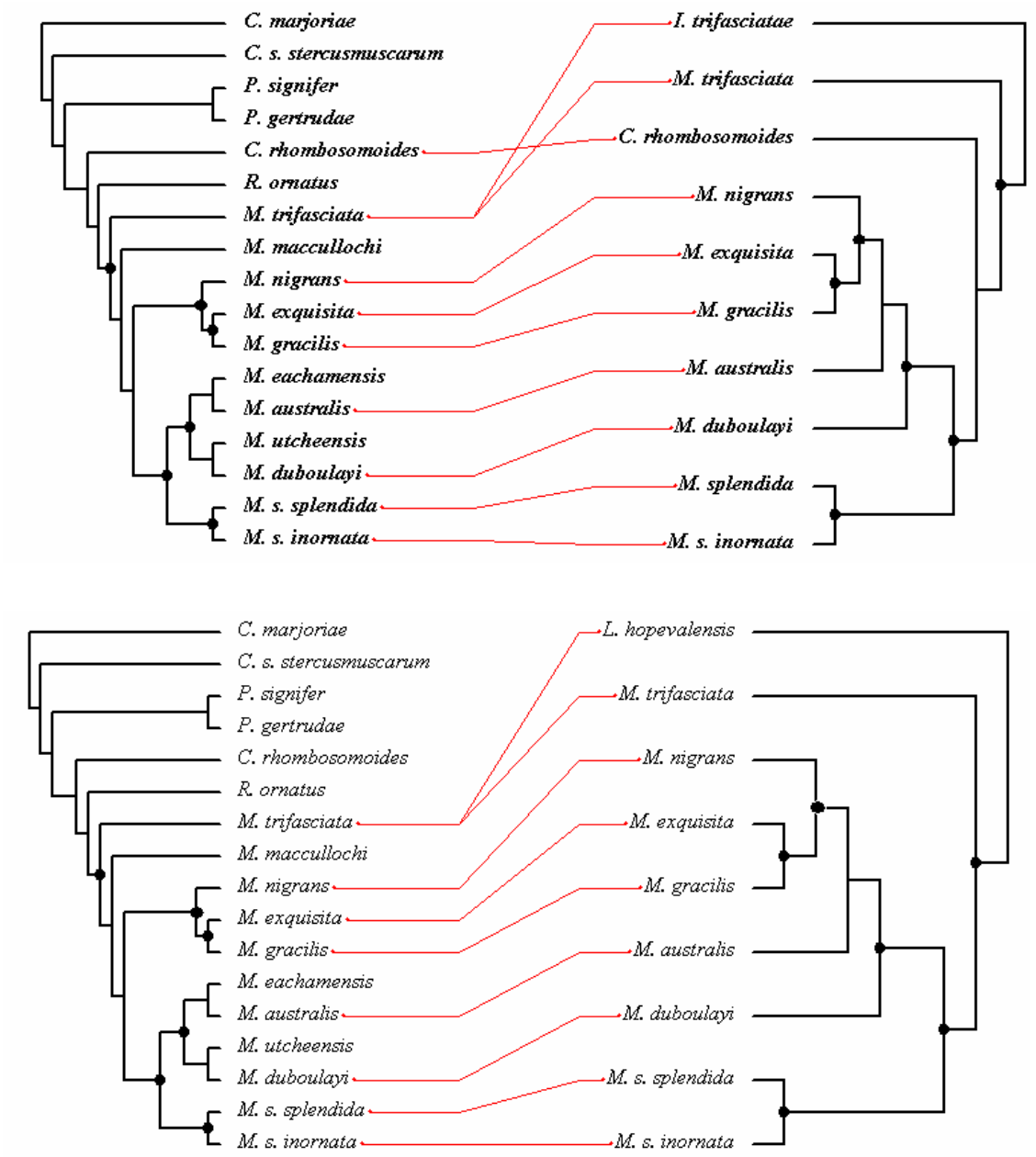


Figure 9.14. Tanglegrams of intraspecific parasite variation (haptoral sclerite) and inferred associations through cospeciation with host phylogeny of *I. iliocirrus* and *L. auripontiformis* and the comparison with the host phylogeny.
Top. For *I. iliocirrus*, *I. trifasciata* used as the outgroup. Bottom. For *L. auripontiformis*, *L. hopevalensis* from *M. trifasciata* used as the outgroup. Dots represent cospeciation events.

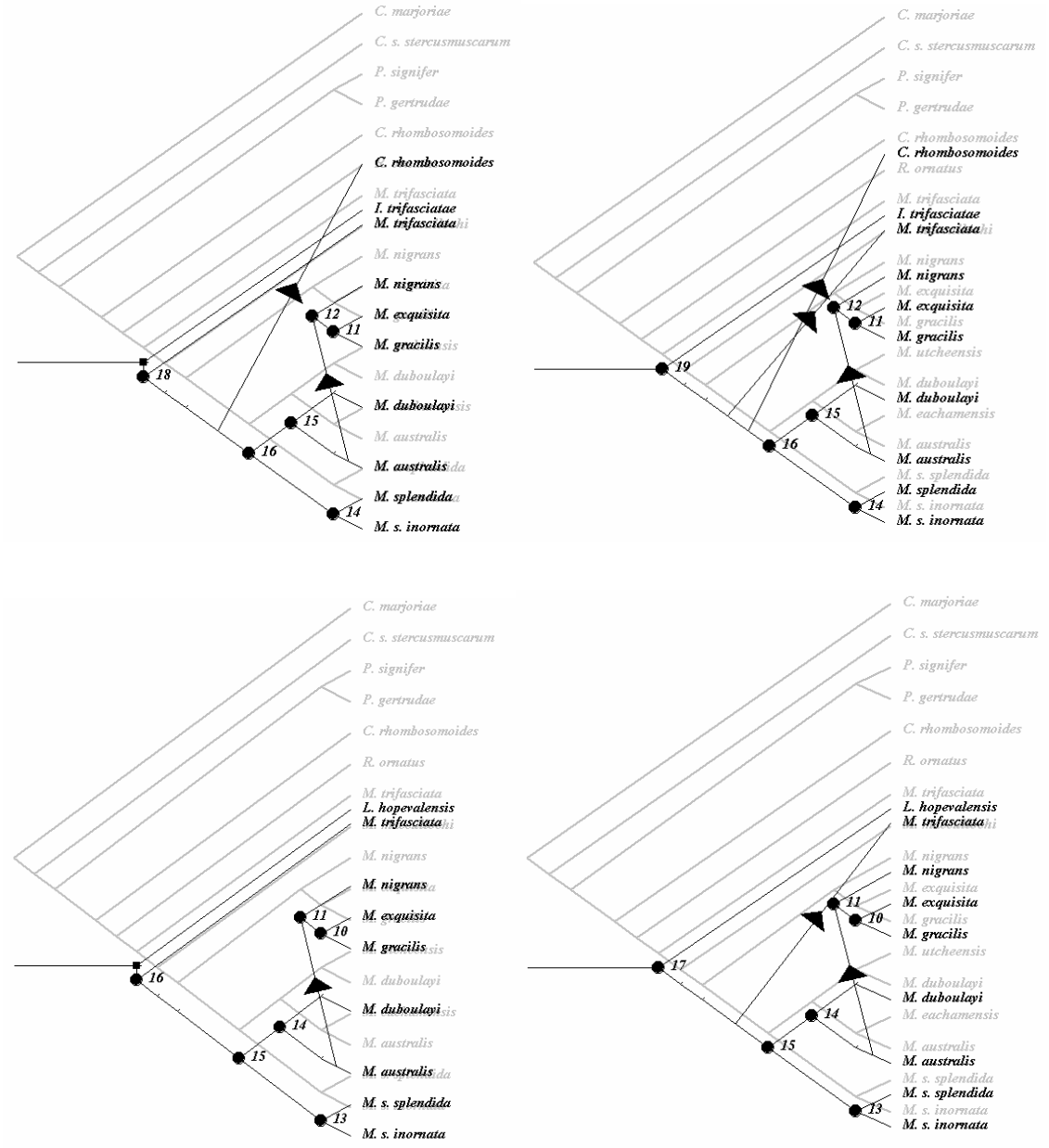


Figure 9.15. Most parsimonious reconciled trees of cospeciation events inferred from parasite intraspecific variation of *I. iliocirrus* (top) and *L. auripontiformis* (bottom) associated with host species infected.
Least *ad hoc* costs are incurred when *L. auripontiformis* and *I. iliocirrus* are treated as host switches/addition on *M. trifasciata* (right). Black dots represent cospeciation nodes. Black square represents a duplication event (sympatric speciation). Arrows represent host switch/addition. Bold type represents parasite phylogeny while grey type represents host phylogeny.

9.7 Discussion

In my study, cospeciation is frequent between hosts and their associated parasites and two forms are identified. So called "synchronous cospeciation" (which could also be delayed cospeciation disguised by the passage of evolutionary time) explains the association between parasite species and all hosts basal to and including *M. maccullochi* (Figure 9.13). Delayed cospeciation (parasite lagging behind host) best explains the association between parasite species and hosts in lineages more recently evolved than the *M. maccullochi* lineage, as exemplified by the congruence of the parasite phylogenies, inferred from intraspecific for the species *I. iliocirrus* and *L. auripontiformis* and the host phylogeny. The alternative, host addition, would result in an extra 34 *ad hoc* assumptions. Cospeciation events were also replicated in species of all four parasite genera. Two major speciation events were identified among parasites infecting species of *Melanotaenia*; the first with the "*goldiei*" (*M. trifasciata*) clade and the second with the "*nigrans*" clade. Parasite speciation events are strongly associated with the host mtDNA clades; each host speciation node being associated with a new parasite or set of parasites.

Generally host clades identified using DNA (Zhu *et al.*, 1994) are congruent with host morphological (Schmida, 1997) and parasite clades. The exception is *M. maccullochi*. Mitochondrial DNA groups *M. maccullochi* within the "*splendida*" clade while morphology places this species in its own clade. The presence of mono-host-specific parasites on *M. maccullochi* and the absence of them from sister species, together with the absence of the two widespread species, would appear to favour the latter.

Melanotaenia maccullochi was sampled from two locations. In the McIvor R, *M. maccullochi* does not co-occur with *M. s. splendida* and no parasite species were common with *M. s. splendida*. This is the only location identified so far that has *H. maccullochi*. For the second sample site, Corduroy Ck., both host species are present and *M. maccullochi* has two species common to *M. s. splendida* at the same site, namely *H. splendidae* and *H. megaloanchor*. Samples of *M. maccullochi* from other regions where subspecies of *M. splendida* are not present need to be examined to confirm or refute host addition of *H. splendida* and *H. megaloanchor*. If host addition is accepted, then *M. maccullochi* and its affinity with the "*splendida*" clade is uncertain as discussed later.

Several *a posteriori* assumptions were used to clarify the history of host-parasite associations. Host addition was included as a category, separate from host switching, to clarify some of the host parasite associations and the reason for this needs to be addressed. Sympatric speciation is considered a rare event and likely to be rare in hypothesised host-parasite associations. TREEMAP inferred much *ad hoc* duplication because of its inability to cope with host addition to older sister or basal lineages (see reconciled trees for *Helicirrus*, Figure 9.8) resulting in the inference of a low number of cospeciation events. An example is the association of parasite speciation events with *R. ornatus*. The inferred occurrence of three sympatric speciation events on a single host species may be possible, but seems highly improbable.

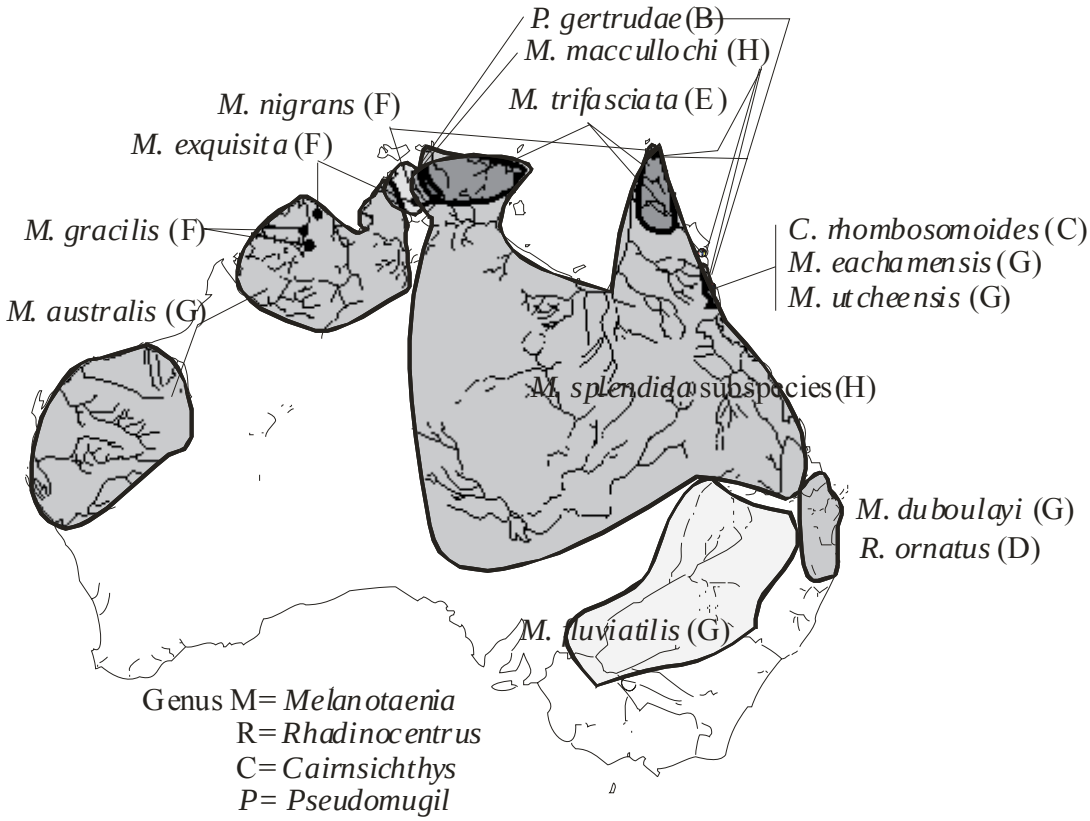


Figure 9.16. Host species distributions showing their disjunctions.
Letter after species name indicates mtDNA species clades; B. "*Pseudomugil*", C. "*rhombosomoides*", D. "*ornatus*", E. "*goldiei*" (*trifasciata*), F. "*nigrans*", G. "*australis*"; H. "*splendida*".

Host additions of parasites on *M. trifasciata*, *M. maccullochi* and *C. rhombosomoides* were suggested to be the result of a close geographical association possible through later invasion of a derived species. This seems plausible since there is growing evidence that *M. s. splendida* invaded its current geographic distribution much later than other host species (Coventry *et al.*, 1980; Crowley & Ivanstoff, 1991; Hurwood & Hughes, 2001; McGuigan, 2001; Unmack, 2001). Evidence for this includes the relict populations of the "*australis*" and "*goldiei*' clades, the *M. maccullochi* distributions associated with the relict populations of *Pseudomugil gertrudae* and *M. nigrans* (see Figure 9.16) and the widespread distribution of *M. splendida* subspecies.

Melanotaenia maccullochi has been grouped with the "*splendida*" clade (DNA) or has been recognised as a separate clade (morphology). As mentioned above, parasite associations suggest the latter. When alternative positions on the phylogenetic tree were examined, a more parsimonious reconciled tree was identified if *M. maccullochi* is placed between the "*goldiei*" and "*nigrans*" clades. This incongruence between DNA and morphology may be due to introgression of mitochondrial DNA from *M. s. splendida* into *M. maccullochi*. Similar occurrences have been identified between *M. s. inornata* and *M. exquisita* (see McGuigan, 2001) and between *M. gracilis* and *M. australis* (Unmack, pers. com).

The sympatric speciation of *L. hopevalensis* and *L. auripontiformis* and of *I. iliocirrus* and *I. trifasciata* are associated with parallel changes in copulum shape. For species of both genera, thick copulum shafts are the common state. However, for the two species mono-host-specific to *M. trifasciata*, copulum shafts are clearly thinner. This difference in copulum morphology may represent sympatric speciation resulting from character displacement and reproductive segregation. The alternative explanation of the parasite associations with *M. trifasciata*, host addition, was tested and resulted in two further cospeciation events and a reduction in costs.

In Chapter 5, I suggested that phylogenetic signal may be present in microvariation of haptoral sclerites associated with host species. This resulted from the need for the parasite to adapt to and track changes in the host attachment structure as these evolved in the radiating fish lineage. From the analysis of the two widespread species (*L. auripontiformis* and *I. iliocirrus*, Section 9.6), this appears to be a valid approach to

examining coevolution. Intraspecific variation, associated with host infected, inferred that multiple low-host-specific parasites can be used to examine phyletic relationships. This was exemplified by the mirroring of the two parasite phylogenies and high level of cospeciation identified with the host phylogenies. However, there are limitations to the more general application of this method. Temperate parasite species show seasonal sclerite variation and hence difficulties of removing this environmental correlation from true host phylogenetic signal would be difficult. Rarely are congeneric host species isolated from each other in regions elsewhere in the world as is common for Australian hosts. Thus intermixing of parasite component populations would reduce the chance of peripheral isolation mechanisms of widespread species.

To sum up, cospeciation is common between atheriniform fishes and their monogenean parasites; interspecific morphometric variation of haptoral sclerites can be used to identify synchronous cospeciation in more basal lineages; delayed cospeciation is evident in the more modern host lineages, which can be resolved using intraspecific variation of parasite species: two major parasite speciation events were identified.

In summary, phylogenies of hosts and parasites were compared. That of the host was adapted from published information. For parasites, phylogenies were inferred, at both inter and intra-specific levels, using morphometric data from haptoral sclerites. Apparent cospeciation between Australian atheriniform fishes and their monogenean parasites is more common in older lineages than in more recently evolved lineages. Two major parasite speciation events were identified, one co-occurred with the speciation event that produced *Melanotaenia trifasciata* and the second with the speciation event

that produced the "*nigrans*" clade. Delayed cospeciation is also evident and is reflected in the distributions of *Longidigitis auripontiformis*, *L. gracilis*, *Iliocirrus iliocirrus*, *Helicirrus splendidae* and *H. megaloanchor* on their hosts.

CHAPTER 10 GENERAL DISCUSSION

In Chapter 1, I asked, “does a phyletic association exist between monogenean parasites and their rainbowfish hosts?” Before this can be answered, aspects of the parasite taxonomy, specificity, interspecific associations, host-parasite associations and host behaviour needed to be addressed.

10.1 Taxonomy

Any study of evolutionary associations between unrelated organisms requires accurate taxonomy. Twenty host species from the Atherinidae, Melanotaeniidae and Pseudomugilidae were examined for monogenean gill parasites. In Chapter 4 and Appendix 1, I recognised and described four new genera and 19 species of monogeneans, while in Chapter 5 the taxonomy was reinforced by a morphometric study. What was the basis for the separation of species and their grouping into genera? Both haptoral and copulatory apparatus structure are recognised as important in defining genera and congruence was shown between the two approaches. However, reproductive morphology appears the most natural taxonomically for grouping species into relevant *Helicirrus*, *Recurvatus, Iliocirrus* and *Longidigitis*. Four types of copulatory apparatus were recognised, each regarded as diagnostic for one of the genera (Section 4.3.1.1). A comparison was made to see if morphometric variation of the haptoral structures also grouped specimens into their respective genera using the statistical techniques of PCA and DFA and clusters were clearly identified and recognised *a posteriori* and *a priori* respectively, as the parasite genera identified by copulatory apparatus morphology. An extended analysis examining 2139 parasite specimens from multiple hosts and sample sites confirmed genus groups with 97.4% correctly classified (Section 5.3.1.2). All

individuals *Longidigitis*, *Iliocirrus*, *Recurvatus* and all individuals of *Helicirrus* with similar length hooks and reduced dorsal anchors were correctly classified to genus. Thus, the genera defined by copulatory apparatus type can also be defined using only morphometric variation of haptoral sclerites. However, no single, or set of haptoral sclerite characters is unique to each genus. Instead, separation is by a set of broadly recognised morphometric variations of characters defined by PCA and DFA. Individuals of *Helicirrus* species possessing elongated hooks and well-developed dorsal anchors were mis-classified as belonging to the genus *Recurvatus*. This represents a break from the association between the haptoral structure and the copulatory apparatus structure. As discussed in Section 4.4, this is most likely an adaptation to a different attachment site due to changes in haptoral structure, hence, *Helicirrus* should not be split based on haptoral structure. Whether elongated hooks represent an apomorphic or plesiomorphic state or homoplasy is discussed later. From the evidence presented above, I consider copulatory apparatus structure more important than haptoral structure for grouping into genera the species described in this study.

All species were clearly defined using PCA and DFA and individuals were identified correctly by cross-validation, except individuals of *Iliocirrus* species. I suggested in Chapter 4 that the nominal species of *Iliocirrus* could be variants of a single polymorphic species, since little apparent variation of copulatory apparatus or haptoral morphology was present. However, species recognition is confirmed (see Section 5.3.1.2) using cross-validation of individuals examined from multiple host species and sample sites with 96.2% of 1077 specimens classified into their correct species. These species are also validated since several sample sites had more than one species present (eg. Barron R., Ithica R.), while some host species had more than one of these species

present (eg. *M. trifasciata* and *"M. eachutchee"*). The highest level of mis-classification was for individuals of *I. trifasciatae*, this species being grouped with *I. iliocirrus*. However, these two species, based on copulatory apparatus structure, are easily separated. Does the co-occurrence of these two species on *M. trifasciata* represent sympatric speciation or host addition? This is discussed later. Because of this work, I am confident that the taxa I have named are real biological entities suitable for the study of phyletic associations.

10.2 Host-Parasite Specificity

Host specificity is an important consideration in studies of phyletic associations. Most parasite species (12 of 19) showed mono-host-specificity (Section 7.6.4). However this may only be a reflection of the sampling intensity since only a small number of the known species were examined from each host family (see Section 1.7.1) especially for Pseudomugilidae and Atherinidae. Sixty percent of parasite species were restricted to one host species, 90% were restricted to one genus and 100 % to one family. Corresponding values from Rohde (1993) were 78%, 89% and 96% respectively. This specificity, at the genus and family level, is comparable to that observed by Rohde (1993). However, at the host species level, this study has identified a much lower specificity that may be explained by several factors. I suggested a low sampling intensity in published studies as a cause of apparent high host specificity of marine monogeneans (Section 1.5). Nearctic freshwater fishes, an intensely studied group, show a much lower specificity: approximately 50% of ancyrocephaline monogenean species infect multiple host species (pers. database). Poulin (1992) also found a lower level of host specificity at the species level (65%) The value is slightly lower for parasites of host species of Centrarchidae (46%) (pers. database). This is comparable to

the specificity identified for parasites from the Melanotaeniidae (50%). It might be instructive to explore the literature published since Rohde's work to see if host-specificity estimates have changed with recent additional studies.

The lower specificity identified in this study may also be due to the recent evolution of the host groups examined (Section 1.7.1) suggesting parasite speciation is lagging host speciation (Chapter 9). A similar lag in speciation is also suggested for monogenean parasites on cichlid fishes from Africa (Section 1.2.). Thus, the higher level of specificity in marine monogeneans implies that marine host species evolved much earlier than host species from freshwaters. This would allow parasite speciation to catch up. However, earlier evolved host species may be more prone to the possibility of host addition by parasites, thus negating the effect of catch-up speciation. If parasite speciation does not lag host speciation, then a high degree of host addition is needed to explain the pattern.

Many situations occur where a host addition by congeneric parasites is possible. Among the Melanotaeniidae species, *M. trifasciata* co-occurs with *M. s. splendida* (McIvor R.), *M. inornata* (Cape York Peninsula) and *M. exquisita* (Moline Rockpool); all species are infected with *L. auripontiformis* and *I. Iliocirrus.* However, the three mono-host-specific species infecting *M. trifasciata* do not occur on other rainbowfish from the same sample sites. Similarly, *H. megaloanchor* does not occur on *M. trifasciata* but is present on sympatric *M. s. splendida* and *M. s. inornata*, while *L. gracilis* found on *M. exquisita* does not infect *M. trifasciata.* Similarly, the mono-host specific species infecting *M. maccullochii* have not transferred to sympatric *M. s. splendida* (Corduroy Ck.). Although I recognised a general absence of host addition, possible exceptions

were presented and used as *a posteriori ad hoc* assumptions for assessing cospeciation (Chapter 9).

Two scenarios, host addition or sympatric speciation, are possible for *L. auripontiformis* and *I. iliocirrus* infecting *M. trifasciata*. These two species have congeners, *L. hopevalensis* and *I. trifasciatae* specific to *M. trifasciata* and show few morphological differences between their haptoral structures. However, differentiation of the copulatory structure is clearly evident. The mono-host-specific species, *I. trifasciatae* and *L. hopevalensis*, have employed the same strategy of differentiation, having thin copulum shafts as opposed to thick copulum shafts for their sympatric congeners. If allopatric speciation produced this difference, then host addition has occurred. However, if sympatric speciation has occurred then a permanent association is indicated. Sympatric speciation has been considered more probable in monogeneans than other parasite groups (Brooks & McLennan, 1993; Kennedy & Bush, 1992) although allopatric speciation is considered the norm. Since sympatric speciation is not evident on any other host species and this model of speciation in Monogenea has never been documented (see Littlewood *et al.*, 1997) the most likely scenario is allopatric speciation and hence this may be considered a case of host addition. These host additions are discussed later.

Since I consider, for reasons outlined above, sympatric speciation and host addition rare in the host species examined, what does the varying host specificity signify? If delayed parasite speciation is assumed, then specificity may represent host group clades. In the Melanotaeniidae, the monotypic *Rhadinocentrus* and *Cairnsichthys* are infected with a mono-host-specific species while in *Melanotaenia* two species have mono-host-specific

parasites: *M. maccullochi* has two while *M. trifasciata* has three. In a host phylogeny, these species might be expected to form separate clades. This is shown to be true (see Chapter 9) and is discussed further (Section 10.6).

The need to sample host species from several sites is evident for *M. maccullochi*. This species has a number of isolated relict populations. Two of these were sampled and the parasite community structures were distinct. Host specimens from McIvor R. were infected with *H. maccullochii* while two other species *H. splendidae* and *H. megaloanchor* were absent. The reverse was observed in specimens from Corduroy Ck. As proposed in Chapter 9, *M. maccullochi* should be placed in a clade distinct from the "*splendida*" and the association with *H. splendidae* and *H. megaloanchor* are the result of host addition, resulting from the invasion of *M. s. splendida* (Section 10.7).

10.3 Host Size and Parasite Associations

Host specificity has been correlated with host length (Sasal & Morand, 1998) where parasites with low host-specificity are more common on smaller hosts while highly host-specific parasite species are more common on larger fishes (Sasal *et al.*, 1999b). The opposite association was evident in the current study. The smaller host species *P. gertrudae*, *P. signifer, R. ornatus* and *M. maccullochi* were infected by mono-host-specific parasites while the largest host species (eg. *M. s. splendida*) were infected with parasites of low host-specificity.

Host body size has often shown a positive relationship with parasite abundance and richness of parasite species infecting a host (Barse, 1998; Chubb, 1979; Guégan & Hugueny, 1994; Kim *et al.*, 2001; Matejusová *et al.*, 2000; Morand *et al.*, 1999). Two

explanations have been proposed (Guégan & Hugueny, 1994). These are the effect of passive sampling of parasite species with increasing gill surface areas and host age.

In this study, parasite abundance and intensity was weakly correlated with host length when all host species were examined together, but this correlation was not present when each host species was analysed separately. This lack of correlation may be due the constraints of available gill surface area or may also be related to the evolutionary history of the host and parasites.

Identifying the pattern of parasite distribution on a host population is important in assessing the sampling effort needed to identify the degree of host specificity and species numbers. Typically, parasites are aggregated among host individuals; most host individuals have few or no parasites while a few host individuals have many (Section 1.6.1). However, I found that the number of parasite species occurring among individuals of each host were random or regular (Chapter 7). The same pattern was observed for parasite species intensities at the metapopulation level. This has implications for future sampling designs as discussed (Section 1.5.1). Consequently, a small number of host individuals should yield the full range of monogeneans.

This departure from an aggregated distribution might be attributed to host gregariousness (Section 1.6.1) and this may be explained by metapopulation theory (see Section 7.8). Therefore, extinction in an individual host is compensated by immigration of parasites from other host individuals within a school. I predict that non-aggregated parasite distributions and high prevalence are also present in aquaculture populations where there is a close physical association between hosts. Among melanotaeniids, I

noted that habitat types where individuals were highly concentrated had the highest parasite intensities, while hosts from small fast flowing streams, which would tend to disperse the hosts and swimming parasite larvae, had a low parasite load.

10.4 Inter- and Intra-Specific Associations

Identifying parasite species associations and the causal mechanisms is important for understanding host specificity and phyletic anomalies. Community structures of parasites or free-living animals have predominantly neutral or positive overall associations (Lotz & Font, 1991, 1994; Schluter, 1984). Here, positive associations between parasite species were identified at several levels. The hierarchy of parasite species abundance was the same for all host species infected with the five most common parasite species (Section 7.6.2): *I. Iliocirrus*>*L. auripontiformis*>*L. gracilis*> *H. splendidae*> *H. megaloanchor*. High positive associations of intensity were also observed, of which the strongest was between *I. iliocirrus* and *L. auripontiformis*. For *L. gracilis* and *L. auripontiformis*, a slightly lower R^2 value with high significance was observed. The association between *I. iliocirrus* and *L. gracilis* was the weakest but still highly significant. These associations appear to indicate that, once *I. iliocirrus* infects a host, infection by other species becomes possible, perhaps via an immune-suppression effect. However, the high degree of prevalence of infection (95%) appears to indicate that immunity to infection is apparently very weak or absent. Competition between parasite species is usually reflected by negative associations. However positive associations and competition can co-occur. The dominance of *I. iliocirrus* in terms of intensity of infection over all other parasite species suggests competition for a resource (e.g. food, attachment site, etc) between monogenean parasite species. Nevertheless,

differences in fecundity may also explain this and this would need to be confirmed by experiment.

Strong positive correlations between parasite species were also present for sclerite size. Increases in size for the same sclerite character were highly correlated between the two species *L. auripontiformis* and *L. iliocirrus*. This may be due to competition between the two parasites where bigger size produces a competitive advantage. It may also be due to an inverse correlation between the number of parasite species infecting an individual host and sclerite size; this association was observed. The smaller sclerites and possibly body size could reduce competition between parasite species for space. A third possibility is a host-induced phenomenon related to body length where larger sclerites are needed for larger gills. This phenomenon was observed when all host-species were analysed together but was highly reduced or absent at the individual host-species and sample site level (Section 6.3.2).

10.5 Specificity and the Identification of Host Hybridisation

The comparison of parasite host-specificity, abundances and intensities of infection on host species has been proposed as a possible method for the identification of host species and of hybrids (Chapter 8). My preliminary study of *M. s. splendida, M. eachamensis* and *M. utcheensis* from the Atherton tablelands identified a possible application of this approach. Pure *M. s. splendida* were characterised by the presence of *L. auripontiformis* and *I. Iliocirrus* and the absence of *I. mazlini* and *L. gracilis*. Pure strains of *Melanotaenia eachamensis* were infected with *L. gracilis* and *I. mazlini* but were not infected with *L. auripontiformis* or *I. iliocirrus*. Hybrids are most easily identified by the co-occurrence of *I. mazlini* and *I. iliocirrus* since these were the most

common species. *Melanotaenia utcheensis* and *M. eachamensis* could not be separated by the presence of a mono-host-specific parasite species. However, a difference in species abundance was observed. The parasite species *H. splendidae* and *L. gracilis* were more abundant than *I. mazlini* on *M. utcheensis*, while *I. mazlini* and *H. splendidae* were most abundant on *M. eachamensis*. There is however, a need for a greater sampling of *M. utcheensis* since only a single sample site was examined. If this method of host species identification is confirmed, then it represents a method complementary to molecular studies. An advantage of using parasites for host identification is that cost is low since often only a single host specimen is needed, as prevalence of infection is usually 100% (Section 7.6.1).

10.6 Host Phylogenies and Parasite Specificity on Hosts

To what extent does the spectrum of hosts infected by each parasite species reflect the phylogenetic relationships among of the hosts? In Sections 7.6.4 and 7.7, I examined parasite species associations and several patterns were identified between the taxonomic levels of the hosts and their parasites. At the host family level, species of Atherinidae and Pseudomugilidae were infected with species of *Recurvatus* and of *Helicirrus* possessing the elongated hook type of haptor. Melanotaeniidae was typically defined by the presence of species of *Longidigitis*, *Iliocirrus* and *Helicirrus* possessing the reduced hook form of haptor (see below).

Parasite specificity did not mirror host species phylogeny, instead specificity generally reflected the major clades observed in the host phylogeny (Section 7.7). Seven melanotaeniid species clades are recognised: "*rhombosomoides*", "*ornatus*", "*goldiei* (*trifasciata*)", "*maccullochi*", "*nigrans*", "*australis*", and "*splendida*". Based on

morphometric categorisation six clades are recognised (from mtDNA, *M. maccullochi* is placed in "*splendida*") (Section 1.7) (Table 10.1). There is a strong congruence in the relative positions of host clades, using parasite presence/absence, and the host phylogeny. Host species (parasite presence/absence) generally clustered in their correct expected phylogenetic clade. The monotypic *Cairnsichthys* and *Rhadinocentrus* were infected with mono-host-specific parasites. *Melanotaenia* species were generally identified by the presence of three low-host-specific species (*H. splendida*, *L. auripontiformis* and/or *I. iliocirrus*) (Figure 10.1). Mono-host-specific parasites separated the "*goldiei*" (*trifasciata*) clades from the other species. The disjunct clades "*australis*" and "*nigrans*" were infected with *L. gracilis* while absence of this species grouped host species as the clade "*splendida*". The "*australis*" and "*nigrans*" clades are separated by the presence of *H. megaloanchor* on the former; however, this division is not very strong due to their low abundance in host populations.

Table 10.1. Morphological and mtDNA clades recognised for species of Melanotaeniidae

Host sp.	*Morphological clade (Schmida, 1997)*	*mtDNA clade (McGuigan et al., 2000)*
M. gracilis	"*nigrans*"	"*nigrans*"
M. exquisita	"nigrans"	"*nigrans*"
M. nigrans	"*nigrans*"	"*nigrans*"
M. australis	"*nigrans*"	"*australis*"
M. eachamensis	"*australis*"	"*australis*"
M. utcheensis	"*australis*"	"*australis*"
M. duboulayi	"*australis*"	"*australis*"
M. trifasciata	"*goldiei*"	"*goldiei*"
M. maccullochi	"*maccullochi*"	"*splendida*"
M. s. splendida	"*splendida*"	"*splendida*"
M. s. inornata	"*splendida*"	"*splendida*"
C. rhombosomoides	"*rhombosomoides*"	"*rhombosomoides*"
R. ornatus	"*ornatus*"	"*ornatus*"

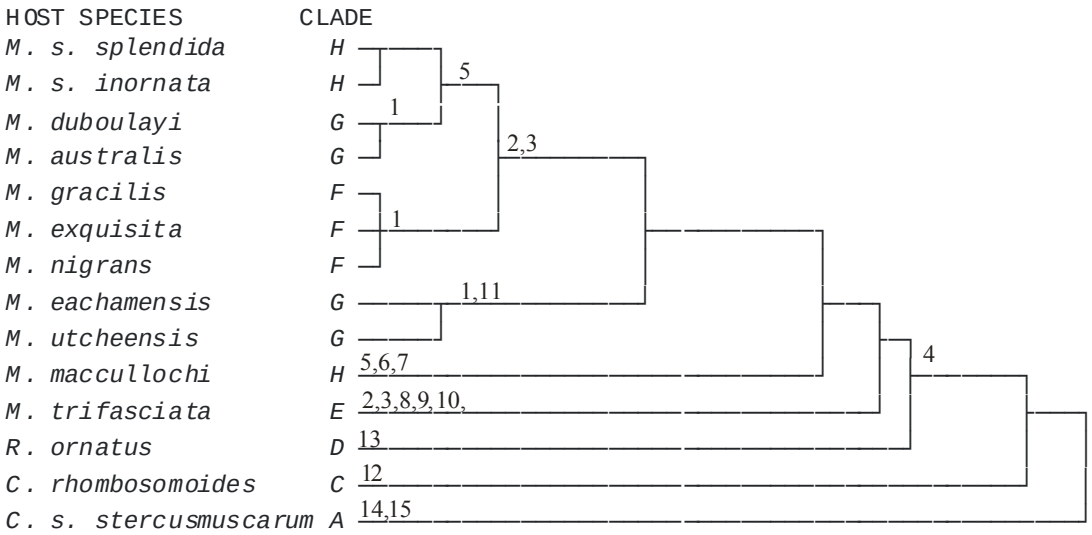


Figure 10.1. Associations between host species using parasite species presence/absence?. Parasite species 1. *L. gracilis*, 2. *L. auripontiformis*, 3. *I. iliocirrus*, 4. *H. splendidae*, 5. *H. megaloanchor*, 6. *L. maccullochii*, 7. *H. maccullochii*, 8. *L. hopevalensis*, 9. *I. trifasciatae*, 10. *H. mcivori*, 11. *I. mazlini*, 12. *L. utcheei*, 13. *I. ornatusi*, 14. *I. rossi*, 15. *R. chelatus*.

Two exceptions were present: *M. maccullochi* did not cluster in the "*splendida*" clade while *M. eachamensis* and *M. utcheensis* did not cluster in the "*australis*" clade. These exceptions may reflect cases of host addition, host switching or an anomaly in the host mtDNA phylogeny. These exceptions are discussed further in the following section.

The identification of ancestral and derived structures is needed in order to present a plausible evolutionary history of speciation for hosts and parasites. Looking at species of *Helicirrus* two types of haptoral morphology are recognised (Section 4.4). The question is which one is considered plesiomorphic. Elongated hooks were considered plesiomorphic using the argument that common characters are primitive and rare characters are advanced (Section 4.4). Therefore, those host-groups infected with *Helicirrus* spp. having the primitive form are considered basal to those host species infected with *Helicirrus* spp. having the advanced haptoral form. Determination of

parasite species groups as ancestral or derived used parasite specificity as criteria (Section 9.2). Those parasite species with high host-specificity are regarded as more primitive than parasite species with medium or low host-specificity. This is implied from the concept of delayed parasite speciation.

Using the arguments presented above, parasite groups can be ranked in order from primitive to advanced and the associated host groups are classified accordingly. The host families Atherinidae and Pseudomugilidae are basal to Melanotaeniidae (Section 9.2). *Cairnsichthys*, *Rhadinocentrus* and the species clades '*goldiei*' (*M. trifasciata*) and '*maccullochi*' are considered basal to the '*splendida*', '*australis*' and '*nigrans*' clades. Because of the presence of *L. gracilis* among the disjunct '*australis*' and '*nigrans*' clades and the widespread continuous distribution of the '*splendida*' clade, the last host clade is considered derived. Molecular studies identified *M. maccullochi* as belonging to the "*splendida*" clade while morphological studies identified *M. maccullochi* as being separate from the "*splendida*" clade. Parasite specificity infers host morphology separation.

Within the Melanotaeniidae an evolutionary scenario is presented (Figure 10.2). The disjunct '*australis*' clade predates the '*splendida*' clade and the two are allopatric. A major isolating event such as an extended dry period (related to lower sea levels, or a rise in sea level which flooded central Australia) could have allowed, from a single host ancestor, the speciation of *M. duboulayi* from S.E. Queensland, *M. eachamensis* and *M. utcheensis* from the Atherton Tablelands in N. Queensland and *M. australis* from the Kimberley region in Western Australia. Subsequently when freshwater became available throughout Northern and central Australia, the invasion of the "*splendida*"

progenitor from the Lake Carpentaria region occurred. The invasion across the Atherton Tablelands produced the admixture lineages of '*splendida*' and *M. utcheensis* (see Hurwood & Hughes, 2001; McGuigan, 2001).

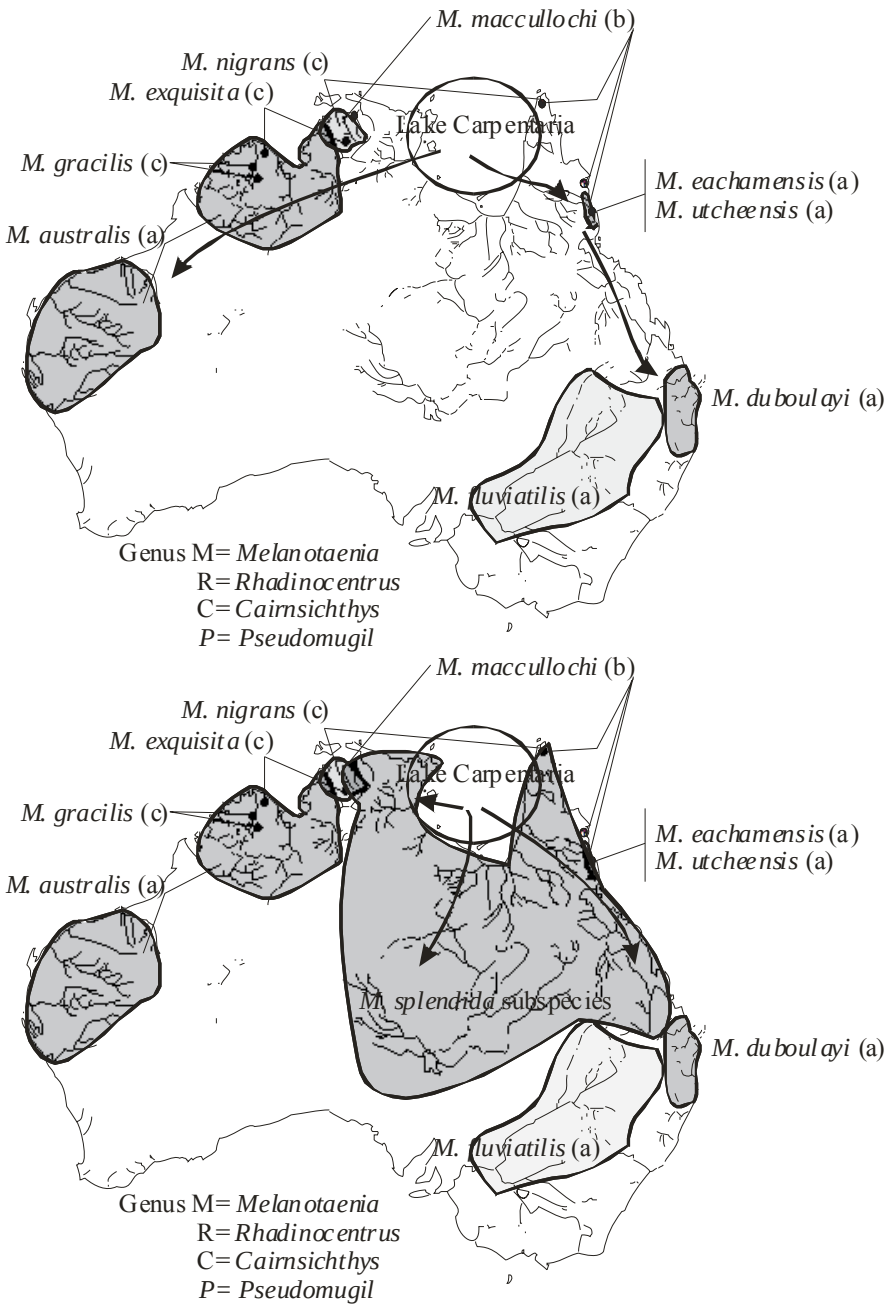


Figure 10.2. Invasion of Australia by *Melanotaenia* progenitors and subsequent speciation. Top. Speciation of "*australis*" clade. Bottom. Speciation of "*splendida*" clade after vicariance event allowing speciation of hosts forming "*australis*" clade was removed.

Several unique aspects of the Australian teleost fauna are identified and these present an interesting situation for the study of host-parasite associations. The teleost fauna of Australia is atypical of fauna from other continents, as the majority of species do not occur in sympatry with their closely related congeners (Unmack, 2001). For example *Craterocephalus* spp. often are sympatric, but the congeners are distinct lineages (Crowley, 1990): species within a single sub-generic lineage are never sympatric. The same situation is found for the species of Melanotaeniidae (McGuigan, 2000). Unmack (2001) has suggested that if congeners come into contact, sympatry is temporary, ending in extinction of one or merging through hybridisation. This segregation of congeners limits the opportunity for parasite host-switching or host-addition.

In this study, I examined only fourteen, all from Australia, of the sixty-five known rainbowfish species. Yet several clear patterns were evident. Based on the strong associations of the parasite species with the host phylogenetic clades a prediction can be made regarding the composition of the parasite communities of rainbowfishes in New Guinea where a further 40 (approx.) species are found. I predict very few additional parasite species will be identified from fishes from New Guinea. The parasite species *H. splendidae I. iliocirrus* and *L. auripontiformis* will be found on all *Melanotaenia* species of southern New Guinea which group with the "*splendida*" clade identified by molecular phylogenies. The *Glossolepsis* clade will be identified by a set of undescribed *Iliocirrus*, *Longidigitis* and *Helicirrus* species. The *Helicirrus* species will have the primitive type haptoral structure, possessing elongated hooks and well developed dorsal anchors. Species of this clade may also host a *Recurvatus* species indicating a link to the Atherinidae and Pseudomugilidae. *Melanotaenia* species from Northern New Guinea will be defined by undescribed species of *Iliocirrus* or *Longidigitis* or both and possibly

a new species of *Helicirrus* most likely with a primitive haptor. For *Melanotaenia* species from western New Guinea a similar parasite situation is likely.

10.7 Host Phylogenies and Morphometric Variation of Parasite Haptor

In any discussion of phylogenetic association, a phylogeny of both taxa of associates is required. For the host molecular trees, I discussed above the notion that host clades can be identified by the distribution and specificity of parasite species. However, another method is required to look at host speciation events. I have introduced the use of morphometric variation for the identification of phylogenetic signal in parasites. Using morphometric patterns of association among parasite species and of species with low specificity, clustering patterns are produced that are similar to those of the host (molecular phylogeny). Cospeciation was recognised between parasite species and host clades basal to the "*maccullochi*" clade (interspecific variation used) while delayed cospeciation is suggested for low-host-specific parasite species infecting the "*splendida*", "*australis*" and "*nigrans*" clades (Section 9.7) (intraspecific variation used).

The use of inter and intra-specific morphometric variation of haptoral sclerites for preparing a parasite phylogeny is novel. This new approach may not be appropriate for other studies although several studies have identified host-associated morphometric variation (Section 6.1). The analysis of intra-specific host-associated variation requires modifications of traditional techniques. Because intra-specific variation rarely produces significant differences in a variable, discriminant function analysis was used to concentrate the sclerite variation associated with a host species. I then used the new eigenvectors to produce a phylogeny of associated variation. The two species *I.*

iliocirrus and *L. auripontiformis* used for this type of analysis produced a similar pattern of host association, which also corresponded with the host clades recognised using molecular analysis. Why does morphometric variation of the haptoral structures show such a strong host phylogenetic signal?

The haptoral structure of the parasite requires an architecture that is compatible with a particular host attachment site. This is seen in the two architectural variations observed among the *Helicirrus* species. Species that attach to the gill filaments had well-developed anchors and hooks while species that attach to the gill rakers had reduced dorsal anchor structures and hooks. In addition, species of other genera that attach to the gill filaments had the well-developed dorsal anchor and hook architecture. For *H. megaloanchor,* a compensatory enlargement of the ventral anchor was suggested as a mechanism that allowed this species to attach to the gill filaments (Section 4.4.). Differences in gill structure should be small between sister species and large between distant lineages. Since attachment to the gill filaments requires a particular type of architectural structure then minor modifications within a single parasite species should also be required for different host species infections. The degree of difference is reflected as phylogenetic signal and is represented as morphometric variation.

10.8 Conclusions

Is a phyletic association present among rainbow fish and their monogeneans from Australian freshwaters? A strict one-host one-parasite cospeciation scenario is clearly not apparent; however, a high congruence between parasite and host clades is clearly present for distribution patterns of parasite species among hosts and morphometric

variation of parasite species occurring on many host species. This congruence may be explained by delayed speciation in the parasites, since the alternative, extensive host addition, should produce a high level of incongruence in host phylogeny especially for morphometric variation-derived phylogenies. Whether the patterns of congruence shown represent coevolution or phylogenetic tracking is uncertain (Section 9.6). There is clearly a parasite response induced by the host in the form of morphometric variation of sclerite variables. Coevolution may be present but the high prevalence of parasite infection appears to counter the idea of a parasite-induced host immune response. Thus, phylogenetic tracking seems to be the process controlling parasite speciation in this system.

I recognised in this study that the use of monogenean parasites and their hosts as a model for cospeciation, when using the assumption of high host specificity, is inappropriate. A much lower level of mono-host-specificity, than previously documented or assumed, has been detected which creates many problems for comparing and interpreting host and parasite phylogenies. Nevertheless, three approaches to aid such studies have been presented. The first is the distribution of parasite species among hosts. The second compares the phylogenies of parasite species generated from interspecific variation of the haptoral morphology with the host phylogeny and infers the history of cospeciation events. The third is the use of intraspecific morphometric variation of the haptoral sclerites of a parasite species possessing low host-specificity within a host genus. The methods produced clustering patterns that appear to reflect a rather high level of congruence with the host clades. These three methods however require a well-planned sampling design to enable collection of sufficient parasites from host species and sample sites. The measurement of morphometric variation is especially

time consuming (10-15min/specimen), however this type of analysis should be included in all taxonomic studies since between-population variation is an important part of defining species boundaries. I identified (see Section 7.6.4) that small sample sets from multiple sites would identify levels of host specificity better than a large sample set from a single site since, in this study, parasite community structures show a high level of homogeneity.

To conclude, if all the factors that have identified relationships between parasites and the host species are integrated what does the integration tell us about the coevolution of the monogenean species and the hosts? If the parasites have not evolved at the same rate, but appear to be one taxonomic level behind the host, then host phylogenetic clades can be identified by the presence of a particular parasite species. Host addition or switching does not appear to be relevant in the monogeneans studied here. Morphometric variation of the parasite haptor can be used in phylogenetic studies. Parasite distribution can reflect host phylogeny. Parasite species associations are strong, especially between low host-specific species. Host length does not appear to affect these interactions. It is clear that when examining phyletic associations of Australian teleosts and their parasites, the host family should be studied as a basic unit.