PHYLOGENETIC SYSTEMATICS AND THE EVOLUTIONARY HISTORY OF SOME
INTESTINAL FLATWORM PARASITES (TREMATODA: DIGENEA:
PLAGIORCHIOIDEA) OF ANURANS

by

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Abstract

Historical structuralism is presented as a research program in evolutionary biology. It uses patterns of common ancestry as initial hypotheses in explaining evolutionary history. Such patterns, represented by phylogenetic trees, or cladograms, are postulates of persistent ancestral traits. These traits are evidence of historical constraints on evolutionary change. Patterns and processes consistent with a cladogram are considered to be consistent with an initial hypothesis of historical constraint.

As an application of historical structuralism, a phylogenetic analysis is presented for members of the digenean plagiorchioid genera Glypthelmins Stafford, 1905 and Haplometrana Lucker, 1931. The eight species studied are intestinal parasites of frogs and toads in North, Central, and South America. In a Wagner parsimony analysis of 21 morphological characters with both the PAUP and PHYSYS computer programs, a single phylogenetic tree with a consistency index of 84.8% can be inferred. This suggests strong historical constraint in the evolution of the characters examined. It is postulated that the eight species form a monophyletic group (clade), consisting of two less inclusive clades. Glypthelmins hyloreus and G. pennsylvaniensis comprise one of these clades; G. robustus, G. shastai, H. intestinalis, G. californiensis, G. quieta, and G. facioi comprise the other. G. robustus, found in Bufo marinus in Colombia, is both the southernmost and the most plesiomorphic member of its clade. Glypthelmins

californiensis, G. quieta, and G. facioi form a clade, and parasitize frogs in the Rana pipiens complex in Mexico, eastern North America, and Central America, respectively. Glypthelmins shastai and H. intestinalis, the latter of which is the only member of its genus, form a western North American clade, and parasitize Bufo boreas and Rana pretiosa, respectively. The phylogenetic analysis includes a redescription of G. shastai, the synonymy of the genus Haplometrana with Glypthelmins, the redescription of H. intestinalis as G. intestinalis, an emended diagnosis of the genus Glypthelmins, and the first account of the life cycle of G. californiensis. Three aspects of phylogenetic analysis are examined in detail. These are the coding of multistate character trees, the use of parasite data to infer host relationships, and the properties of the Consistency Index and the F-Ratio. It is proposed that the Consistency Index be calculated without non-homoplasious autapomorphic characters. For the present study, this modification gives a value of 76.9%.

Using the phylogenetic tree as a general reference system of patterns of common ancestry, it is inferred from developmental studies that (1) there is no conflict between the phylogenetic relationships indicated by only larval or only adult characters, and that (2) the evolution of some of the characters involved certain types of heterochrony. Paedomorphic heterochrony is inferred to have occurred in the evolution of the uterus in G. shastai, H. intestinalis, G. californiensis, G. quieta, and G. facioi. Peramorphic heterochrony is inferred

to have occurred in the evolution of the penetration glands in
G. facioi, and of the hindbody in H. intestinalis. The
relatively longer hindbody of H. intestinalis was experimentally
induced to show paedomcrphic development by raising specimens of
H. intestinalis in Bufo boreas, which is the host of G. shastai,
its sister-species. By one year after infection, the relative
length of the hindbody is shorter, and is equal to that of the
primitive state found in G. shastai.

The phylogenetic relationships among the anuran hosts are
re-analyzed. There is 80% congruence between them and the
postulated phylogenetic tree for their parasites, suggesting
strong historical association between the parasite and host
groups. This inference of coevolution is further supported by
the concordance of the present geographical distributions of the
parasites and their hosts with the historical geology of the
areas in which they occur. This implies an historical
association between the areas and the organisms.

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I. INTRODUCTION

Despite their apparent complementarity, evolutionary theory and systematic theory have long been operating on different conceptual bases. That is, not only have they been interested in different things - the former in processes, the latter in patterns - but they have each been assuming the operation of different forces in the production of organic form. One reason for this disparity is that humans were categorizing organisms before there was any thought that those organisms might have evolved from one another. Plato, with his concept of natural kinds as immutable, discrete, and eternal essences, wanted to "cut nature at her joints". Others, such as Linnaeus, wanted to catalogue their god's handiwork. Regardless of the causative factor presumed, these efforts amounted to grouping organisms by phenetic similarity, that is, by overall similarity of form. It could have been little else, given that there was no concept of genealogical relationship. Systematic theory, then, has an ancestry of non-evolutionary thought. It is important to note that the concept of homology introduced by Owen (1848) was based upon similarity of form, and not upon evolutionary relationship. Homology did not acquire its evolutionary connotation until workers such as Darwin (1859), Haeckel (1866), Lankester (1870) and Gegenbaur (1878). Once this connotation was recognized, systematists began seeing homologues as indicators of kinship among organisms. Thus, the evolutionary relationships among organisms came to be inferred from structural criteria.

Evolutionary theory, on the other hand, has operated predominantly with functional criteria. I am referring to Darwin's theory, and its derivative, neo-Darwinism. For various historical reasons (see, e.g., O'Grady, 1984), these consider the primary force of evolution to be natural selection acting upon relative functional differences among organisms. Thus, while systematic theory considers structure before function, current evolutionary theory considers function before structure. This disparity creates a conflict of interest because it is systematic theory that reconstructs the genealogies whose origin evolutionary theory tries to explain. For most of the twentieth century, the result has been a systematics that attempts to represent both genealogical relationships (pattern) and degree of functional change (presumed process) at the same time, within the same evolutionary tree (see Mayr, 1981). This creates problems when the descendants of a common ancestor are split into two or more groups of equal taxonomic rank because of differences in their functional properties. Such an operation can obscure more relationships than it clarifies because it disrupts the hierarchical internesting of the pattern of inherited homologues. The apparent loss of pattern can direct evolutionary explanations away from structural properties and towards functional attributes.

Phylogenetic systematics is one attempt to base systematic theory on structural evolutionary terms. Developed by Hennig (1950; 1966), it has been refined extensively in the last twenty years (e.g., Camin and Sokal, 1965; Kluge and Farris, 1969;

Farris, 1970, 1982, 1985; Maddison et al., 1984; Swofford and Maddison, in review). Procedures for inferring homology have been improved, and there are now a number of computer programs capable of analyzing data sets quickly, efficiently, and in large volume.

This dissertation is offered as an application of phylogenetic systematics to the study of evolution. It begins with a preliminary chapter (Chapter II) that attempts to bring the concerns of evolutionary theory and systematic theory together into an approach called historical structuralism. This is offered as an ontological justification of phylogenetic systematics. Chapter III presents the empirical core of the dissertation, a phylogenetic analysis of eight species of digenean trematodes, a group of flatworms (Platyhelminthes) parasitic in vertebrates and invertebrates. The species are members of two genera in the Superfamily Plagiorchioidea Dollfus, 1930: Glypthelmins Stafford, 1905 and Haplometrana Lucker, 1931. They are intestinal parasites of anurans in North, Central, and South America. The analysis is based upon 21 morphological characters. The animals examined consisted of type material, specimens from private collections, and specimens from my field and laboratory studies.

The study of type material and the recent availability of additional specimens has allowed a redescription of Glypthelmins shastai. In addition, Haplometrana intestinalis is redescribed as G. intestinalis n. comb., and the genus Haplometrana is synonymized with the genus Glypthelmins. Field collections have

established new localities for some of the parasite species. Laboratory work has resulted in the first description of the life cycle of G. californiensis and the collection of various developmental stages of G. quieta and H. intestinalis. The phylogenetic relationships of the taxa are inferred with computer-assisted parsimony techniques, which construct genealogical trees by maximizing the number of postulated homologous series of characters. The phylogenetic tree, or cladogram, is then used as a general reference system with which to examine the occurrence of certain evolutionary events. This analysis is presented in Chapter IV.

Chapter IV begins with a demonstration of the congruence of the trees inferred from either the larval or the adult characters of the worms. Included in this section is a discussion of why some earlier evolutionary explanations, drawing implicitly or explicitly from concepts of Haeckelian recapitulation, did not expect this congruence to exist. This discussion leads into an examination of the evolution of the life cycles in the study group. Next, there is an analysis of the heterochronic development of certain morphological properties of Glypthelmins and Haplometrana. This analysis uses Fink's (1982) methodology for analyzing heterochrony from a phylogenetic perspective. It not only looks at three characters of the worms that are the possible result of different types of heterochrony, but it also reports on the experimentally-produced heterochronic development of the hindbody in one of the species, H. intestinalis.

The remaining sections of Chapter IV assess the extent of host-parasite coevolution, as well as the biogeographic and speciation patterns involved. For this study, I found it necessary to re-analyze the data of some earlier phylogenetic studies on the relationships of the anuran hosts. These comparisons are further demonstrations of the uses to which an hypothesis of phylogenetic relationships can be put. The digenean trematodes are good organisms for such a multi-level study because of the number of levels of relationships that exist. These are the result of the parasites' complex life cycles, in different environments, with an intimate association with different types of hosts.

Finally, this dissertation contains two appendices, F and G, which explore certain methodological procedures in systematics. Appendix F evaluates the strengths and weaknesses of the methods that can be used to represent the shape of an evolutionary tree with a numerical code, so as to allow various forms of computer analysis. Applications include multistate character trees (an evolutionary transformation series of more than two homologous character states) and the use of parasite phylogenies to infer host relationships. Appendix G examines the properties of two commonly-used optimality measures in systematics: the Consistency Index (Kluge and Farris, 1969; Farris et al., 1970) and the F-Ratio (Farris, 1972). These measures are used to assess the goodness-of-fit of a tree to the data. Each has what I believe are previously unnoticed properties that affect its performance with certain types of

character distributions. Some of these properties are shortcomings of the measures, and in an effort to avoid such problems, I describe the calculation of a modified Consistency Index.

II. HISTORICAL STRUCTURALISM AS A RESEARCH PROGRAM IN EVOLUTIONARY BIOLOGY

Any physical entity can be examined with or without consideration of the means by which it came to exist. An approach that takes etiology into account can be called historical, meaning that events that occurred in the past are considered to be of use in explaining current properties. The alternative perspective can be called nonhistorical. [1] Some questions, such as "What is it made of?" and "What does it do?" are nonhistorical; they are interested in the properties of the thing now. Other questions, such as "How did it come to be?" are historical. Still others, such as "What causes it to be as it is?" and "Why is it as it is?" look for either nonhistorical or historical answers, depending upon the completeness of explanation desired. (The use of "why" questions should be minimized because of the ambiguity of that adverb; see the section, "WHY" QUESTIONS.) Consider a window broken by a falling rock. The nonhistorical question is "What breaks the window?". The corresponding historical questions can address many levels of past causality, such as "How did the rock come to be moving?", "How did it come to be a rock?", and "How

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[1] O'Grady (1984) referred to the nonhistorical as "ahistorical". As noted in O'Grady (1986), it has since become necessary to distinguish between those views that simply do not consider historical phenomena in biology (nonhistorical), and those that actively deny their importance (ahistorical). Only the former is discussed here.

did gravity come to have such an effect upon rocks?"

With respect to biological systems, I use the term "history" in a phylogenetic sense. That is, I use it to refer to the evolutionary history of organisms. I do not use this term to refer to simple persistence through time, or to intragenerational change over time, such as that occurring during ontogeny. Ontogeny is, of course, a product of evolution. Nevertheless, I consider it separately here because, unlike phylogenesis, it need not necessarily be viewed as the product of such a process.

Historical and nonhistorical perspectives can be examined with respect to investigations of two basic organic properties: structure and function. I suggest that many disagreements over the validity of biological explanations are the result of insufficient delineation of these perspectives. In the following pages, I examine hierarchic causality in physical systems, and then use this framework to assess the explanatory strengths and weaknesses of structuralist and functionalist approaches. I hope to demonstrate the advantages to evolutionary biology of historical structuralism.

BIOLOGICAL SYSTEMS

Biological systems are specifically configured physical systems, and so they possess, minimally, the same physical properties as an inanimate object. Cats, after all, fall from heights just as rocks do. But there are also properties unique

to biological systems. The two with which I am concerned here are their functionality and their evolution.

At any moment of an organism's existence, a particular organization of its components must be maintained in the face of potentially disruptive forces (heat, cold, energy flows, etc.). Living systems must always be "doing" something. They cannot passively exist, as do inanimate objects. Any physical object has structure, and thus some degree of order. A living system must have its structure organized in a manner that performs particular functions that result in the continued existence of the organism. Organisms do not function in order to survive. Rather, it is trivially true that they will not survive if they do not function. Organisms must actively exist. If they die, their constituent elements will still exist and still possess basic physical properties - dead cats fall from heights just as live cats do - but the organisms will no longer have the organizational properties of living things.

The second property of organisms that is of concern here is their evolution. An ancestor-descendant continuum is established through reproduction. As this continuum is produced, whether intraspecifically or trans-specifically, ancestral properties become incorporated into the ontogeny of the descendants. Every new descendant, does not, so to speak, start from scratch. It inherits many of its properties from its ancestors, which inherited many of theirs from their ancestors - and so on. This has two consequences. The first is that a property that arises for the first time in a species (i.e.,

unique, and contingent upon previously unrealized circumstances) may become a fixed, inherited property in its descendants. Because of this, an investigation of an organism's existence must deal with more than questions about ultimate origins, that is, about the origin of life on earth. It is also necessary to ask questions about any subsequent evolution, for a series of events, spanning millions of years, is responsible for the organism appearing as it does now. A phylogeny is thus not a passive "trace" of organisms' existence through time, but a record of the relative times and levels of generality at which the causes of various organic properties evolved.

For example, a cat has three properties caused by three historical events during its evolution: it has inherited a nuclear membrane from the common ancestor of eukaryotes, it has inherited an internal skeleton from the common ancestor of vertebrates, and it has inherited fur from the common ancestor of mammals. Each of these properties originated as a unique, contingent character of a particular species, and each became an inherited character through the subsequent evolution of descendent species.

END-ATTAINING ACTIVITY

The functionality and the evolution of biological systems are often considered together, in that it is asked whether the fact that organisms must function in a particular manner if they are to survive has any causal connection with the means through

which they came to possess the structures that perform those functions. This introduces the subject of teleology, or goal-directed activity. It is at this point that a distinction between historical and nonhistorical explanations is important. To assist in this distinction, discussed in the next section, it is helpful first to recognize three types of processes that attain end-states.

First, natural entities or systems (i.e., not made by humans) may attain end-states because of the operation of processes whose existence depends not on the entity being alive or purposefully designed, but on the properties of its constituent matter. Some of these properties, such as gravity or entropic decay, operate universally. Others, such as radioactive decay and reaction gradients, are more restricted in their operation. All of this is teleomatic activity (Mayr, 1974; Wicken, 1981a,b). The end-state, such as a rock coming to rest at the bottom of a cliff, can simply be said to result from the properties of the entity. There is no "control" or "purpose" involved. I refer to this activity as end-resulting.

Second, there is the functionality of living systems that is dependent upon the operation of inherited genetic and epigenetic factors. These factors determine the end-states of processes such as homeostasis (maintenance of a species-specific physiological state), ontogeny (development into an adult of the species), and reproduction (production of new members of the species). This is teleonomic activity (Pittendrigh, 1958; Mayr, 1961, 1974). The end-states are reached because of inherited,

internal, controlling factors. It is end-directed.

The third category is that of purposeful behavior, in which certain outcomes occur because events were deliberately brought about so as to produce them. This behavior requires some degree of cognition. It is most prevalent in humans, and exists to various extents in other animals. The end-state is deliberately sought, and is therefore a goal (i.e., one type of end-state). O'Grady (1984) referred to this activity as goal-directed; it is perhaps better to call it goal-seeking (O'Grady, 1986), so as to make it clear that such behavior is the result of a consciousness capable of some amount of premeditation and choice. I think that only this type of end-attaining activity should be called teleological. My position is a departure from earlier usage of the term. Traditionally (see, e.g., Nagel, 1984; Wicken, 1985), teleology has been used as a general term for all activities that achieved an end - which was not distinguished from a goal. Thus, not only have the teleomatic and the teleonomic been considered to be types of teleology, but so, too, have biological function and organization. From this position, for example, Gray (1874) was correct in saying that Darwin had "wedded morphology to teleology" - simply because Darwin's theory deals with functionality. I suggest that such terminology interferes with the search for, and the study of, different end-attaining processes in biological systems.

With respect to Aristotle's concept of universal causality, which is in some ways responsible for much of the uncertainty over the status of teleological explanations (see, e.g., Nagel,

1984; Jaki, 1966), it seems that the following observations apply. (1) All three types of end-attaining activities have material causes (that in which change occurs) and efficient causes (that by which change occurs). (2) Teleonomic and teleological activity also have formal causes (internal representations: that into which something changes). (3) Only teleological activity also has final causes (that for the sake of which change occurs). Of course, the nature of the internal representations in teleonomic and teleological activity is quite different. In the former, it consists of inherited developmental factors; in the latter it is a cognitive act. Nevertheless, in contradistinction to teleomatic activity, it can be said that in both there is something within the organism that has a representation of the end-state and is involved in the taking of steps that attain that state.

While not all physical entities show all three types of end-attaining activity, those that do are a subset of those that do not (Fig. 1). All physical entities show some type of teleomatic activity; a subset of these also shows teleonomic activity (biological systems); and a subset of these also shows teleological activity (cognitive biological systems). Thus, while the act of a rock falling to earth is teleomatic (gravity) and the act of a kitten becoming a cat is teleonomic (ontogeny), the act of a kitten falling to earth is also teleomatic (gravity). To complete the example, a human parachutist teleologically reaches for the ripcord as his teleonomically-maintained body plummets teleomatically to the ground below.

If the etiology of the entities involved in this internesting is to be addressed, it is necessary to introduce an historical perspective. It is at this point that there is a risk of introducing inappropriate teleology - i.e., an inappropriate assumption of goal-seeking behavior - by incorporating the attainment of the end-state into the causal explanation for the entity coming to exist. Evolutionary theory seeks to provide mechanistic explanations for the etiology of organic structure and function, and to keep such ideas distinct from teleological explanations for the existence of man-made objects. Critics of evolution, such as William Paley (1802), blurred this distinction when they presented their arguments from design. Paley's teleology of the designer appealed to external, imposed causality. Hull (1973) has called this Platonic teleology. Others writers, such as those orthogeneticists who approved of teleology (discussed later), appealed to internal, immanent causality. Hull (1973) has called this Aristotelian teleology. I think that it results from mistaking formal causation for final causation. That is, the properties of the state into which something changes are seen as the reason for the change occurring. Later in this section I suggest that this line of argument can be partly attributed to an inappropriate analogy of evolution with ontogenetic development. Both Platonic and Aristotelian teleology assume that the existence of functionality in organisms is indicative, as it is in human works, of purpose. They assume that it must have come to exist for some reason.

For evolutionary theory, the recognition of teleonomic activity provides a mechanistic nonhistorical explanation for organic functionality, and thus deals with the "organic teleology" of design discussed by writers such as Russell (1916) and von Bertalanffy (1962). (See also Pittendrigh's letter to Mayr, in Mayr, 1974.) But this does not address the question of where those factors came from. What produced teleonomic systems in the first place, and what causes the evolution of one species-specific teleonomic system into another? Unless a mechanistic explanation can be found for these historical questions, teleonomy will mean nothing more than "purposefully designed but currently unattended organic activity", like a machine with servo-mechanisms.

The solution seems to lie in seeing teleonomic activity as the result of certain types of teleomatic activity. With respect to the origin of teleonomic systems – the origin of life on earth – this means that the living emerged from the nonliving through processes of self-organization, such as those proposed by Eigen (1971) (see also Wicken, 1979). With respect to the transformation of one teleonomic system into another – the evolution of species – this means that evolution is caused by teleomatic changes in inherited teleonomic properties during reproduction and ontogeny. Genetic drift (King and Jukes, 1969; Kimura and Ohta, 1971) and molecular drive (Dover, 1982a, b) are examples of such changes. More generally, I am simply referring to mutations, which are changes in the reproductive message from one generation to the next. The impact of this activity upon

the teleonomic system in which it occurs may exceed that system's homeostatic capabilities enough to cause the death of the individual, or even the extinction of the species. If the system remains together, but nevertheless changes to a certain degree, then speciation occurs. This view appears to be consistent with Paterson's (1980, 1981, 1982) argument that speciation is an incidental effect of breakdowns in species-maintaining systems. Brooks and Wiley (1986) have argued that this process is the result of entropic dissipation of species-cohesive factors.

There is no immutable <u>telos</u> in the teleonomic activity of biological function. There is only an end-state, sufficiently stable for generations of intraspecific reproduction, that eventually evolves into the end-state of a descendent species. The teleomatic processes responsible for this transformation attain their end-states because of the natural properties of the physical entities involved. They do not do so <u>for</u> the sake of the organism in which they happen to occur. Teleomatic activity does not occur <u>for</u> anything. The teleomatic produces the teleonomic (origin of life), whereupon the teleonomic sets the context of operation for, but does not direct or control, further teleomatic activity (evolution).

Teleonomic activity is an <u>intraspecific</u> phenomenon. I see no evidence for <u>trans-specific</u> teleonomic activity, in which controlling factors direct the attainment of an end-state that is evolutionarily beyond the maintenance of the existing species. The trans-specific process of evolution is therefore

not analogous to the intraspecific process of ontogeny, for only the latter is directed towards an end-state. Ontogeny, a teleonomic activity, operates with an internal representation of the end-state(s) to be attained. It cannot be said that evolution operates with a similar, controlling representation of what is to be. Such an analogy between evolution and ontogeny has been drawn a number of times (e.g. Polanyi, 1968), and it would seem to be responsible for many of the shortcomings of orthogenetic theory at the turn of the century. As with any end-attaining activity, this analogy has also attracted teleological interpretations of evolutionary change, such as Bergson's (1911) "creative evolution" and the "progressive evolution" of some neo-Lamarckians (see Bowler, 1983).

HISTORICAL AND NONHISTORICAL APPROACHES IN BIOLOGY

As products of evolution, organisms are historical entities. They can, however, be examined from either historical or nonhistorical perspectives. The intrusion of teleological explanations into what should be mechanistic explanations in biology is prevented by maintaining a distinction between these perspectives. Even though the questions may be phrased in similar ways, each is looking for a different type of answer. Each is usually associated with particular biological disciplines, and disagreements over what constitutes a valid explanation of a phenomenon may arise because historical and nonhistorical perspectives are being mixed. In addition, some

disciplines are more interested in structural properties, while others are more interested in functional properties. These criteria give four ways of looking at biology: nonhistorical structuralism, nonhistorical functionalism, historical structuralism, and historical functionalism.

NONHISTORICAL STRCUTURALISM AND FUNCTIONALISM: Studies of this type take the existence of an organism for granted and ask questions such as "What is it made of?" and "What does it do?". They are usually known as form-and-function studies. Anatomy, physiology, molecular biology, biochemistry, biomechanics, and medicine, for example, are nonhistorical disciplines. By this I mean not that one cannot do evolutionary research from within these areas of study, but that one does not have to do such research in order to study these subjects. Embryology can also take a nonhistorical perspective, for even though it studies organic development through time, it need not address the evolutionary origin of those ontogenies. Even much of population biology is nonhistorical, for the same. reason that it does not address evolutionary origins. For example, Kettlewell's (1955, 1961, 1965) classic studies on industrial melanism in Biston betularia were concerned with changes in the relative frequencies of pigmentation patterns in a given system, and not with how the pigmentation properties themselves came to exist. (But see Lambert et al., 1986, for comments on the validity of Kettlewell's conclusions.)

By disassociating themselves from the question of historical origin, nonhistorical studies can take either a

mechanistic or a teleological approach without creating any problems. The questions "What is it made of?" and "What does it do?" are mechanistic. But it may be possible to discover more about the properties of the organism by asking the teleological "What is this for?". From a nonhistorical perspective, "what for" questions are perfectly legitimate. This is the proper place of teleology in biology. But there is a price paid, for it must be acknowledged that such teleological causation plays no role in the origin of the properties. Nagel (1984) considered teleological explanations in biology to be acceptable when they simply refer to functional attributes. Even though such explanations contain phrases such as "in order to" and "the purpose of", they create no problems because they are not intended as an etiological explanation. I concur.

An anatomist studies the circulatory system of vertebrates, asks "What is the heart for?", and answers "For pumping the blood." An endocrinologist studies nervous transmission, asks "What is acetylcholinesterase for?", and answers "For maintaining proper levels of acetylcholine." This approach treats organisms as if they had been purposefully designed; it looks for explanations for functions and structures in the same way that a mechanic would examine an oil pump or the electrical system of an engine. It treats the end-directed activity of teleonomic systems as if it were the goal-seeking activity of a teleological system. The teleology that this introduces is not problematic because it is divorced from mechanistic causal explanations for the existence of organisms and is used as only

an explanatory analogy.

This decoupling is one reason why centuries of data on the properties of organisms can still be used today, even though most of them were collected not only before evolutionary theory was developed, but under implicit or explicit assumptions of purposeful design. Biology today makes use of the findings of Harvey, Vesalius, Owen, and Agassiz – and it does not matter how those people thought living systems came to be.

HISTORICAL STRUCTURALISM: I apply this term to the view of evolution advocated here. In this view, function is an effect of structure, and has no causal power of its own; evolutionary change is structural change, from which functional change emerges; and any end-attaining aspects of evolutionary change comes from teleomatic processes operating within, but not because of, a teleonomic context. In no sense of the word does evolution occur for something. "What for" questions are not applicable in historical structuralism. Instead, the questions asked are of the type, "How did it come to be?", or, "Through what means did it come to have these properties?".

HISTORICAL FUNCTIONALISM: This approach looks to function, rather than to structure, for the explanation of how things came to be. In doing so, it looks for causal powers in what are, in fact, effects. Other authors have noted the shortcomings of such a viewpoint in morphological (e.g., Lauder, 1981, 1982; Smith, 1982) and behavioral studies (Jamieson, 1986). The inability to find mechanistic evolutionary causes in

<u>functionality</u> <u>results</u> <u>in</u> <u>the</u> extension <u>of</u> <u>the</u> <u>teleological</u> <u>causality</u> <u>of</u> <u>nonhistorical</u> <u>studies</u> <u>into</u> <u>the</u> <u>historical</u> <u>realm</u>. This extension is not valid because such teleological causality does not actually exist. If the extension is made, the result is an historical teleology that asks a <u>second</u> type of "what for" question. This type is unacceptable. It asks "What did this <u>originate</u> for?", or "For what <u>purpose</u> did this come to be?"

Historical functionalism does not necessarily recognize a process of evolution through descent with modification. If it does not, then its teleology comes from nothing more than an argument from design: this performs a function, therefore, it came to exist so as to perform that function. If historical functionalism does recognize evolution, the question then becomes "What did this <u>evolve</u> for?", and the historical teleology of the answers becomes an evolutionary teleology of the type "The heart evolved in order to circulate the blood." As discussed below, an over-reliance on natural selection in evolutionary explanations can produce a variant of this evolutionary teleology that I have termed <u>adaptational</u> <u>teleology</u> (O'Grady, 1984, 1986).

NATURAL SELECTION

Although I have suggested that the transformational causes recognized by historical structuralism are necessary and sufficient for <u>some</u> kind of biological evolution to occur, I am not suggesting that they are the only causes affecting the

appearance of the organisms that have actually evolved on earth. Functionality <u>does</u> play a role in certain evolutionary phenomena. This is the basis of Darwin's theory, and of the derivative theory, neo-Darwinism. [1]

In addition to being <u>effected</u> by the <u>generative</u> processes of historical structuralist causes, biological systems can be <u>affected</u> by imposed <u>eliminative</u> processes. Natural selection is an eliminative force. It involves the survival of a subset of organisms from a heterogeneous grouping according to the relative functional efficiency of the organisms in a particular environment. Those organisms that function best (attain and maintain their teleonomic end-state best) will be in a position to leave more offspring than those that perish.

This view of natural selection appears to agree with that of Brandon (1981), who identified three differential processes at work: (1) <u>differential reproduction</u> resulting from (2) the <u>differential survival</u> of potential progenitors, such differential survival resulting from (3) <u>differences in the organisms' relative functional efficiencies</u>. Brandon considered all three factors to be necessary for natural selection. This is because subsets of one or two of the factors can occur in

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[1] For current purposes, this is defined as those evolutionary explanations that look to natural selection as the <u>primary</u> cause of evolution, that is, the explanation of <u>first choice</u>. Neo-Darwinism is thus not a label that should be applied to every view that recognizes natural selection somewhere in its framework. The nature of a theory depends upon the way in which its components are structured, and not on a simple enumeration of the components contained.

other circumstances. For example, differential reproduction can also be caused by accidental events, unrelated to functional efficiency, that kill off potential breeders. Differential "survival" can also occur among inanimate objects, such as granite and sandstone deposits on a mountainside. In the face of erosional forces, the granite will maintain its form for a longer time than will the sandstone. One cannot call this process natural selection and expect that term to retain its usefulness for biological studies, especially with respect to concepts of adaptation (discussed below).

Lewontin (1980) gave three factors that he considered necessary and sufficient for evolution by natural selection: (1) variation, (2) heritability of that variation, and (3) differential fitness of the inheritors of that variation (I suggest that the third factor is the net result of Brandon's three aspects of natural selection). I think that this triad can be restated as: the processes that produce novelty (factor 1), the processes that perpetuate that novelty (factor 2), and the processes that eliminate some of that novelty (factor 3). Stated in this way, I suggest that factors 1 and 2 are sufficient not only for some kind of biological evolution to occur, but for its products to possess sufficient organization to survive. This is because of the phylogenetically internested nature of the causes of biological organization. A newly evolved organism does not develop from a state of disorder which must be somehow ordered by an imposed force such as natural selection. Instead, the continuum of inheritance provides

historically accumulated properties, and thus sets the <u>initial</u> <u>conditions</u> that result in the production of descendent order from ancestral order.

Natural selection can thus be said to act as a proximate cause operating <u>among</u> biological systems, each of which is a hierarchy of ultimate and proximate <u>within</u>-system causality (Fig. 2). Selection may operate during the evolutionary history of a lineage, but because its among-system causality is extrinsic to any particular organism, it cannot become incorporated into the historical causality of the lineage. Investigations of selection may therefore address the history of the evolution of an organism, but not the historical, transformational processes that caused the organism to exist.

I am not suggesting that any evolutionary biologist today would argue that all biological order is the result of natural selection operating upon totally unordered variation. Futuyma (1984), for example, considered such an unqualified concept to be "simplistic". But there can be a tendency to lean towards modified versions of this position. This tendency appears to be a relict of the controversies of the 1860s to the 1920s. There was at that time a struggle, first for the acceptance of Darwinian evolutionary theory, and then for the primacy of neo-Darwinian, mutationist, orthogeneticist, or neo-Lamarckian explanations (see Bowler, 1983). One of the major disputes was whether evolution was in any way predetermined or directed towards a goal. Orthogeneticists and neo-Lamarckians had difficulty keeping their proposed mechanistic explanations free

from teleology. In addition, some critics of evolution offered blatantly teleological alternative explanations. The concept of purposeless change with which the Darwinians and neo-Darwinians attempted to introduce mechanistic causality advocated natural selection acting upon undirected variation. This means that variation is not directed towards the future needs of organisms. It can thus be said to be "random" with respect to those needs, but it cannot be said to be random with respect to the sources that produced it. Restricted variation, persistence of ordered states, and morphological trends in evolution are not indicative of predetermination; they result from unique historical events. When change must take place within the initial conditions established by these past events, it can be said to be past-determined. This is not the same as future-directed change that serves the needs of the organism.

ADAPTATION

Darwin's theory recognized a particular type of organic functionality, the relative efficiency of which allowed survival from the eliminative forces of natural selection. This functionality is adaptation. (I do not address the application of the term "adaptation" to refer to intra-generational adjustments to new conditions, other than to say that its use in such instances should be avoided.) Some workers (e.g., Bock, 1967, 1979, 1980), have taken the view that "On theoretical grounds, all existing features of animals are adaptive." (1967:

63). Others, such as Williams (1966) and Gould and Vrba (1982), have argued that the designation of adaptation should be reserved for features that have been affected by natural selection. I agree with Brandon (1981), van der Steen (1983), and van der Steen and Voorzanger (1984) that the tight coupling of cause and effect in the latter approach is preferable. However, I do not accept Williams' (1966: 9) argument that only adaptive characters have "functions", while any remaining characters have "effects". All function, I suggest, is an effect of structure, regardless of whether selection and adaptation are involved. Adaptive functionality is simply one type of organic functionality, and adaptive structural changes must occur within the context of organic structural changes.

Adaptations are "useful" to the organism in that they have allowed its survival from selection because of a relative superiority of function. But no matter how useful a character is, it has not <u>necessarily</u> become that way because of selection. It could have been produced by genetic and epigenetic associations with other characters that have been selected. (Brandon, 1981, termed these epiphenomenal traits, and included with them gene linkage and pleiotropic effects.) A useful character could also have evolved as a monomorphic derivative of an ancestral condition, or it could have been inherited, unchanged, from an ancestor.

Selection can be cited as a contributing cause of character usefulness only when there are grounds to believe that there has been a process of differential survival from a polymorphic stage

because of relative functional differences in that character. Otherwise, there is the risk of trivializing the concept of adaptation by synonomizing it with practically any aspect of biological organization. This would cause some of the perceived evidence for natural selection to come, in fact, from the fallacy of Affirming the Consequent: from the statement "if adaptation through selection, then usefulness and functionality", it is concluded "usefulness and functionality, therefore adaptation through selection."

Some authors appear to have adopted this line of argument. Bock (1967: 63), for example, stated "If [all existing features of animals] were not adaptive, then they would be eliminated by selection and would disappear". Ayala (1976) and Simpson (1958) have presented similar arguments that equate biological organization and functionality with adaptation. Once the state of adaptation has been synonymized with biological organization, the process of adaptation may then become synonymized with the process that produces that organization, namely, evolution (see Eldredge, 1985: 107). Such a perspective confounds studies of causes and their effects. It also ensures a specious supply of confirmations of the efficacy of neo-Darwinian theory.

When natural selection is taken to be the primary cause of evolution, its effect, adaptation, becomes incorporated into explanations of why organisms, or parts of organisms, evolved. The result is a teleological causality that is relied upon to explain the phenomena concerned. This teleology is a form of historical functionalism, and it is not difficult to find

examples of it in the research literature (see O'Grady, 1984). Organisms are imbued with powers of foresight and action. Questions such as "Why does this frog have green skin?" are given answers such as "In order to be better camouflaged" and "So that it could not be seen by predators". I agree with Nagel (1984) that sentences of this type are acceptable for explaining contemporary functional properties (i.e., from what I have termed a nonhistorical perspective). But I suggest that they are positively misleading when they are used in evolutionary explanations.

One undesirable aspect of the reliance on teleological causality that historical functionalism produces is that it gives a false sense of security about how well an evolutionary phenomenon has been explained. That is, if some kind of causal explanation seems to have been provided, then there will be less incentive to study the matter further.

A SINGLE EXPLANATORY FRAMEWORK

Natural selection and historical structuralism can be brought together in a protocol that factors out the relative contributions of causes by considering the more general causes first, and then the less general causes only if necessary. As an extreme example, one would not look to a selection level cause to explain why a cat walks upon the ground (selection against those that disobeyed gravity and rose into the air?). By extension, it may not be justifiable to immediately look to

selection when trying to explain why cats have four limbs, or a placenta, or retractile claws. The internesting of causes in historically-produced systems means that there is a _primacy of action_ of some causes over others. It follows that explanations attributing phenomena to more general causes have _logical primacy_ over those employing less general causes. The more general explanation should thus be the explanation of _first choice_, or the _initial hypothesis_. Its acceptance gives the least departure from the data and the greatest consistency with the causes known to be capable of producing the effects in question. It is to be retained so long as additional data do not show it to be inadequate.

It must be noted that in the internested hierarchy of evolutionary causality, the initial hypothesis for any particular level appeals to the causal properties of the immediately lower level. Thus, for a hierarchy of n levels, there are $n-1$ initial hypotheses. As will be shown in the following four subsections, this internesting of explanation results in a sequence of evolutionary analyses, each of which begins with the results of an analysis of the immediately lower level of causality.

1. INTRASPECIFIC INVESTIGATIONS

Suppose one wanted to provide an evolutionary explanation for a species of frog having strictly green skin coloration. Biotic or abiotic selection may have acted as a proximate cause (C1) by eliminating non-green frogs from a polymorphic state produced by a non-green immediate ancestor (Fig. 3a). If so, the developmental alteration that first produced the color change is the ultimate cause (C2), and the complete explanation for green coloration must refer to both the developmental and selectional causes. There are, however, at least three other means by which the species could come to be green and only green. (1) The non-green immediate ancestor may have produced only green descendants that were not subject to any selection for color (Fig. 3b), in which case only the ancestral developmental alteration would need to be cited as a cause. (2) Selection may have acted upon a polymorphic state produced by a green immediate ancestor (Fig. 3c). (3) The current green species may simply be descended from a green immediate ancestor, and be unaffected by selection (Fig. 3d). The last two cases do not address the question of the origin of green coloration, and so the answer must be sought further back in the phylogeny, in even more general levels of causality (C3). However, for the species whose characters prompted the study, the cause of green coloration has been determined.

In all four cases above, the complete causal explanation for the condition of the species includes a component of historical causality. In the absence of data suggestive of

selection, this historical causality should be accepted as a sufficient explanation. Historical causality is thus the explanation of first choice, while historical plus selectional causality is the explanation of second choice. This relationship among causes in an internested system differs from the more traditional either-or method of analysis, which is derived from Aristotle's Law of the Excluded Middle. It is not a question of history or selection, but one of whether selection has acted upon a system already affected by more ultimate causes.

2. PHYLOGENETIC INVESTIGATIONS

At the next level up, the inference of the evolutionary relationships of a group of organisms takes as its initial hypothesis the supposition that similarities among organisms are caused by descent from a common ancestor. These similarities are homologues. The application of Hennigian methodology (Hennig, 1966) then constructs a putative genealogy by internesting the organic similarities attributable to shared derived homologues (synapomorphies) within those attributable to shared primitive homologues (symplesiomorphies). This attempt to minimize the number of postulated instances of character evolution is a parsimony argument, hence, the connection in the systematics literature between cladistics and parsimony (e.g., Sober, 1983; Felsenstein, 1982, 1984; Farris, 1982, 1985). An analysis of, say, four taxa (Fig. 4a) begins with any basal

synapomorphies and autapomorphies (derived traits on terminal branches of the tree). Then, through the application of Hennigian Argumentation (Hennig, 1966), the tree is resolved with the addition of other character data (Fig. 4b). As each character is added, its causality is, whenever possible, attributed to a single historical origin. The result is an internesting of characters and their causes (Fig. 4d).

It is crucial to note that, as an application of historical structuralism, phylogenetic systematics constructs genealogies from internested patterns of inferred homologous traits regardless of the perceived functional importance or lack thereof of those traits. The focus is on inherited traits as indicators of kinship, and it does not matter to what use those traits may have been put. Thus, no character of an organism, no matter how useless or trivial it may seem, is to be considered a priori to be uninformative of phylogenetic relationships. Conversely, the retention of a character through an evolutionary lineage is not to be immediately taken as indicative of its functional importance to the organisms. It may be important, but that should not be be concluded merely from its continued existence. Historical functionalism can produce an argument of the form, "If it is here, it must be doing something; and if it has been here for a long time, it must be doing something indispensable".

3. LIFE HISTORY INVESTIGATIONS

Because a phylogenetic tree postulates patterns of common descent, it can be used to investigate the evolution of properties other than those used to construct the tree. Life history traits are one such type of property. The digenean trematodes are an interesting group for such a study because of the numerous life history components involved in their complex life cycles in an invertebrate intermediate host and a vertebrate final host. The origin of such a condition can be inferred from a phylogenetic tree for the parasitic flatworms. Figure 5a gives the result of such a study (Brooks et al., 1985a). This is a cladogram[1] for the parasitic platyhelminths, a group that includes digenean and monogenean "trematodes", as well as the Cestoidea, or tapeworms. There are three types of life cycles: direct in an invertebrate host (DI), direct in a vertebrate host (DV), or indirect (complex), involving both host groups (CB). These properties can be mapped onto the tree, and the ancestral states can be inferred by optimizing the nodal values so as to give the most parsimonious (i.e., most homologous) interpretation of character evolution. The optimization procedures of both Farris (1970) and of Swofford and Maddison (in review) give the nodal values in Figure 5b. This posits that there were three changes in an ancestral direct

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[1] An hypothesis of phylogenetic relationships, constructed from inferred synapomorphic characters, and consisting of an internested set of monophyletic groups sensu Hennig (1966), or clades. See Chapter III.

life cycle in an invertebrate: the addition of a vertebrate host in the Digenea, the addition of a vertebrate host in the common ancestor of the Cercomeromorphae (the four taxa on the right), and the addition of an invertebrate host in the Cestoidea.

This analysis was first presented in Brooks (1982; see also Brooks and Wiley, 1984, and Brooks et al., 1985a). O'Grady (1985) discussed at length the means by which the cladogram could be used to infer the evolutionary events that were involved in the production of a complex life cycle in both the Digenea and the Cestoidea. The relationships in the cladogram suggest that certain characters in the adults of the Monogenea, Gyrocotylidea, and Amphilinidea are homologous with those in the larvae of the Cestoidea. For example, a hook-bearing adhesive disc at the posterior of the body, which is a synapomorphy for the Cercomeromorphae, is present only in the early larval stages of the Cestoidea. The characters usually associated with tapeworms, such as strobilization into multiple body segments, and the formation of an anterior holdfast organ, called a scolex, are adult characters that seem to have been added onto the ancestral ontogeny by terminal addition. Furthermore, whereas the other three members of the Cercomeromorphae develop in vertebrate hosts, the comparable ontogenetic stage in the tapeworms, the larva, develops in an invertebrate, which is inferred to be a more recently acquired host. The plesiomorphic host, the vertebrate, contains the apomorphic ontogenetic stage, the adult. This suggests an intercalation, or a nonterminal addition, of an invertebrate host into the ancestral life cycle.

The evolution of the Digenea appears to have involved just the opposite processes. The cladogram supports an inference of homology between certain characters of the adult stages of digeneans and their sister group, the Aspidocotylea. The sequence of digenean larval stages (miracidia - sporocyst - redia - cercaria) associated with the complex life cycle has no homologues in the Aspidocotylea. This suggests that they have been intercalated, by nonterminal addition, into the ancestral ontogeny. Conversely, the invertebrate host present in the Dalyelloidea, Temnocephalidea, and Aspidocotylea life cycles holds the apparently more recently evolved digenean larval stages. Thus, it is the more recently acquired vertebrate host that holds the plesiomorphic, adult ontogenetic stage of the Digenea. This suggests a terminal addition of a vertebrate host into the ancestral life cycle.

This analysis suggests that the complex life cycles of tapeworms and digeneans arose through different means. Tapeworms show terminal addition of ontogenetic stages and nonterminal addition of a host. Digeneans show nonterminal addition of ontogenetic stages and terminal addition of a host. The generalized term "complex life cycles" would thus appear to be an instance of functional equivalence in the face of structural differences.

4. COMMUNITY STRUCTURE INVESTIGATIONS

Logical primacy and explanations of first choice have also been examined in community structure analyses (e.g., Quinn and Dunham, 1983; Roughgarden, 1983; Simberloff, 1983; Strong, 1983). The question has been whether one hypothesis has primacy of consideration over another, or whether there is such a multiplicity of impinging causes of equal status that one hypothesis is as reasonable a starting point as any. Brooks (1985; see also Brooks and Wiley, 1986) suggested that neither of the hypotheses discussed so far, random dispersal (Roughgarden, 1983) or competition effects (Simberloff, 1983; Strong, 1983) is a suitable initial hypothesis for biological systems. I concur.

The assumption of historically determined relationships appeals to the most general causes in community structure analysis. Of course, this approach does not deny that factors such as colonization and competition may have affected communities. It simply argues that the historical contribution must be factored out first (Fig. 6). Simberloff (1983) and Strong (1983) presented arguments that can be interpreted to support this approach. They noted the need to examine intraspecific, autecological, factors, such as vagility, before dealing with interspecific, synecological, factors, such as competition. Strong (1983: 639) came close to attributing this requirement to the historical nature of biological systems. It would seem that these goals are best met by formulating initial hypotheses of historical causality (e.g., Brooks, 1979a, 1979b,

1980, 1981a, 1985; see also Brooks and Mitter, 1984; Mitter and Brooks, 1983; Cressey et al., 1983; Collette and Russo, 1985).

"WHY" QUESTIONS

The recognition of the different types of biological questions discussed here is made all the more difficult when the adverb "why" is used. Questions are sometimes categorized according to the interrogative adverb they use. I suggest that there are no distinct classes of "what" questions, "how" questions, or "why" questions in biology, but that it is the form of the sentence that is more important in determining what kind of question is being asked. The adverb "why" is problematic because the same sentence can be used to ask questions that anticipate historical, nonhistorical, selectional, or even teleological answers. For example, the question, "Why do cats have fur?" can be answered with "epidermal derivation", "common inheritance from a mammalian ancestor", "common inheritance from a mammalian ancestor whose fur gave it a relative functional advantage during a period of selection", or "so that they can keep warm".

In addition, in comparative biological studies a question such as "Why does it have this?" is often intended simply to mean "Is there a function for this?" or "Is there a function associated with this being different in this particular organism?". The ambiguity produced by using "why" questions as abbreviated forms of mechanistic causal questions can introduce

the teleology of historical functionalism. Mayr (1961) avoided
this ambiguity by noting that the "why" questions of
evolutionary biology (= the historical perspective, discussed
herein) should be taken to mean the mechanistic "how come", and
not the finalistic "what for". I recommend the approach put
forward in this dissertation because it recognizes two types of
"what for" questions - one of which is valid, one of which is
not - and because it avoids the use of "why" questions
altogether.

SUMMARY

The causes responsible for the existence of an organism are
internested in levels of generality. As specific configurations
of physical elements, organisms are subject to general, physical
causes, and then to particular, biological causes. Within this
hierarchy are evolutionary causes that were once contingent for
a particular species, but have subsequently been incorporated
into the historical causality of its descendent species. This
internesting of proximate and ultimate causality is best studied
with a theory that appeals to similarly internested causes. The
more general, historical causes have operative and thus logical
priority over the less general causes.

The interactions of the structures in biological
hierarchies produce functions that contribute to the survival of
the organism. Organisms must function if they are to continue
existing as organisms, rather than as collections of structured,

but inanimate, matter. Function is an effect of structure.

There are three types of end-attaining activity that can exist in biological systems: teleomatic, or end-resulting; teleonomic, or end-directed; and teleological, or goal-seeking. Organic functionality attains end-states, but these are not goals, and the functionality is not teleological.

An historical lineage of organisms persists over generations of teleonomic activity until teleomatic disruptions in species cohesion result in the evolution of new species with derived, but altered, structural properties. Function may change also, but this is not a necessary consequence. Teleomatic processes occur because of the physical properties of the matter constituting the organism, and not because of any effects they might have on the teleonomic system in which they take place.

At any point in this evolutionary process, natural selection can eliminate those organisms whose relative functional efficiency is inadequate for survival in their environment. Although it can become part of the evolutionary history of a lineage, selection acts as a proximate cause operating among organisms; it is not responsible for the within-organism historical causality that produces changes in organic form.

The resulting adaptation of the surviving organisms is an effect, not a cause, of their evolution. Only natural selection produces adaptation. An adaptive character is useful to the organism that possesses it, in that it has allowed survival of

selection, but not all useful characters, no matter how indispensible to continued survival, are necessarily adaptations. Selection and adaptation are not necessary for the existence of functionality, usefulness, organization, or teleonomic activity.

As products of evolution, organisms are historical entities. They can, however, be studied from either a nonhistorical or historical perspective. The first is interested in current properties and does not ask where they came from. The second is interested in how organisms came to be, and is thus evolutionary. <u>Nonhistorical</u> <u>studies</u> <u>can</u> <u>be</u> <u>structuralist</u> <u>or</u> <u>functionalist,</u> and can ask mechanistic or teleological questions. The latter are a form of "what for" question, but the teleology creates no problems because it comes from a machine analogy and is not intended to be extended to historical explanations.

<u>Historical structuralism</u> is the term given to the view advocated in this dissertation: evolutionary change is structural change, from which functional change emerges. In no sense of the word does evolution occur <u>for</u> something; "what for" questions are not applicable in historical structuralism.

<u>Historical functionalism</u> is the result of attempting to use current functions to explain the origins of the structures that make those functions possible. This places effect before cause in the explanatory sequence, and produces an unacceptable teleology. In an evolutionary context, this can be termed evolutionary teleology, a variant of which can be termed

adaptational teleology. This has its own type of "what for" question: it asks "What did this evolve for?", and it answers "In order to adapt the organism to x".

Adaptational teleology is not the inevitable result of selection-adaptation explanations, for such explanations are mechanistic as long as they are restricted to dealing with proximate among-system causality. Adaptational teleology is the result of improperly extending selection-adaptation explanations to the realm of historical within-system causality. Neo-Darwinian approaches can encounter problems distinguishing this error because, on the one hand, natural selection acts upon the same functional properties with which teleological nonhistorical studies deal, and, on the other hand, its explanatory framework does not include those causes responsible for the production and transformation of organic form. The recognition of these types of questions is made all the more difficult by the ambiguity produced with the use of the adverb "why".

CONCLUSIONS

I have argued here that historical structuralism is a productive way of studying evolution. Its use seems to allow the examination of both pattern and process from within a single explanatory framework. The following two chapters provide an application of historical structuralist methodology. The study begins with the construction of a phylogenetic tree.

III. PHYLOGENETIC ANALYSIS OF <u>HAPLOMETRANA</u> LUCKER, 1931 AND

SPECIES OF <u>GLYPTHELMINS</u> STAFFORD, 1905 (DIGENEA:

PLAGIORCHIOIDEA) IN NORTH, CENTRAL, AND SOUTH AMERICA

As discussed in the previous chapter, historical structuralism in phylogenetic systematics postulates that historical processes produce a set of traits that covary with phylogeny. These are homologues. As each character is brought into a phylogenetic analysis, the existing tree acts as an hypothesis that predicts that the new character will show the same patterns of relationship as do the previously-considered characters. The existing tree can be supported, modified, or rejected. This chapter presents such a procedure.

INTRODUCTION

<u>Glypthelmins</u> Stafford, 1905 and <u>Haplometrana</u> Lucker, 1931 are members of the Superfamily Plagiorchioidea Dollfus, 1930. Approximately 19 species have at one time or another been placed in the genus <u>Glypthelmins</u> (see below). They are parasitic in the intestine, rarely the gall bladder, of amphibians in the New and Old World. The seven species of <u>Glypthelmins</u> studied in this dissertation are all parasitic in the intestine of anurans, primarily ranids, in North, Central, and South America. The eighth species studied here, <u>Haplometrana</u> <u>intestinalis</u> Lucker, 1931, is parasitic in the intestine of ranids in western North America and is the only member of that genus.

The familial and generic status of <u>Glypthelmins</u> has been revised a number of times since Stafford (1905) erected the genus to receive <u>Distomum quietum</u> Stafford, 1900. On the basis of two characters, excretory vesicle shape and cercarial tail morphology, the genus has been placed in either the Plagiorchiidae Luhe, 1901 (Fishthal and Kuntz, 1967; Martin, 1969; Ulmer, 1970; Sullivan, 1976), or the Macroderoididae McMullen, 1937 (Schell, 1962a; Odening, 1964; Sullivan and Byrd, 1970; Sullivan, 1976). On the basis of four characters, excretory vesicle shape, uterine extent, vitellarian extent, and presence of pharyngeal glands, there have been seven revisions of the genus (Miller, 1930; Olsen, 1937; Caballero, 1938; Cheng, 1959; Byrd and Maples, 1963; Nasir and Diaz, 1970; Sullivan, 1976). Brooks' (1977) phylogenetic analysis[1] of a number of genera of plagiorchioid trematodes (see the section, CHARACTER ANALYSIS) concluded that <u>Glypthelmins</u> and <u>Haplometrana</u> are each other's closest relatives, that is, they are sister taxa.

In a study of species that have at one time or another been placed in the genus <u>Glypthelmins</u>, Brooks (1977) examined 11 morphological characters and presented a cladogram (Fig. 7) containing four monophyletic lineages. The first two lineages are the subject of this dissertation, and consist of all the species in North America, and some of those in Central and South

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[1] The study by Brooks (1977) used Camin-Sokal parsimony to construct the tree and the common-equals-primitive criterion to polarize characters. See the section, THE INFERENCE OF PHYLOGENETIC RELATIONSHIPS.

America. Lineage I consists of <u>G</u>. <u>hyloreus</u> and <u>G</u>. <u>pennsylvaniensis</u>. Lineage II consists of <u>G</u>. <u>robustus</u>, <u>G</u>. <u>shastai</u>, <u>G</u>. <u>californiensis</u>, <u>G</u>. <u>quieta</u>, and <u>G</u>. <u>facioi</u>. Members of the remaining two clades[1] are found in South America, Africa, Asia, and the western Pacific. Brooks (1977) postulated the paraphyletic[2] or polyphyletic[3] nature of all of the genera to which some of the species had at some point been assigned. These are: <u>Glypthelmins</u> Stafford, 1905; <u>Choledocystus</u> Pereira and Cuocolo, 1941; <u>Rauschiella</u> Babero, 1951; and <u>Repandum</u> Byrd and Maples, 1963. Brooks' cladogram supported Miller's (1930) transference of <u>Margeana</u> <u>californiensis</u> Cort, 1919 to <u>Glypthelmins</u>, and Nasir's (1966) transference of <u>Reynoldstrema</u> <u>africana</u> (Dollfus, 1950) Cheng, 1959 to <u>Glypthelmins</u>. Brooks proposed that the entire monophyletic assemblage be referred to as <u>Glypthelmins</u>. This provided systematic support for Nasir's (1966) proposal to subsume the genera <u>Choledocystus</u>, <u>Reynoldstrema</u>, and <u>Repandum</u> in <u>Glypthelmins</u>.

The question asked in this part of the present study was whether the application of more recently developed phylogenetic analytic methods to a larger set of character data for

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[1] A monophyletic group <u>sensu</u> Hennig (1966): containing an ancestor and all of its descendants (see also Farris, 1974).

[2] A group that includes a common ancestor and some, but not all, of its descendants (Farris, 1974).

[3] A group in which the most recent common ancestor is assigned to some other group, and not to the group itself (Farris, 1974).

Haplometrana and the seven species of Glypthelmins concerned would continue to support the existing cladogram. The reanalysis used morphological characters observed through the examination of type material, specimens from private collections, specimens from field surveys, and specimens from laboratory studies. As an hypothesis of common ancestry and homologous character inheritance, the resulting cladogram provided the basis of the further comparative studies reported in Chapter IV.

TAXA STUDIED

All of the members of the glypthelminth lineages I and II postulated by Brooks (1977) (Fig. 7) were examined (Table I). The genus Haplometrana was included in the analysis because of (1) its postulation as the sister taxon to Glypthelmins by Brooks (1977), (2) its phenetic similarity to species of Glypthelmins, and (3) its parasitism of R. pretiosa, a ranid closely related to the ranid hosts of the species of Glypthelmins in western North America (see Chapter IV, COEVOLUTION ANALYSIS).

Table I - SPECIES STUDIED

Glypthelmins hyloreus Martin, 1969

G. pennsylvaniensis Cheng, 1961
  [=Choledocystus pennsylvaniensis (Cheng, 1961)
  Byrd and Maples, 1963]

G. robustus Brooks, 1976

G. shastai Ingles, 1936

G. californiensis (Cort, 1919) Miller, 1930
  [=Margeana californiensis Cort, 1919]

G. quieta (Stafford, 1900) Stafford, 1905
  [=Distomum quietum Stafford, 1900]
  [=Glypthelmins subtropica Harwood, 1932]

G. facioi Brenes et al., 1959

Haplometrana intestinalis Lucker, 1931
  [=Haplometrana utahensis Olsen, 1937]

MATERIALS AND METHODS

SPECIMENS EXAMINED

1. MUSEUM SPECIMENS

USNM Helm. Coll. refers to the U.S. National Museum, Helminthological Collection, Beltsville, Maryland.

Glypthelmins hyloreus Martin, 1969

Specimens:	USNM Helm. Coll. No. 70463: holotype; No. 70464: paratypes; additional specimens from the collection of D.R. Brooks, Dept. of Zoology, Univ. of British Columbia (collected in Nebraska, see Brooks, 1976a)
Type Locality:	Near Corvallis, Oregon
Type Host:	Hyla regilla Baird and Girard

Glypthelmins pennsylvaniensis Cheng, 1961

[=Choledocystus pennsylvaniensis (Cheng, 1961)

Byrd and Maples, 1963]

Specimens: USNM Helm. Coll. No. 59515: holotype and
                  paratype

Type Locality: Lake Warren, Pennsylvania

Type Host: Hyla crucifer Weid

Glypthelmins robustus Brooks, 1976

Specimens: USNM Helm. Coll. No. 73482: holotype; No.
                  73483: paratype

Type Locality: 15 km west of Neiva, Huila, Colombia

Type Host: Bufo marinus L.

Glypthelmins shastai Ingles, 1936

Specimens:	USNM Helm. Coll. No. 8925: holotype; additional specimens from the collection of J.C. Holmes, Dept. of Zoology, Univ. of Alberta (collected in British Columbia and Alberta)
Type Locality:	Glenburn, Shasta County, California
Type Host:	Bufo boreas Baird and Girard

Glypthelmins californiensis (Cort, 1919) Miller, 1930
[=Margeana californiensis Cort, 1919]

Specimens:	USNM Helm. Coll. No. 51701: syntypes; additional specimens from the collection of D.R. Brooks, Univ. of British Columbia (collected in Langley, British Columbia)
Type Locality:	San Francisco area, California
Type Host:	Rana aurora Baird and Girard

Glypthelmins facioi Brenes et al., 1959

Specimens: USNM Helm. Coll. No. 72275: deposited by
                      Sullivan (1976); additional specimens from
                      the collection of J.J. Sullivan, CDC,
                      Atlanta, Georgia (collected in Costa Rica;
                      see Sullivan, 1976)

Type Locality: Coris, Cartago Province, Costa Rica

Type Host: Rana pipiens Schreber

Glypthelmins quieta (Stafford, 1900) Stafford, 1905
    [=Distomum quietum Stafford, 1900]
    [=Glypthelmins subtropica Harwood, 1932]

Specimens: USNM Helm. Coll. No. 72268-72271:
                      deposited by Sullivan (1976); additional
                      specimens from the collection of D.R.
                      Brooks, Univ. of British Columbia
                      (collected in Nebraska; see Brooks, 1976a)

Type Locality: Eastern Canada;[1] Toronto area presumed

Type Host: <u>Rana</u>   <u>virescens</u>   Garman   (= <u>R</u>. <u>pipiens</u>),
                   <u>R</u>. <u>catesbeiana</u>   Shaw,   and   <u>Hyla</u> <u>pickeringii</u>
                   Kennicott (= <u>H</u>. <u>crucifer</u> Weid)

<u>Haplometrana</u> <u>intestinalis</u> Lucker, 1931

    [=<u>H</u>. <u>utahensis</u> Olsen, 1937]

Specimens: USNM Helm. Coll. No. 29903: holotype; No.
                   29904: paratypes; No. 9025: holotype of <u>H</u>.
                   <u>utahensis</u> Olsen, 1937; No. 9026: paratype
                   of <u>H</u>. <u>utahensis</u>; additional specimens from
                   the collection of J.C. Holmes, Univ. of
                   Alberta (collected in Kelowna, British
                   Columbia: originally identified as
                   <u>Glypthelmins</u>   sp.;   identified   as
                   <u>H</u>. <u>intestinalis</u> by the present author)

Type Locality: Bothell, King County, Washington State

Type Host: <u>Rana</u> <u>pretiosa</u> Baird and Girard

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[1] Stafford's (1900, 1905) reports specified neither locality nor
type specimens. I have recently discovered that there are five
of Stafford's specimens deposited at the National Museum of
Natural Sciences, Ottawa, Ontario, Canada (Invertebrate Zoology
Division, nos. NMCP 1900-1694 to 1900-1698). It may thus be
possible to designate a lectotype for <u>G</u>. <u>quieta</u>. I thank Dr.
Gordon G. Gibson, NMNS, for his help with this search.

2. FIELD COLLECTIONS

During the spring and summer of 1983 to 1986, anurans were collected in the following areas (see also Chapter IV, HOST AND DISTRIBUTION DATA):

1) Southern British Columbia, along an east-west transect remaining within 50 km of the Canada - U.S. border, from Vancouver Island to the British Columbia - Alberta border (Fig. 8). The species of anurans collected, and the pertinent species of parasites found, were: (a) Rana aurora: G. californiensis; (b) R. pretiosa: H. intestinalis; (c) Bufo boreas: no intestinal digeneans found; and (d) Hyla regilla: no intestinal digeneans found.

2) Siskiyou, Shasta, Modoc, Lassen, Plumas, and Sierra Counties in northern California (Fig. 9). Collections in Shasta, Modoc, and Lassen Counties centered around the drainage basin of the Pit River. This river runs through Glenburn, the type locality of G. shastai in B. boreas. The species of anurans collected were R. cascadae and B. boreas. No intestinal digeneans were found.

3) Southeast Nebraska, in the region of Lincoln.

4) Northwest Wyoming.

The Nebraska collections acquired specimens of G. quieta from

R. pipiens, so as to obtain early developmental stages of the parasite for laboratory studies. Collections of R. aurora from Langley, British Columbia yielded specimens of G. californiensis for use in laboratory studies. Collections in Wyoming were directed towards the identification of "Glypthelmins sp.", reported by Turner (1958) from R. pretiosa at Fishing Bridge.

3. EXAMINATION OF SPECIMENS

All of the specimens examined from public and private collections were whole mounts, stained with either hematoxylins or acetocarmine. Worms obtained from dissections of anurans collected in the field were flattened under a coverslip, fixed for 24 hours in Alcohol-Formalin-Acetic Acid, and then stored in 70% EtOH. They were stained with hematoxylins, acetocarmine, or Fast Green, then whole-mounted. Developmental stages obtained from laboratory infections were processed in the same manner. Living specimens were studied with vital stains (Nile Blue and Methylene Red). Unless stated otherwise, observations were taken from fixed specimens. Illustrations from microscope work were done with the aid of a drawing tube.

THE INFERENCE OF PHYLOGENETIC RELATIONSHIPS

Hennig (1950, 1966) developed the methodology of phylogenetic systematics (= cladistics) as a formalized procedure for inferring phylogenetic relationships by grouping organisms together on the basis of their shared possession of derived homologous characters.[1] Such hypotheses of common ancestry can serve as general reference systems for other studies in comparative biology.

1. HOMOLOGY

Two characters (= attribute, or trait) are considered to be homologues (= "special homology": Russell, 1916; Riedl, 1978; Wiley, 1981a) of one another if one is evolutionarily derived, through trans-specific reproductive links, from the other. These two characters are referred to as a transformation series.

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[1] As introduced by Owen (1848), the concept of homology had no evolutionary connotations. Later workers, such as Darwin (1859), Haeckel (1866), Lankester (1870), and Gegenbaur (1878) used the term to refer to structural similarities that were the result of common ancestry. The criteria of structural similarity used today are generally those delineated by Remane (1956): (1) similarity of position, (2) degree of resemblance, and (3) continuity through intermediate species. To these, Hennig (1950, 1966) added the criterion of concordance between hypotheses of individual characters' homologies and the phylogenetic relationships that such homology suggests. If two structures are inferred to be homologues sensu Remane, and if they are also inferred to be shared, derived characters from a common ancestor, then the genealogical grouping of taxa supported by that shared character will continue to be supported when other characters are examined. This criterion introduced the concept of evolution producing a covarying suite of inherited characters.

The character that existed first is termed the <u>plesiomorphic</u> ("near the form"), or ancestral state, of the series, and the character that evolved from it is termed the <u>apomorphic</u> ("away from the form"), or derived state, of the series. In the same manner, three or more characters can be homologues of each other, with the added possibility that the character states in the series can be derived from one another in a linear sequence, such as:

A -- B -- C

or in a branching sequence, termed a <u>multistate character tree</u>, such as that given below (see Chapter IV and Appendix F for further discussion of multistate character trees):


```
        C        D
        |        |
        ----B----
             |
             A
```


A character found in two or more taxa is homologous in all of those taxa when the most recent common ancestor can also be inferred to have had the character. If, in such a case, the character can be considered to have originated in that most recent common ancestor, it is termed a <u>synapomorphy</u>, a shared

derived trait. If it is considered to have originated in an earlier ancestor, it is termed a symplesiomorphy, a shared ancestral trait. If a character is the derived state of an homologous series, but is present in only one of the taxa in the analysis, it is termed an autapomorphy. Cladistic methodology attempts to reconstruct the phylogenetic relationships among organisms by grouping them on the basis of inferred synapomorphic traits. This minimization of the number of postulated evolutionary events is a parsimony argument (e.g., Farris, 1982, 1985; Sober, 1983; Felsenstein, 1982, 1984)

Plesiomorphy and apomorphy refer to the relative degree of evolutionary derivation, and every species is a composite of the different homologue types. This is because a new evolutionary character can originate as an autapomorphy, after which, through the evolution of descendent species, it can become first a synapomorphy and then a symplesiomorphy. For example, hair is an autapomorphy of mammals when addressing the evolution of the Tetrapoda within the Vertebrata, a synapomorphy of mammals when addressing the evolution of the Mammalia within the Tetrapoda, and a symplesiomorphy of mammals when addressing the evolution of Felis within the Felidae.

Characters that cannot be inferred to be homologues on a cladogram are termed homoplasies (Lankester, 1870). These characters do not satisfy Hennig's criterion of phylogenetic congruence with other characters. They are considered either to have originated independently of one another, that is, from two different pre-existing characters (convergence), or to have

originated from the same character at different times or in different taxa (parallelism). In the latter case, homoplasious characters may satisfy Remane's (1956) criteria of similarity of position, resemblance, and continuity. As with homologues, homoplasies show structural similarities. They are thus not equivalent to Owen's (1848) other type of comparative anatomical property, an analogue. This latter category refers to similarity in function regardless of similarity in form. Rather, homoplasies are structurally similar attributes that are considered to be non-homologues only after a phylogenetic analysis shows that they cannot be inferred to be synapomorphic for all of the taxa in which they occur. [1]

2. OUTGROUPS

A number of protocols have been proposed for determining the relative plesiomorphy and apomorphy of character transformation series (e.g., Stevens, 1980), a procedure termed polarization. To date, they have been found either to give incorrect estimates (such as the principle of common-equals-primitive: e.g., Estabrook, 1978; Crisci and Steussy, 1980), or

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[1] Homoplasious characters can be synapomorphic, i.e., homologous, for less inclusive clades on the tree. This follows from the recognition that regardless of whether a character arises as a parallelism, convergence, or reversal, it is capable of being inherited in descendent species. One consequence of this is that if a paraphyletic group possesses, say, the "1" state in a 0-1-0 or 0-1-2 transformation series, then that state is an homologous trait of that group.

to be special cases of the more general method of "outgroup" comparisons (see Lundberg, 1972). This method is based upon the assumption that a trait found in at least one member of the study group and in a taxon outside the group (the outgroup - a close relative, preferably the sister taxon) is plesiomorphic. Such a trait is hypothesized to have been inherited by the species that is now the ancestor of the study group.

Because outgroups can themselves evolve (i.e., what is taken to be a plesiomorphy is actually an apomorphy of the outgroup taxon), it is necessary to use more than one outgroup to determine the plesiomorphy of a character (see Maddison et al., 1984). One must establish the character state of the outgroup node, which is the ancestral state on the tree immediately below (= evolutionarily prior to) the group of taxa being studied (= the ingroup, or study group). This is done by examining at least two outgroup taxa that are not themselves monophyletic. For this reason, a cladogram will, depending upon the characters used in the study, often be rooted at a composite outgroup (i.e., at a node consisting of plesiomorphic character states, rather than at an existing taxon). This follows from Hennig's (1966) argument that any organism is a composite of plesiomorphic and apomorphic traits.

The necessity to polarize characters by examining at least two non-monophyletic outgroup taxa continues to hold as an analysis attempts to resolve relationships within the ingroup. This is because a member of the ingroup can be an outgroup to the remaining ingroup members at a less inclusive level of

analysis. Thus, the criteria of Maddison et al. (1984) are to be applied in the Functional Outgroup Analysis of Watrous and Wheeler (1981), which deals with ingroup character polarizations.

3. MULTISTATE CHARACTERS

If a character occurs in only two states in the taxa studied, it is termed binary, and its polarization into plesiomorphic and apomorphic states will concurrently establish its order of transformation. That is, if one state is ancestral, then the other state must be the derived state in a linear transformation series. This concurrence does not apply when a character has more than two states. These are called multistate characters. When one of their states is polarized as the ancestral state, there is still ambiguity as to the subsequent transformations among the remaining states. There has long been a tendency in systematics to order these states into an intuited transformation series. Much of traditional evolutionary taxonomy is based upon choosing an appropriately "key" character, and then postulating the evolution of its states according to various functional or structural criteria. [1] At the very least, this approach suffers simply because it uses so few characters that actually test the phylogenetic

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[1] A commonly used criterion is a linear increase or decrease in size or number.

hypothesis. As discussed in the following subsection, it is possible to analyze multistate characters cladistically, in a manner comparable to that for binary characters.

4. COMPUTER-ASSISTED PARSIMONY ANALYSIS

A number of algorithms have been developed for constructing phylogenetic trees on the basis of inferred synapomorphies. The Wagner method (Kluge and Farris, 1969; Farris, 1970) places no restrictions on the kinds of character changes that are postulated so as to produce a minimum length tree. The Camin and Sokal (1965) algorithm prohibits character reversals (a return to a more primitive state). The Dollo algorithm (Farris, 1977) minimizes multiple origins (parallelisms and convergences) of characters. Wagner parsimony is the most defensible method because it makes the fewest a priori assumptions about evolutionary events. The method does not deny the existence of reversals or multiple origins: it simply makes no initial assumptions and then lets the distribution of the characters on the tree indicate a posteriori postulations of such types of character change (see Farris, 1985; Sober, 1983). This form of tree-wide, or global, parsimony can produce trees that are shorter than those produced by Camin-Sokal or Dollo parsimony criteria.

With respect to evolutionary "reversals", it should be noted that similar codes for various characters in a data matrix can refer to completely different organic properties. For

example, in a two-state character, the plesiomorphic state is usually coded as "0", and the derived state is coded as "1" (i.e., a "binary" character). But the "0" code can refer to (1) the primitive absence of a character whose derived state is its presence, (2) the primitive presence of a character whose derived state is its absence, or (3) the primitive morphology of a character whose derived state is an altered morphology. If a computer program for phylogenetic analysis prohibits evolutionary reversals, the only thing actually being prohibited may be a "0 - 1 - 0" transformation in the coding.

The two programs implementing Wagner parsimony that were used in this study are PAUP (version 2.4, 1985, developed by D.L. Swofford) and PHYSYS (1983 version, developed by J.S. Farris and M.F. Mickevich). Both PAUP and PHYSYS contain algorithms that build trees by the step-wise addition of taxa (often referred to in this context as OTUs: Operational Taxonomic Units) to a less inclusive tree containing all of the character data such that the number of postulated changes for all of the character states is minimized.[1] Another approach is simply to contruct <u>all</u> possible trees for the taxa, and then pick the shortest one. This may rather inelegantly eliminate any problems concerning the appropriate manner of step-wise addition of OTUs, but it <u>is</u> guaranteed to find the optimal tree

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[1] Although the goal is clear enough, the manner in which an algorithm can and should accomplish this task is the subject of much discussion in the systematics literature (see, e.g., Swofford and Maddison, in review; Fink, 1986).

according to whatever optimality criterion is being applied. Such an operation is only practical when a computer is available to carry out the extensive calculations (for nine taxa, there are 135,135 trees). PAUP's ability to do this for a limited number of taxa was utilized in this study.

Parsimony procedures have been developed for multistate characters (Fitch, 1971; Mickevich, 1982; Swofford and Maddison, in review). Mickevich's technique is termed Transformation Series Analysis, and is distributed with the PHYSYS computer program. The Swofford and Maddison technique, which is a form of Fitch Optimization (Fitch, 1971), is the Unordered Analysis procedure of the PAUP computer program. All of the techniques attempt to find the most parsimonious arrangement of character states on a tree regardless of any directionalities of transformation implied by the coding of the character states. The main differences lie in the criteria of homology, and the means by which ambiguity over the character state at an internal node is handled. I will not investigate these differences here, other than to say that PAUP's Unordered Analysis was used because I consider it to make fewer a priori assumptions about character evolution.

CHARACTER ANALYSIS

Twenty-one morphological characters were used in the analysis of Haplometrana intestinalis and the seven species of Glypthelmins. The listing below indicates the inferred states of each character, and the relative plesiomorphy postulated from outgroup comparisons. These comparisons utilized the results of cladistic analyses of digenean familial relationships (Brooks et al., 1985b), certain plagiorchioid genera (Brooks, 1977), and specific relationships within Glypthelmins (Brooks, 1977). In addition to Glypthelmins and Haplometrana, the plagiorchioid genera included in the analysis by Brooks (1977) were: Brachycoelium (Dujardin, 1845) Stiles and Hassall, 1898, Tremiorchis Mehra and Negi, 1926, Mesocoelium Odhner, 1911, Haplometroides Odhner, 1911, Ostioloides Odening, 1960, Xenopodistomum MacNae et al., 1973, Metaplagiorchis Timofeeva, 1962, Laiogonimus Vercammen-Grandjean, 1960, Opisthioglyphe Looss, 1899, and Astiotrema Looss, 1900.

The criteria of Maddison et al., (1984) for eliminating ambiguity as to the plesiomorphic state were applied whenever possible. The polarization procedures applied here use the term "widespread character state" in the sense of Kluge and Farris (1969) (see also Farris, 1982). Although outgroup taxa from outside the study group were examined for each character, the partial resolution of the tree by some characters rendered these comparisons moot for other characters. This is because the former gave sufficient ingroup resolution to provide functional outgroups (sensu Watrous and Wheeler, 1981) that established the

plesiomorphy of the latter characters <u>at the level of generality</u> <u>encompassed by the study group</u>.

In the listing below (summarized in Table II), plesiomorphic states are coded with a "0". The derived states of binary characters are coded with a "1", while those of multistate characters are given letter codes. Such alphanumeric coding was done so as to indicate that the order of transformation in the multistate characters was inferred <u>a posteriori</u> in the cladistic analysis. This coding does not affect the manner in which multistate characters are treated in the Unordered Analysis of PAUP. Missing data or logically inapplicable character states are coded with a question mark (?) in Table II.

(1) <u>Tegumental projections</u> (2 states) (Fig. 10):

(0) spines; (1) scales

<u>Character States</u>: Although only Miller (1930) actually illustrated scaled projections on the tegument of <u>G. quieta</u>, "scale-like spines" have been noted on <u>G. quieta</u> by Rankin (1944), on <u>G. californiensis</u> by Cort (1919), and on <u>G. facioi</u> by Sullivan (1976). Spines are approximately 9 μm long, 4 μm wide, and do not overlap one another. Scales are approximately 18 μm long, 8 μm wide, and overlap. Scales appear to be expanded spines, since the centers of the bases of both are approximately 18 μm apart

from one another on the surface of the tegument.

Polarity: Based upon an analysis of digenean familial relationships (Brooks et al., 1985b), spines are postulated to be symplesiomorphic for the level of this study.

(2)   Proportion of body bearing tegumental projections (3 states):

(0) from anterior end to posterior fifth of body; (A) from anterior end to level of pharynx; (B) from anterior end to middle of hindbody[1]

Character States: The character states reported here agree for the most part with published accounts. Differences lie with reports of projections to the level of the testes in G. shastai (by Ingles, 1936), G. facioi (by Sullivan, 1976), and H. intestinalis (by Olsen, 1937). As Cort (1919) noted for G. californiensis, the density of the projections begins to thin posterior to the testes, most likely as a result of the growth of the hindbody (see Chapter IV, HETEROCHRONIC DEVELOPMENT). Nevertheless, large specimens of H. intestinalis and G. shastai, which

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[1] The region from the anterior edge of the ventral sucker to the posterior end of the body.

are the largest species studied, bear projections to the posterior of the body.

Polarity: Based upon an analysis of digenean familial relationships (Brooks et al., 1985b), the plesiomorphic state of spination in the Digenea is postulated to be state (0).

(3) Diameter of oral sucker / diameter of ventral sucker (2 states):

(0) 1:0.40 - 1:0.75; (1) ≥1:0.75

Character States: Ranges and (means), all specimens:
G. hyloreus, n = 10, 1:0.80 - 1:0.84 (1:0.82)
G. pennsylvaniensis, n = 2, 1:0.75 - 1:0.78 (1:0.77)
G. robustus, n = 2, 1:0.45 - 1:0.50 (1:0.48)
G. shastai, n = 19, 1:0.58 - 1:0.74 (1:0.69)
G. californiensis, n = 20, 1:0.52 - 1:0.67 (1:0.60)
G. facioi, n = 10, 1:0.58 - 1:0.65 (1:0.61)
G. quieta, n = 15, 1:0.50 - 1:0.68 (1:0.58)
H. intestinalis, n = 25, 1:0.47 - 1:0.68 (1:0.62)

Polarity: State (0) is established as the plesiomorphic state because of its widespread distribution throughout Glypthelmins lineages III and IV (sensu Brooks, 1977; see

Fig. 7) and other plagiorchioid genera parasitic in anurans.

(4) <u>Penetration glands</u> (2 states) (Fig. 11):

(0) present in cercaria, absent in adult; (1) present in cercaria, present in adult

<u>Character States and Polarity</u>: Many digenean cercariae possess penetration glands whose ducts empty near the oral sucker. The glands usually degenerate when adulthood is reached, although the ducts can sometimes still be seen in young adults. Although cercariae of <u>G</u>. <u>facioi</u> have not been examined, the position of the glands and their ducts in the adults suggests that these glands are penetration glands persisting from the cercarial stage.

(5) <u>Medial glands</u> (2 states) (Figs 11, 12, 13 and 47)

(0) at level of prepharynx; (1) at level of pharynx and esophagus

<u>Character States</u>: This character and character no. 6 comprise what have been previously referred to collectively in <u>Glypthelmins</u> as the pharyngeal or the peripharyngeal

glands. They lie on each side of the body in the region of the pharynx. Byrd and Maples's (1963) caution against the use of these glands for systematic purposes was directed against Cheng's (1959) attempt to re-establish the genus Margeana Cort, 1919 for species lacking such glands. My work on the study group indicates that there is always at least one type of gland, with two types present in G. quieta. The first are herein referred to as the medial glands (from Leigh, 1946, see below), the second as the pharyngeal glands. All of the species possess the more anterior, and smaller, medial glands. They were difficult to observe in H. intestinalis, and the vital staining of live specimens was necessary. Only G. quieta has the larger, more postero-lateral pharyngeal glands. The two types of glands are located in different areas, and, although they often overlap each other when both are present, each has ducts with different orientations. Those of the medial glands enter the prepharynx dorso-laterally, while those of the pharyngeal glands enter ventro-medially. Leigh (1946) first reported medial glands in G. quieta, although he concluded that they degenerated as the animal matured. Rankin (1944) can also be considered to have observed them in G. quieta, since he noted that the pharyngeal glands (sensu lato) were sometimes arranged in an anterior group and a posterior group on each side of the body.

Polarity: Although polarity is established by ingroup
relationships, the relative plesiomorphy of state (0) is
not known. Accordingly, it is coded as a missing datum for
the composite outgroup in the analysis (a question mark in
Table II).

(6) Pharyngeal glands (2 states) (Fig. 13):

(0) absent; (1) present

Character States: As noted in the remarks for character
(5), only G. quieta possesses these large and conspicuous
glands. They lie postero-lateral to the pharynx, with
ducts that enter the prepharynx ventro-medially.

Polarity: This is established by ingroup relationships.
Pharyngeal glands are absent in the outgroup taxa.

(7) Position of ovary (2 states):

(0) sinistral: on left side of body; (1) dextral: on right
side

Character States: Brooks (1976b) illustrated the dextral
condition in G. robustus. Examination of the type

specimens confirmed this character. It should be noted that amphitypy (i.e., in this case, a sinstral or dextral ovary position in different specimens of the same species) is not uncommon in digeneans, and that only the holotype and the paratype of G. robustus were available for examination. Nevertheless, such amphitypy has not been reported for any of the species studied here.

Polarity: The dextral position also occurs in the South American species, Choledocystus hepaticus Lutz, 1928 (= G. hepaticus, lineage III sensu Brooks, 1977) and Rauschiella palmipedes Lutz, 1928 (= G. palmipedes, lineage IV) (see Sullivan, 1977a,b). Nevertheless, the widespread distribution of the sinistral condition among other glypthelminths and plagiorchioids establishes the sinistral state as plesiomorphic.

(8) Position of Laurer's canal (2 states)

(0) arising between seminal receptacle and common vitelline duct; (1) arising distal to common vitelline duct

Character States: Brooks (1976b) illustrated the distal location of the canal in G. robustus. I have not ascertained the condition in Central and South American members of Choledocystus and Rauschiella.

Polarity: This is established by ingroup relationships. The relative plesiomorphy of state (0) is not known, and it is coded as a missing datum for the composite outgroup in the analysis (Table II).

(9) Anterior extent of anterior vitelline field (3 states) (Figs. 14, 15, 16, 17, 18)

(0) anterior vitelline duct present, with anterior vitelline field extending to level of bifurcation; (A) anterior vitelline duct present, with anterior vitelline field extending to level of pharynx; (B) anterior vitelline duct absent, no anterior vitelline field

Character States: The vitelline system is herein considered to consist of five characters, presented here as characters (9) to (13). The vitelline glands on each side of the body are composed of anterior and posterior fields, each of which empties into its own duct. These ducts join and form a single duct that runs mediad towards the ootype region. The single ducts from each side of the body unite into the common vitelline duct. I define the anterior and posterior fields of vitellaria with respect to the duct from which they arise. Although the fields often overlap along the sides of the body, the anterior field comprises the majority of the vitellaria anterior to the ootype region,

while the posterior field comprises the majority of the vitellaria posterior to that region. Figure 14 presents a schematic diagram of the distribution of the vitellaria as a reference for characters (9) to (13). Figure 15c is an illustration of a specimen of G. quieta with reduced vitelline development that clearly shows the anterior and posterior ducts and fields. Figures 15a and b show the usual condition in G. quieta. The vitelline distributions for some of the other species are illustrated in: Figs 15d and e, for G. facioi; Figs 16a and b, for G. californiensis; Figs 17a and b, for G. shastai; and Figs 17c and d, for H. intestinalis. The absence of the anterior vitelline fields in H. intestinalis is associated with the absence of the anterior vitelline ducts (Fig. 18a). On the basis of anterior vitelline distribution, Rankin (1944) synonymized G. californiensis with G. quieta. Sullivan (1976) concurred with this position. I disagree. Only G. quieta possesses pharyngeal glands (sensu character 6), and its anterior vitellaria only occasionally extend to the level of the esophagus, whereas those of G. californiensis almost always do. Furthermore, there is a difference in the posterior extent of the vitellaria (character 11).

Polarity: This is established by ingroup relationships. State (0) is widespread among other glypthelminths and plagiorchioids.

(10) <u>Dorso-medial</u> <u>confluence</u> <u>of</u> <u>anterior</u> <u>vitelline</u> <u>fields</u>
(2 states) (Figs. 14, 15, 16)

(0) absent; (1) present

<u>Character</u> <u>States</u>: In all of the species studied, the vitellaria lie dorsal, lateral, and ventral to the intestinal ceca (Fig. 14). In some of the species, the vitellaria from each side extend mediad and become confluent, or nearly so, with each other. The four vitelline areas that may be involved are the dorsal or ventral parts of the anterior or posterior vitelline fields. Dorsal confluence of what I herein term the anterior field has been noted in G. quieta, by Rankin (1944) (see Figs 15a,b), G. facioi, by Sullivan (1976) (see Figs 15d,e), and G. californiensis, by Cort (1919) (see Figs 16a,b). Rankin (1944) synonymized G. subtropica Harwood, 1932 with G. quieta on the basis of this character.

<u>Polarity</u>: This is established by ingroup relationships. State (0) is widespread among other glypthelminths and plagiorchioids.

(11) <u>Posterior</u> <u>extent</u> <u>of</u> <u>posterior</u> <u>vitelline</u> <u>field</u> (4 states)
(Figs. 15, 16, 17, 18)

(0) to posterior third of hindbody (= mid-way between posterior testis and posterior end of body; (A) to posterior end of body; (B) to within approximately one testis diameter posterior to testes; (C) no further posterior than testes

Character States: As noted in character (9), this character is another in which G. californiensis differs from G. quieta. The former has state C (Figs 16a,b), while the latter has state B (Figs 15a,b). However, there are two discrepancies in the literature. First, in the original description of G. californiensis, Cort (1919) presented an illustration of a living specimen in which the vitellaria extend slightly posterior to the testes. But in both the diagnosis and the syntypes, the vitellaria possess state C, as above. In my observations on living specimens of G. californiensis collected from R. aurora in British Columbia, I found no differences in the distribution of the vitellaria in living and fixed specimens. As shown in Figure 18b, this restricted distribution is associated with the reduced development of the posterior vitelline ducts and their accompanying vitelline fields. The other discrepancy lies with Stafford's (1900) original description of G. quieta (= Distomum quietum), in which the vitellaria are illustrated as extending to mid-way between the posterior testis and the posterior end of the body (i.e., state 0 herein). The diagnosis is unclear on this

point: it is not specified whether a living or fixed specimen is illustrated, and no type specimens were deposited. (But see the section, SPECIMENS EXAMINED, for comments on Stafford's specimens.) Subsequent studies on G. quieta have occasionally found the same state of vitelline extent (e.g., Brooks, 1976a), but the usual condition is that of state B.

Polarity: State (0) is established as the plesiomorphic state because of its widespread distribution throughout Glypthelmins lineages III and IV (sensu Brooks, 1977) and other plagiorchioid genera parasitic in anurans.

(12) Dorso-medial confluence of posterior vitelline fields (2 states) (Figs 15, 17)

(0) absent; (1) present

Character States: The derived state of this character has been previously noted in G. facioi, by Sullivan (1976) (see Figs 15d,e) and H. intestinalis, by Olsen (1937) (see Figs 17c,d).

Polarity: This is established by ingroup relationships. State (0) occurs in most of the outgroup taxa.

(13) <u>Ventro-medial</u> <u>confluence</u> <u>of</u> <u>both</u> <u>vitelline</u> <u>fields</u>
(2 states) (Figs 14, 17)

(0) absent; (1) present

<u>Character</u> <u>States</u>: In the derived state of this character, the fields are confluent, or nearly so, ventrally at the level of the ootype, where the vitelline ducts empty. Although this condition has occasionally been reported in <u>G. quieta</u> (by Rankin, 1944) and <u>G. californiensis</u> (by Cort, 1919), it occurs regularly only in <u>G. shastai</u>.

<u>Polarity</u>: This is established by ingroup relationships. State (0) occurs in the outgroup taxa.

(14) <u>Lateral</u> <u>extent</u> <u>of</u> <u>uterine</u> <u>loops</u> (2 states)

(0) extracecal, overlapping intestinal ceca ventrally; (1) intercecal, lying between intestinal ceca

<u>Character</u> <u>States</u>: The early uterine development of both states is intercecal (Sullivan and Byrd, 1970; Olsen, 1937; see also Appendix E, and the section, HETEROCHRONIC DEVELOPMENT). Additional lateral growth of the uterus produces the extracecal condition (Sullivan and Byrd, 1970).

Polarity: State (0) is established as the plesiomorphic state because of its widespread distribution throughout Glypthelmins lineages III and IV (sensu Brooks, 1977) and other plagiorchioid genera parasitic in anurans.

(15) Anterior extent of uterine loops (2 states)

(0) throughout hindbody, with pretesticular loops present;
(1) throughout hindbody, with pretesticular loops absent

Character States: On the basis of characters (14) and (15), Byrd and Maples (1963) distinguished among Glypthelmins (no extracecal loops, no pretesticular loops), Repandum (no extracecal loops, pretesticular loops present), and Choledocystus (extracecal loops present, pretesticular loops present).

Polarity: State (0) is established as the plesiomorphic state because of its widespread distribution throughout Glypthelmins lineages III and IV (sensu Brooks, 1977) and other plagiorchioid genera parasitic in anurans. It is possible that future studies on G. robustus will result in its removal from the group of glypthelminths studied here, and its grouping with some of the other South American species of Glypthelmins previously placed in the genera Choledocystus and Rauschiella. This possiblity exists for

two reasons. First, the holotype and paratype of G. robustus are not fully mature specimens, and it cannot be known for the moment whether the mature uterine condition exhibits extracecal and pretesticular loops. Second, the dextral ovary position possessed by G. robustus also occurs in Choledocystus hepaticus (= G. hepaticus) and Rauschiella palmipedis (= G. palmipedis) (see Character 7).

(16) Relative position of testes (3 states) (Figs 15, 16, 17, 35)

(0) oblique; (A) symmetrical; (B) tandem

Character States: Symmetrical testes are lateral to one another (Figs 15a,b; 16a,b), oblique testes are diagonal to one another (Figs 15c,d; 17a,b), and tandem testes are arranged one posterior to the other (Figs 35a,b). One of the characters by which Olsen (1937) considered H. utahensis to differ from H. intestinalis was the oblique testes position in the former and the tandem testes position in the latter. In his synonymization of the two species, Waitz (1959) considered the two states to be the extremes of a continuum. In most of the specimens of H. intestinalis examined in the present study, the testes are tandem. In some, they are oblique. Within the study group, tandem testes occur only in H. intestinalis.

Polarity: State (0) is established as the plesiomorphic state because of its widespread distribution throughout Glypthelmins lineages III and IV (sensu Brooks, 1977) and other plagiorchioid genera parasitic in anurans.

(17) Seminal vesicle (3 states)

(0) internal, unipartite, coiled; (A) internal, unipartite, straight; (B) internal, bipartite, straight

Character States: The character states reported here agree with published accounts. The bipartite condition of G. shastai has not been reported before (see Appendix D).

Polarity: State (0) is established as the plesiomorphic state because of its widespread distribution throughout Glypthelmins lineages III and IV (sensu Brooks, 1977) and other plagiorchioid genera parasitic in anurans.

(18) Length of cirrus sac / length of forebody[1] (2 states)

(0) ≤0.5:1; (1) >0.5:1

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[1] The region from the anterior edge of the ventral sucker to the anterior end of the body.

Character States: This character quantifies the noticeably greater cirrus sac length in G. shastai and H. intestinalis. In all of the species studied, the cirrus sac overlaps the ventral sucker, and the genital pore is immediately anterior to the ventral sucker. Longer cirrus sacs extend further posteriad.

Polarity: This is established by ingroup relationships. The plesiomorphic state is widespread among glypthelminth lineages III and IV (sensu Brooks, 1977) and other plagiorchioids parasitic in anurans.

(19) Mean egg length (3 states)

(0) <30μm; (A) 30-40 μm; (B) >40μm

Character States: Ranges and (means), in μm, all specimens:

G. hyloreus, 46-52 (50)

G. pennsylvaniensis, 21-43 (34)

G. robustus, 27-37 (31)

G. shastai, 42-50 (45)

G. californiensis, 42-50 (45)

G. facioi, 28-32 (29)

G. quieta, 42-50 (45)

H. intestinalis, 45-53 (50)

Brooks (1976b) reported a range of 23-26 μm for the length

of the eggs in G. robustus. Brenes et al. (1959) reported
a range of 33-47 μm for the length of the eggs in their
specimens of G. facioi (not examined). The values for
G. facioi in the current study agree with those of Sullivan
(1976).

Polarity: State (0) is established as the plesiomorphic
state because of its widespread distribution throughout
Glypthelmins lineages III and IV (sensu Brooks, 1977) and
other plagiorchioid genera parasitic in anurans.

(20) Cercarial stylet (2 states)

(0) present; (1) absent

Character States and Polarity: The xiphidiocercarial
condition (presence of stylet) is symplesiomorphic for the
study group, being postulated by Brooks et al. (1985b) as
a synapomorphy for the Order Plagiorchiiformes. The
gymnocephalous condition (no stylet) occurs in G. hyloreus
and G. pennsylvaniensis.

(21) Shape of excretory vesicle (2 states) (Fig. 19)

(0) bifurcating anterior to level of testes; (1)

bifurcating at, or posterior to, level of testes

Character States: I consider these inferred character states to be of some help in circumventing problems with traditional terminology for the shape of the excretory vesicle in digeneans. This terminology has referred to V, Y, or I shapes. Another traditional descriptive term is that of a "tubular" vesicle, primarily for reference to an I-shaped vesicle. Manter (1969) noted the ambiguity of this term, since all three shapes of vesicles are tubular in some sense of the word. On the basis of a Y or I-shaped vesicle, certain members of Glypthelmins have been assigned to the Plagiorchiidae (Y-shaped), while others have been assigned to the Macroderoididae (I-shaped) (see Sullivan, 1976). Schell (1961) placed H. intestinalis in the Macroderoididae on the basis of its I-shaped vesicle. With the exception of H. intestinalis, which was not included in the analysis, and of G. shastai, for which there was at the time insufficient information, Sullivan (1976) placed all of the species included in this present study into the genus Glypthelmins on the basis of their I-shaped excretory vesicle. The following can be said of vesicle shape in general. Entering the vesicle postero-medially from each side of the body is an excretory tubule. The shape of the vesicle is determined by (a) the relative width of the tubules in the area of this bifurcation, and (b) the point along the length of the vesicle at which its body

bifurcates. If the vesicle bifurcates towards its base at the excretory pore, it is V-shaped. If it bifurcates further anteriad, it is Y-shaped. If the bifurcation involves only the unexpanded tubules, it is I-shaped, with the tubules forming the thin arms of a Y. All of the species studied here have an I-shaped excretory vesicle in which the bifurcation occurs at, or posterior to, the level of the testes (Fig. 19a) (reported here in G. shastai for the first time, see Appendix D). Although Brooks (1976b) reported that the excretory vesicle of G. robustus is I-shaped, this is not clear in the specimens examined (all available specimens). Accordingly, this character is coded as a missing datum for that species (Table II).

Polarity: Glypthelminths in lineages III and IV (sensu Brooks, 1977), in Central and South America, possess a Y-shaped vesicle in which the bifurcation occurs anterior to the level of the testes (Fig. 19b) (see Sullivan, 1977a,b). This state is widespread throughout plagiorchioid genera parasitic in anurans.

1. CHARACTERS EXCLUDED FROM ANALYSIS

Metraterm: The metraterm has been described as muscular in
G. pennsylvaniensis, by Byrd and Maples (1963), and in
G. shastai, by Ingles (1936). I observed the same condition in
specimens of G. robustus, G. quieta (both stained with
hematoxylin), G. californiensis, and H. intestinalis (both
stained with Fast Green). In all cases, longitudinal muscle
fibers run the length of the metraterm in the region of the
surrounding gland cells. The observation of the muscle fibers
proved to be highly dependent upon the preparation of the
individual worm, and was greatly facilitated by the use of Fast
Green. I did not include this character in the analysis because
I was unable to examine all of the species in a comparable
manner.

Vas deferens: I observed that the two vasa efferentia were
joined into a vas deferens before they entered the cirrus sac in
older adults of H. intestinalis, as well as in some specimens of
G. shastai and G. facioi (presence not related to body size).
The vasa efferentia were observed to enter the cirrus sac
separately in all available specimens of G. hyloreus,
G. pennsylvaniensis, and G. robustus, as well as in young adults
of H. intestinalis, G. californiensis, and G. quieta (although
Rankin, 1944, illustrated a vas deferens in a specimen of
G. quieta). Given that the presence of a vas deferens is age-

dependent in H. intestinalis, the lack of sufficient specimens of known ages for all of the species studied necessitated the exclusion of this character from the analysis.

Table II presents the codings assigned to the 21 characters listed above. A "0" code in the listings below is an arbitrary coding value for the plesiomorphic state; it does not necessarily indicate absence.

Table II - CHARACTERS ANALYZED

Character Number

									1	1	1	1	1	1	1	1	1	1	2	2	
1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	
0	0	0	0	?	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	0	composite outgroup
0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	A	A	0	B	1	1	G. hyloreus
0	0	1	0	0	0	0	0	0	0	B	0	0	0	0	0	0	A	1	1		G. pennsylvaniensis
0	A	0	0	0	0	1	1	0	0	A	0	0	1	1	0	0	0	A	0	?	G. robustus
0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	1	0	B	1	B	0	1	G. shastai
0	0	0	0	1	0	0	0	B	C	0	1	0	1	1	B	B	1	B	0	1	H. intestinalis
1	0	0	0	0	0	0	0	A	1	C	0	0	1	1	A	B	0	B	0	1	G. californiensis
1	0	0	0	0	1	0	0	0	1	B	0	0	1	1	A	B	0	B	0	1	G. quieta
1	B	0	1	0	0	0	0	0	1	B	1	0	1	1	0	B	0	A	0	1	G. facioi

All analyses were conducted on the Amdahl 5850 computer at the University of British Columbia. Initial runs of PAUP used the

MULPARS step-wise addition of OTUs algorithm with global branch swapping (SWAP=GLOBAL). This algorithm examines all minimum-length trees that are obtained for a data set. Each of these trees is, in turn, input to the branch-swapping procedure. In global branch-swapping, the fit of each OTU at each position on the tree is examined. In later computer runs, when ambiguities in character coding had been eliminated as much as possible, the branch-and-bound (BANDB) algorithm was used. This approaches an exhaustive inspection of all possible topologies of a tree through a modification of the methodology of Hendy and Penny (1982). As a final analysis, the ALLTREES algorithm was used. For small numbers of taxa, this examines every possible topology of a tree for minimum-length representations. In the Unordered Analysis of the multistate characters with PAUP, a CSPOSS output was requested. This lists the nodes for which there is ambiguity of the character state after optimization.

From the resulting cladogram, the inferred transformation series of each multistate character was represented with a numerical code. The coding method is herein referred to as Nonredundant Linear Coding, and its properties are examined in Appendix F. The recoded multistate characters were then reinserted into the data matrix. This new matrix was then reanalyzed with both the ALLTREES algorithm of PAUP (Ordered Analysis on all characters) and the WAGNER.S algorithm of PHYSYS. The latter performs step-wise addition of OTUs with Wagner parsimony and subsequent global branch-swapping.

RESULTS AND DISCUSSION

PHYLOGENETIC ANALYSIS

The cladogram for the eight species, rooted with a composite outgroup (ROOT=ANCESTOR), is presented in Figure 20. The postulated homologous series for both binary and multistate characters can be interpreted by reading the tree in conjunction with the character list in the preceding section. With an Unordered Analysis of the multistate characters, nos. 2, 9, 11, 16, 17, and 19, the same tree is obtained with MULPARS SWAP=GLOBAL, BANDB, and ALLTREES. The computer runs for ALLTREES are given in Appendix A.

The goodness-of-fit of a parsimony tree to the data from which it is inferred is usually measured with the Consistency Index (CI) (Kluge and Farris, 1969; Farris et al., 1970). This is a ratio of the minimum number of evolutionary changes (character steps) required by the data, divided by the number of changes postulated by the tree. A perfect fit of the tree to the data gives a CI of 1.0. The CI of the cladogram in Figure 20 is 0.848. The standard calculation of the CI does not distinguish between autapomorphies and synapomorphies. It can be increased by the addition of either type of character to a tree. Since only the latter strengthen a phylogenetic hypothesis, the inclusion of autapomorphies in the calculation can produce a misleadingly high goodness-of-fit value. For this

reason, it is herein suggested that non-homoplasious autapomorphies be excluded from the calculation of the CI. For the present study, this procedure gives a CI of 0.769. In association with this proposed modification, the properties of the CI are examined in Appendix G.

Each multistate transformation series was recoded (Nonredundant Linear Coding, see Appendix F) for ordered analysis with both PAUP and PHYSYS. This recoding assigned a numerical value to each state of the multistate character such that the multistate tree, which had been inferred through parsimony criteria, was unambiguously represented as a set of linearly coded transformation series. In addition to providing an example of the multistate coding methods discussed in Appendix F, this procedure allowed an analysis of the full matrix with the WAGNER.S algorithm of PHYSYS, which operates only on ordered data.

Multistate characters 2, 9, 16, 11, and 17 have no ambiguities as to their nodal values, and their recoding is given in Figure 21. Character 19, the mean egg length, shows ambiguity at some nodes (Fig. 22). As noted in the documentation for PAUP (version 2.4, 1985), the resolution of this ambiguity for the purpose of the program's output is completely arbitrary (thus, the importance of requesting a CSPOSS listing). In the Unordered ALLTREES analysis given in Appendix A, state 19(B) was chosen. These nodal assignments are outside any parsimony considerations. Their alternative resolutions do not affect the length of the tree.

So as to provide a tentative resolution for character 19, additional criteria were considered. The character is that of mean egg length, with state 0 <30μm, state A = 30-40 μm, and state B >40μm. With an assumption of linear increase of egg length, state A was given precedence in the transformation series. However, at the node uniting G. shastai, H. intestinalis, G. californiensis, G. quieta, and G. facioi, state B was given precedence because there is some uncertainty as to whether the mean egg length of G. facioi is state A or B (see the section, CHARACTER ANALYSIS).

The a posteriori recoding of the six multistate characters produced five new character columns in the data matrix. The reassigned character numbers are given in Table III. When run through an Ordered ALLTREES analysis in PAUP, as well as a WAGNER.S analysis in PHYSYS, the new matrix produced the same cladogram as that in Figure 20 (see Appendix A).

Table III - CHARACTERS RENUMBERED FROM UNORDERED ANALYSIS

Character No. in Unordered Analysis	Character State Transformations	Character No. in Ordered Analysis
1	0 - 1	1
2	0 - A(1)	2
	0 - B(1)	3
3	0 - 1	4
4	0 - 1	5
5	0 - 1	6
6	0 - 1	7
7	0 - 1	8
8	0 - 1	9
9	0 - A(1)	10
	0 - B(1)	11
10	0 - 1	12
11	0 - A(1)	13
	0 - B(1) - C(2)	14
12	0 - 1	15
13	0 - 1	16
14	0 - 1	17
15	0 - 1	18
16	0 - A(1)	19
	0 - B(1)	20
17	0 - A(1)	21
	0 - B(1)	22
18	0 - 1	23
19	0 - A(1) - B(2)	24
20	0 - 1	25
21	0 - 1	26

As a final analysis, the expanded matrix was mapped onto the Brooks (1977) cladogram for Glypthelmins (see Appendix A). Thus, the hypothesis of relationships postulated by the earlier cladogram was compared to the present hypothesis by seeing how well all of the present character data supported it. The earlier analysis used 11 characters, and differed in its placement of G. facioi and by its non-inclusion of H. intestinalis. The mapping-on can be done by hand, but it is more easily accomplished with the DIAGNOSE routine of PHYSYS. The new data fit onto the earlier cladogram with an unmodified CI of 0.788.

TAXONOMIC CONSIDERATIONS

The higher level monophyly of Brooks' (1977) analysis of North, Central, and South American species of Glypthelmins is supported by the results of the present study. The clade consisting of G. hyloreus and G. pennsylvaniensis corresponds to the genus Hylotrema, proposed by Sullivan (1972). Brooks' analysis also supported this correspondence. Despite its use by Schell (1985), Hylotrema is not a valid genus. This is because it has been proposed in no place other than a dissertation (Article 9, International Code of Zoological Nomenclature, 3rd edition).

The results presented in Figure 20 postulate that Haplometrana is not the sister taxon of Glypthelmins, but is in fact a member of one of the clades (lineage II sensu Brooks,

1977) within <u>Glypthelmins</u>. Thus, <u>Glypthelmins</u> will not be monophyletic if the monotypic genus <u>Haplometrana</u> is excluded from it. It is therefore proposed that <u>Haplometrana</u> be synonymized with <u>Glypthelmins</u> as a junior subjective synonym. Appendix D presents this synonymy in conjunction with a redescription of <u>H</u>. <u>intestinalis</u> and the necessary emendation of the generic diagnosis of <u>Glypthelmins</u>. Also in Appendix D is a redescription of <u>G</u>. <u>shastai</u>, made possible by the examination of the additional specimens used in this study.

Such classificatory changes are directed towards maintaining consistency between classifications and the evolutionary trees whose relationships they are intended to communicate (Hull, 1964; Wiley, 1979, 1981b). This consistency can be lost when certain characters are considered, <u>a priori</u>, to be more important than others as criteria of group membership. See Figure 23a. The tree at the top is a cladogram for five taxa with four characters, each of which occurs in either a "U" or "X" state. The cladogram postulates the evolution of six character states (i.e., it is six character steps long), including the parallel evolution of character 4 and the reversal of character 1.

In a departure from the criterion of grouping by monophyly, some of the characters might be considered more important because of their functional role, as discussed in Chapter II. Or, some might be considered more important as, say, generic or familial level characters. In such cases, trees such as those in Figures 23b-d would result. Tree 23b results from putting

taxa A and B in their own group because they do not possess the derived state of character 3 that makes taxa C, D, and E look different. The tree is seven character steps long, and is an example of grouping by shared primitive characters. Tree 23c results from putting A, D, and E together because of their common possession of the derived state of character 4. It is seven steps long, and is an example of grouping by convergent traits. Tree 23d results from excluding taxon E from the group because it does not possess the derived state of character 1. It is eight steps long, and is an example of failing to distinguish between plesiomorphic and apomorphic absence. All three types of character interpretation above distort phylogenetic relationships and interfere with the use of a phylogenetic tree as a general reference system in comparative biology.

IV. USING THE PHYLOGENETIC TREE TO STUDY EVOLUTIONARY EVENTS

Once a cladogram has been constructed, it can be used as a general reference system for further comparative biological studies. This chapter presents such studies of five types of events inferred to have occurred during the evolution of the species of _Glypthelmins_ and _Haplometrana_ studied here. For the remainder of this dissertation, this group will be referred to as the _Glypthelmins_ clade. Although the synonymization of _H. intestinalis_ as _G. intestinalis_ n. comb. is supported by the analysis in Chapter III (see Appendix D), the former name is retained here for continuity.

The five evolutionary events considered here are: (1) the phylogenetic concordance of larval and adult characters, (2) life cycle evolution, (3) heterochronic character development, (4) coevolution of parasites and hosts, and (5) biogeographic and speciation patterns. In addition, heterochronic development is studied further, with a report on the experimentally-produced heterochronic development of the hindbody in _H. intestinalis_.

PHYLOGENETIC CONCORDANCE OF LARVAL AND ADULT CHARACTERS

INTRODUCTION

Cladograms constructed from the characters of different developmental stages can be compared in order to determine whether the different character sets indicate the same phylogenetic relationships. This comparison is important for two reasons. First, the ontogenetic stages (= larval + adult stages) of digeneans occur, to varying extents, in different environments. At the very least, there are three such environments: the mollusc intermediate host, in which the miracidia, sporocysts, rediae, and cercariae develop (see next section); the water, in which the cercariae disseminate; and the vertebrate final host, in which the adult digeneans develop. If phylogenetic analyses of the different developmental stages produce the same hypothesis of relationships, then there is evidence of constraints on character evolution that produce similarly covarying sets of characters regardless of environmental differences and presumed selection pressures.

Second, a priori assumptions of recapitulation can be used to promote or discount the usefulness of certain characters in phylogenetic inference. By this I mean the following. There are six basic types of sequence changes that can occur in an ancestral developmental sequence (say, A-B-C-D). There can be an addition of a stage that is terminal (A-B-C-D-X) or

nonterminal (A-B-X-C-D). There can be a deletion of a stage that is terminal (A-B-C) or nonterminal (A-C-D). And there can be a substitution of a stage that is terminal (A-B-C-X) or nonterminal (A-B-X-D). As discussed in O'Grady (1985b), a study of life cycle evolution in the parasitic flatworms, so long as there is some sort of inherited temporal series involved, a "developmental sequence" can refer to both ontogenetic stages and life history stages. Thus, as discussed in Chapter II (A SINGLE EXPLANATORY FRAMEWORK)., it was postulated that the evolution of the Digenea involved a nonterminal addition of ontogenetic stages and a terminal addition of a host.

To the extent that evolutionary transformations are added to and retained in developmental sequences, information about those evolutionary events will be retained in individual developmental sequences. Conversely, to the extent that the sequences are altered, such information will be lost. Haeckelian recapitulation (Haeckel, 1866) exists when there has been terminal addition to an ancestral sequence (see Fink, 1982; Gould, 1977; Lovtrup, 1978); it allows for the strongest inference of phylogenetic relationships from developmental sequence observations. Von Baerian recapitulation (von Baer, 1828) exists when there has been terminal substitution to an ancestral sequence (see Fink, 1982); it allows for a weaker inference of phylogenetic relationships from developmental sequence observations. Nonterminal alterations to a developmental sequence will disrupt the parallel between evolutionary history and individual sequences, and thus violate

both Haeckelian and von Baerian criteria of recapitulation. Using Haeckel's terminology, sequence characters that are recapitulations (sensu lato: the term will henceforth be used here to refer to both Haeckelian and von Baerian recapitulation), are palingenetic, while those that are not are cenogenetic. Cenogenetic changes have come to be often attributed to the effects of natural selection (e.g., Russell, 1916).

Some parasitologists have attempted to infer phylogenetic relationships strictly from characters that are considered to be palingenetic. Chitwood and Chitwood (1974: 213), for example, considered certain larval characters of nematodes to be cenogenetic adaptations associated with larval selection pressures, and thus not indicative of phylogenetic relationships. Goodchild (1943) presented a similar argument for the cercariae of phyllodistome digeneans. Cable (1974) considered the larval stages of digeneans to be recapitulations of ancestral adult stages, and so based his phylogenetic inferences on the larvae. Conversely, Gibson (1981) considered the larval stages to be cenogenetic adaptations, and so based his phylogenetic inferences on the adults. There are two drawbacks to such a reliance on recapitulatory phenomena: (1) the perception that recapitulatory developmental sequences are the only source of information on phylogenetic relationships, and (2) the application of this perception in conjunction with some a priori assumption about which of the characters in an analysis are recapitulatory.

Recapitulatory characters are not the only source of information for phylogenetic inference because sequence analysis is not a primary source of such information. [1] The study on the parasitic flatworms by Brooks et al. (1985a), discussed above, is not only an example of other types of character data being used to draw phylogenetic inferences, it also shows that a cladogram can be used to come to a posteriori decisions about the types of sequence changes involved in the evolution of a clade. Thus, an initial assumption of recapitulation as the primary criterion with which to infer phylogeny encounters the same problems as does any evolutionary interpretation based upon a single transformation series: namely, without a phylogenetic hypothesis corroborated by other characters, it is not possible to determine the polarity or the order of transformation. Just as a phylogenetic hypothesis is necessary to distinguish primitive absence from secondary absence, so too is it necessary to distinguish whether, say, in a study of three taxa, the presence of the developmental sequence A-B-C in one of them and the presence of the sequence A-B-C-D in the other two is indicative that the latter two taxa are apomorphic.

The cladistic analysis of the Digenea by Brooks et al. (1985b) was, in part, a test of previous arguments that only larval or adult characters are of use in inferring the

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[1] Nelson (1978, 1985) and Nelson and Platnick (1981) argued for the utility of ontogenetic sequence data for polarizing characters. Brooks and Wiley (1985) and Kluge (1985) offered counter-arguments.

relationships of these worms (see Cable, 1974; Gibson, 1981). This question was especially interesting because our earlier study (Brooks et al., 1985a) had hypothesized that the larval stages of digeneans are nonterminal additions to an ancestral ontogeny. By recapitulationist criteria, these cenogenetic characters should be phylogenetically uninformative. Furthermore, by selectionist criteria, they should instead correlate with the different environmental conditions experienced by the larvae, as compared to the adults. The study found that (1) larval characters alone resolved 74% of the tree, (2) adult characters alone resolved 76% of the tree, (3) there was no disagreement in phylogenetic inferences drawn from only larval or adult characters, and (4) ecological characters resolved 26% of the tree. The concordance of larval and adult characters was taken as evidence that there are evolutionary constraints capable of producing a single, phylogenetically informative, covarying set of characters despite the nonterminal intercalation - i.e., the non-recapitulatory modification - of ontogenetic stages, and despite development in different environments.

Such comparisons of phylogenetic hypotheses drawn from different data sets involve the comparison of the branching patterns, or topologies, of two or more trees. The terms used in systematics for the degrees of similarity of trees are congruence (Sokal and Sneath, 1963; Farris, 1971; Mickevich, 1978) and consistency (Hull, 1964; Wiley, 1981b). When trees have the same topology, as do Figures 24a and b, they are said

to be completely congruent (solid arrows) with each other. When a tree is ambiguous about the relationships involved, as is the polytomy in Figure 24c, then all possible resolutions of that tree, such as Figures 24a, b, and d, are said to be consistent (dashed arrows) with it. Complete congruence requires that trees have identical topologies, consistency only requires that there are no disagreements among the trees. Any intermediate condition (i.e., only parts of trees are identical) is referred to as partly congruent (e.g., Mickevich, 1978; Miyamoto, 1981; Crother et al., 1986). Trees with mutually exclusive topologies, such as Figures 24b and d, are said to be incongruent with each other (= the incompatiblity criterion of Camin and Sokal, 1965; see Farris, 1971).

LIFE HISTORY DATA

Figure 25 shows a generalized digenean life cycle. It is a complex life cycle, involving one or more intermediate hosts in addition to the final, or definitive host, in which the parasite develops to maturity. Eggs pass into the water. The miracidium emerges from the egg and enters the first intermediate host, usually a gastropod mollusc, sometimes a polychaete. Within the host tissues, the miracidium metamorphoses into a relatively undifferentiated stage called a sporocyst. Asexual reproduction then occurs, as numerous rediae develop from germinal cells in each sporocyst. This reproduction may be preceded by a second generation of sporocysts. There can also be a second generation

of rediae. Rediae are somewhat more differentiated than sporocysts, possessing a mouth, pharynx, and gut. Again, asexual reproduction occurs, as numerous cercariae develop from germinal cells in each redia. A cercaria is the juvenile of the adult digenean, in that its body develops into the adult body. It possesses a complete digestive and osmoregulatory system, and often has the genital primordia as well. In addition, there is usually a tail. In some groups, there is also a stylet in the oral sucker. The cercaria emerges from the snail host and enters the body of the next host either through penetration or ingestion. If a two-host life cycle is involved, the parasite will develop to maturity in this second host. If a three-host life cycle is involved, the parasite develops into a metacercaria in the tissues of the second intermediate host, and then infects the final host when the second intermediate host is eaten.

1. PREVIOUS STUDIES

There are published reports of the life histories of the following species: G. hyloreus, by Martin (1969), G. pennsylvaniensis, by Sullivan and Byrd (1970), G. quieta, by Rankin (1944) and Leigh (1946), and H. intestinalis, by Olsen (1937). In all of these species, there is a three-host life cycle in which the frog is both second intermediate host and final host. Glypthelmins hyloreus develops in Lymnaea stagnalis L.; the cercariae possess a finfold, but no stylet; they enter

the nares of H. regilla tadpoles, remain as unencysted metacercariae in the coelom, then enter the gut when the host metamorphoses. Glypthelmins pennsylvaniensis develops in Physa gyrina Say; the cercariae possess a finfold, but no stylet; they penetrate the body of H. crucifer tadpoles and remain as unencysted metacercariae beneath the epidermis; the host becomes infected when it ingests the sloughed epithelium sometime after metamorphosis. Glypthelmins quieta develops in P. gyrina and P. integra Haldeman; the cercariae possess a finfold and stylet; they penetrate and encyst in the epithelium of R. pipiens adults, which become infected when they ingest the sloughed epithelium. Haplometrana intestinalis has been reported to develop in P. utahensis (Clench), by Olsen (1937), in P. gyrina and P. ampullacea Gould, by Schell (1961), and in Lymnaea stagnalis wasatchensis (Hemphill), L. bulimoides Lea, and Helisoma trivolvis (Say), by Current and Lang (1975). The cercariae possess a finfold and stylet; they penetrate and encyst in the epithelium of R. pretiosa adults, which become infected when they ingest the sloughed epithelium. The cercariae will encyst in, but not develop to maturity in, adults of R. pipiens, R. clamitans Latreille, and R. areolata (see Olsen, 1937).

2. <u>NEW</u> <u>DATA</u>

The life cycles of three parasite species were maintained in the laboratory: <u>G</u>. <u>californiensis</u>, <u>G</u>. <u>quieta</u>, and <u>H</u>. <u>intestinalis</u>. This is the first report of the life cycle of <u>G</u>. <u>californiensis</u> (see Appendix E). For each of the three species, the appropriate snail and anuran host were maintained in the laboratory. Uninfected populations were established for the following species of locally-collected snails (identifications based on Clarke, 1981): <u>Physa</u> <u>gyrina</u> Say, <u>P</u>. <u>propinqua</u> Tyron, <u>P</u>. <u>lordi</u> Baird, <u>Stagnicola</u> <u>elodes</u> (Say) [=<u>Lymnaea</u> <u>palustris</u> (Muller)], and <u>Pseudosuccinea</u> <u>columella</u> (Say). This last species is indigenous to eastern North America and has been introduced to the western part of the continent (Clarke, 1981). Difficulties were encountered in rearing anurans from eggs to adults. Five species were involved: <u>R</u>. <u>aurora</u>, <u>R</u>. <u>pretiosa</u>, <u>R</u>. <u>cascadae</u>, <u>R</u>. <u>pipiens</u> and <u>B</u>. <u>boreas</u>. Each suffered high mortality as tadpoles and during the transition from tadpoles to adults. As a result, only <u>R</u>. <u>pretiosa</u> was completely reared in the laboratory. For the remaining four species, young adults were collected from areas where there had been no reports of the parasite species involved. Surveys were conducted of these areas to support the conclusion of an absence of parasitism. The areas were:

<u>R</u>. <u>aurora</u>: Streams associated with Hicks and Moss Lakes, Sasquatch Provincial Park, British Columbia (approximately 20 km NE of Chilliwack) (clear, fast moving water, no snails seen)

R. cascadae: Dead Fall Lakes, Siskiyou County, California
(clear, glacial lakes at approximately 2200m elevation, no
snails seen)

R. pipiens: Elk Point, Union County, South Dakota (flooded
gravel pit, no snails seen)

B. boreas: Meadows north of Kangaroo Lake, Siskiyou County,
California (no R. pretiosa and no H. intestinalis found)

The life cycles were maintained as follows. For G. quieta, the
procedures of Rankin (1944) and Leigh (1946) were used for
reference: Physa gyrina and P. propinqua were fed overnight on
eggs that had been teased out of mature worms and had been
allowed to sit in filtered pond water at 15°C for at least a
week. Approximately one month later, cercariae began to emerge
from the snail. Adult R. pipiens were placed for two hours in
finger bowls containing shedding snails in filtered pond water.
The frogs were then kept in a common tank at 15°C. For
H. intestinalis, Olsen's (1937) study was used for reference:
P. gyrina and P. propinqua were fed overnight on H. intestinalis
eggs that had been prepared in the above manner. Approximately
one month later, cercariae began to emerge from the snails.
Adult R. pretiosa were exposed to them as above and then kept in
a common tank. Attempts were made to establish infections of
H. intestinalis in the adults of R. cascadae, R. aurora, and
B. boreas. Frogs were either exposed to H. intestinalis

cercariae or were fed metacercariae encysted in the skin of
R. pretiosa. In addition, the penetration behavior of cercariae
of H. intestinalis was observed on pithed adults of R. cascadae,
R. aurora, R. clamitans, R. catesbeiana, R. pipiens, and
B. boreas. The penetration behavior of cercariae of G. quieta
was observed on pithed adults of R. cascadae and R. aurora. In
an attempt to produce an infection, live adults of R. aurora
were also exposed to cercariae of G. quieta.

The miracidia, daughter sporocysts, and cercariae of
G. californiensis were obtained. The results, presented in
Appendix E, show that members of this species develop as
xiphidiocercariae in sporocysts in the physid snails, Physa
gyrina and P. propinqua. The cercariae encyst in the skin of
adults of R. aurora, and develop in the small intestine of the
frog when the host eats the shed skin. Cercariae of G. quieta
and H. intestinalis were also obtained from infections of
P. gyrina and P. propinqua. Metacercariae of both species were
obtained encysted in the skin of specimens of R. pipiens and
R. pretiosa, respectively. Adults of G. quieta were obtained
from R. pipiens at one, four, eight, and twelve months after
exposing adult frogs to cercariae. Adults of H. intestinalis
were obtained at the same ages from R. pretiosa and R. cascadae
after exposing adult frogs to cercariae. Attempts to infect
adults of R. aurora with cercariae and metacercariae of
H. intestinalis were unsuccessful. Adults of H. intestinalis
were obtained at one, four, and twelve months from B. boreas
after feeding the adult toads metacercariae encysted in the skin

of <u>R</u>. <u>pretiosa</u>. Changes in the relative body dimensions of these specimens are examined in the section, HETEROCHRONIC DEVELOPMENT.

The infection of <u>B</u>. <u>boreas</u> with <u>H</u>. <u>intestinalis</u> proved to be difficult. Exposure to cercariae was unsuccessful. The cercariae lost their tails, penetrated the skin, and balled up under the epithelium within approximately 30 minutes. Not only is this penetration process twice as long as that shown by <u>H</u>. <u>intestinalis</u> when penetrating ranids (see below), but the encysted metacercariae on the toads were not as deeply embedded in the epithelium, and could be dislodged fairly easily. Alternatively, feeding the toads metacercariae encysted in the skin of <u>R</u>. <u>pretiosa</u> produced an infection on the third trial. The infection of tadpoles of <u>B</u>. <u>boreas</u> was not attempted.

Olsen (1937) reported that the cercariae of <u>H</u>. <u>intestinalis</u> were capable of encysting in, but not developing to maturity in, adults of <u>R</u>. <u>areolata</u>, <u>R</u>. <u>pipiens</u>, and <u>R</u>. <u>clamitans</u>. The present study observed cercarial encystment in adults of <u>R</u>. <u>pipiens</u>, <u>R</u>. <u>clamitans</u>, <u>R</u>. <u>catesbeiana</u>, and <u>R</u>. <u>aurora</u>. Of these, only <u>R</u>. <u>aurora</u> individuals were subsequently studied for development of the worm in the gut. The results were negative. In all four ranid species, as with <u>R</u>. <u>pretiosa</u> and <u>R</u>. <u>cascadae</u>, cercariae took approximately 15 minutes to penetrate the epithelium. In each case, the cercaria would stop swimming upon contacting the frog. It would then crawl over the surface for a short distance, penetrate the skin, lose its tail as it entered, and ball up. The longer penetration time shown by specimens of

H. intestinalis when exposed to B. boreas results from both extended crawling and penetration periods.

It was also observed that cercariae of G. quieta could encyst in adults of R. pretiosa, R. aurora, and R. cascadae. Penetration behavior and timing is the same as that for H. intestinalis with the ranids noted above. Subsequent study of R. aurora individuals found G. quieta to develop for one month, but no more, in the gut.

ANALYSIS OF LARVAL CHARACTERS

Figure 26 presents a cladogram for the five species of Glypthelmins or Haplometrana for which there are larval data. It is constructed from the following five cercarial traits: (1) cercarial stylet, (2) dorso-ventral finfold, (3) tandem testes, (4) symmetrical testes, and (5) tegumental scales. Observations for G. californiensis, G. quieta, and H. intestinalis were conducted on specimens reared in the laboratory during the course of this research. Observations for G. hyloreus and G. pennsylvaniensis were taken from the studies by Martin (1969) and Sullivan and Byrd (1970), respectively. The tree is congruent with the cladogram in Figure 20 with respect to its support of the G. hyloreus + G. pennsylvaniensis clade, and the G. californiensis + G. quieta clade, and it is consistent with respect to the placement of H. intestinalis (the dashed line shows that species' placement in Figure 20.

There is thus no disagreement between phylogenetic

inferences drawn from the available adult and larval data. This conclusion can be contrasted with the comments by Martin (1969) and by Sullivan and Byrd (1970) that the cercariae of G. hyloreus and G. pennsylvaniensis appear to be instances of larval divergence from the usual condition in Glypthelmins. In conjunction with life history data, discussed in the next section, the latter authors supported the interpretation of this divergence as a phylogenetically uninformative cenogenetic property. The present study has shown that although G. hyloreus and G. pennsylvaniensis may, in certain respects, be highly derived members of the clade, this derivation does not obscure their phylogenetic relationships.

ANALYSIS OF LIFE CYCLE EVOLUTION

Seven life cycle properties can be examined: (1) a physid snail as at least one of the first intermediate host types, (2) a lymnaeid snail as at least one of the first intermediate host types, (3) a lymnaeid snail as the only first intermediate host type, (4) a helisomid snail as at least one of the first intermediate host types, (5) an anuran as both second intermediate host and final host, (6) development as an unencysted metacercaria in anuran tadpoles, and (7) development as an encysted metacercaria in anuran adults.

The tree supported by these characters is presented in Figure 27. Again, there is no disagreement with the primary cladogram in Figure 20. And again, any larval divergence in

G. hyloreus and G. pennsylvaniensis does not obscure phylogenetic relationships. Physid snails are concluded to be the plesiomorphic first intermediate host, with lymnaeids replacing them in G. hyloreus, and augmenting them in H. intestinalis. The latter species is also postulated to have acquired the ability to develop in helisomid snails. Development as an unencysted metacercaria in anuran tadpoles is concluded to have arisen as a synapomorphy for the G. hyloreus + G. pennsylvaniensis clade, while development as an encysted metacercaria in anuran adults is concluded to have arisen as a synapomorphy for the H. intestinalis + G. californiensis + G. quieta clade.

HETEROCHRONIC DEVELOPMENT

INTRODUCTION

Heterochrony exists when there are differences in the relative developmental timing of characters. It is a comparative phenomenon: developmental timing and its effects must be different with respect to something, and that something can be the ancestral, or plesiomorphic, state. Heterochrony in a descendant can result in a derived condition that is, with respect to some criterion of change, less developed or more developed than the plesiomorphic condition. If it is less developed, the heterochrony involved is termed paedomorphosis

(e.g., Gould, 1977; Alberch et al., 1979). If it is more developed, it is termed peramorphosis (Alberch et al., 1979).

Alberch et al. (1979) gave three parameters of developmental change that could produce heterochrony. These are illustrated in Figure 28, in which the degree of development (gamma) of the ancestral state (A) and the derived state (D) is plotted against the time period over which development occurs. The parameters of change are: the rate of development (k), the time at which development starts (alpha), and the time at which it stops (beta). Paedomorphosis can be produced by (1) a slower developmental rate: neoteny;[1] (2) a later initiation of development: postdisplacement ; and (3) an earlier cessation of development: progenesis. Peramorphosis can be produced by (1) a faster rate: acceleration; (2) an earlier initiation: predisplacement ; and (3) a later cessation: hypermorphosis.

These are the six basic types of heterochrony that occur when the three parameters change one at a time. Although Alberch et al. (1979) did not discuss them, additional types of heterochrony would result if more than one parameter changed. For example, a slower rate in conjunction with an earlier cessation of development would produce paedomorphosis. It is even possible for a process that is usually associated with paedomorphosis, such as a slower rate, to produce a peramorphic

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[1] In this usage, this term is disassociated from particular reference to gonadal development. The terminology here treats all development equally, and thus does not give special weight to gonadal maturation.

result through a sufficiently early initiation or a sufficiently delayed cessation of development, or both. It should also be noted that peramorphosis is only possible when the apomorphic development is capable of developing beyond the plesiomorphic state. If there is no such capability, acceleration or predisplacement will simply result in the derived developmental trajectory reaching the plesiomorphic final state relatively sooner and then levelling off with respect to gamma. Thus, the six developmental changes in Figure 28 should be seen as the processes that will produce the types of heterochrony described under conditions of ceteris paribus.

Heterochrony may or may not involve alterations in developmental sequences. When it does, it provides the processes by which some of the sequence changes discussed in the first section of this chapter can evolve. With respect to Figure 28, all three types of paedomorphosis involve a descendant not attaining the plesiomorphic state because of a terminal deletion to its developmental sequence, and all three types of peramorphosis involve a descendant evolving beyond the plesiomorphic state because of a terminal addition to its developmental sequence. Peramorphosis therefore exhibits Haeckelian recapitulation. It appears that the third type of terminal change, substitution, is not produced by heterochronic processes. As for nonterminal sequence changes, it appears that heterochrony can be involved sometimes, but if it is, it will not be one of the six types outlined by Alberch et al. (1979). This is partly because in a nonterminal change, the

plesiomorphic terminal state is still attained. There may, however, be alterations in the relative time at which the descendant attains that state. For example, a nonterminal deletion (1-2-3-4 to 1-2-4) may result in state 4 being attained sooner. Conversely, a nonterminal addition (1-2-X-3-4) or even substitution (1-2-X-4) may result in state 4 being attained later. Finally, there can be heterochrony that does not involve any terminal or nonterminal changes to a developmental sequence. In this, the plesiomorphic sequence still occurs in the descendant, but it simply takes place faster or slower (i.e, changes in k that cannot be categorized as acceleration or neoteny because there is no change in development with respect to gamma).

Fink (1982) demonstrated that a cladogram could be used to detect the types of heterochrony noted by Alberch et al. (1979). He showed that a cladogram is needed to distinguish between paedomorphic and symplesiomorphic morphologies (Figs. 29a and b), and that an inspection of developmental sequences may be necessary to distinguish between paedomorphosis and peramorphosis (Fig. 29c).

An example from Fink's (1982) study will help to clarify the methodology before examining heterochrony in Glypthelmins and Haplometrana. Figure 30 presents Figure 5, a contrived example from Fink (1982). The cladogram in Figure 30a is assumed to be supported by a number of characters. One of these is a possible instance of heterochrony because its less complex form (state 0) is present not only in the outgroups and the most

plesiomorphic taxon of the study group, taxon A, but also in one of the more derived taxa, C. Taxon B, on the other hand, has the more complex form (state 1). The question asked in the analysis is whether character state 0 in taxon C is symplesiomorphic, paedomorphic, or peramorphic. Figure 30b gives one case in which this question could be answered. A and C could both have state 0, but the developmental rate of the character may be slower in C than in either A or B, and the later cessation of development in B may coincide with that of C. In such a case, the inference is that the common ancestor of B and C evolved character state 1 through hypermorphosis (cessation of development occurred later), subsequent to which C evolved state 0 through neoteny (slower rate of development).

As noted by Fink (1982), there can also be instances in which a cladogram cannot help to detect heterochrony. An example is given in Figure 30c. The common ancestor of taxa B and C could have evolved character state 1 through hypermorphosis, subsequent to which C evolved state 0 through progenesis (earlier cessation of development). Because the plesiomorphic and apomorphic developmental rates are the same, and because the time of cessation of development in A and C is the same, the parsimony criterion of phylogenetic systematics would produce the spurious conclusion that the common occurrence of state 0 in A and C is symplesiomorphic.

ANALYSIS

Of the 21 characters analyzed, three were examined for heterochrony because their apomorphic states exhibit morphologies that are conspicuously less developed or more developed than those of the plesiomorphic states. These three characters are not presented as an exhaustive collection of the possibly heterochronic characters in the data set, but simply as those that were chosen for examination. A fourth character, relative hindbody length, was also examined. The apomorphic states of the first three characters (refer to Chapter III, CHARACTERS ANALYZED, and the cladogram in Fig. 20) are: the absence of extracecal uterine loops (character 14, state 1); the absence of pretesticular uterine loops (character 15, state 1); and the persistence of penetration glands in the adult (character 4, state 1);

The apomorphic state of the fourth character, relative hindbody length, that is of interest here is that of an especially long hindbody (HBL) in relation to total body length (TBL) in H. intestinalis. The mean values for HBL/TBL in each species, for all available adult specimens, are as follows: G. hyloreus: 0.68; G. pennsylvaniensis: 0.71; G. robustus: 0.62; G. shastai: 0.75; G. californiensis: 0.69; G. quieta: 0.71; G. facioi: 0.74; H. intestinalis: 0.80. (For comments on statistically significant differences, see the section, EXPERIMENTALLY-PRODUCED HETEROCHRONY IN HAPLOMETRANA INTESTINALIS.)

Comparisons involving G. californiensis, G. quieta, and

<u>H</u>. <u>intestinalis</u> made use of observations on specimens reared in the laboratory during the course of this research (see the section, LIFE HISTORY DATA). Comparisons involving <u>G</u>. <u>hyloreus</u> and <u>G</u>. <u>pennsylvaniensis</u> made use of the data of Martin (1969) and of Sullivan and Byrd (1970), respectively. There are no developmental data for <u>G</u>. <u>robustus</u>, <u>G</u>. <u>shastai</u>, and <u>G</u>. <u>facioi</u>.

<u>The</u> <u>absence</u> <u>of</u> <u>extracecal</u> <u>and</u> <u>pretesticular</u> <u>uterine</u> <u>loops</u>: Sullivan and Byrd (1970) observed that during the development of <u>G</u>. <u>pennsylvaniensis</u>, the uterus remains intercecal and post-testicular until it has grown to the posterior of the body. Subsequent laterad and anteriad growth produces the extracecal and pretesticular loops, respectively. In my observations of young specimens of <u>H</u>. <u>intestinalis</u> and <u>G</u>. <u>quieta</u>, I found that during both its growth to the posterior of the body and its subsequent enlargement, the uterus remains intercecal and post-testicular. These observations for <u>H</u>. <u>intestinalis</u> agree with those of Olsen (1937). The conclusion is therefore that the absence of extracecal and pretesticular uterine loops in mature adults is an instance of paedomorphosis. There are insufficient data to determine whether this is produced through alterations in the rate, time of initiation, or time of cessation of the developmental trajectories.

<u>The</u> <u>presence</u> <u>of</u> <u>penetration</u> <u>glands</u> <u>in</u> <u>the</u> <u>adult</u>: These glands, located in the forebody and emptying into the oral region, are found in many digenean cercariae. They usually degenerate and

disappear in adulthood. They persist, however, in the adults of
G. facioi. Using the traditional usage of the term (see Gould,
1977), this property might be termed neotenic because it is a
larval character appearing in an adult - comparable to the
retention of gills in sexually mature salamanders. However,
according to the criteria for heterochrony discussed herein, it
is most likely peramorphic. The availability of only mature
adult specimens of G. facioi forces a tentative conclusion, but
two observations can nevertheless be made. First, the
persistence of the glands in the adult indicates that the
operation of whatever homeostatic mechanisms maintain them has
been extended into the adult stage. Second, if the glands in
the adults of G. facioi are compared to the glands in the
cercariae of the two sister species, G. californiensis and
G. quieta, they are seen to be larger. Therefore, not only are
the glands maintained in adulthood, but they also continue to
grow. This is peramorphosis, presumably through hypermorphosis.

Hindbody length: The development of a longer hindbody in
H. intestinalis was followed by examining specimens at the
metacercarial stage, and at 1, 4, 8, and 12 months after
development in the gut of the final host, R. pretiosa. Figure
31 presents the growth curve, plotted as hindbody length/total
body length (HBL/TBL) vs. time. Growth data are also given for
specimens of G. quieta reared in the laboratory in R. pipiens.
This is the only other species in the study group for which such
data are available. Both species show the same growth pattern

up to four months after infection (MAI). Subsequent to that, the rate of development of G. quieta slows down, while that of H. intestinalis continues at approximately the same rate as during the 1 MAI to 4 MAI period. By 12 MAI, specimens of H. intestinalis have attained a mean HBL/TBL value of 0.79 (n = 10; s = 0.03), while those of G. quieta have attained a mean value of 0.71 (n = 10; s = 0.03). Figure 31 also includes the mean HBL/TBL values for mature adults, of unknown ages, of the other species of Glypthelmins that are in the same clade as H. intestinalis and G. quieta. These values fall within the 0.62 to 0.75 range. The value for H. intestinalis is significantly different at the 0.01 level (difference of two means from independent samples) from that of the closest species, G. shastai, which is 0.75 (n = 14; s= 0.03).

The plesiomorphic value of the HBL/TBL ratio for H. intestinalis (i.e., the value of the node uniting H. intestinalis and G. shastai) can be estimated by setting the nodes on the cladogram to the median values of the ratios (Fig. 32). This gives a value of 0.75. [1]

The conclusion for H. intestinalis is that given the equality of its early HBL/TBL values to those of G. quieta (i.e., the time of initiation of development is the same), and given the equality of the time at which final measurements were taken (i.e., the time of cessation of development is the same),

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[1] As noted in Chapter III, the available specimens of G. robustus are not fully mature; this species was therefore omitted from the calculations.

a longer hindbody evolved through peramorphosis by acceleration of the rate of development subsequent to 4 MAI.

ALLOMETRY AS HETEROCHRONY

Huxley (1932) introduced the concept of allometry for reference to relative differential growth of body parts. It is best considered as a phenomenological label that can be applied to certain patterns of character change brought about by various developmental processes. As such, if heterochrony involves differences in the relative developmental timing of characters affecting body dimensions, then the result will show an allometric growth pattern.

Huxley (1932) demonstrated how an allometric growth constant can be calculated by double logarithmic plots of the relative growth in any two body parts. This allowed the quantification of instances in which body features were observed to become longer/shorter, wider/thinner, bigger/smaller, etc. than certain other features. If the growth of the two parameters is equal, then the allometric growth constant has a value of 1.0, and the condition is referred to as isometric. If the growth of body feature X is greater than that of body feature Y over the same period of time, then the constant will be greater than 1.0, and the growth of X is referred to as positively allometric with respect to that of Y. If the converse holds, the growth of X is negatively allometric.

Studies of allometric growth of various body parts in

digeneans or monogeneans have been conducted by: (1) Thomas (1965), for <u>Mesocoelium monodi</u> Dollfus, 1929; (2) Rohde (1966), for <u>Anchitrema sanguineum</u> (Sonsino, 1894) Looss, 1899, <u>Platynosomum fastosum</u> Kossack, 1910, <u>Zonorchis</u> sp., <u>Mesocoelium</u> sp., <u>Diaschistorchis multitesticularis</u> Rohde, 1962, <u>Maxbraunium baeri</u> Rohde, 1964, <u>Odeningotrema hypergenitalis</u> Rohde, 1962, <u>Novotrema nyticebi</u> Rohde, 1962, <u>Renschetrema malayi</u> Rohde, 1964, <u>Kaurma intermedia</u> Rohde, 1963, <u>Parorientodiscus magnus</u> Rohde, 1962, <u>Opisthorchis viverini</u> Poirier, 1886, <u>Polystomoides malayi</u> Rohde, 1963, and <u>P. renschi</u> Rohde, 1965; (3) Fischthal and Kuntz (1967), for <u>Pleurogenoides taylori</u> (Tubangui, 1928) Travassos, 1930; (4) Sullivan (1977a), for <u>Choledocystus hepaticus</u>; and (5) Fischthal (1978a,b), for <u>Apocreadium mexicanum</u> Manter, 1937, <u>Pseudocreadium lamelliforme</u> (Linton, 1907) Manter, 1946, <u>Paracryptogonimus americanus</u> Manter, 1940, <u>Multitestis rotundus</u> Sparks, 1954, <u>Stenopera equilata</u> Manter, 1933, <u>Leurodera decora</u> Linton, 1910, and <u>Prosorhynchus pacificus</u> Manter, 1940. In all of these species, the hindbody shows positive allometric growth in relation to total body length. The observations on <u>G</u>. <u>quieta</u> and <u>H</u>. <u>intestinalis</u> reported herein also indicate such positive allometry of the hindbody.

Rohde (1966) supported the suggestion by Huxley (1932) and Needham (1964) that allometric relationships be used as taxonomic characters. Thus, the allometric growth constant would be seen as a taxon-specific character determined by taxon-specific growth patterns. Fischthal and Kuntz (1967) cautioned against the weighting of such a character over any other

characters of the taxa involved. I concur, and I also note that (1) allometric growth constants can change during the ontogeny of an organism, and (2) since allometry is not necessarily produced by a single process, there is the danger, as with any character, of mistaking homoplasy for homology.

Just as Fink (1982) demonstrated that heterochrony can be placed in a phylogenetic context, so too can allometry. Thus, with reference to Figure 31 and the phylogenetic analysis presented herein, the following can be said. The growth of the hindbody in H. intestinalis and G. quieta is equally positively allometric with respect to total body length for the first four months of adult development. Subsequent to this, the apomorphic character of H. intestinalis is expressed when, through peramorphosis of the plesiomorphic growth rate, the hindbody continues to increase in length such that its growth is positively allometric not only with respect to its total body length, as before, but now also with respect to the plesiomorphic value of the same growth parameter, represented in G. quieta.

EXPERIMENTALLY-PRODUCED HETEROCHRONY IN HAPLOMETRANA INTESTINALIS

INTRODUCTION

As an hypothesis of common ancestry, a cladogram can be used as a general reference system not only for making inferences about evolutionary events, but also for experimental manipulations of character development. In this context, the development of the hindbody in H. intestinalis was investigated. I postulated in the previous section that the relatively longer hindbody in this species evolved through peramorphosis of the plesiomorphic developmental rate. In an attempt to see whether this autapomorphic trait could be perturbed, the development of H. intestinalis in anurans other than R. pretiosa was studied.

ANALYSIS

The choice of experimental hosts was based upon an optimization of the Glypthelmins cladogram for hosts (Fig. 33). The eight parasite species studied develop in ranid (R), hylid (H), or bufonid (B) anurans. Optimization of the nodal values of the tree indicates an ambiguity at the node uniting G. shastai and H. intestinalis. The host of the common ancestor of these species is inferred to have been either a bufonid or a ranid. Following the procedures in the section, LIFE HISTORY

DATA, attempts were made to infect adults of <u>Bufo</u> <u>boreas</u>, <u>Rana</u> <u>cascadae</u> and <u>R</u>. <u>aurora</u> with cercariae or metacercariae of <u>H</u>. <u>intestinalis</u>. <u>Bufo</u> <u>boreas</u> was chosen because it is the host of <u>G</u>. <u>shastai</u>, which is postulated to be the sister species of <u>H</u>. <u>intestinalis</u>. If <u>B</u>. <u>boreas</u> is the apomorphic host, that is, a newly acquired host colonized by <u>G</u>. <u>shastai</u> during that parasite's evolution, then the development of <u>H</u>. <u>intestinalis</u> in that host may somehow be abnormal. Conversely, if <u>B</u>. <u>boreas</u> is the plesiomorphic host of the two species, then the development of <u>H</u>. <u>intestinalis</u> in that host should proceed as it does in <u>R</u>. <u>pretiosa</u>.

Of the two ranid experimental hosts, <u>R</u>. <u>cascadae</u> is parapatric with <u>R</u>. <u>pretiosa</u> in eastern Washington and Oregon (Fig. 37), and <u>R</u>. <u>aurora</u> is parapatric with <u>R</u>. <u>pretiosa</u> in British Columbia (Fig. 38).[1] Cladistic analysis of ranid relationships (see Fig. 42a, and the section, COEVOLUTION ANALYSIS) postulates that <u>R</u>. <u>aurora</u> and <u>R</u>. <u>cascadae</u> are the closest relatives of <u>R</u>. <u>pretiosa</u>.

Of the three experimental host species, infections developed in <u>R</u>. <u>cascadae</u> and <u>B</u>. <u>boreas</u>. Specimens of <u>H</u>. <u>intestinalis</u> were obtained from adults of each of these two host species at 1, 4, and 12 months after infection (MAI). Changes in the ratio of hindbody length to total body length

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[1] Licht (1969, 1974) reported sympatric populations of <u>R</u>. <u>aurora</u> and <u>R</u>. <u>pretiosa</u> in Langley, British Columbia. My recent collections in the same area found only <u>R</u>. <u>aurora</u>. The only digenean gut parasites obtained from these frogs were <u>G</u>. <u>californiensis</u> and <u>Megalodiscus</u> <u>microphagus</u>.

(HBL/TBL) over time are given in Figure 34. This plot, which can be compared to that of the HBL/TBL growth curve of specimens of H. intestinalis in R. pretiosa (Fig. 31), gives the values for H. intestinalis developing in R. cascadae (upper dashed line), the values for H. intestinalis developing in B. boreas (solid line), and the values for G. quieta developing in R. pipiens (lower dashed line: from Fig. 31).

Specimens of H. intestinalis developing in R. cascadae follow the same growth curve as when development occurs in R. pretiosa. Figure 35a is an illustration of one of the specimens from R. pretiosa at 12 MAI. [1] In B. boreas, however, the rate of development slows after 4 MAI, such that the mean value of HBL/TBL at 12 MAI, 0.74 (n = 6; s = 0.02), is within the range of values present in the other species in the clade. Figure 35b is an illustration of one of the specimens from B. boreas at 12 MAI. [2] Testing for the difference between means from independent samples shows that there is no significant difference between the HBL/TBL value for H. intestinalis from B. boreas at 12 MAI and that for mature adults of its postulated sister species, G. shastai (mean HBL/TBL = 0.75, n = 14; s = 0.03).

Recall that the plesiomorphic HBL/TBL value for the node uniting G. shastai and H. intestinalis can be estimated to be

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[1] [2] Voucher specimens of H. intestinalis at 12 MAI in R. pretiosa (HWML no. 23659) and B. boreas (HWML no. 23660) have been deposited at the Harold W. Manter Laboratory, University of Nebraska State Museum, 529-W Nebraska Hall, University of Nebraska, Lincoln, NE 68588-0514.

0.75 (Fig. 32). Thus, it can be said that when H. intestinalis develops in B. boreas it exhibits not the apomorphic HBL/TBL value of approximately 0.80, but the plesiomorphic value of approximately 0.75. Because the altered growth parameter that brings this change about is a slowing of the rate of development after 4 MAI, the heterochrony involved - with respect to the development of H. intestinalis in its natural host, R. pretiosa - is that of paedomorphosis through neoteny. Heterochrony can thus be studied from a phylogenetic perspective both as an evolutionary event and as an experimentally-produced alteration in development.

DISCUSSION

There are at least three terms that might be applied to the altered heterochronic growth of the hindbody in H. intestinalis. First, although it is still positively allometric with respect to total body length, it is not as positively allometric as it is in development in R. pretiosa or R. cascadae. Second, it could correctly be termed "retarded growth", for this term is in fact a reference to heterochrony. Third, and most relevant to digenean systematics, it could be cited as an instance of "host-induced variation". Such variation has been reported for a number of digeneans developing in other than their usual host: e.g., (1) Beaver (1937), for Echinostoma revolutum (Froelich, 1802); (2) Boddeke (1960), for Prosthogonimus ovatus Rudolphi, 1803; (3) Grabda-Kazubska (1967), for Opisthoglyphe ranae

Froelich, 1791; (4) Watertor (1967), for _Telorchis bonnerensis_ Waitz, 1960; (5) Blankespoor (1974), for _Plagiorchis noblei_ Park, 1936; and (6) Palmieri (1977), for _Posthodiplostomum minimum_ (MacCallum, 1921). Studies such as these have concentrated on determining the characters of the worms that would vary under such developmental conditions, but only in so far as such variation interfered with the correct identification of the species of parasite. Thus, such characters were considered to be not as taxonomically useful as those that remained constant. Typically, the characters affected include body dimensions, vitelline distribution, relative sucker sizes, and gonad location.

In the context of such studies, it is possible to say that the alteration of hindbody growth in _H. intestinalis_ developing in _B. boreas_ is yet another example of host-induced variation. (There are still two autapomorphies - tandem testes and the absence of anterior vitelline fields - that allow identification of the species.) Nevertheless, this term and the aspects of comparative biology it represents are of limited use in studying the developmental properties of _H. intestinalis_ discussed here. This is because host-induced variation is traditionally considered with respect to the _host_, and not to the _parasite_. Character variation is seen as something that a host "induces" in a parasite, and a parasite's "plasticity" in the face of such induction is seen as problematic for evolutionary biology. Phylogenetically determined limits to this variation are recognized, but this recognition is not explicit enough to avoid

the perception that such characters are not of much use in parasite systematics.

Without denying the importance of establishing ranges of character variation and the hosts in which it can occur, and without denying the problems that highly variable characters create for parasite taxonomy, the studies herein demonstrate that variable characters may be relevant to parasite systematics and evolutionary biology. The reduced hindbody development in specimens of H. intestinalis developing in B. boreas is not just any variation - it is the plesiomorphic state. From within the context of an hypothesis of phylogenetic relationships, changes in characters can be investigated. Some of these changes may be environmentally-triggered, and since the environment of a parasite is usually another organism, that triggering will be what is called host-induced variation.

COEVOLUTION AND BIOGEOGRAPHY OF PARASITES AND HOSTS

INTRODUCTION

A concordance between the phylogenetic relationships of parasites and of their hosts has been recognized since the nineteenth century (von Ihering, 1891). Hennig (1966) discussed this concordance and the resultant possibility of inferring host phylogenies from parasite data. The continued discovery of covarying associations between parasites and their hosts has led

to the formulation of various "rules" of evolution, of which the best known is probably Farenholz's Rule: Parasite Phylogeny Mirrors Host Phylogeny (for reviews, see Inglis, 1971; Brooks, 1979a, 1981a, 1985). Brooks (1981a) developed this concept of coevolution[1] in terms of phylogenetic systematics and demonstrated that host-parasite coevolution can arise through processes comparable to the evolution of homologous and homoplasious characters. Symplesiomorphic parasitism occurs when a host lineage speciates, its parasites do not, and a paraphyletic distribution of retained primitive parasites in derived hosts results. Synapomorphic and autapomorphic parasitism occurs when hosts and parasites cospeciate, and their phylogenies do in fact "mirror" each other. Homoplasious parasitism occurs when ecological association results in parasites colonizing hosts other than those with which they have been evolutionarily associated. This homoplasy can occur in a parallel or a convergent manner.

Analysis of the biogeography and speciation patterns of organisms relates their distribution patterns to their postulated phylogenetic relationships and the geographical history of the areas in which the organisms occur. As with other levels of phylogenetic analysis, the intent is to

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[1] This term is used here in the sense of Brooks (1979a), who applied it to those instances of a common evolutionary history of lineages of hosts and their parasites. This results in congruent, or at least consistent, host and parasite cladograms. The term coevolution has also been used to refer to reciprocal adaptive responses between hosts and parasites during evolution (e.g., Ehrlich and Raven, 1964).

determine the contribution of historical processes to contemporary phenomena (e.g., Wiley, 1980). Any group of organisms can be analyzed with respect to its geographical distribution (e.g., Nelson, 1974; Rosen, 1978). When a host-parasite system is involved, there is an additional level of relationships that can be examined, namely, parasite relationships <u>with</u> <u>respect</u> <u>to</u> host relationships <u>with</u> <u>respect</u> <u>to</u> geographical relationships (see Brooks, 1985).

<u>HOST AND DISTRIBUTION DATA</u>

1. <u>PREVIOUS STUDIES</u>

The following are either published reports in the literature or personal communications, as indicated. New reports arising from the current study are given in the next section. Figures refer to distribution maps.

<u>G. hyloreus</u>: (Fig. 36)
Type locality: near Corvallis, Oregon, from <u>Hyla</u> <u>regilla</u>
                   Baird and Girard

Other reports: Nebraska, from <u>Pseudacris</u> <u>triseriata</u> Weid (by
                   Brooks, 1976a)
                   Colorado, from <u>P</u>. <u>triseriata</u> (by Ubelaker <u>et</u>
                   <u>al</u>., 1967: reported as <u>G</u>. <u>pennsylvaniensis</u>,

identified as G. hyloreus by Brooks, 1976a)

Spokane County, Washington State, from
H. regilla (by B. Lang, pers. comm.: Dept.
of Biology, Eastern Washington State Univ.,
Cheney, Wash.)

George Lake, Alberta, from P. triseriata (by
J.C. Holmes, Dept.of Zoology, Univ. of
Alberta, pers. comm.) [1]

G. pennsylvaniensis: (Fig. 36)

Type locality: Lake Warren, Pennsylvania, from Hyla crucifer
Weid

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[1] These specimens were originally identified as "G. quieta from Pseudacris nigrita" by the collector, Z. Hameed. Given the locality, in southwestern Alberta, the host is more likely to have been P. triseriata. Even though the worms are immature specimens, their possession of tegumental spines, rather than scales, indicates that they are not specimens of G. quieta. Also, the peripharyngeal glands are of the medial type, rather than of the pharyngeal type. The testes are oblique, rather than symmetrical, and the I-shaped excretory vesicle extends to the level of the posterior testes. Both of these are characteristic of G. pennsylvaniensis. The length of the eggs, however, is characteristic of G. hyloreus, being 45 - 53 (mean of 49) μm. The specimens are tentatively identified as G. hyloreus.

Other reports: Clarke and Chatham Counties, Georgia, from H.

crucifer and Pseudacris nigrita (Le Conte)

(by Byrd and Maples, 1963)

Oconee County, Georgia, from H. crucifer. (by

Sullivan and Byrd, 1970)

G. robustus: (Fig. 36)

Type locality: 15 km west of Neiva, Huila, Colombia, from

Bufo marinus L.

Other reports: None

G. shastai: (Fig. 37)

Type locality: Glenburn, Shasta County, California, from Bufo

boreas

Other reports: Gorge Creek, R.B. Miller Biological Station,

60 km SW of Calgary, Alberta, from B. boreas

(by J.C. Holmes, Dept. of Zoology, Univ.

of Alberta, pers. comm.)

Nelson, British Columbia, from <u>B</u>. <u>boreas</u>
(ibid.)

<u>G</u>. <u>californiensis</u>: (Fig. 38)

Type locality: San Francisco area, California, from <u>Rana</u>
                   <u>aurora</u> Baird and Girard

Other reports: San Diego and Butte Counties, California, from
                   <u>R</u>. <u>boylii</u> Baird (by Ingles, 1936)

                   Marin and Sonoma Counties, California, from
                   <u>R</u>. <u>boylii</u> (by Lehmann, 1960)

                   Cienaga de Lerma, and Lago de Xochimilco,
                   Mexico, from <u>R</u>. <u>montezumae</u> Baird, and from
                   <u>R</u>. <u>pipiens</u> Schreber (by Caballero Y.C.,
                   1942; Caballero Y C. and Sokoloff, 1934)

                   Langley, British Columbia (approximately 65 km
                   SE of Vancouver), from <u>R</u>. <u>aurora</u> (by R.
                   Douthwaite and D.R. Brooks, pers. comm.)

<u>G</u>. <u>facioi</u>: (Fig. 36)

Type locality: Coris, Cartago Province, Costa Rica, from <u>Rana</u>

pipiens (by Brenes et al., 1959)

Other reports: Turrialba, Cartago Province, Costa Rica, from
                  R. pipiens (by Sullivan, 1976)

G. quieta: (Fig. 38)

Type locality: Eastern Canada (Toronto area presumed),[1] from
                  Rana catesbeiana Shaw, R. virescens Garman
                  (=R. pipiens), and Hyla pickeringii
                  Kennicott (=H. crucifer Weid)

Other reports: Over sixty, primarily throughout eastern North
                  America, from members of the R. pipiens and
                  R. catesbeiana groups (see the section,
                  COEVOLUTION ANALYSIS). See Figure 38, in
                  conjunction with the Index Catalogue of
                  Medical and Veterinary Zoology, Oryx Press,
                  Phoenix, Arizona, U.S.A.

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[1] Stafford's (1900, 1905) reports specified neither locality nor type specimens. However, as noted in the section, SPECIMENS EXAMINED, there are specimens from Stafford's collection deposited at the National Museum of Natural Sciences, Ottawa, Ontario, Canada.

Haplometrana intestinalis: (Fig. 37)

Type locality: Bothell, King County, Washington State, from

Rana pretiosa Baird and Girard

Other reports: Springville, Utah, from R. pretiosa (Olsen,

1937)

Idaho,   from   R. pretiosa,   R. pretiosa   X

R. sylvatica, and B. boreas (Waitz, 1961)

Spokane County, Washington State; cercariae

from lymnaeid and helisomid snails (Current

and Lang, 1975); adults from R. pretiosa (B.

Lang, pers. comm.)

Coleman, Alberta, from R. pretiosa   (J.C.

Holmes, Univ. of Alberta, pers. comm.)

Lake County and Flathead County, Montana, from

R. pipiens (ibid.)

Gorge   Creek,   Alberta,   from R. sylvatica

(ibid.)

Postill Lake, Kelowna, British Columbia, from

R. pretiosa (ibid.; originally identified as

Glypthelmins sp;   identified   as

H. intestinalis by the present author)

2. NEW DATA

From 1983 to 1986, surveys were conducted in southern British Columbia. The study area lay along an east-west transect remaining within 50km of the Canada - U.S. border, from Vancouver Island to the British Columbia - Alberta border (Fig. 8: see Appendix C for field collection sites). The following data on parasitism by species of Glypthelmins and Haplometrana were obtained.

G. hyloreus: Negative, in 76 Hyla regilla examined

G. californiensis: Langley, British Columbia, in area of Little
                      Campbell River; from Rana aurora; prevalence
                      = 62% (35 frogs examined)

    New Localities:

                      Bonsall Creek, Duncan, Vancouver Island,
                      B.C.; from R. aurora; prevalence = 42% (26
                      frogs examined)

Remarks: 25 R. catesbeiana and 18 H. regilla from the same areas in Langley were examined and found to be negative for infections by any species of Glypthelmins.

<u>H</u>. <u>intestinalis</u>:

New Localities:

Little Muddy Pond, Manning Park, B.C.; from <u>Rana</u> <u>pretiosa</u>; prevalence = 83% (15 frogs examined)

Okanagan Falls, B.C.; from <u>R</u>. <u>pretiosa</u>; ; prevalence = 80% (15 frogs examind)

Wilgress Lake, B.C.; from <u>R</u>. <u>pretiosa</u>; (1 frog examined)

Champion Lakes, B.C.; from <u>R</u>. <u>pretiosa</u>; prevalence = 74% (31 frogs examined)

Creston, B.C.; from <u>R</u>. <u>pretiosa</u>; prevalence = 50% (6 frogs examined)

Loon Lake, B.C.; from <u>R</u>. <u>pretiosa</u>; (1 frog examined)

Remarks: (1) Other than Holmes' unpublished report of <u>H</u>. <u>intestinalis</u> from <u>R</u>. <u>pretiosa</u> in Kelowna (see PREVIOUS STUDIES), these are the first reports of <u>H</u>. <u>intestinalis</u> in British Columbia. (2) Although the distribution of <u>R</u>. <u>pretiosa</u> is primarily throughout the Columbia and Snake River Plateaus, there are populations occurring further

north and further west (see Fig. 37). Licht (1969, 1974) reported sympatric populations of R. pretiosa and R. aurora along the Little Campbell River, in Langley, B.C.. I have examined Licht's data and deposited specimens (Cowan Vertebrate Museum, University of British Columbia; R. aurora: nos. 461, 1250, 1251, 1307; R. pretiosa: nos. 477, 478, 415), and I agree with the identification of the two species. During the course of the present study, from 1983 to 1986, 35 R. aurora were collected at and around the areas surveyed by Licht. No R. pretiosa were observed, and no specimens of H. intestinalis were recovered from R. aurora, as might happen if there had been a host transfer when R. pretiosa were present. (Note: as reported in the section, LIFE HISTORY DATA, attempts to infect R. aurora with H. intestinalis in the laboratory were unsuccessful.) (3) Two specimens of B. boreas were collected from Little Muddy Pond, Manning Park, B.C., but were found to be free of digenean parasites.

G. quieta: Negative, no R. pipiens collected in British Columbia

Remarks: Three of the four localities for R. pipiens in B.C. reported by Carl (1949) were surveyed: Osoyoos, Creston, and Loon Lake. No specimens were found. Two specimens of R. catesbeiana, another host of G. quieta,

were collected in Osoyoos, but were negative for digenean gut parasites. Green (1978) reported a population of R. pipiens on Vancouver Island that was established in the 1930s (see also Orchard, 1984), but this was not examined.

From 1983 to 1986 surveys were conducted in northern California. The study area covered Siskiyou, Shasta, Modoc, Lassen, Plumas, and Sierra Counties (Fig. 9). Collections in Shasta, Modoc, and Lassen Counties centered around the drainage basin of the Pit River. This river runs through Glenburn, Shasta County, the type locality of G. shastai in B. boreas. Collections in Sierra County were directed towards obtaining specimens of R. muscosa; while those in Siskiyou County were directed towards obtaining specimens of R. cascadae. Both of these ranids have areas of sympatry with B. boreas (Fig. 37), and are thus potential hosts of G. shastai. The following data on parasitism by species of Glypthelmins and Haplometrana were obtained.

(1) 82 specimens of R. cascadae were examined from areas in Siskiyou and Shasta Counties where this species is sympatric with B. boreas. All were free of intestinal digenean parasites.

(2) No specimens of R. muscosa were collected from the Gold Lakes area, Sierra County.

(3) No specimens of R. pretiosa were collected from Medicine Lake, Siskiyou County; Upper Mud Lake, Modoc County; and Blue Lake, Lassen County. This was unexpected, given the report by Hayes and Jennings (pers. comm.) that this species was collected at these sites in 1980. R. pretiosa is a slow, basking frog, and there is little risk of missing it in a survey. [1]

(4) 25 specimens of B. boreas were examined and found to be free of intestinal digenean parasites. Four of these toads were collected from Medicine Lake, Siskiyou County; two from Little Bear Flats, Shasta County; and 19 from Brown Rd., in Glenburn, Shasta County: the type locality of G. shastai in B. boreas.

(5) Ingles (1933) reported that no digenean parasites had been collected in California from the introduced species, R. catesbeiana, despite its sympatry with R. aurora and R. boylii, and despite the presence of suitable snail hosts. This led him to suggest that bullfrogs were incapable of acquiring the digenean parasites of the indigenous frogs. Three years later, Ingles (1936) reported Megalodiscus temperatus, a digenean parasite of a number of western anurans, from R. catesbeiana in Butte

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[1] Marc. P. Hayes, Dept. of Biology, University of Miami, Coral Gables, Florida; Mark R. Jennings, U.S. Fish and Wildlife Service, Los Banos, Calif.

County, California. As will be reported elsewhere, in my work in California I recovered gorgoderid and haematoloechid digeneans from specimens of R. catesbeiana.

From North American survey sites other than those in British Columbia and California, the following data on parasitism by species of Glypthelmins and Haplometrana were obtained.

G. quieta:

New Localities:

White Earth River, Mountrail County, North Dakota; from R. pipiens; prevalence = 17% (6 frogs examined)

H. intestinalis:

New Localities:

Pelican Creek, Wyoming; from R. pretiosa; prevalence = 50% (2 frogs examined)

3. DISCUSSION

The following can be concluded from the information given in the previous two sections.

1) Glypthelmins hyloreus parasitizes hylid frogs west and east of the Rockies, but not east of the Missouri River; G. pennsylvaniensis parasitizes hylids east of the Missouri (Fig. 36).

2) The distribution of G. shastai in B. boreas in North America is disjunct, occurring in northeast California and southwest Alberta (Fig. 37). Surveys of B. boreas in Idaho (Waitz, 1961), Utah (Frandsen and Grundmann, 1960), and southern B.C. (present study) have not found H. intestinalis.

3) Glypthelmins californiensis parasitizes western ranid species along the west coast of North America, with additional occurrence in allopatric members of the Rana pipiens group (Hillis et al., 1983, see the section, COEVOLUTION ANALYSIS) in Mexico (Fig. 38).

4) Glypthelmins quieta parasitizes members of the R. pipiens and R. catesbeiana groups (see the section, COEVOLUTION ANALYSIS) in eastern North America, with additional occurrence in some sympatric hylids and bufonids (Fig. 38). Glypthelmins quieta can be considered to be a parasite primarily of the R. pipiens group, rather than of the R. catesbeiana group. This is because

it occurs in members of the R. pipiens group where there are no members of the R. catesbeiana group, but it does not occur in members of the R. catesbeiana group where there are no members of the R. pipiens group. It is also of interest that the reports of G. quieta in Seattle, Washington (Rankin, 1944) and in Cuba (Odening, 1968) were both from R. catesbeiana, a species indigenous to southeast North America and frequently transplanted because of its commercial value. [1]

5) Haplometrana intestinalis parasitizes R. pretiosa in western North America, with occurrence in a sympatric bufonid, B. boreas, as well as in populations of R. pipiens and R. sylvatica (Fig. 37).

6) Turner (1958) reported "Glypthelmins sp." from R. pretiosa at Fishing Bridge, Wyoming. My collection of H. intestinalis from this species of frog at Pelican Creek, which is within 10 miles of Fishing Bridge, suggests that Turner actually collected H. intestinalis.

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[1] Hayes and Jennings (in press) report that R. catesbeiana was introduced to western North America in California in 1896. Green (1978) reported a population of R. catesbeiana introduced on Vancouver Island, British Columbia, in the 1930s (see also Orchard, 1984).

COEVOLUTION ANALYSIS

1. FAMILY LEVEL

The species of anurans parasitized by the eight parasite species studied here are members of the Hylidae, Bufonidae, or Ranidae. These families were mapped onto the parasite cladogram in Figure 33 for the heterochrony study, reported above. That study eliminated some of the ambiguity of the nodal values by demonstrating disrupted development of H. intestinalis in B. boreas, the host of G. shastai. Disrupted development in a bufonid provides evidence that the plesiomorphic host for the G. shastai + H. intestinalis clade was a ranid. With this inference, the nodal values on the cladogram are as in Figure 39a. These values imply the host relationships shown in Figure 39b. Because it is a trichotomy (all three taxa united at a single node), the tree's topology would not change if, as discussed in Chapter III, G. robustus were placed as the sister taxon to the other seven species. The conclusion, therefore, is that all of the parasite species except G. shastai show coevolution with their hosts at the family level. Glypthelmins shastai appears to have colonized B. boreas.

Comparison of the host tree in Figure 39b to familial level cladistic analyses of anurans shows that, with respect to the Hylidae, Bufonidae, and Ranidae, those studies have also postulated a trichotomous relationship. There have been three

such studies, using primarily morphological data. That of Kluge and Farris (1969) presented a cladogram (their Figure 5) that, interpreted in the conventional manner, seems to place the hylids and bufonids in a separate clade. However, the text and their Figure 4 make it clear that a trichotomy is being postulated. The study by Lynch (1973) also placed the hylids and bufonids in a clade separate from the ranids. This was done on the basis of three characters: the absence of transverse processes on the coccyx, the presence of an accessory head on the adductor magnus muscle, and the presence of an axillary amplectic position. These characters were interpreted in this manner because of the use of Camin-Sokal parsimony in the analysis. By prohibiting character state reversals, this method of phylogenetic inference causes the postulation of an additional four instances of character state evolution for the first two characters (see Figure 3.7, Lynch, 1973), and it eliminates an equal-length postulation of reversal in the third character. The postulation of a trichotomous relationship is therefore better supported by the data. Lastly, in an analysis performed with the WAGNER78 computer program developed by J.S. Farris, Duellman and Trueb (1986: Fig. 17.3) postulated a trichotomous relationship for the hylids, bufonids, and ranids. My re-analysis of their data with both PAUP (Unordered Analysis on multistate characters) and PHYSYS (WAG.S) did not alter the phylogenetic conclusions pertaining to these three families.

2. SPECIES LEVEL

A study of parasite-host coevolution at the species level was done for the ranid hosts. Figure 40 presents the Glypthelmins cladogram with the species of ranid hosts mapped on. These are: R. pretiosa (pr), R. aurora (au), R. boylii (bo), R. montezumae (mo), R. pipiens (pi), R. blairi (bl), R. sphenocephala (sp), R. palustris (pa), and R. palmipes (pp).

With the exception of R. catesbeiana and R. clamitans (see the section, HOST AND DISTRIBUTION DATA), all of the hosts of G. quieta have been postulated to be members of the R. pipiens group (Hillis et al., 1983), a clade containing at least 20 species endemic to areas in Mexico and eastern North America (Fig. 38). With respect to the hosts of G. californiensis, R. pipiens is put in inverted commas because given the locality of its collection by Caballero (1942) and Caballero and Sokoloff (1934), on the Mexican Plateau, it was most likely not R. pipiens, but some other member of the group. The other host of G. californiensis in that area, R. montezumae, is also a member of the R. pipiens group. The host of G. facioi is marked as R. palmipes, rather than as R. pipiens, as reported by Brenes et al. (1959) and Sullivan (1976). This change was made because the locality from which the host was collected, Costa Rica, indicates that it was most likely R. palmipes, a member of the R. tarahumarae group (Webb, 1977; Case, 1978; Hillis et al., 1983). This group contains about four species endemic to areas of Central and South America.

The remaining hosts on the cladogram, R. pretiosa,

R. aurora, and R. boylii, are considered to be members of a clade of ranids endemic to western North America (Figs 37, 38). The monophyly of this group, whose other members are R. cascadae, and R. muscosa, has been postulated by morphological (Chantell, 1970), immunological (Wallace et al., 1973; Case, 1978), electrophoretic (Case, 1978; Green, 1986a), and karylogical (Green, 1986b) analyses. Farris et al. (1979, 1982) reanalysed the data of Case (1978) and Post and Uzzell (1981) and concluded that the European species, R. temporaria, should also be included in this group. This conclusion has been disputed by Green (1986a), with electrophoretic data, and by Uzzell and Post (1986), with immunological data.

A summary cladogram of the higher level relationships of ranids in the Americas is given in Figure 41. The tree presents two species: R. temporaria and R. sylvatica, and four clades of species: the R. boylii group, the R. tarahumarae group, the R. pipiens group, and the R. catesbeiana group (with respect to the monophyly of this last group, endemic to eastern North America, see Case, 1978, and Lynch, 1965). The sister relationship of the R. tarahumarae and R. pipiens groups has been postulated by Case (1978) and Hillis et al. (1983), and that clade's sister relationship to the R. catesbeiana group has been postulated by Case (1978). The monophyly of the clade containing R. sylvatica, R. temporaria, and the R. boylii group has been postulated by Farris et al. (1979) and Green (1986b). The monophyletic status of the entire assemblage is unclear. Savage (1973) considered all ranids in the Americas to be

descended from an ancestral stock entering western North America via Beringia during the Eocene. Case (1978) considered the immunological data to support the conclusion that only the R. boylii group arrived in this manner, while the R. tarahumarae, R. pipiens, and R. catesbeiana groups entered eastern North America via a Laurasian land connection that existed until the mid-Eocene (McKenna, 1975). Regardless of the resolution of this question, there appear to be two main lineages of ranids in the Americas: a north-west clade and a south-east clade.

The relationships of the species within the R. boylii group have been cladistically analyzed a number of times. The most recent studies are those with allozyme (Green, 1986a) and karyological (Green, 1986b) data. Green's analysis of the electrophoretic properties of the allozyme data followed the coding procedures of Mickevich and Mitter (1981) and Buth (1984). Both studies used Camin-Sokal parsimony, in conjunction with a priori transformation series for the multistate characters. I have re-analyzed the data sets with the Wagner parsimony algorithms of PAUP, using Unordered Analysis for the multistate characters (see Appendix A for the data matrices). Such treatment of these types of data is supported by similar studies on leptodactylid frogs (Miyamoto, 1983, 1984, 1986) and xantusiid lizards (Crother et al., 1986). These authors analyzed the multistate characters with either Swofford's Unordered Analysis or Mickevich's (1982) Transformation Series Analysis.

The trees I obtained are given in Figures 42a (karyological data) and 42b (electrophoretic data). Green (1986b) presented a single tree for the karyological data, the topology of which is the same as that shown here.[1] Although no goodness-of-fit statistics for this tree were given, analysis of Green's original 13-character data set with WISS, the Camin-Sokal parsimony algorithm in PHYSYS, gives a consistency index (CI) of 0.94.[2] If Wagner parsimony is used and the author's _a priori_ multistate transformation series are maintained, analysis with the BANDB algorithm of PAUP gives two trees with a CI of 0.94, one of which is the tree published by Green. When, in conjunction with BANDB, Unordered Analysis is performed on the multistate characters, the single tree shown in Figure 42a is obtained. It is the same tree published by Green (1986b), and it has a CI of 1.0, indicating a perfect fit to the data.

In my re-analysis of the electrophoretic data of Green (1986a), I collapsed the populations of _R. aurora_ and _R. pretiosa_ used by Green to a single OTU (Operational Taxonomic Unit) each. Analysis of the original 47-character data set with the Camin-Sokal parsimony WISS algorithm of PHYSYS gives the

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[1] The tree has the same topology whether or not _R. aurora_ is taken as the outgroup, as it was by Green. If this species is not taken as the outgroup, then there will be a composite outgroup, consisting of zero states, in the data matrix in Appendix A.

[2] Unless otherwise noted, CI values presented here are calculated as originally proposed by Kluge and Farris (1969). Appendix G of this dissertation presents a modified CI, in which non-homoplasious autapomorphies are not included in the calculations.

tree he published, with a CI of 0.58. Analysis with the Wagner parsimony BANDB algorthm of PAUP while maintaining the a priori multistate transformation series gives the same tree again, with a CI of 0.61. Analysis with BANDB and Unordered Analysis on the multistate characters gives eight trees with a CI of 0.80 (modified CI of 0.69; according to the criteria in Appendix G). None of these trees is the same as the tree published by Green (1986a). Figure 42b gives the two topologies of the multiple trees, as well as the consensus tree. The latter differs from Green's results primarily in the paraphyletic nature of the two species of "stream frogs": R. boylii and R. muscosa (see Zweifel, 1955; Chantell, 1970; Green, 1986a).

The phylogenetic inferences drawn from the karyological and electrophoretic data differ primarily in the placement of R. aurora. It is either placed as the sister species to the rest of the clade, or it is placed further up in the tree, grouped with R. cascadae and R. boylii. The immunological and electrophoretic studies by Case (1978), re-analyzed by Farris et al. (1979) also place R. aurora with R. boylii.

The tree inferred from the karyological data is consistent with the morphological data that are available (overlap of dorsal flange and ventral crest on shaft of humerus, vastus prominence of ilium: Chantell, 1970; skin texture and coloration: Dumas, 1966). It is also consistent with the biogeographical study by Dumas (1966), in which a conclusion of vicariant speciation associated with late-Cenozoic orogeny in western North America was reached. The species on the tree fall

into three groups previously recognized on the basis of phenetic similarity. These are: the "wood frogs" (Dumas, 1966): R. sylvatica (one of the outgroups, see Fig. 41) and R. aurora; the "pond frogs" (Dumas, 1966): R. pretiosa and R. cascadae; and the "stream frogs" (Zweifel, 1955; Chantell, 1970; Green, 1986a,b): R. boylii and R. muscosa. Of these three groups, only the third is postulated to be monophyletic. The first two groups are components of a larger, apparently paraphyletic assemblage, the Palearctic "brown frogs". Green (1986a,b) postulated that this assemblage is the result of a geographically and systematically widespread symplesiomorphic morphology.

I consider the tree inferred from the karylogical data to be the preferred hypothesis of the phylogenetic relationships of the species in the R. boylii group. This conclusion is based on the tree's optimal CI value and on its support by other types of data. Its acceptance necessitates the conclusion that in the electrophoretic and immunological data analyzed so far, there has been convergent evolution that results in the grouping of R. aurora with R. boylii.

When the plesiomorphic states for the ranid hosts are optimized on the Glypthelmins cladogram (Fig. 40; i.e., host relationships inferred from parasite relationships), and the applicable portions of the host cladogram from Figures 41 and 42a (dashed lines) are superimposed, four conclusions can be drawn. The first is that the plesiomorphic host of the G. californiensis + G. quieta + G. facioi clade was a member of

the R. pipiens group. The inference of a common "R. pipiens" node for all three of the parasite species involved indicates coevolution of hosts and parasites. A coevolutionary pattern continues to be found when the host and parasite phylogenies are examined at a lower level. Figure 43a presents the cladogram of Hillis et al. (1983) for the R. pipiens group. The reported occurrences of parasitism by G. californiensis (ca), G. quieta (qu), and G. facioi (fa) are noted. In a process comparable to generating a reduced area cladogram in biogeographic analysis (see Rosen, 1978), Figure 43b shows the cladistic relationships of the parasitized members of the R. pipiens clade. The postulated sister group relationships are congruent with those of the parasite cladogram (see Fig. 40). That is, sister parasite taxa are found in sister host taxa.

The second conclusion of this analysis is that G. californiensis subsequently colonized two members of the R. boylii group: R. aurora and R. boylii. The occurrence of G. californiensis in only R. aurora and R. boylii is evidence of either (a) the descent of these two species of frogs from a common ancestor colonized by G. californiensis, or (b) G. californiensis colonizing first one and then the other host species. Interpretation (a) is consistent with the host phylogeny inferred from immunological and electrophoretic data (Fig. 42b), while interpretation (b) is consistent with the host phylogeny inferred from the karyological and morphological data (Fig. 42a). Given the better support for the latter tree, the tentative conclusion is that the common parasitism of

R. aurora and R. boylii by G. californiensis is a result of
sequential or independent colonization. In light of this
conclusion, it is interesting to note that these two species of
frogs share a number of immunological characters (thus, their
grouping in Figure 42b). It is not unreasonable to expect
hosts' immunological properties to play some role in their
common susceptibility to the same species of parasite.

The third conclusion that can be drawn from comparing the
host and parasite cladograms is that H. intestinalis coevolved
with R. pretiosa, another species in the R. boylii group.
Because only one species of parasite and one species of host are
involved, this relationship is minimally coevolutionary.
Nevertheless, H. intestinalis can only be said to have colonized
R. pretiosa in the sense that every coevolutionary association
must begin with a species of parasite colonizing a species of
host.

The fourth conclusion is that the parasite data are not of
assistance in determining whether the north-west clade and
south-east clade of ranids in the Americas form a monophyletic
group (i.e., the dotted line in Fig. 40). This is because,
with only two converging nodal values, it is not possible to
eliminate ambiguity at the bottom node of the host tree. An
appeal to outgroups cannot help because the remaining species of
Glypthelmins analyzed are not parasites of ranids.

BIOGEOGRAPHIC ANALYSIS

As noted above, phylogenetic analysis of species of Rana in the Americas postulates a south-east lineage consisting of the R. tarahumarae, R. pipiens, and R. catesbeiana groups, and a north-west lineage consisting of R. sylvatica, R. temporaria, and the R. boylii group. Rana palmipes has the most southern distribution of all the ranids in the Americas. Its range extends from Central America to the northern areas of South America, 'where it is the only ranid on that continent. The hypothesis of a northern entry of the ranids into the Americas is supported by the occurrence of ranid fossils in North America only after the mid-Miocene (Estes, 1970), and their occurrence in South America not until the Holocene (Baez and de Gasparini, 1979), despite their appearance in the Old World, particularly in Africa, in the Oligocene, (Estes, 1970; Savage, 1973). The grouping of the R. boylii group with the European species, R. temporaria, by Farris et al. (1979, 1982) is consistent with an hypothesis of northern entry. As discussed by Savage (1973), ranids may have entered South America only after the re-establishment of a land connection between North and South America in the Pliocene, following the disappearance of such a connection in the Cretaceous (e.g., Coney, 1982; but see Carey, 1976, and H.G. Owen, 1976 for geophysical analyses that suggest that the two continents have never been apart).

Bufonids and hylids have been postulated to have a South America-Africa origin (Savage, 1973), with fossils of both families occurring in Paleocene deposits in South America

(Estes, 1970). The appearance in North America of hylid fossils in the Oligocene and bufonid fossils in the Miocene (Estes, 1970) would seem to be evidence against their entry into North America from South America (Savage, 1973) so long as there was no land connection between the continents for most of the Tertiary. Cladistic analyses of bufonids and hylids for their relationships with Old World species would help to clarify this matter.

The Glypthelmins cladogram can be mapped onto a map of the Americas (Fig. 44) following the method of Brundin (1972; see Brooks, 1977). With respect to those species parasitic in ranids and bufonids, there is, as with the ranids themselves, a north-west lineage, consisting of G. shastai and H. intestinalis, and a south-east lineage, consisting of G. facioi, G. californiensis, and G. quieta. This pattern suggests vicariant speciation in association with mid-Cenozoic orogeny and climactic changes in northern Mexico and western North America (see Axelrod, 1975, Rosen, 1978). The Central America / Mexico / eastern North America vicariance shown by the sister species, G. facioi, G. californiensis, and G. quieta, respectively, is a pattern found among their ranid hosts within the R. pipiens group (Hillis et al., 1983), as well as among certain plants, fishes, salamanders, snakes, birds, and mammals (see Rosen, 1978).

The presence of the relatively plesiomorphic parasite species, G. robustus, in B. marinus in Colombia does not suggest a plesiomorphic association of Glypthelmins with bufonids. This

is because, as discussed in the familial level coevolution analysis above, there is an ambiguity in that part of the tree (Fig. 39) as to whether the plesiomorphic host was a hylid or a bufonid. Another consequence of this ambiguity is that it is difficult to interpret the association of G. hyloreus and G. pennsylvaniensis with hylid frogs in only North America. These two parasite species are postulated to be not as closely related to the North American species of Glypthelmins as is G. robustus. If, as discussed in Chapter III, the examination of more mature specimens of G. robustus warrants that species' relocation on the cladogram to a position below G. hyloreus and G. pennsylvaniensis, this question would be partially resolved. In his analysis of these species of Glypthelmins, Brooks (1977) considered that the placement of the G. hyloreus and G. pennsylvaniensis lineage to the sister taxon position below G. robustus supported an interpretation of dispersal of a common glypthelminth ancestor before vicariant speciation events took place (Nelson, 1974). I concur.

Given that the distributions of R. aurora and R. boylii are disjunct from that of R. montezumae (Fig. 45), the postulated colonization of the first two species by G. californiensis would have to (a) have occurred during a time when the distributions were not disjunct, or (b) involve the parasitism of a closely related host species whose contemporary distribution lies between those of the other three host species. Rana tarahumarae and R. chiricahuensis are two such species, being found in the Sierra Madre Occidental. The analysis by Hillis et al. (1983)

places the first species as the sister taxon to the R. pipiens group, and the second species in the same clade as R. montezumae (Fig. 43). These species have not been examined for parasitism.

The remaining lineage to be examined is the G. shastai + H. intestinalis clade, for which I have postulated a colonization of B. boreas by G. shastai from a common ranid ancestor. While H. intestinalis has been reported from R. pretiosa throughout the Columbia and Snake Rivers Plateaus, from southern British Columbia-Alberta to Utah, the distribution of G. shastai in B. boreas is strongly disjunct (Fig 37). The species has been collected in northeastern California and southwestern Alberta. Surveys of B. boreas in Idaho (Waitz, 1961), Utah (Frandsen and Grundmann, 1960), and southern British Columbia (present study) have not found G. shastai. A biogeographic pattern in this lineage suggestive of vicariance is not evident. An alternative explanation is that the divergence of G. shastai and H. intestinalis from a common ancestral parasite of ranids took place within a sympatric association of the hosts, and may have involved an allohospitalic condition (parasites restricted to different host species: analagous to allopatry) created through the colonization by G. shastai of B. boreas.

SUMMARY

The phylogenetic hypothesis of the relationships among Glypthelmins and Haplometrana that was produced in Chapter III serves in this chapter as a general reference system for the study of five evolutionary processes and one laboratory manipulation.

With respect to the phylogenetic relationships that they support, the larval and adult characters studied are found to be consistent with one another. This suggests the existence of constraints on character evolution that produce a similarly covarying set of characters regardless of environmental differences and assumed selection pressures.

Life cycle properties are mapped onto the cladogram, and are found to be consistent with the phylogenetic relationships supported by the larval and adult characters.

The cladogram is used to study possible instances of heterochronic character development. It is concluded that heterochrony has occurred in the evolution of three characters: (1) paedomorphosis, in the retention of intercecal and post-testicular uterine loops in G. shastai, H. intestinalis, G. californiensis, G. quieta, and G. facioi; (2) hypermorphic peramorphosis, in the retention of penetration glands in adults of G. facioi; and (3) accelerated peramorphosis, in the development of a longer hindbody in H. intestinalis.

A coevolutionary and biogeographic analysis, which involves re-analyses of data from earlier studies on anuran

relationships, makes the following conclusions. (1) There is a north-west clade and a south-east clade in the phylogenetic tree for the glypthelminth species in North and Central America, as there is for their ranid hosts. (2) _Glypthelmins facioi_, G. _californiensis_, and G. _quieta_ have coevolved with host species of the _Rana_ _pipiens_ group in a manner that shows a Central America / Mexico / southeastern U.S. vicariance pattern, respectively. This pattern is also shown by their ranid hosts. (3) _Glypthelmins californiensis_ subsequently colonized two members of the R. _boylii_ group in western North America: R. _aurora_ and R. _boylii_; this common susceptibility to parasitism may be associated with the apparent convergence of some of the immunological properties of these two frog species. (4) _Haplometrana_ _intestinalis_ coevolved with R. _pretiosa_. (5) The common ancestor of H. _intestinalis_ and G. _shastai_ was a parasite of ranids, subsequent to which, G. _shastai_, in its speciation, colonized a bufonid, _Bufo_ _boreas_.

Experimental infections of adults of B. _boreas_ with specimens of H. _intestinalis_ produced an altered development of the hindbody of H. _intestinalis_ after 12 months of development in the host. This alteration is considered to be an instance of neotenic paedomorphosis, produced through a decrease in the rate of growth of the hindbody after 4 months of development. The altered morphology is that of the plesiomorphic state, which is exhibited by G. _shastai_, the postulated sister species of H. _intestinalis_. Heterochrony can thus be studied from a phylogenetic perspective both as an evolutionary event and as an
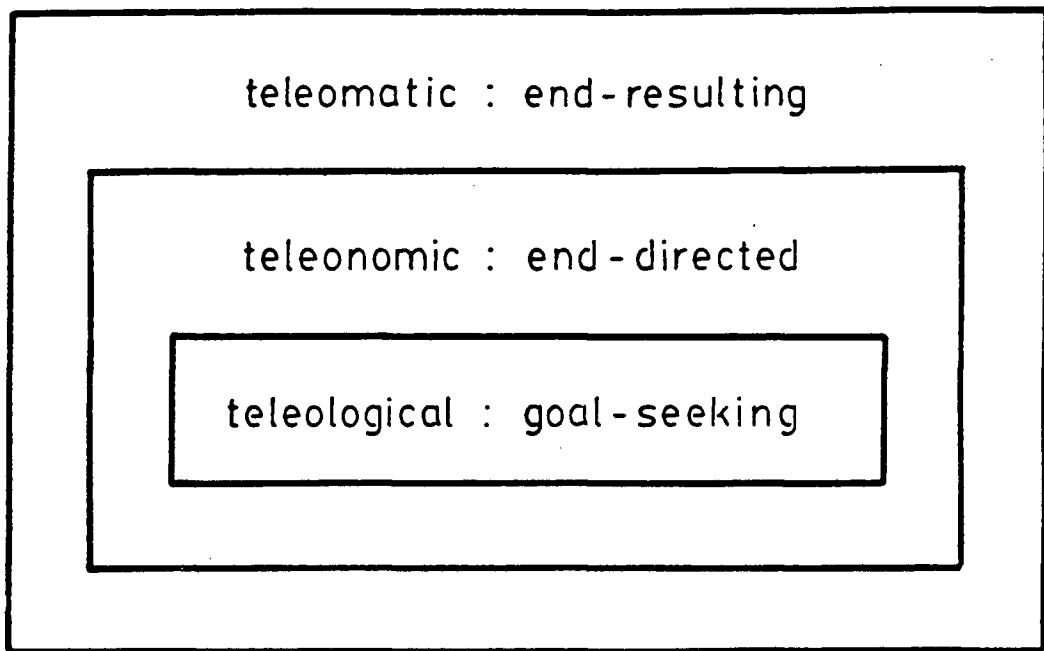
experimentally-produced alteration in development.

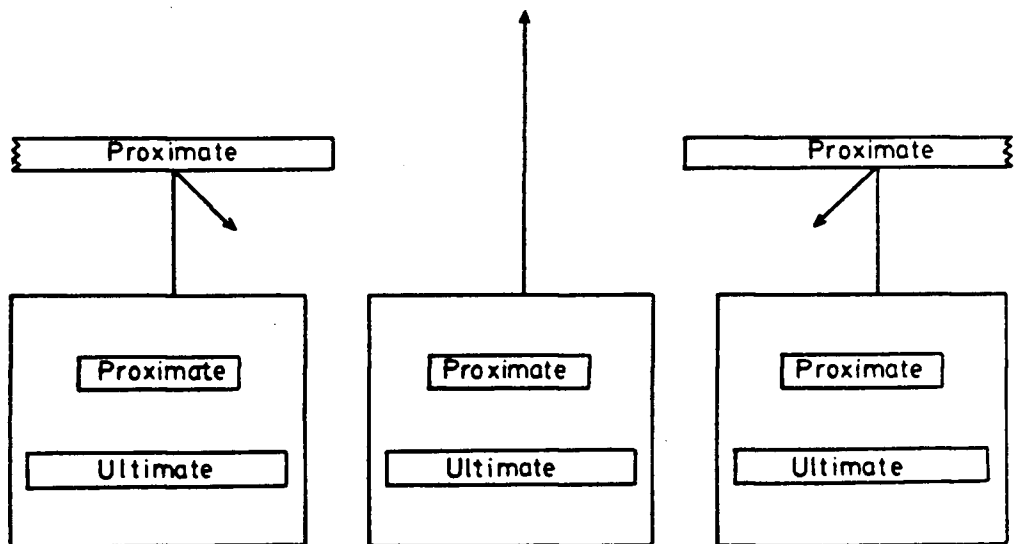Figure 1 - End-Attaining Activity in Biological Systems

Three types of activity are involved. They are internested,
rather than mutually exclusive.   All physical systems show
teleomatic activity; a subset (biological systems) also shows
teleonomic activity; a subset of these (cognitive systems) also
shows teleological activity.   (From O'Grady, 1986. Can. J.
Zool. 64:1010.)

Figure 2 - Ultimate and Proximate Causality

Three organisms composed of a hierarchy of within-system
ultimate and proximate causality.   These are subsequently
differentially eliminated by an among-system proximate cause:
natural selection.   (From O'Grady, 1986.   Can.   J.   Zool.
64:1010.)

teleomatic : end-resulting

teleonomic : end-directed

teleological : goal-seeking

1

Proximate

Proximate

Proximate

Proximate
Ultimate

Proximate
Ultimate

Proximate
Ultimate

2

Figure 3 - Types of Evolutionary Explanations

Four alternative evolutionary histories for four skin colors of
a species of frog: brown (Br), yellow (Ye), red (Re),and green
(Gr). The causes involved are: natural selection (C1), and
inheritance at two levels (C2, C3). (From O'Grady, 1986. Can.
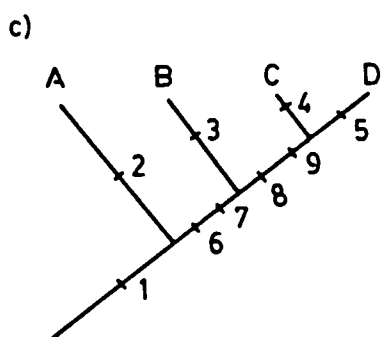J. Zool. 64:1010.)

Figure 4 - Historical Structuralism in Phylogenetic
Systematics

Hennigian phylogenetic analysis of four taxa. As the tree is
resolved with the addition of character data, the analysis
incorporates as much internested historical cauality as
possible. (From O'Grady, 1986. Can. J. Zool. 64:1010.)

3 a)

b)

c)

d)

4 a)

b)

	6	7	8	9
A	–	–	–	–
B	♦	♦	–	–
C	♦	♦	♦	♦
D	♦	♦	♦	♦

c)

d)
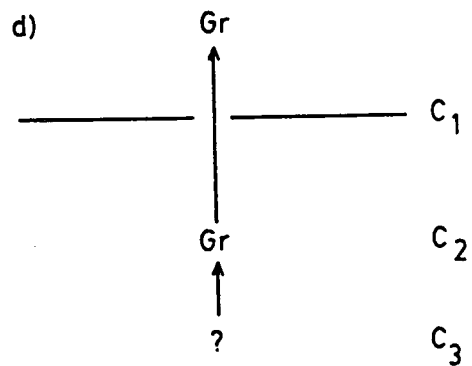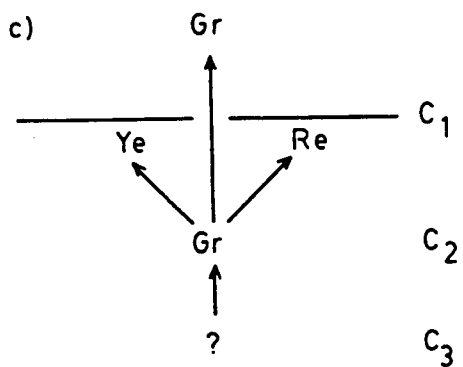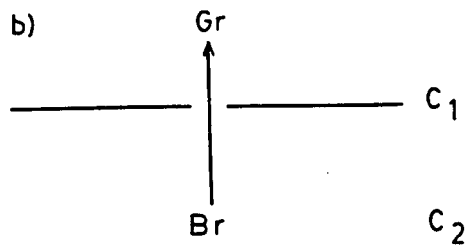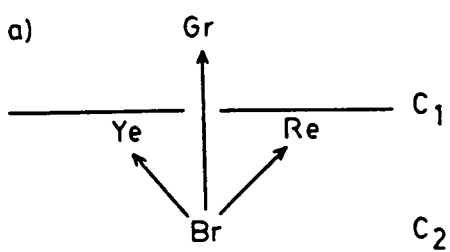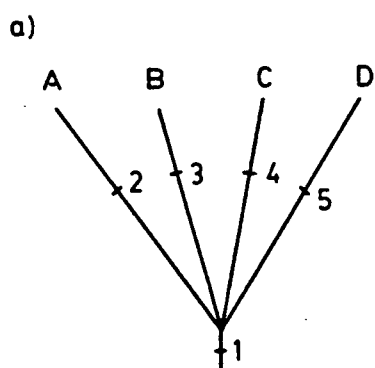
Figure 5 - Using a Cladogram to Study the Evolution of
Life History Traits

Top figure: a phylogenetic tree for the parasitic platyhelminths
(Brooks, 1982; Brooks et al., 1985a; O'Grady, 1985).   DI
indicates a direct life cycle in an invertebrate host, DV
indicates a direct life cycle in a vertebrate host, CB indicates
a complex life cycle involving both host groups.   Bottom figure:
the same tree, with arrows indicating the postulated changes in
the type of life cycle. . The changes are inferred with the
optimization procedures of Farris (1970) and Swofford and
Maddison (in review).

DALYELLOIDEA - DI
TEMNOCEPHALIDEA - DI
ASPIDOCOTYLEA - DI
DIGENEA - CB ★
MONOGENEA - DV
GYROCOTYLIDEA - DV
AMPHILINIDEA - DV
CESTOIDEA - CB ★

★ COMPLEX LIFE CYCLES

DI   DI   DI   CB   DV   DV   DV   CB
Da   Te   As   Di   Mo   Gy   Am   Ce
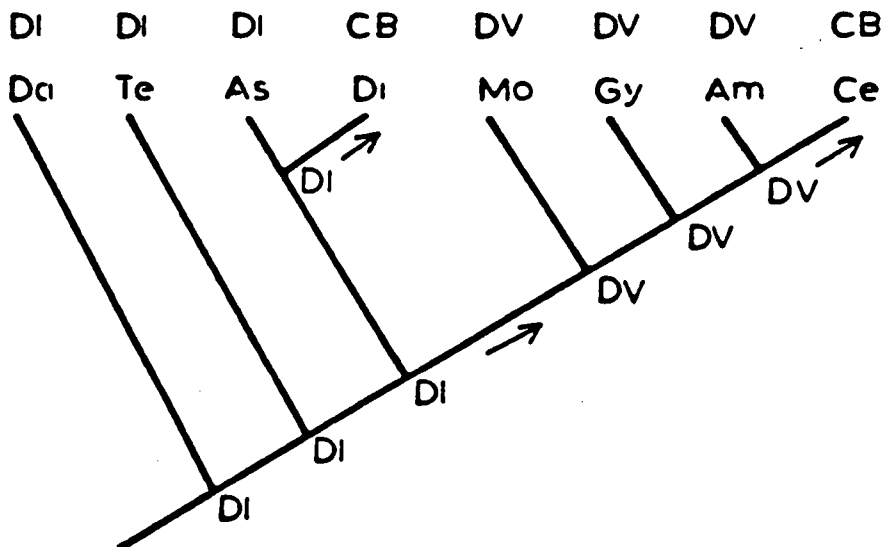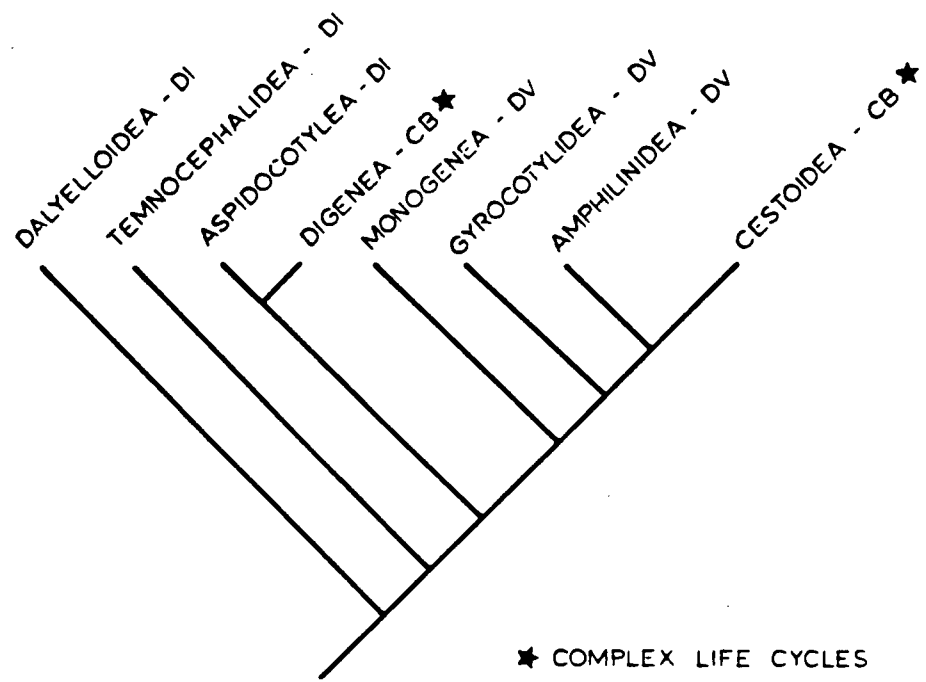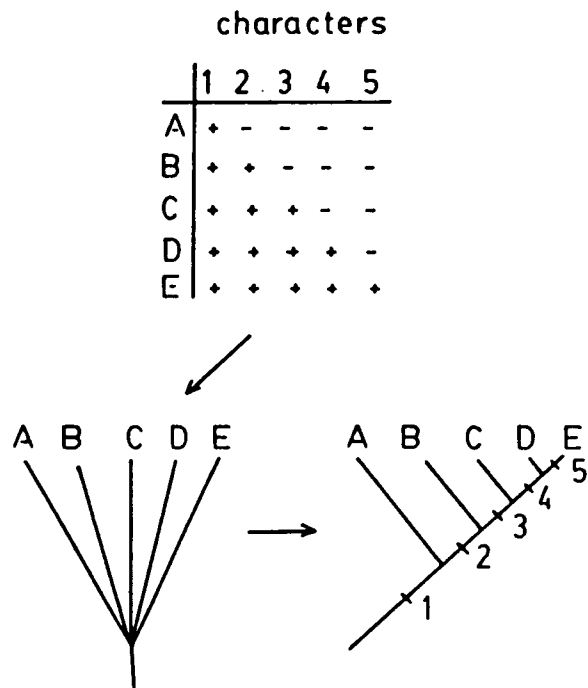
DI

Dv

Dv

Di

Di

DI

165

Figure 6 - The Application of Historical Structuralism to
the Study of Community Structure

The pattern of inheritance suggested by a phylogenetic analysis
can be used as the initial hypothesis in explanations of
ecological traits. Departures from congruence between the
postulated phylogeny and such traits can be postulated to be
indicative of nonhistorical events, such as colonization. (From
O'Grady, 1986. Can. J. Zool. 64:1010.)

PHYLOGENETIC ANALYSIS

characters

	1	2	3	4	5
A	+	-	-	-	-
B	+	+	-	-	-
C	+	+	+	-	-
D	+	+	+	+	-
E	+	+	+	+	+

COMMUNITY STRUCTURE ANALYSIS

ecology

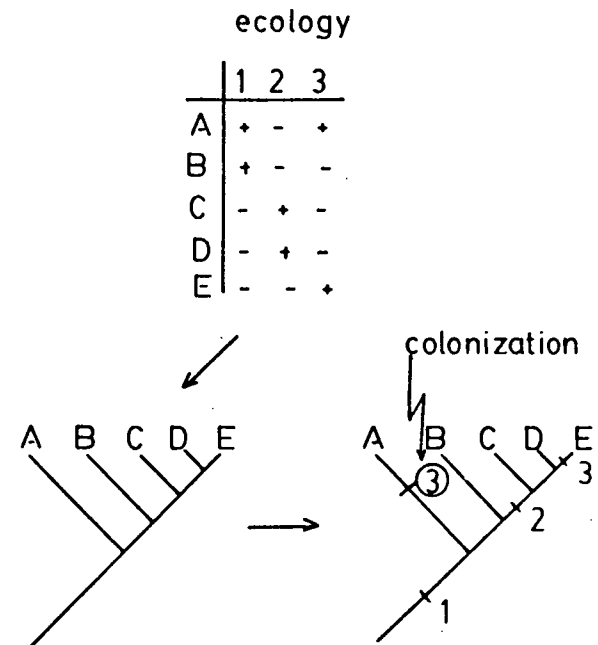|	1	2	3
A	+	-	+
B	+	-	-
C	-	+	+
D	-	+	-
E	-	-	+

colonization

Figure 7 - Postulated Relationships among <u>Glypthelmins</u>,
from Brooks (1977)

Species: a, <u>hyloreus</u>; b, <u>pennsylvaniensis</u>; c, <u>robustus</u>; d,
<u>facioi</u>; e, <u>shastai</u>; f, <u>quieta</u>; g, <u>californiensis</u>; h,
<u>vitellinophilum</u>; i, <u>incurvatum</u>; j, <u>linguatula</u>; k, <u>hepatica</u>; l,
<u>palmipedes</u>; m, <u>proximus</u>; n, <u>repandum</u>; o, <u>tineri</u>; p, <u>africana</u>; q,
<u>diana</u>; r, <u>staffordi</u>; s, <u>rugocaudata</u>. The lower figure
(reproduced from Brooks, 1977. Syst. Zool. 26:277) shows the
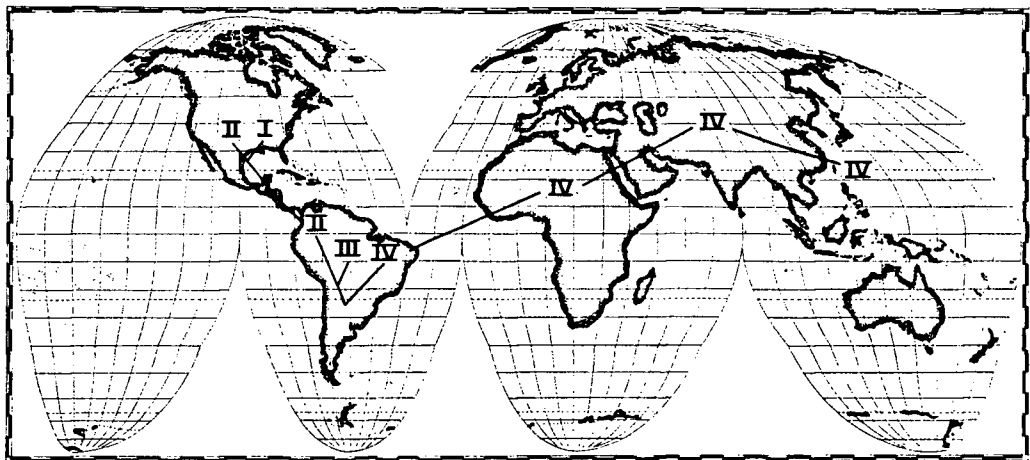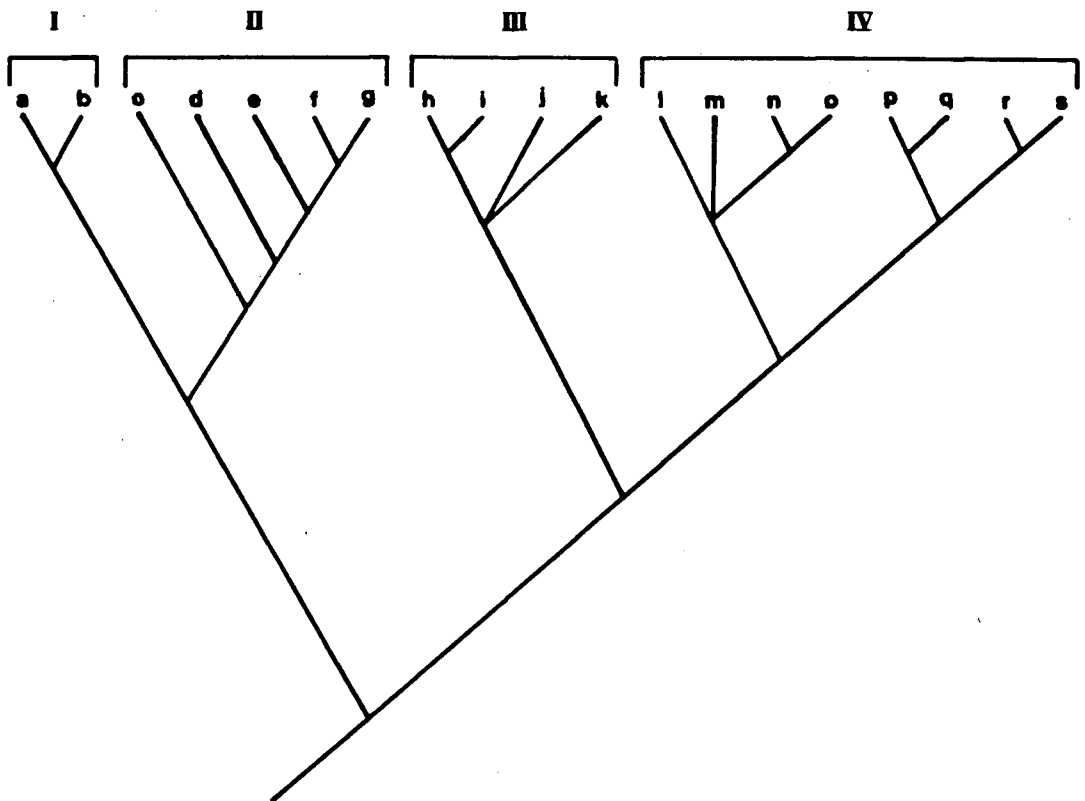biogeographic distributions of the four lineages.

Figure 8 - Collection Sites of Anurans in British Columbia
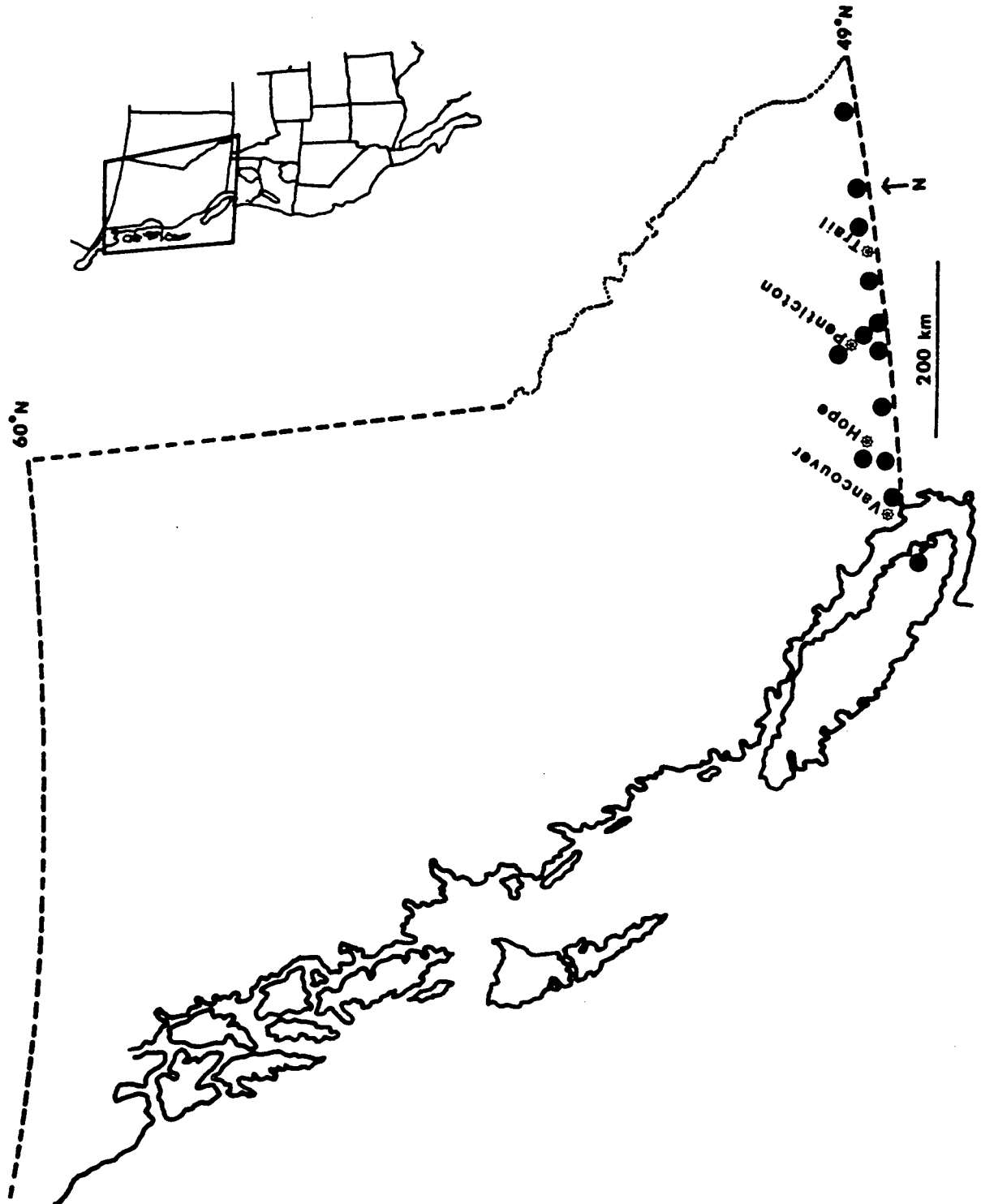
See Appendix C for details.

Figure 9 - Collections Sites of Anurans in California

See Appendix C for details. Solid squares indicate collections
of R. cascadae; small solid triangles indicate collections of
B. boreas; the large solid triangle indicates the collection of
B. boreas at Glenburn, the type locality of G. shastai; open
diamonds indicate localities at which no anurans were found.
The course of the Pit River is indicated; it flows from the
north-east of the state towards Redding, Shasta County, joining
with the Sacramento and McCloud Rivers at Shasta Lake, north of
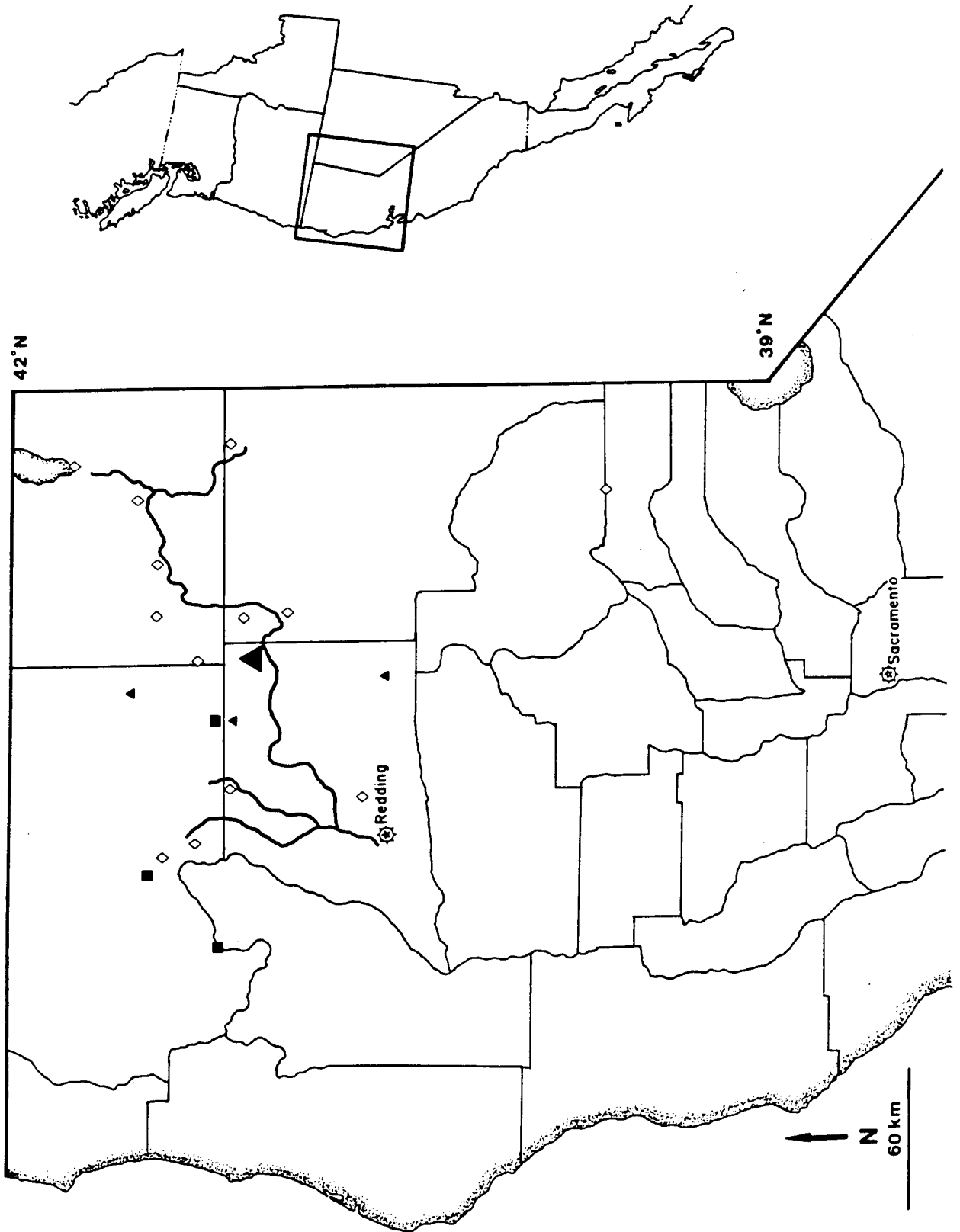Redding.

Figure 10 - Tegumental Projections

The two types of projections that occur among the species
studied, drawn from the ventral surface of the forebody of a
specimen of (a) H. intestinalis, and (b) G. quieta. (The
anterior of the worm is towards the top of the plate.) The
projections in (a) are termed spines, those in (b) are termed
scales; (bar = 0.1 mm).

Figure 11 - Penetration Glands in Adult Glypthelmins facioi
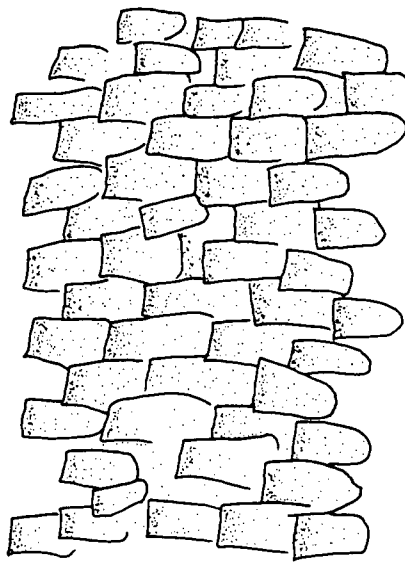
A ventral view of specimen no. 222, from the collection of J.J.
Sullivan,[1] showing penetration glands (pen. gl.) and medial
glands (med. gl.); (bar = 0.2 mm).

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[1] Center for Disease Control, Atlanta, Georgia.
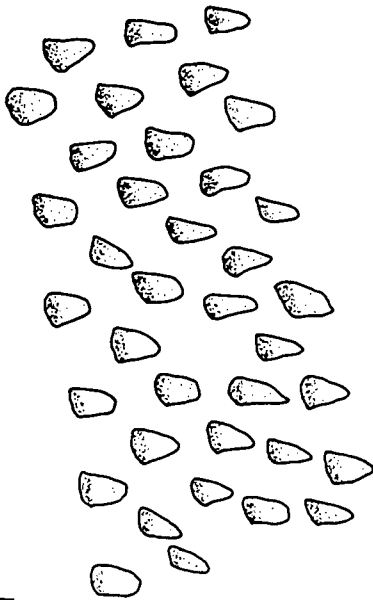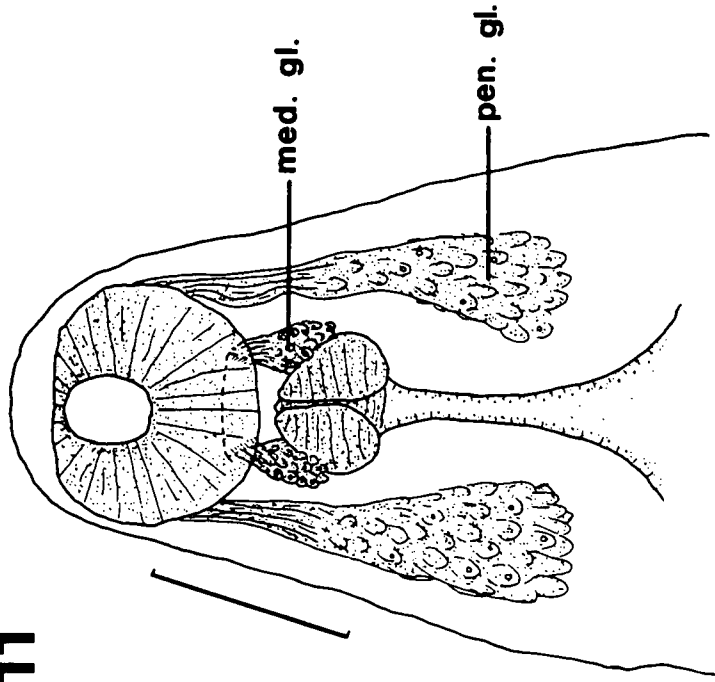
Figure 12 - Medial Glands in <u>Haplometrana</u> <u>intestinalis</u>

A ventral view of a specimen from the collection of the author, showing medial glands (med. gl. & mgl) and ducts; (bars = 0.2 mm).

Figure 13 - Pharyngeal Glands in <u>Glypthelmins</u> <u>quieta</u>

A ventral view of a specimen from the collection of D.R. Brooks, Univ. of British Columbia, showing pharyngeal glands (ph. gl.), medial glands (med. gl.), and their ducts; (bar = 0.2 mm).

12

mgl

med. gl.

13

med. gl.

ph. gl.
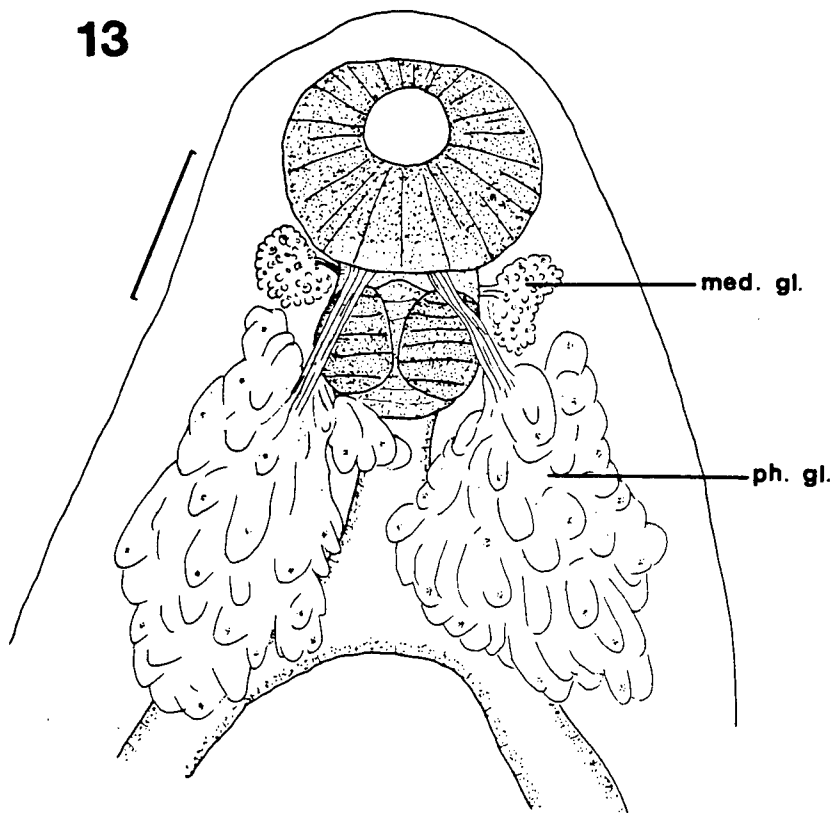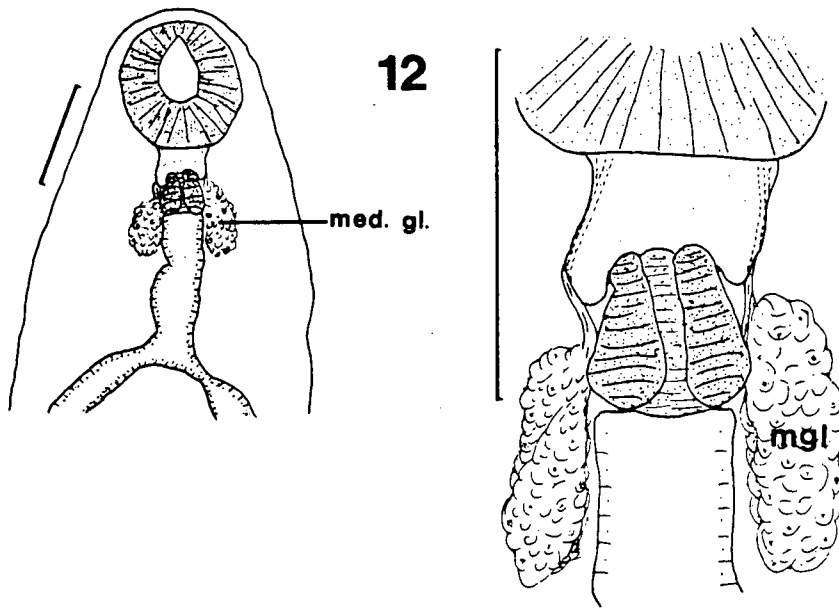
Figure 14 - Schematic Representation of the Distribution of
the Vitellaria in Glypthelmins and Haplometrana

Representations for some of the species studied, showing the
different regions in which medial confluence of the vitellaria
occurs. The view is antero-dorsal, i.e., with the anterior of
the animal at the bottom of the plate. The dark cylinders
represent the intestinal ceca, the light areas represent the
vitellaria, and the stippled lines represent the vitelline
ducts. The species illustrated are: a, H. intestinalis; b,
G. shastai; c, G. quieta; d, G. californiensis; e, G. facioi.
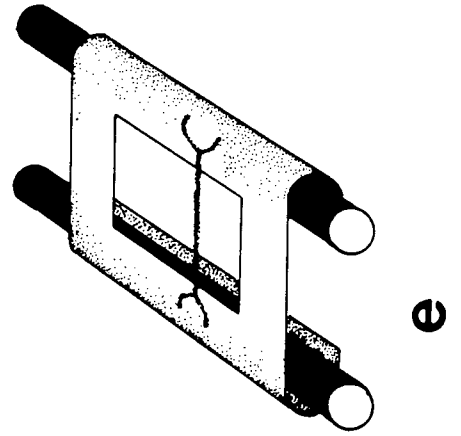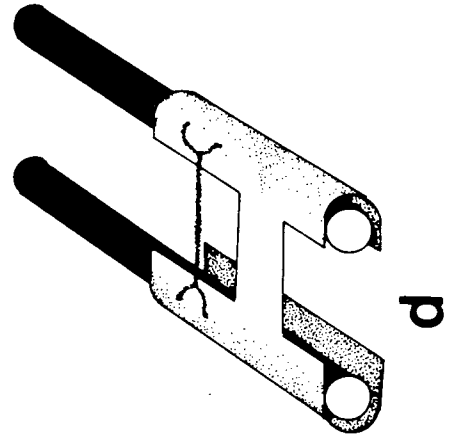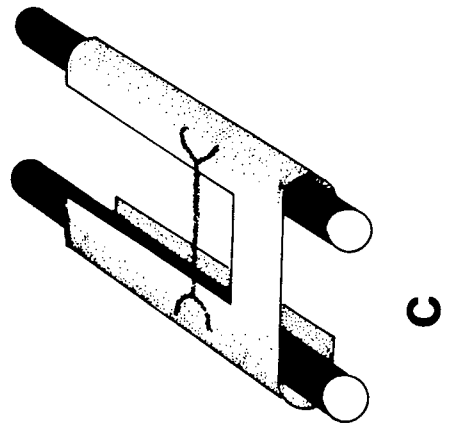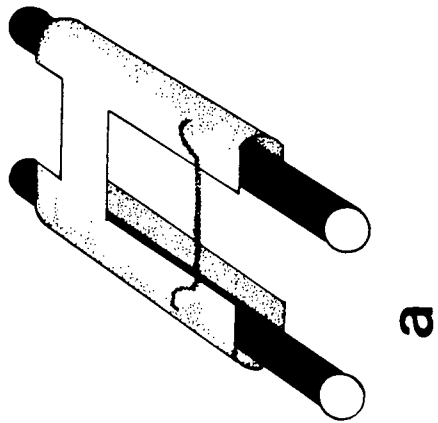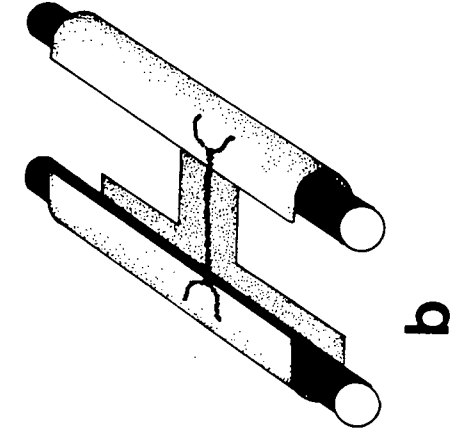
Figure 15 - Distribution of Vitellaria in <u>Glypthelmins</u>
<u>quieta</u> and <u>G</u>. <u>facioi</u>·

(a) and (b): Ventral views of a specimen of <u>G</u>. <u>quieta</u> from the
collection of D.R. Brooks, Univ. of B.C. The dark shaded
areas represent (a) the distribution of the vitellaria dorsal to
the gut and gonads, and (b) the distribution of the vitellaria
ventral to the gut and gonads. (c): A dorsal view of a specimen
of <u>G</u>. <u>quieta</u> from the collection of D.R. Brooks, in which the
reduced development of the vitellaria allows the anterior and
posterior fields to be seen clearly. (d) and (e): Ventral views
of <u>G</u>. <u>facioi</u> specimen no. 200, from the collection of J.J.
Sullivan. The dark shaded areas represent (d) the distribution
of the vitellaria dorsal to the gut and gonads, and (e) the
distribution of the vitellaria ventral to the gut and gonads;
(all bars = 1.0 mm).
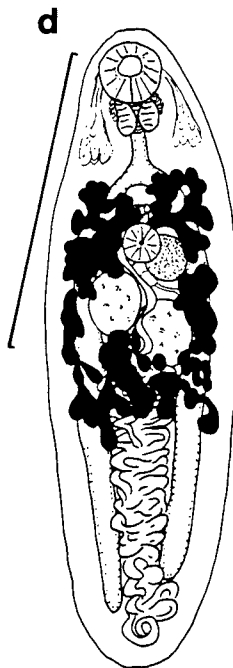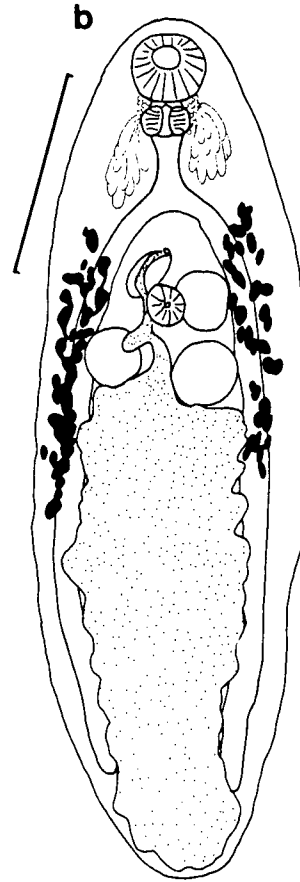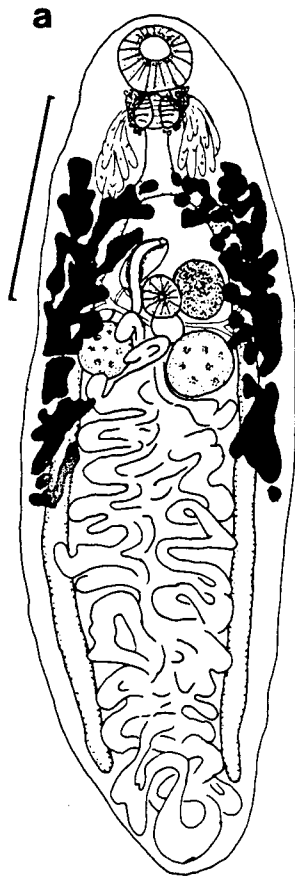
Figure 16 - Distribution of Vitellaria in <u>Glypthelmins</u>
<u>californiensis</u>

Ventral views of a specimen of <u>G</u>. <u>californiensis</u> from the
collection of D.R. Brooks, Univ. of B.C. The dark shaded
areas represent (a) the distribution of the vitellaria dorsal to
the gut and gonads, and (b) the distribution of the vitellaria
ventral to the gut and gonads; (bars = 1.0 mm).

Figure 17 - Distribution of Vitellaria in <u>Glypthelmins</u>
<u>shastai</u> and <u>Haplometrana</u> <u>intestinalis</u>

(a) and (b): Ventral views of <u>G</u>. <u>shastai</u> specimen no. UAPAR
720, from the collection of the Dept. of Zoology, Univ. of
Alberta. The dark shaded areas represent (a) the distribution
of the vitellaria dorsal to the gut and gonads, and (b) the
distribution of the vitellaria ventral to the gut and gonads;
(bars = 1.0 mm). (c) and (d): Ventral views of a specimen of
<u>H</u>. <u>intestinalis</u> from the collection of the author. The dark
shaded areas represent (c) the distribution of the vitellaria
dorsal to the gut and gonads, and (d) the distribution of the
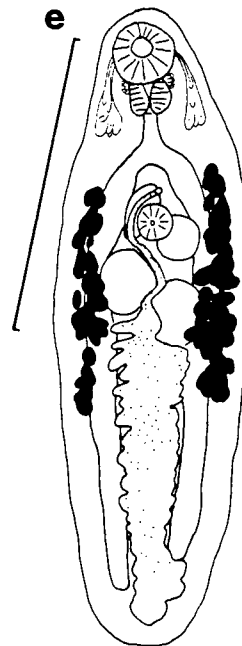vitellaria ventral to the gut and gonads; O, ovary; T, testis;
(bars = 0.5 mm).
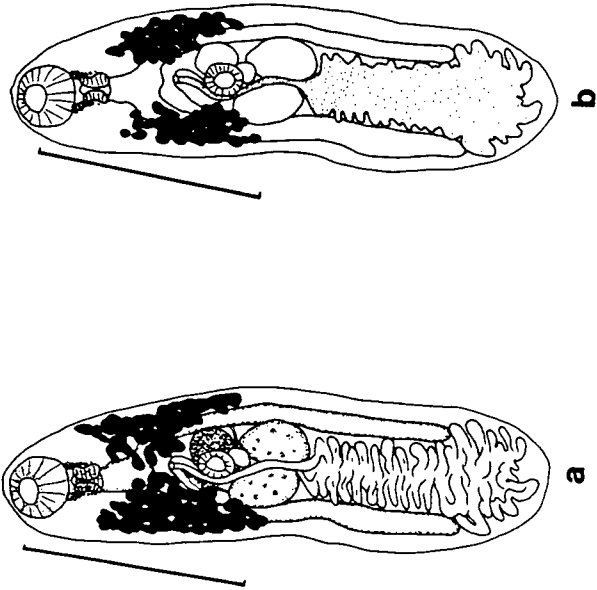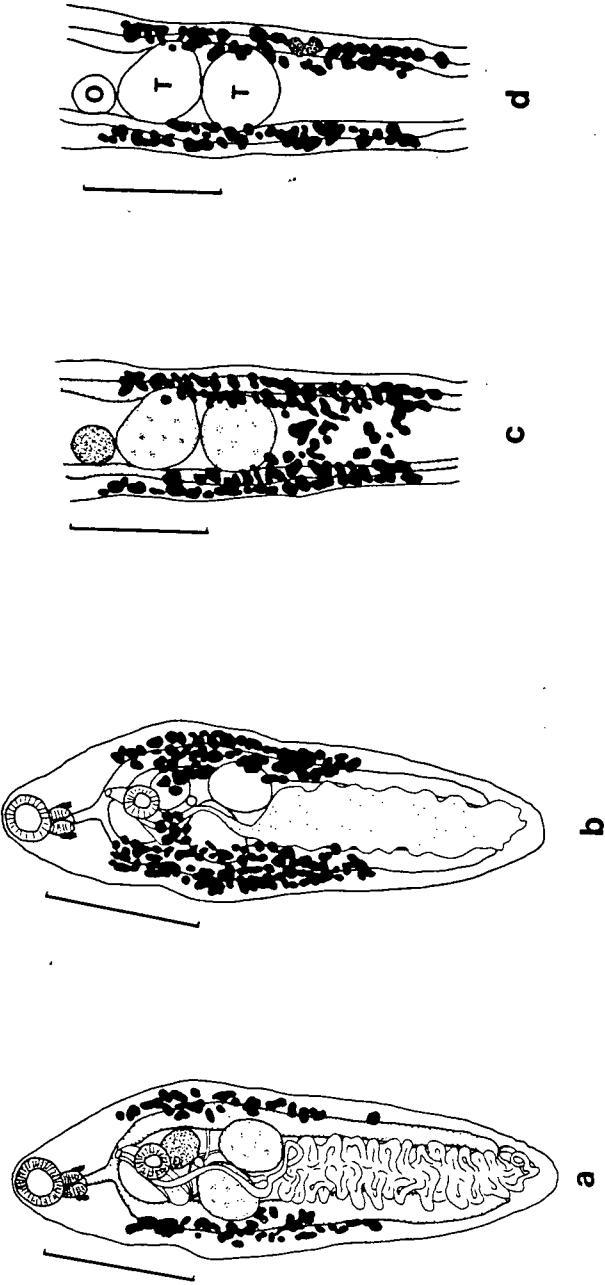
Figure 18 - Vitelline Ducts in <u>Haplometrana</u> <u>intestinalis</u>
and <u>Glypthelmins</u> <u>californiensis</u>

(a) A ventral view of a specimen of <u>H</u>. <u>intestinalis</u> from the
collection of the author, showing the single vitelline duct on
each side; (bar = 0.2 mm).

(b) A dorsal view of a specimen of <u>G</u>. <u>californiensis</u> from the
collection of the author, showing the reduced development of the
posterior vitelline duct (asterisk); (bar = 0.2 mm).

T, testis; SR, seminal receptacle; O, ovary; LC, Laurer's canal;
MG, Mehlis' gland; U, uterus; VD, vitelline duct.
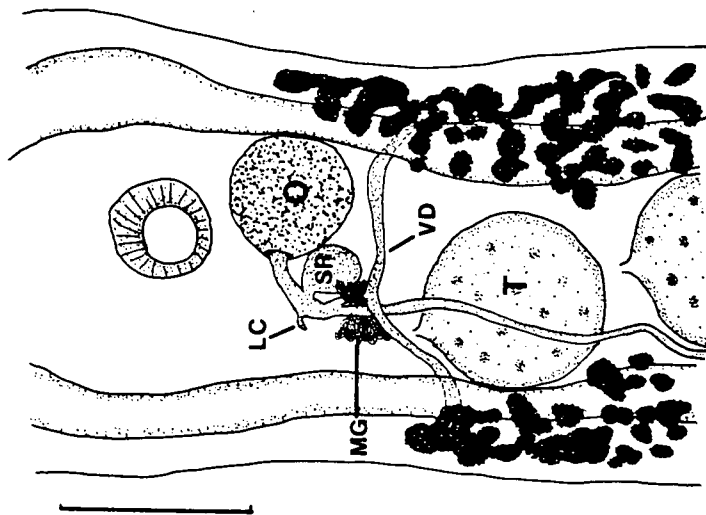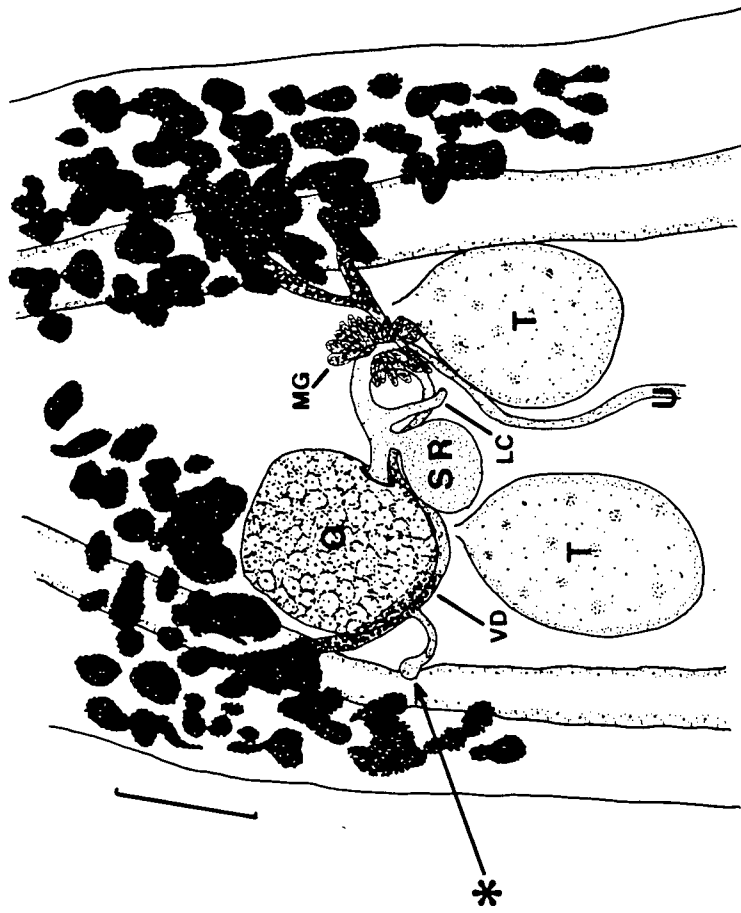
Figure 19 - Shape of the Excretory Vesicle

(a), I-shaped vesicle bifurcating at, or posterior to, the level of the testes; (b), Y-shaped vesicle bifurcating anterior to the level of the testes.
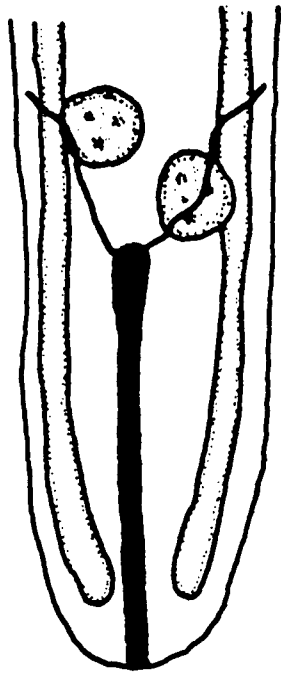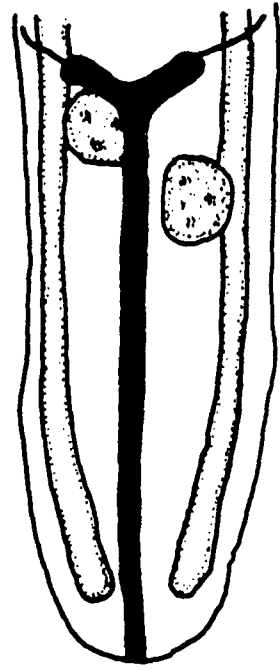
a   b

Figure 20 - Postulated Phylogenetic Relationships among
Glypthelmins and Haplometrana

The cladogram obtained from a phylogenetic analysis of seven
species of Glypthelmins and the single species of Haplometrana
parasitic in the intestines of anurans in North, Central, and
South America. Twenty-one morphological characters are studied.
The Consistency Index of the tree is 0.848. When non-
homoplasious autapomorphic characters are not included in the
calculation (see Appendix G), the Consistency Index is 0.769.
Character states are given in brackets, with binary characters
having a numeric code, and multistate characters having an
alphabetic code. Asterisks indicate characters for which there
is ambiguity as to their state at certain nodes on the tree.
See the section, CHARACTER ANALYSIS, as well as Table II and
Appendix A.
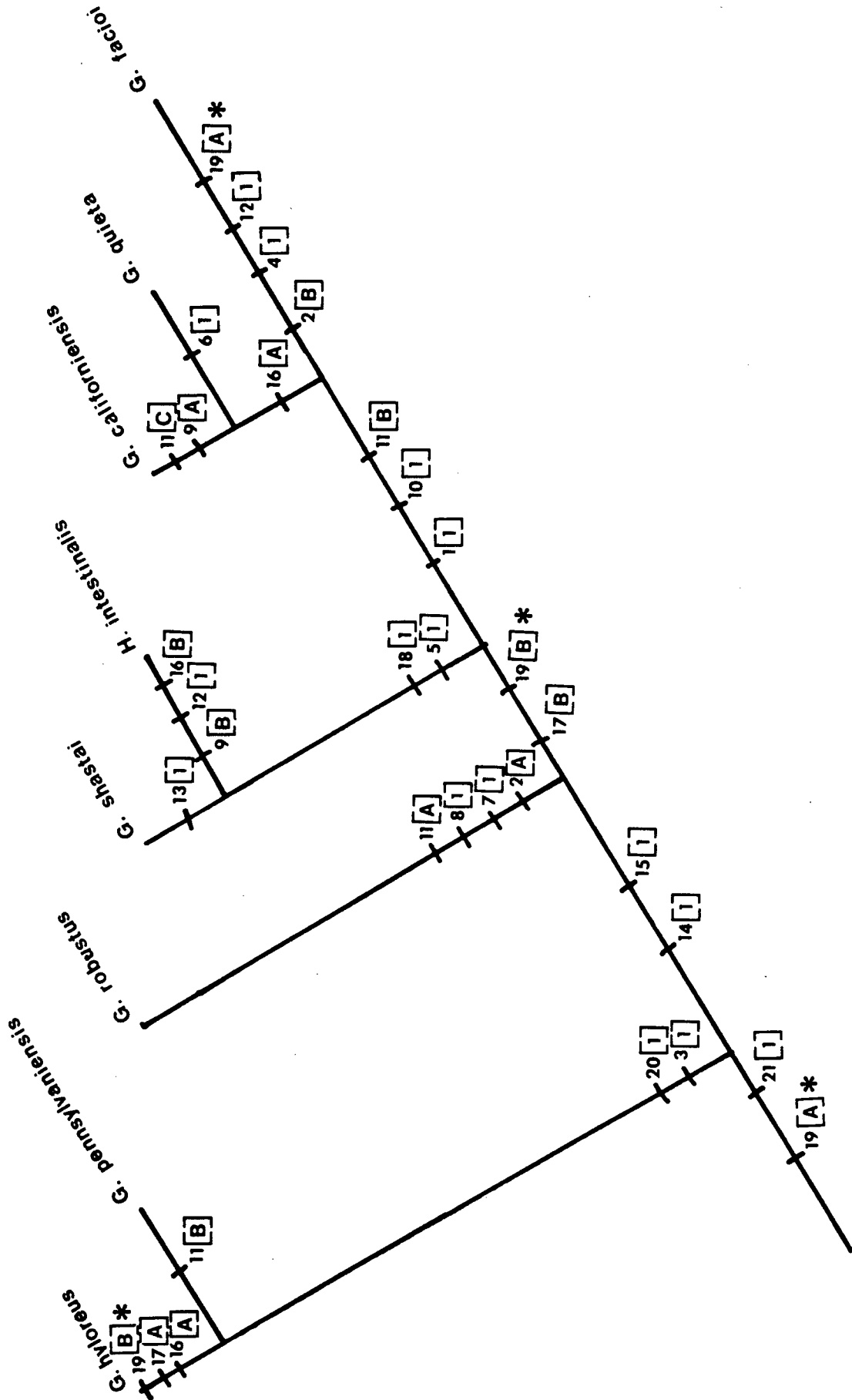
Figure 21 - Multistate Character Trees: One

The multistate transformation series for characters no. 2, 9, 11, 16, and 17, as postulated by an Unordered Analysis with the PAUP computer program (version 2.4, 1985), developed by D.L. Swofford.

#2

O——A
B

CI = 1.00

#9

O——A
B

CI = 1.00

#16

O——A
B

CI = 0.60

#17

O——A
B

CI = 1.00

#11

O——A
B——C

CI = 0.75
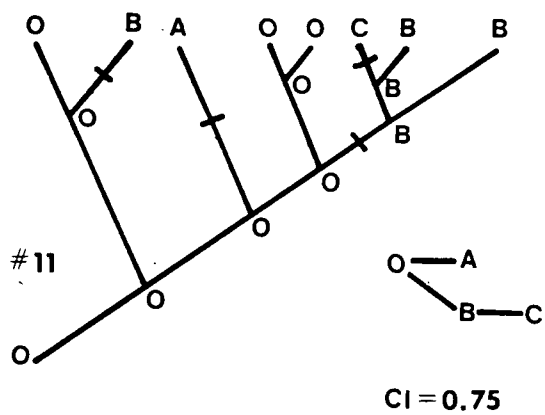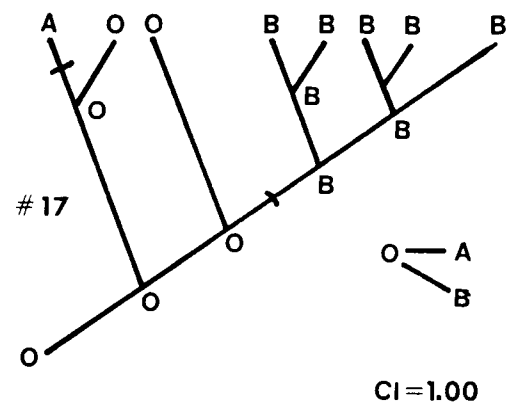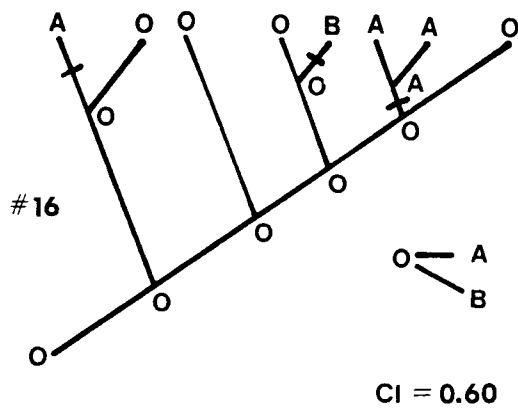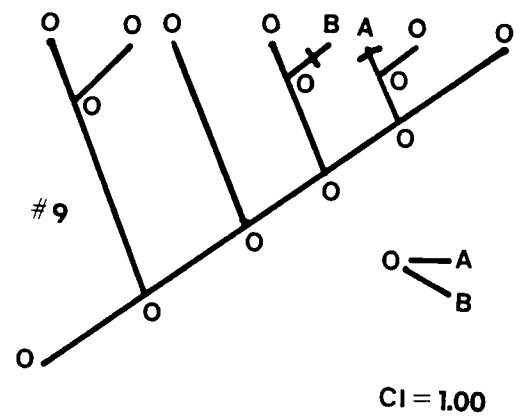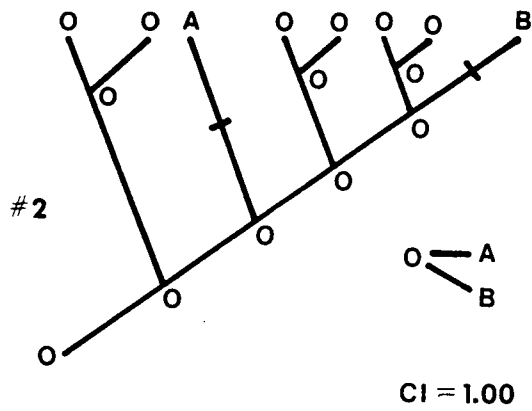
Figure 22 - Multistate Character Trees: Two

Left: the multistate transformation series for character no. 19 (mean egg length), as postulated by an Unordered Analysis with the PAUP computer program (version 2.4, 1985), developed by D.L. Swofford. Right: a possible resolution of the ambiguous nodal values, based upon an assumption of a linear increase in mean egg length.

#19

CI = 0.50
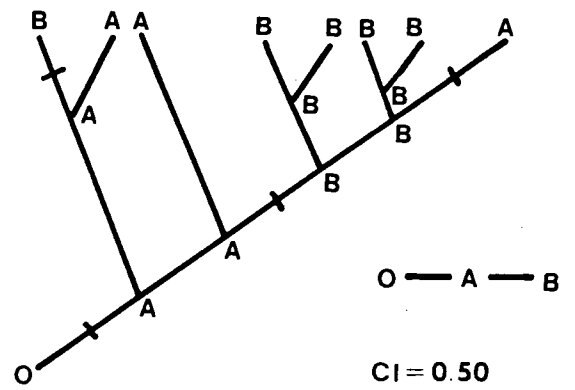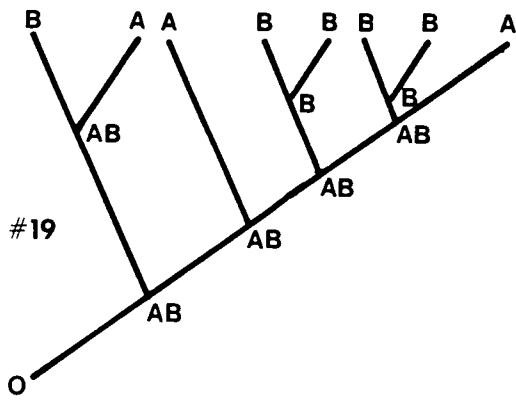
Figure 23 - Disruptive Grouping Criteria in Systematics

Figure (a) gives the most parsimonious cladogram that can be constructed with respect to the distribution of four characters, in which the plesiomorphic states are coded as "U", and the apomorphic states are coded as "X". Character steps on the tree mark the appearance of the apomorphic state. This tree is six character steps long. If one character is given precedence over the others when grouping the taxa, the result can be trees such as those in (b) to (d). These are incongruent (see Figure 24) with the tree in (a). (b): If taxa A and B are grouped together because they both lack state X of character 3 (grouping by symplesiomorphy), the tree is seven steps long. (c): If all of the taxa possessing state X of character 4 are grouped together (grouping by convergent traits), the tree is seven steps long. (d): If all of the taxa possessing state X of character 1 are grouped together (failing to recognize a reversal), the tree is eight steps long.

Character States

4	X	U	U	X	X
3	U	U	X	X	X
2	U	X	X	X	X
1	X	X	X	X	U

a

b

c

d
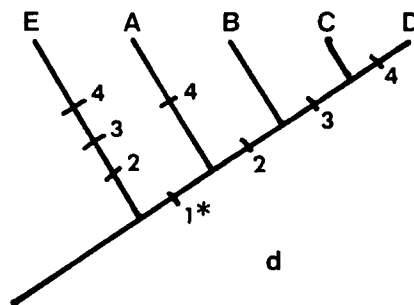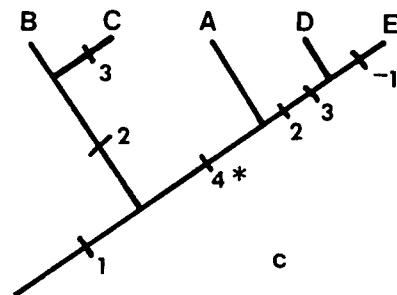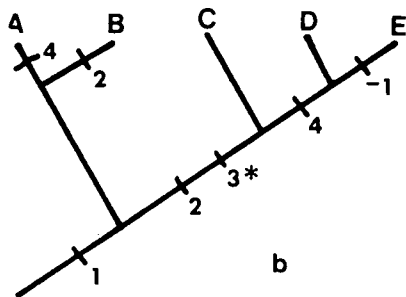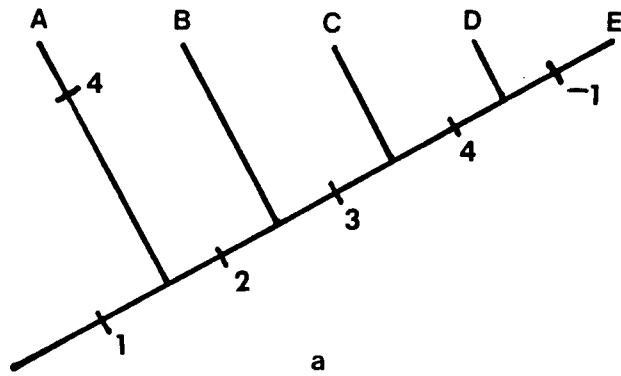
Figure 24 - Congruence and Consistency in Tree Topologies

Trees (a) and (b) are congruent with one another (solid arrows); each of these is consistent with tree (c) (dashed arrows); tree (d) is also consistent with tree (c), but it is incongruent with trees (a) and (b).
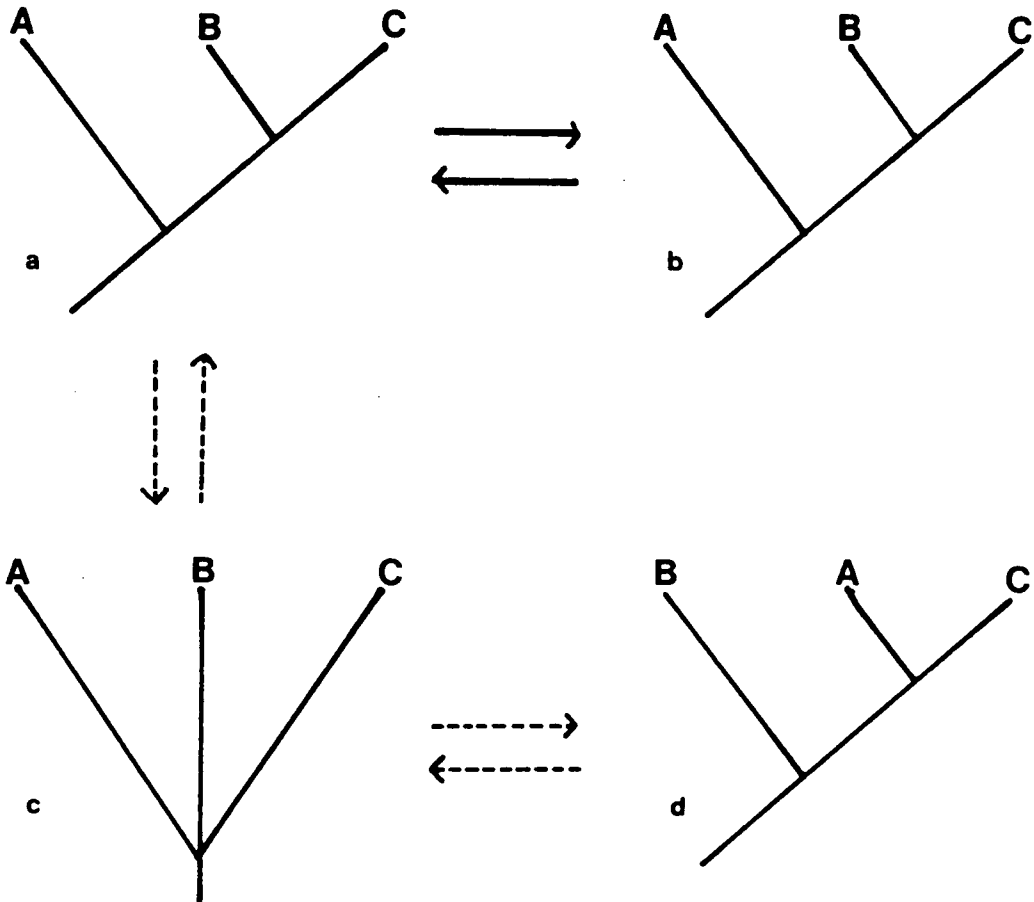
Figure 25 - Generalized Life Cycle of a Digenean Flatworm

The double-headed arrows indicate instances of asexual reproduction. The sequences at lower left indicate the permutations of the developmental sequence of miracidium (M), sporocyst (S), redia (R), and cercariae (C) stages that have been reported in the literature. (Figure reproduced from O'Grady, 1985. Cladistics 1:165.) See O'Grady (1985) and Brooks et al. (1985b) for phylogenetic analyses that suggest that the sporocyst stage is always present.

DEFINITIVE   HOST
(VERT.)

adult

metacercaria   egg

2 nd INTERMEDIATE
HOST
(VERT. OR)   WATER
(INVERT.)

cercaria   miracidium

S→C   redia←—— sporocyst
M   S→R→C
R→C

1 st   INTERMEDIATE   HOST
(MOLLUSC)
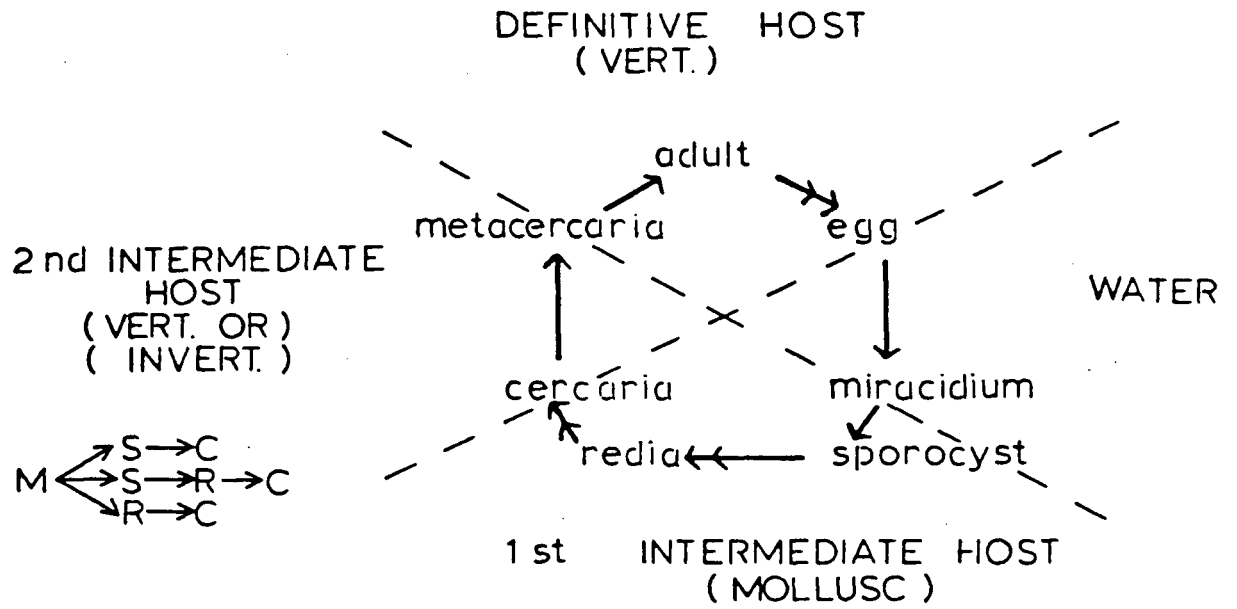
Figure 26 - Phylogenetic Analysis of Larval Characters of
Species of <u>Glypthelmins</u> and <u>Haplometrana</u>

The species included are all of·those in the study group for
which there are data: hy, <u>G</u>. <u>hyloreus</u>; pe, <u>G</u>. <u>pennsylvaniensis</u>;
in, <u>H</u>. <u>intestinalis</u>; ca, <u>G</u>. <u>californiensis</u>; qu, <u>G</u>. <u>quieta</u>. All
of the character states are traits of the cercariae, and are: 1,
a stylet; 2, a dorso-ventral finfold; 3, tandem testes; 4,
symmetrical testes; 5, tegumental scales. Character states
placed on the vertical line at the bottom of the tree are
considered to be symplesiomorphic for the level of the study
group. The dashed line shows the placement of <u>H</u>. <u>intestinalis</u>
in the primary cladogram in Figure 20.
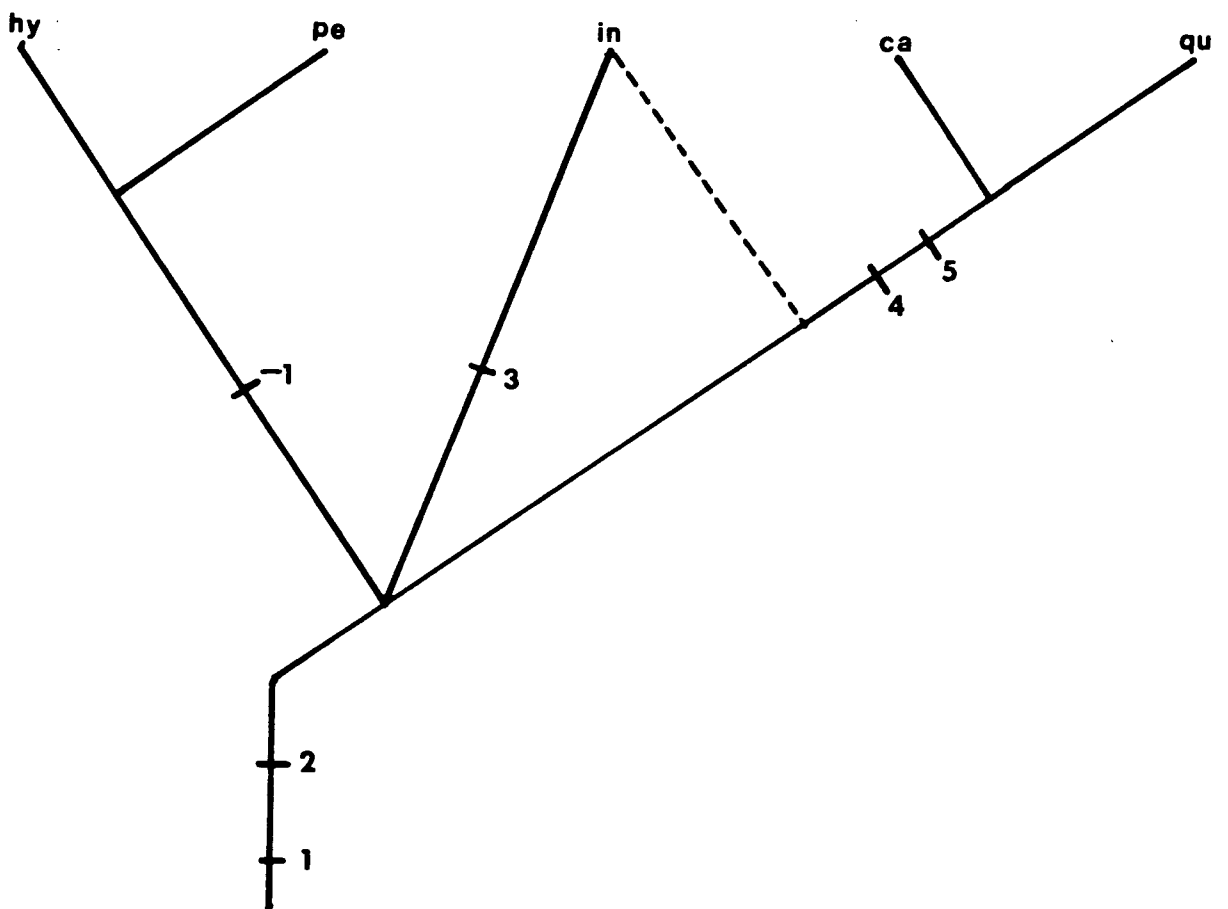
Figure 27 - Phylogenetic Analysis of Life Cycle Evolution
in Species of <u>Glypthelmins</u> and <u>Haplometrana</u>

The species included are all of those in the study group for which there are data: hy, <u>G. hyloreus</u>; pe, <u>G. pennsylvaniensis</u>; in, <u>H. intestinalis</u>; ca, <u>G. californiensis</u>; qu, <u>G. quieta</u>. The character states are: 1, a physid snail as at least one of the first intermediate host types; 2, a lymnaeid snail as at least one of the first intermediate host types; 3, a lymnaeid snail as the only first intermediate host type; 4, a helisomid snail as at least one of the first intermediate host types; 5, an anuran as both the second intermediate and the final host; 6, development as an unencysted metacercaria in anuran tadpoles; 7, development as an encysted metaceraria in anuran adults. Character states placed on the vertical line at the bottom of the tree are considered to be symplesiomorphic for the level of the study group. The dashed line indicates the placement of <u>G. californiensis</u> in the primary cladogram in Figure 20.
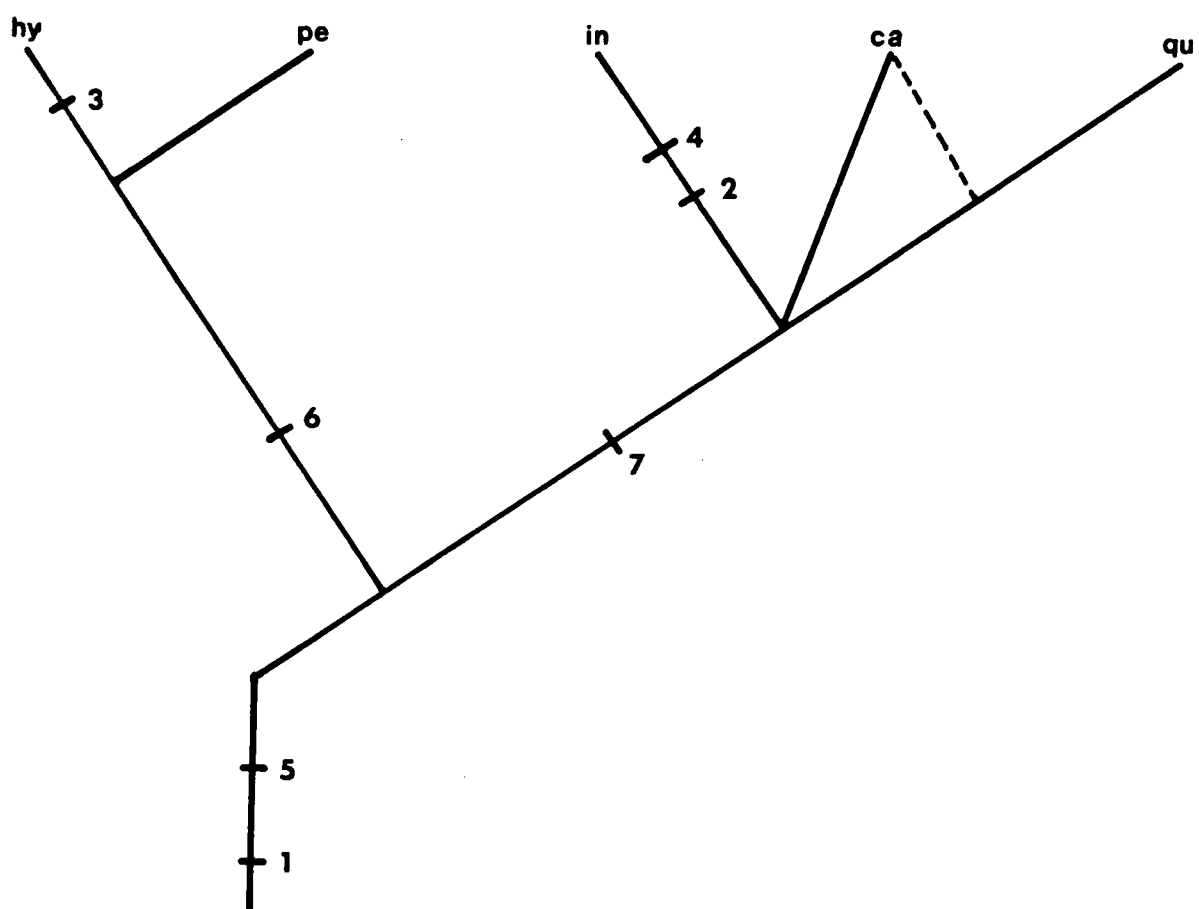
Figure 28 - Categories of Heterochronic Change

Alterations in the rate of development (k), its time (t) of
initiation (alpha), or its time of cessation (beta) can produce
changes in the morphology (gamma) of the descendant (D), as
compared to that of its ancestor (A). There are six categories
of change; three are paedomorphic, in that the descendant's
morphology is less developed than that of the ancestor; three
are peramorphic, in that the descendant's morphology is more
developed than that of the ancestor. (From Alberch et al.,
1979, and Fink, 1982)
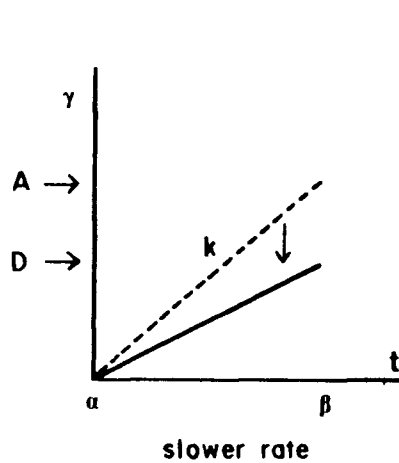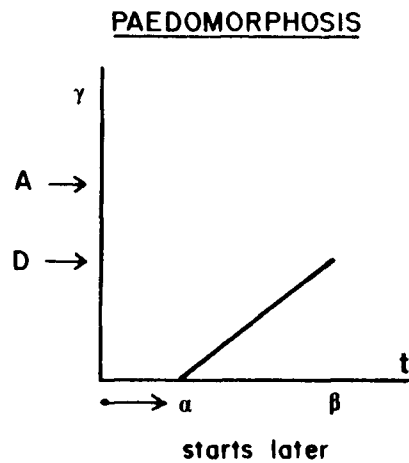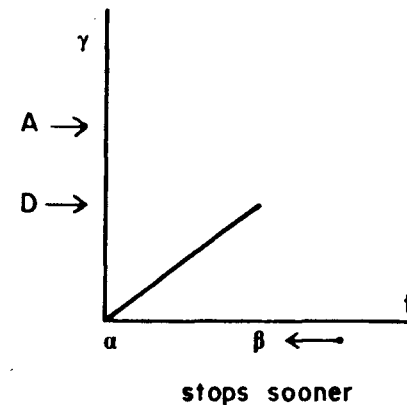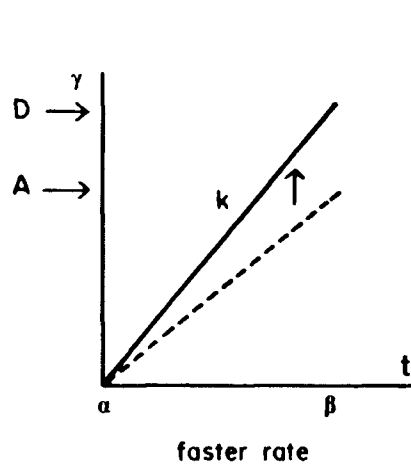
PAEDOMORPHOSIS

NEOTENY — slower rate

POSTDISPLACEMENT — starts later

PROGENESIS — stops sooner

PERAMORPHOSIS

ACCELERATION — faster rate

PREDISPLACEMENT — starts sooner

HYPERMORPHOSIS — stops later

204
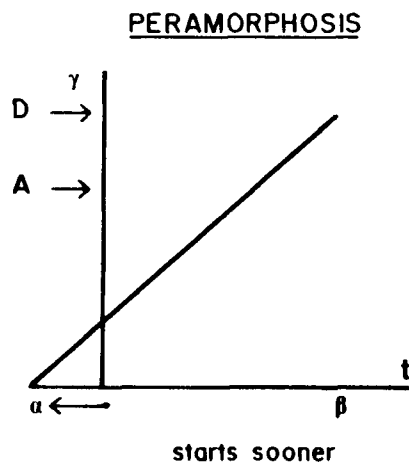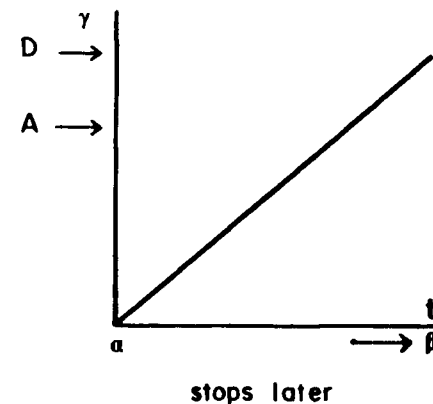
Figure 29 - Using a Cladogram to Distinguish Paedomorphic,
Peramorphic, and Symplesiomorphic Morphologies

The trees show the developmental sequences of the taxa involved.
In all three trees, taxa A and D exhibit terminal state 2, even
though D is more closely related to taxa B and C, which exhibit
terminal state 3. In tree (a), this is due to symplesiomorphy
(retained primitive trait, state 3 is a synapomorphy of B and
C); in tree (b), it is due to paedomorphosis (a less developed
descendent   morphology);   and in tree (c), it is due to
peramorphosis (a more developed descendent morphology, the
terminal state of which (state 2') is similar to the earlier
state 2.

Figure 30 - Some Limits to the Use of a Cladogram to Detect
Heterochrony

An example taken from Fink (1982; Fig. 5).   The taxa in the
cladogram in (a) possess either state 0 or 1 of a character. If
the growth patterns of the taxa are as in (b), it can be
concluded that the common ancestor of B and C evolved state 1
through hypermorphosis (later cessation of development),
subsequent to which C evolved state 0 through neoteny (slower
rate of development). However, if the growth patterns are as in
(c), in which taxon C has evolved state 0 through progenesis
(earlier cessation of development), then it will not be possible
to detect such paedomorphosis.   Instead, it will be wrongly
concluded that state 0 in taxon C is symplesiomorphic (refer to
Figure 28 for the parameters of the growth plots).

29

a

b

c

30

a

b

c
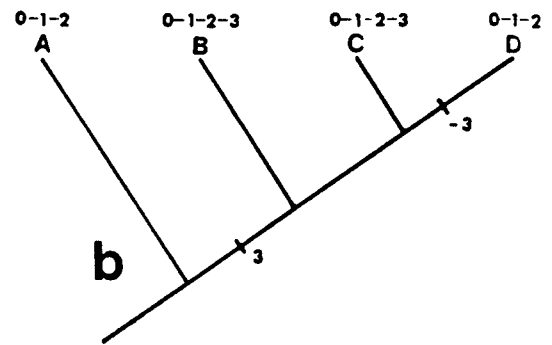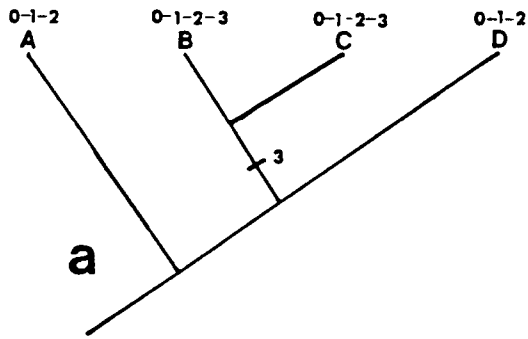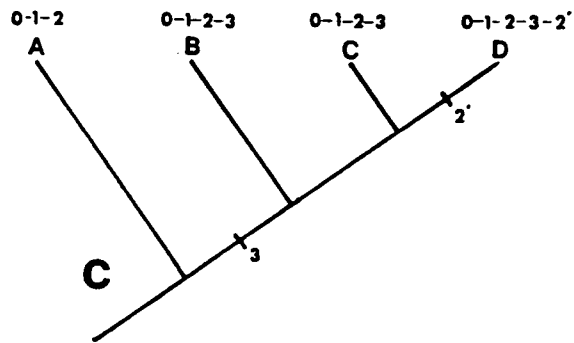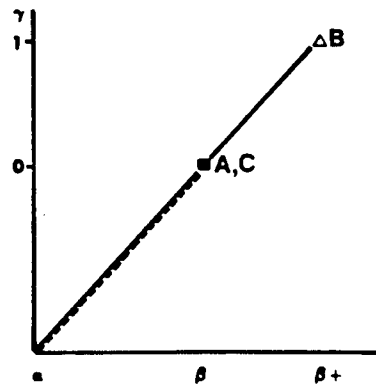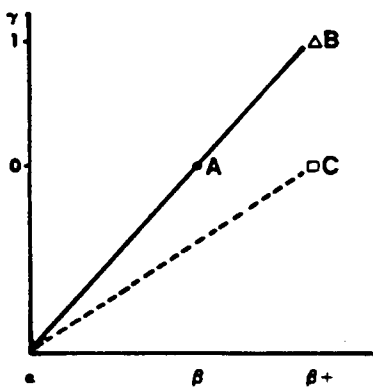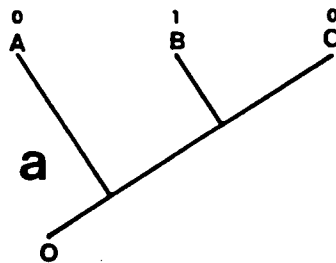
Figure 31 - The Growth of the Hindbody in <u>Haplometrana</u>
<u>intestinalis</u> and <u>Glypthelmins</u> <u>quieta</u>

Values for the mean ratio (with ranges) of hindbody length to
total body length (HBL/TBL)[1] in specimens of the two species as
metacercariae (META) and at 1, 4, 8, and 12 months after
infection (MAI) in <u>Rana</u> <u>pretiosa</u>, for <u>H</u>. <u>intestinalis</u> (in), and
in <u>R</u>. <u>pipiens</u>, for <u>G</u>. <u>quieta</u> (qu). The values for mature adults
of other species of <u>Glypthelmins</u>, of unknown ages, are given at
12+ MAI (see the section, HETEROCHRONIC DEVELOPMENT, for
comments on <u>G</u>. <u>robustus</u>).

_____

[1] The hindbody is the distance from the anterior edge of the
ventral sucker to the posterior end of the body.
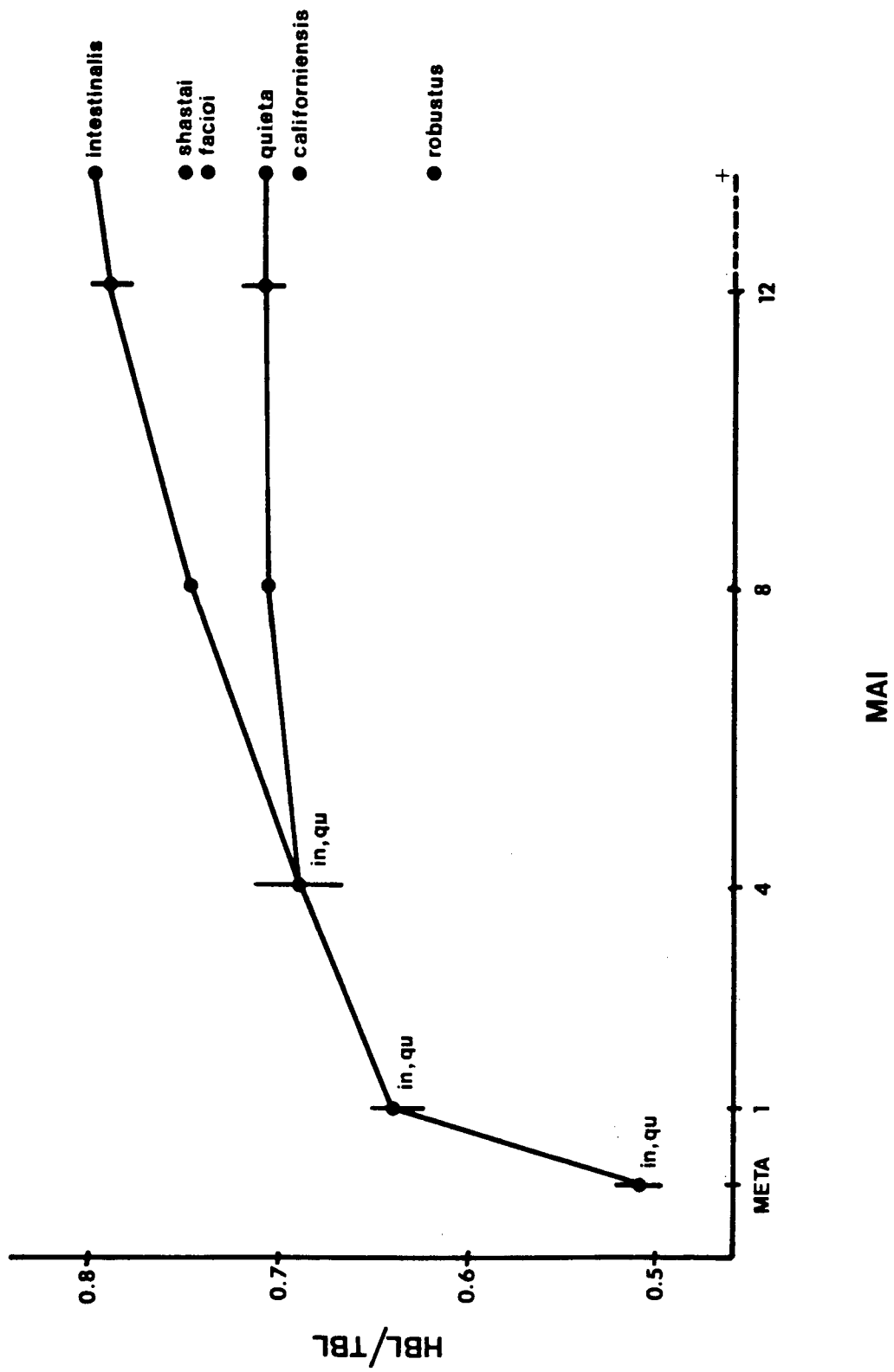
Figure 32 - An Estimation of the Plesiomorphic State of the
Hindbody for Haplometrana intestinalis

The mean values for the ratio of hindbody length to total body
length in the species studied are given across the top of the
figure: hy, G. hyloreus; pe, G. pennsylvaniensis; ro,
G. robustus; sh, G. shastai; in, H. intestinalis; ca,
G. californiensis; qu, G. quieta; fa, G. facioi. These are
mapped onto the cladogram of the species' relationships, from
Figure 20 (see the section, HETEROCHRONIC DEVELOPMENT, for
comments on G. robustus). The plesiomorphic state of the ratio
for H. intestinalis can be estimated by setting the nodal values
on the tree to the median values of the ratios. This yields a
value of 0.75 for the node uniting H. intestinalis and
G. shastai. The value of 0.80 in fully mature specimens of
H. intestinalis is significantly different from this value
(p<0.01).
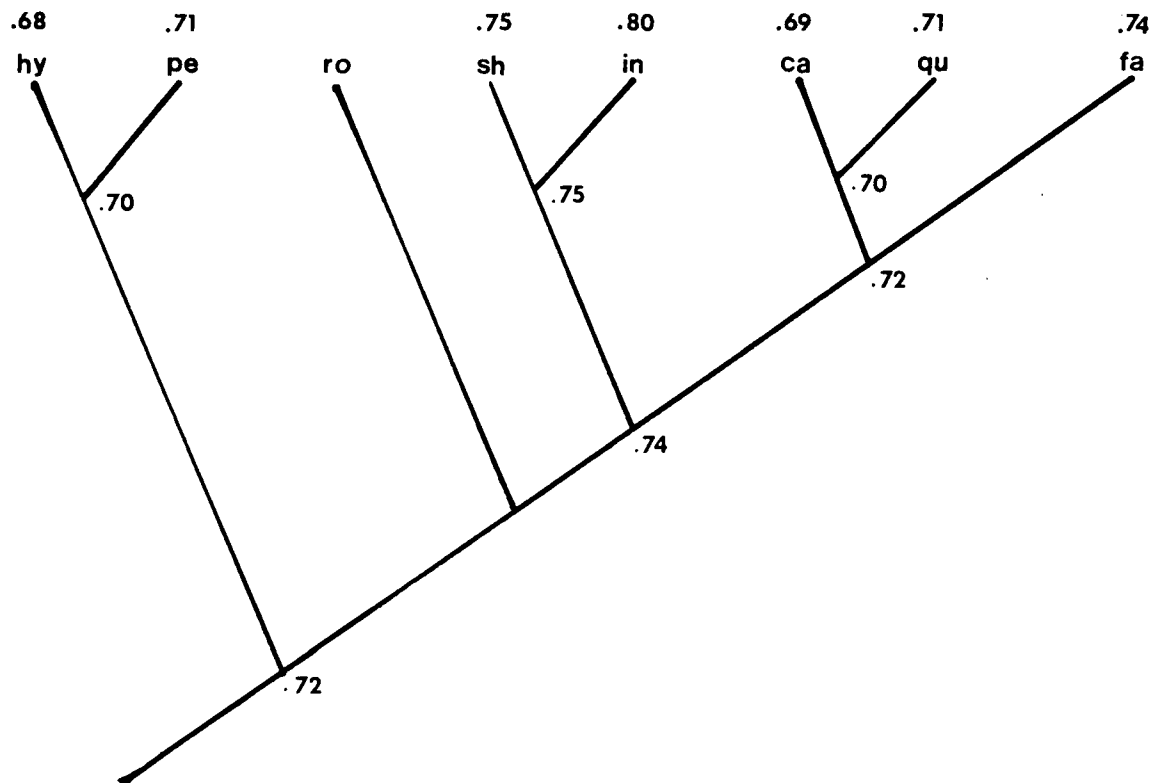
Figure 33 – Inference of Plesiomorphic Hosts for Species of
Glypthelmins and Haplometrana: One

The parasite species indicated are: hy, G. hyloreus; pe,
G. pennsylvaniensis; ro, G. robustus; sh, G. shastai; in,
H. intestinalis; ca, G. californiensis; qu, G. quieta; fa,
G. facioi. The anurans host families indicated are: H, Hylidae;
B, Bufonidae; R, Ranidae. Optimization according to the
criteria of Swofford and Maddison (in review) yields the
indicated nodal values.
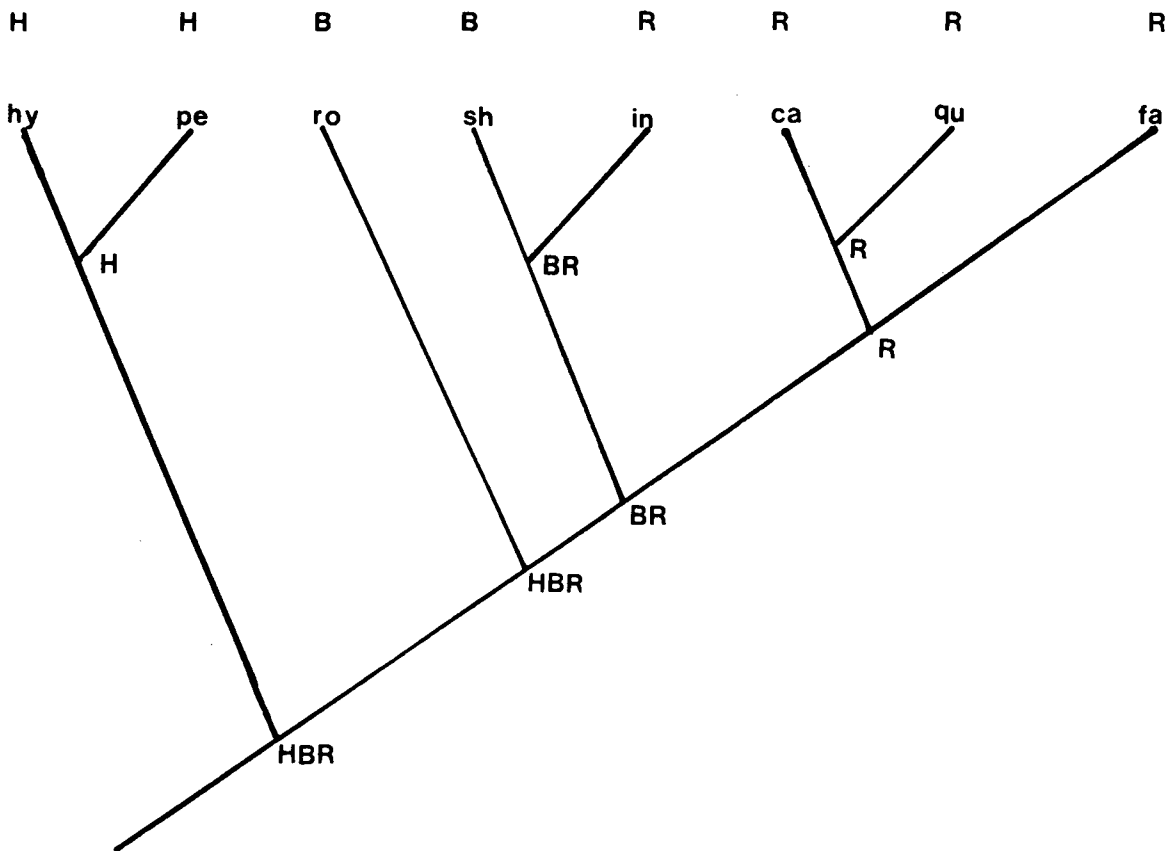
Figure 34 - Experimentally-Produced Heterochronic
Development of the Hindbody in Haplometrana intestinalis

The ratio of hindbody length to total body length (HBL/TBL) is
plotted against time, for specimens of H. intestinalis (in) as
metacercariae (META) and at 1, 4, 8, and 12 months after
infection (MAI). The upper dashed line gives the mean values
for specimens of H. intestinalis developing in either Rana
pretiosa or R. cascadae. The middle solid line gives the values
for specimens of H. intestinalis developing in Bufo boreas. The
lower dashed line, giving the values for G. quieta developing in
R. pipiens, is repeated from Figure 31, as are the values for
mature adults of other species of Glypthelmins (see the section,
HETEROCHRONIC DEVELOPMENT, for comments on G. robustus). The
HBL/TBL value at 12 MAI for specimens of H. intestinalis
developing in B. boreas is (1) significantly different (p<0.01)
from the same measure for specimens developing in R. cascadae
and R. pretiosa, and (2) not significantly different from the
same measure for mature adults, of unknown age, of G. shastai.
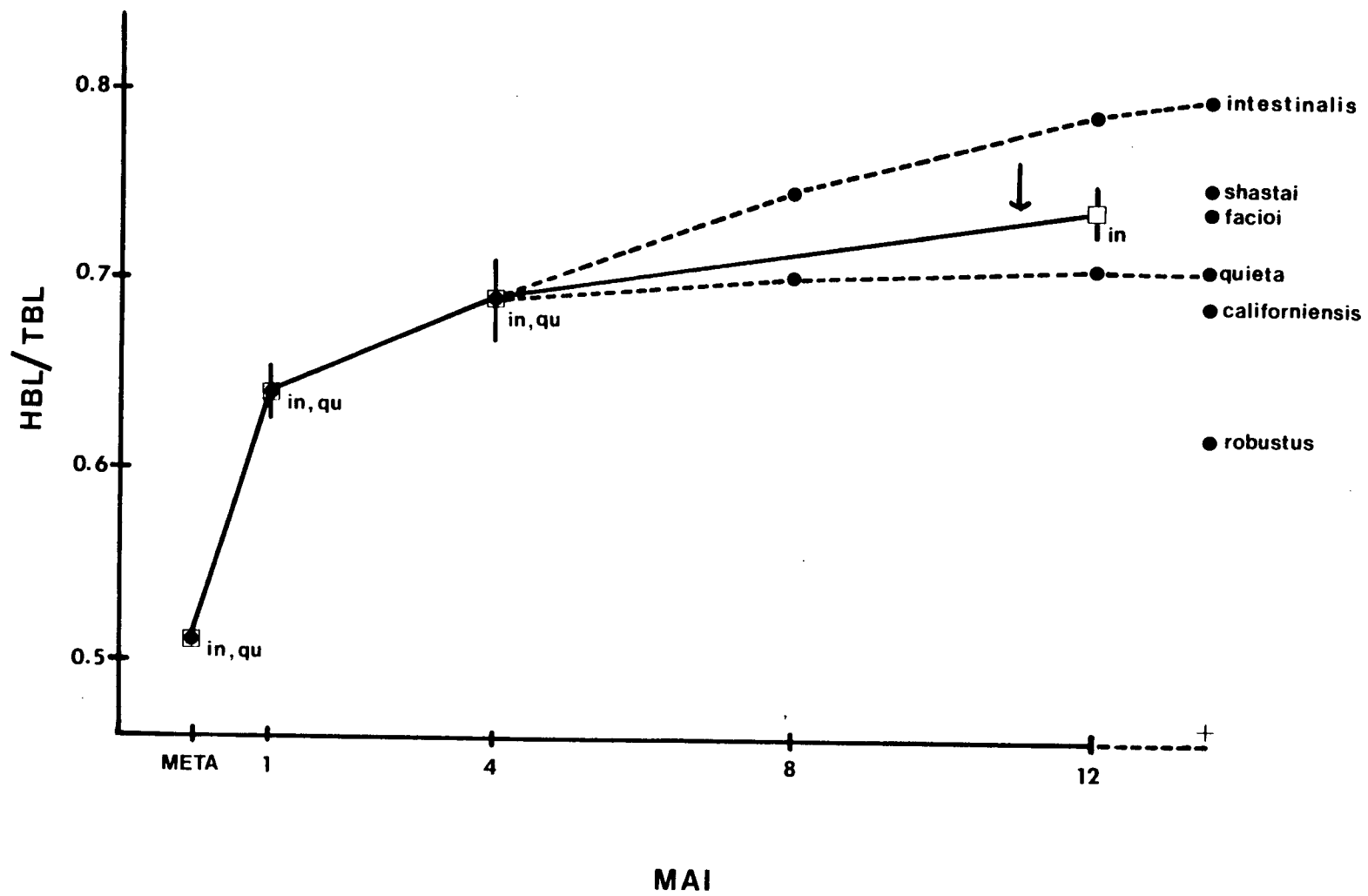
Figure 35 - Altered Growth of the Hindbody in <u>Haplometrana</u>
<u>intestinalis</u>

(a) Ventral view of specimen of <u>H</u>. <u>intestinalis</u> from <u>Rana</u>
<u>pretiosa</u>, 12 months after infection; (b) ventral view of
specimen of <u>H</u>. <u>intestinalis</u> from <u>Bufo</u> <u>boreas</u>, 12 months after
infection; (bars = 1.0 mm)

a b
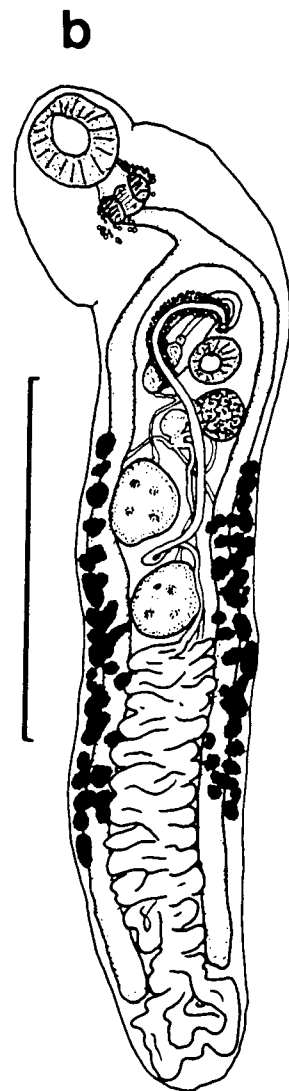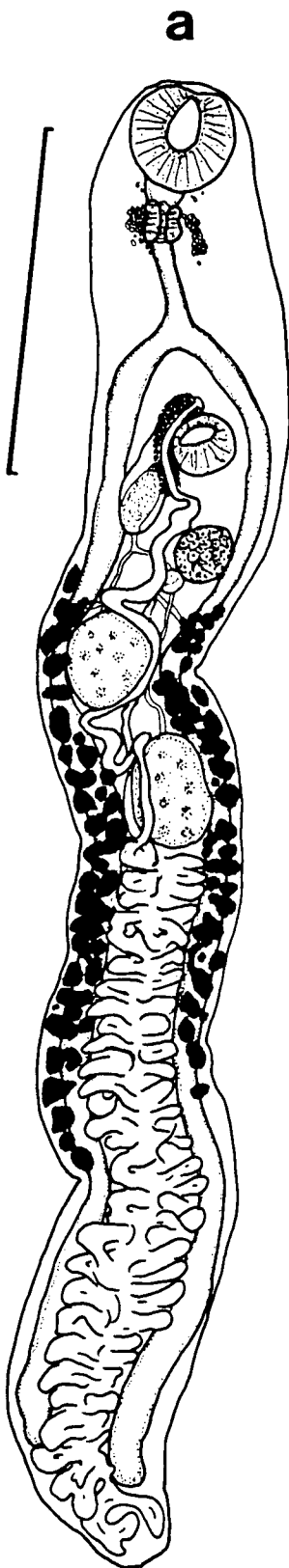
Figure 36 - Distribution in the Americas of the Digenean
Species, G. hyloreus, G. pennsylvaniensis, G. facioi, and
G. robustus

Symbols: dots, G. hyloreus; squares, G. pennsylvaniensis;
triangle, G. facioi; diamond, G. robustus.

1000 km

Figure 37 - Distribution in the Americas of the Digenean Species, _Haplometrana intestinalis_ and _Glypthelmins shastai_, and of the Anuran Species, _Bufo boreas_, _Rana pretiosa_, _R. cascadae_, and _R. muscosa_

Bufo boreas

Rana pretiosa

R. cascadae

R. muscosa

500 km

● H. intestinalis

■ G. shastai
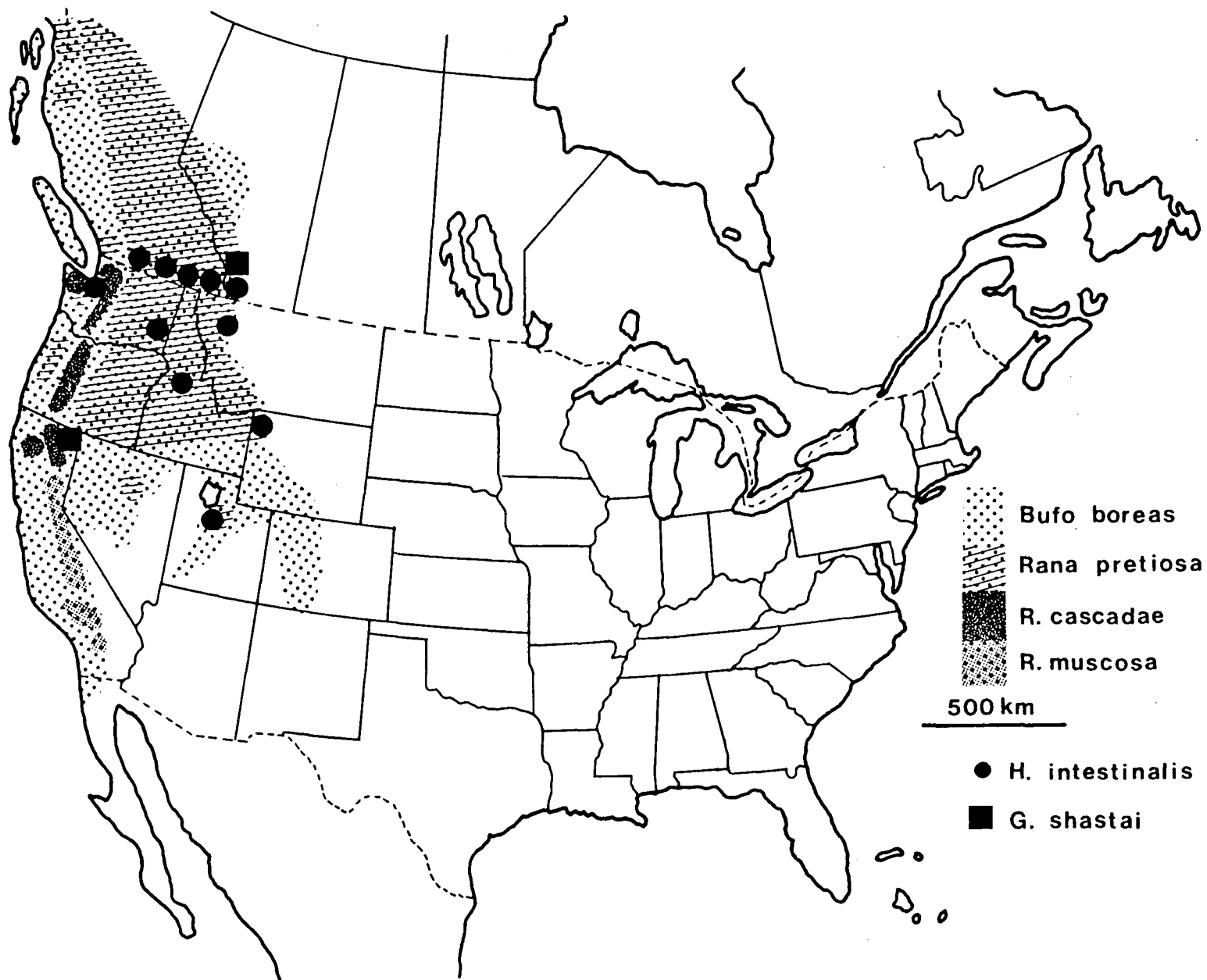
Figure 38 - Distribution in the Americas of the Digenean
Species, <u>Glypthelmins</u> <u>quieta</u> and <u>G</u>. <u>californiensis</u>, and of the
Anuran Species, <u>Rana</u> <u>aurora</u>, <u>R</u>. <u>boylii</u>, and the species of the
<u>R</u>. <u>pipiens</u> Group

See the section, COEVOLUTION ANALYSIS, for comments on the
species composing the <u>R</u>. <u>pipiens</u> group.

Legend:

Rana pipiens GROUP

R. aurora

R. boylii

500 km

● G. quieta
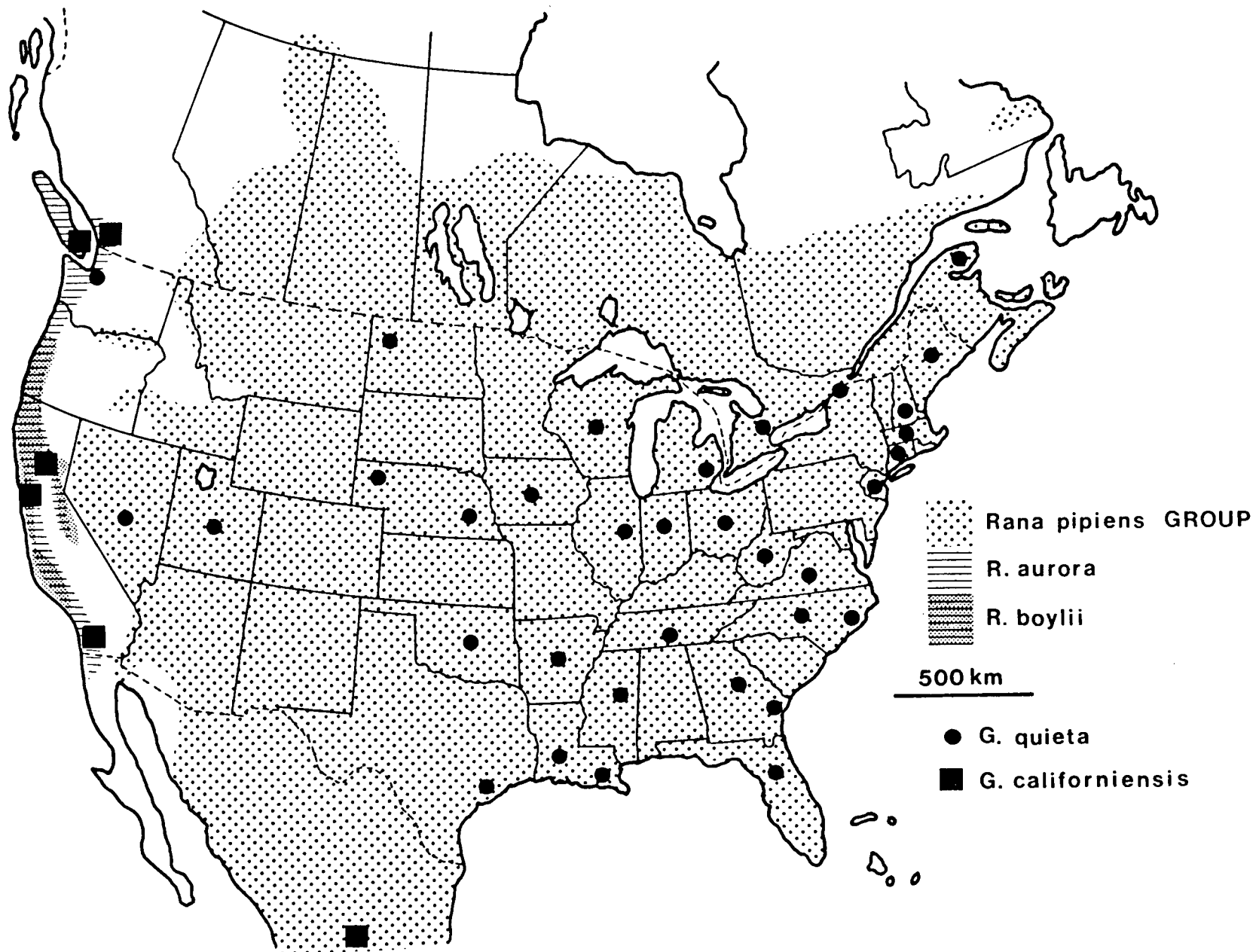
■ G. californiensis

Figure 39 - Inference of Plesiomorphic Hosts for Species of
Glypthelmins and Haplometrana: Two

(a) The species of parasites are: hy, G. hyloreus; pe,
G. pennsylvaniensis; ro, G. robustus; sh, G. shastai; in,
H. intestinalis; ca, G. californiensis; qu, G. quieta; fa,
G. facioi. The host taxa are the anuran families: H, Hylidae;
B, Bufonidae; R, Ranidae. The nodal optimizations are obtained
with the Swofford and Maddison (in review) method. The
bufonid/ranid ambiguity that, in Figure 33, is assigned to the
node uniting G. shastai and H. intestinalis is tentatively
removed here. This is done on the basis of the infection
studies reported in the section, EXPERIMENTALLY-PRODUCED
HETEROCHRONY IN HAPLOMETRANA INTESTINALIS. These suggest that
G. shastai colonized a bufonid, Bufo boreas. Figure (b) gives
the relationships among the host groups that are indicated by
their distribution in tree (a).
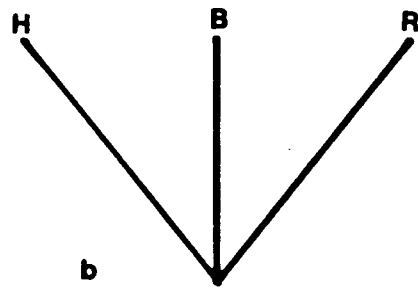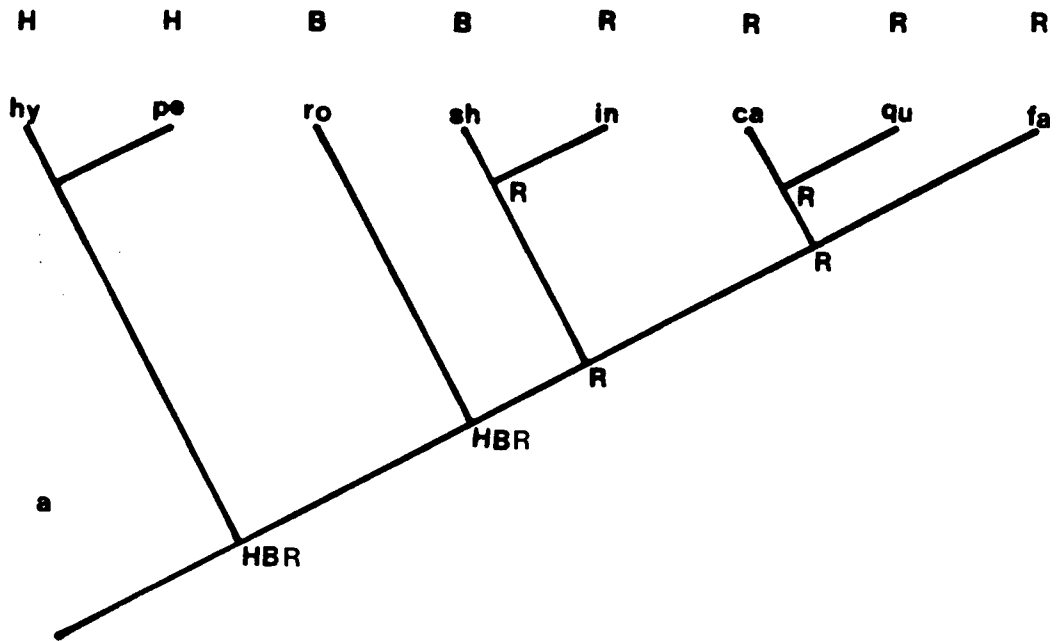
Figure 40 – Coevolutionary Analysis of Species of
Glypthelmins and Rana in North and Central America

The parasite species are: in, H. intestinalis; ca,
G. californiensis; qu, G. quieta; fa, G. facioi. The host
species are: pr, R. pretiosa; au, R. aurora; bo, R. boylii; mo,
R. montezumae; pi, R. pipiens; bl, R. blairi; sp,
R. sphenocephala; pa, R. palustris; pp, R. palmipes. The
notation of "pi" refers to other members of the R. pipiens group
(see the section, COEVOLUTION ANALYSIS). The arrow indicates a
postulated colonization event. The solid lines indicate the
phylogenetic relationships among the parasite species (from Fig.
20). The dashed lines indicate the phylogenetic relationships
among the host species (from Fig. 42a).
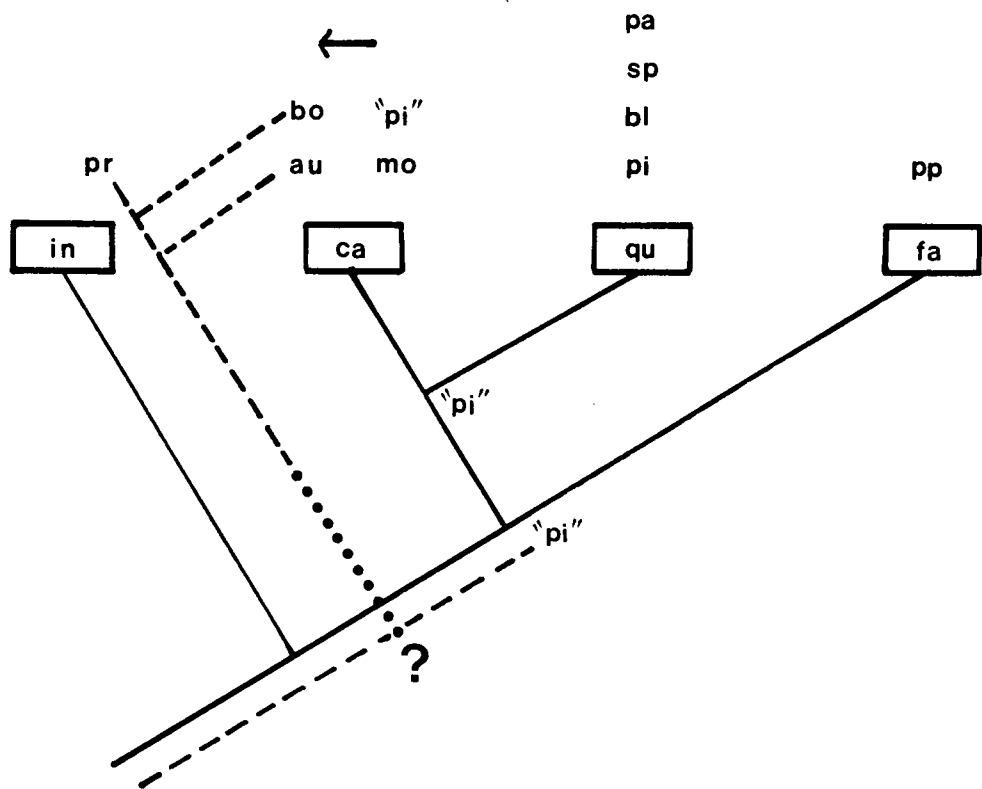
Figure 41 - Summary Cladogram of Higher Level Relationships
among Ranids in the Americas

Compiled from various sources (see the section, COEVOLUTION
ANALYSIS).
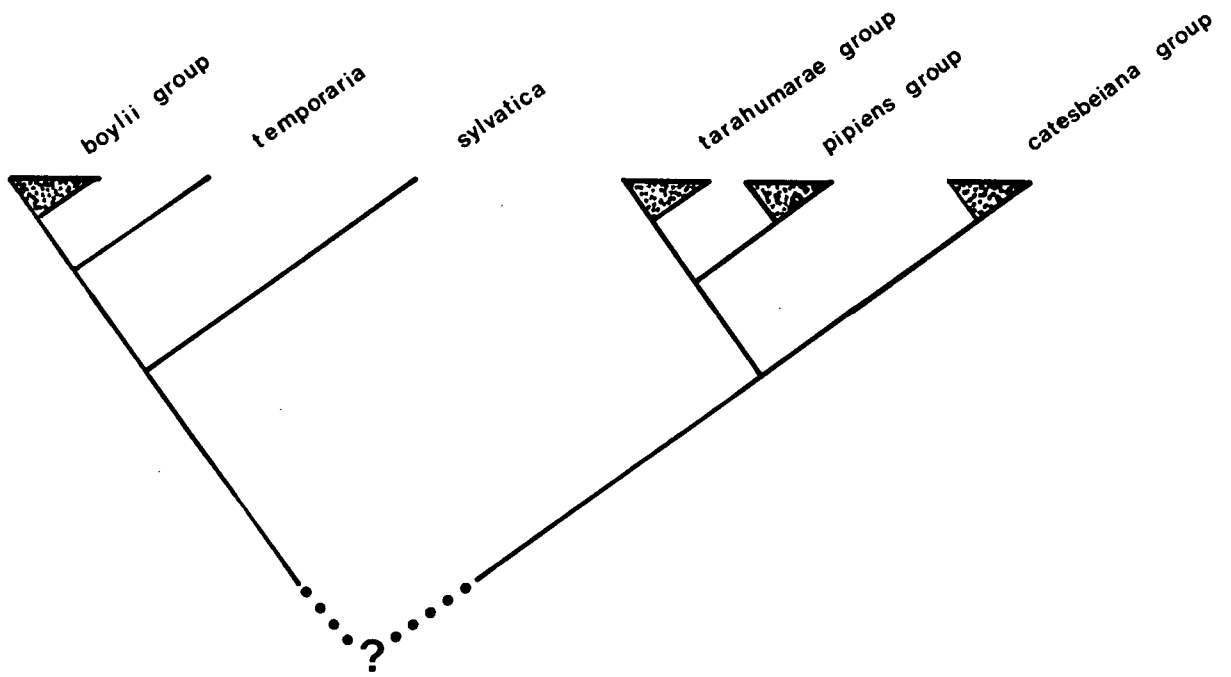
Figure 42 - Phylogenetic Relationships among Ranids in
Western North America

The species are: au, R. aurora; pr, R. pretiosa; ca,
R. cascadae; bo, R. boylii; mu, R. muscosa. Figure (a) gives
the results of a re-analysis of the karyological data of Green
(1986b); Figure (b) gives the results of a re-analysis of the
allozyme data of Green (1986a). The latter is a consensus tree,
the two resolutions of which are shown at bottom left. Re-
analyses used Wagner parsimony and Unordered Analysis of
multistate characters. See Appendix A for the data matrices
from which these trees were constructed.
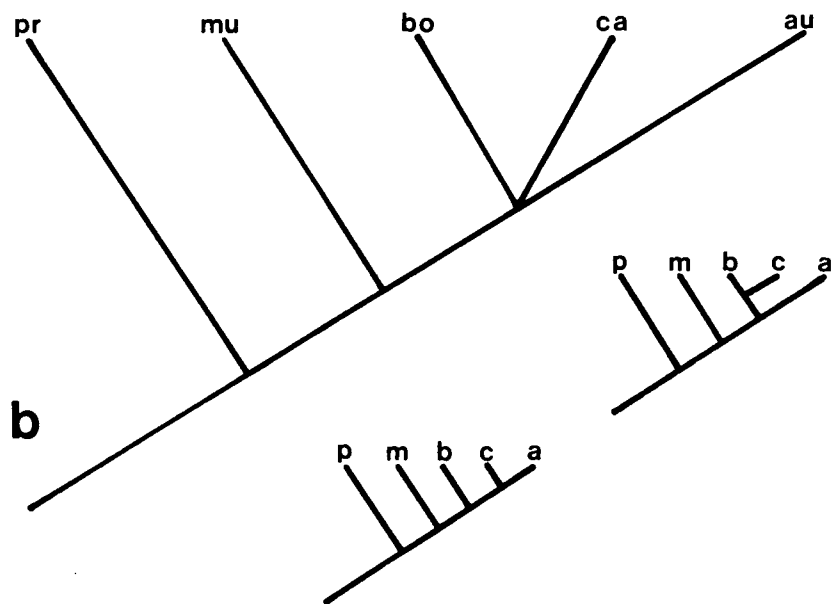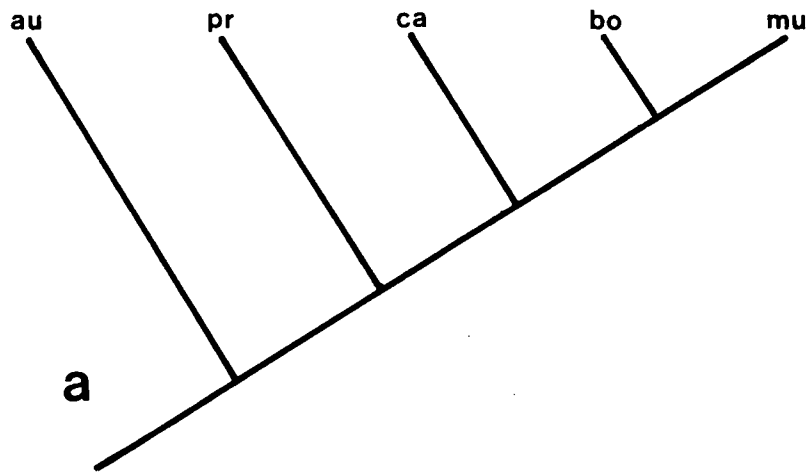
Figure 43 - Postulated Phylogenetic Relationships among
Members of the <u>Rana</u> <u>pipiens</u> Group, from Hillis <u>et al</u>. (1983)

The occurrence of <u>G</u>. <u>facioi</u> (fa), <u>G</u>. <u>californiensis</u> (ca), and
<u>G</u>. <u>quieta</u> (qu) is noted on the ranid cladogram in (a). Figure
(b) shows the relationships among the parasites that are implied
by the relationships among their hosts. This tree is congruent
with the cladogram for these species, in Figure 20.

torreri

Colima

Arcelia

papagayo

berlandieri

Hidalgo

Xochimilco

Chapala

Atenquique Short

magnaocularis

Lowland

sphenocephala [qu]

blair/ [qu]

pipiens [qu]

palustris [qu]

areolata

chiricahuensis

dunni

megapoda

montezumae [ca]

tarahumarae Group [fa]

a

b

fa

ca

qu

qu

qu
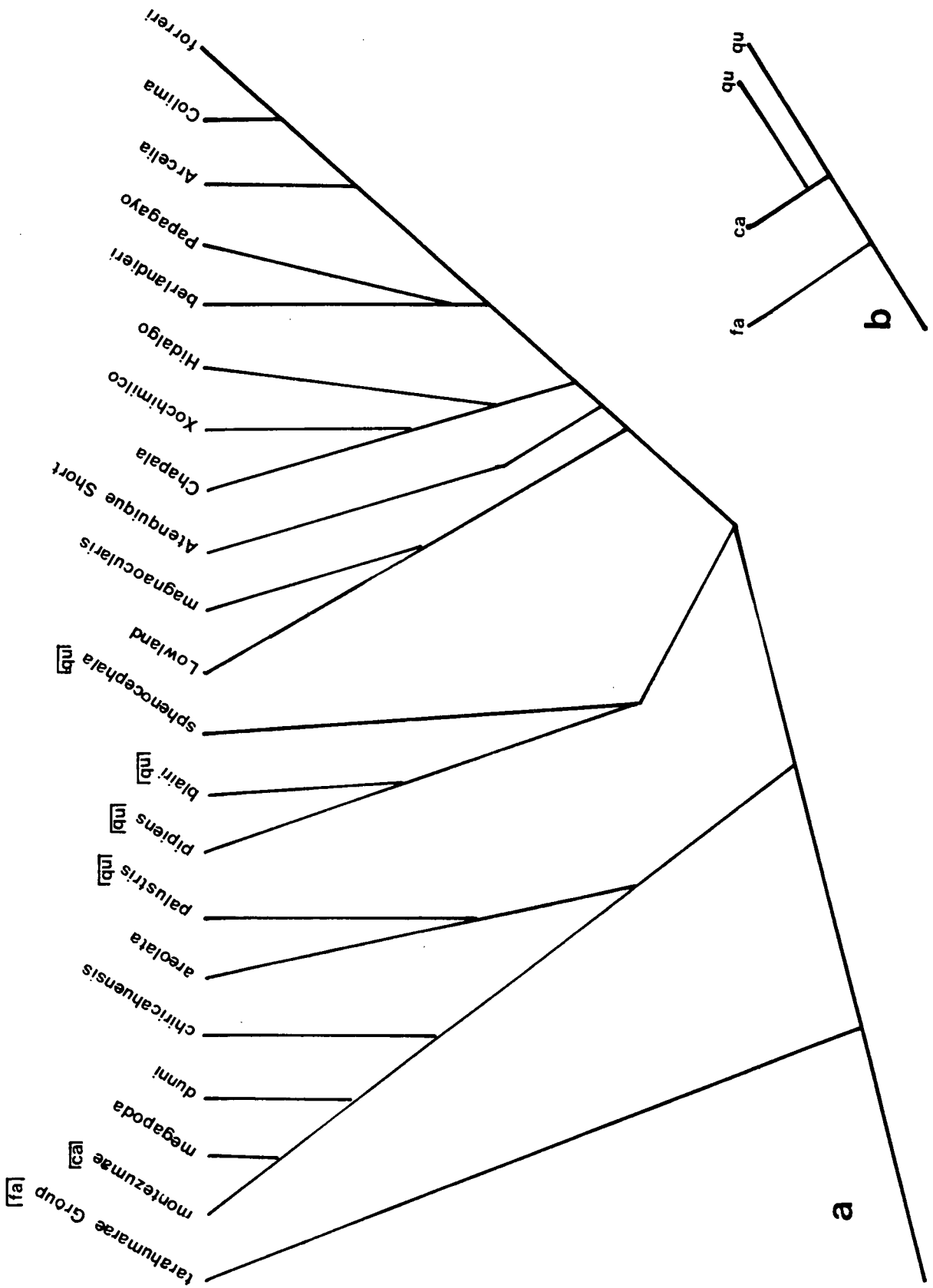
Figure 44 - Biogeographic and Phylogenetic Patterns for
Species of Glypthelmins and Haplometrana in the Americas

The cladogram for the species of Glypthelmins and Haplometrana,
Figure 20, fitted onto a contemporary map of the Americas
according to the species' distribution: hy, G. hyloreus; pe,
G. pennsylvaniensis; ro, G. robustus; sh, G. shastai; in ,
H. intestinalis; ca, G. californiensis; qu, G. quieta; fa,
G. facioi.
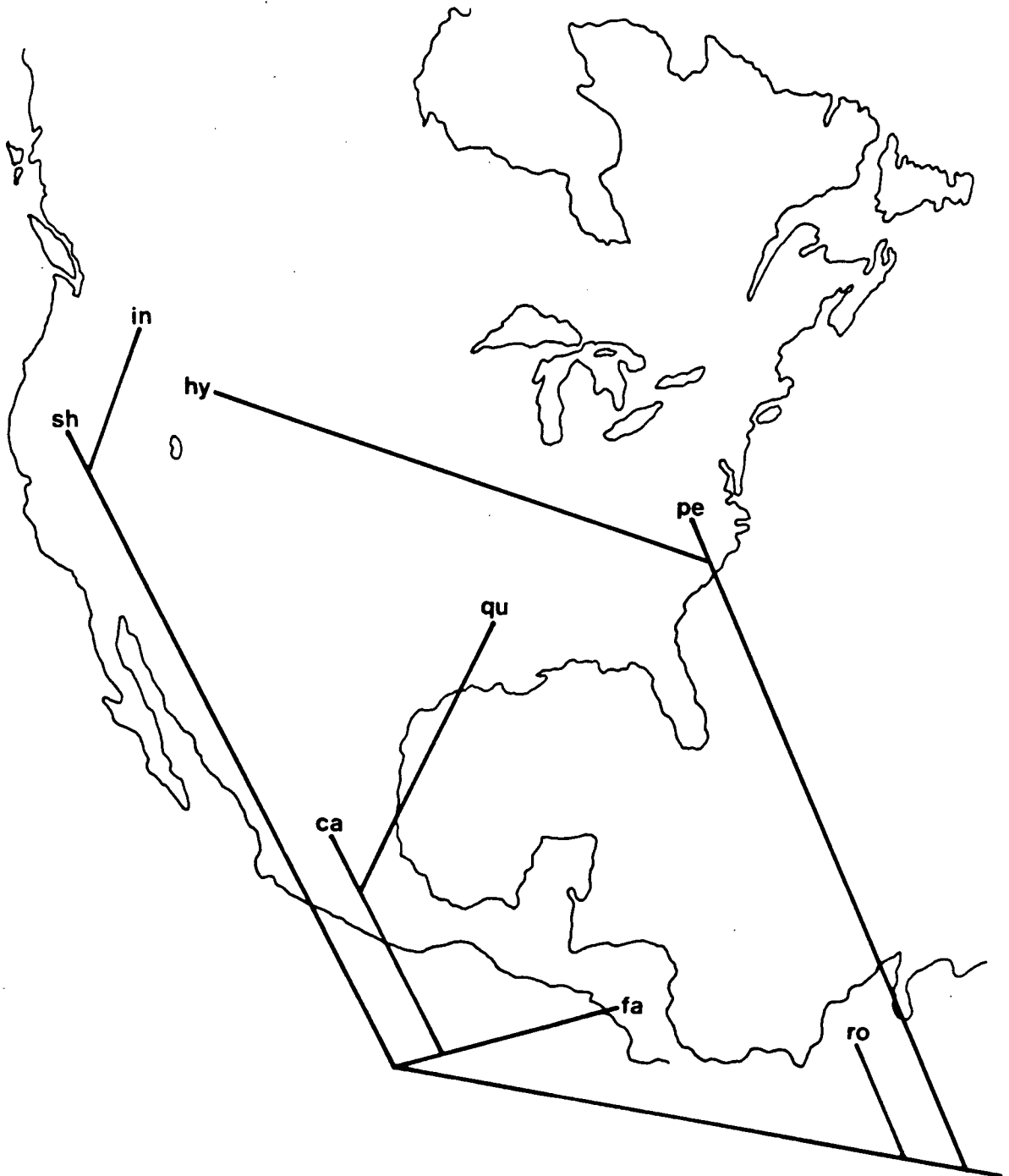
Figure 45 - Distribution of the Hosts of <u>Glypthelmins</u>
<u>californiensis</u>

The parasite species are: ro, <u>G</u>. <u>robustus</u>; sh, <u>G</u>. <u>shastai</u>; in,
<u>H</u>. <u>intestinalis</u>; ca, <u>G</u>. <u>californiensis</u>; qu, <u>G</u>. <u>quieta</u>; gf,
<u>G</u>. <u>facioi</u>. The cladistic relationships are as in Figure 44.

1. Rana aurora
2. R. boylii
3. R. montezumae
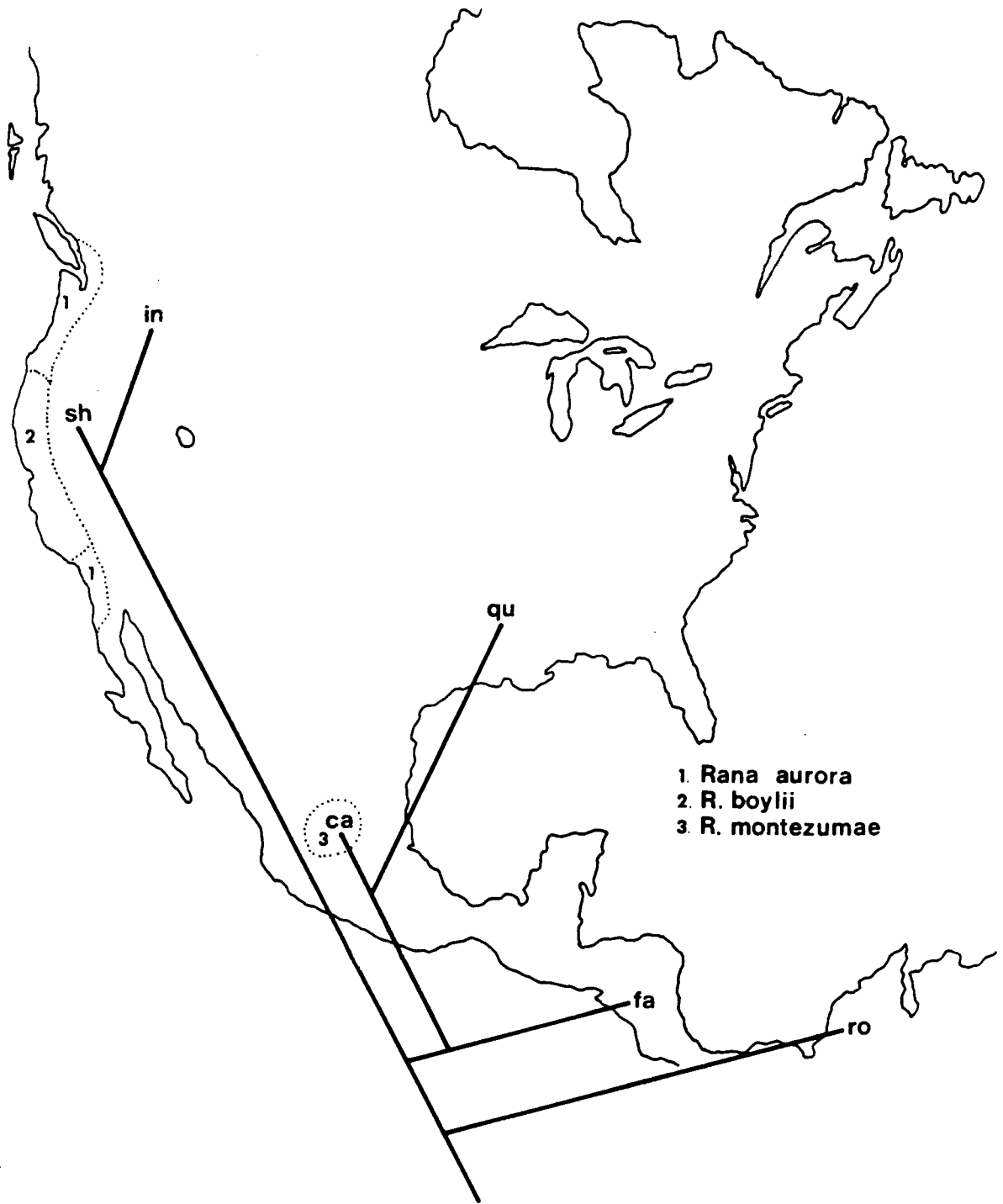
Figure 46 - Morphology of <u>Glypthelmins</u> <u>shastai</u>: One

(a) Ventral view of specimen no. UAPAR 692, Univ. of Alberta;
(b) illustration of the holotype of <u>G</u>. <u>shastai</u>, USNM Helm.
Coll. [1] no. 8925, reproduced from Ingles (1936); (bars = 1.0
mm).

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[1] U.S. National Museum Helminthological Collection, Beltsville,
Maryland.

a

b

cir. sac.
gen. p.
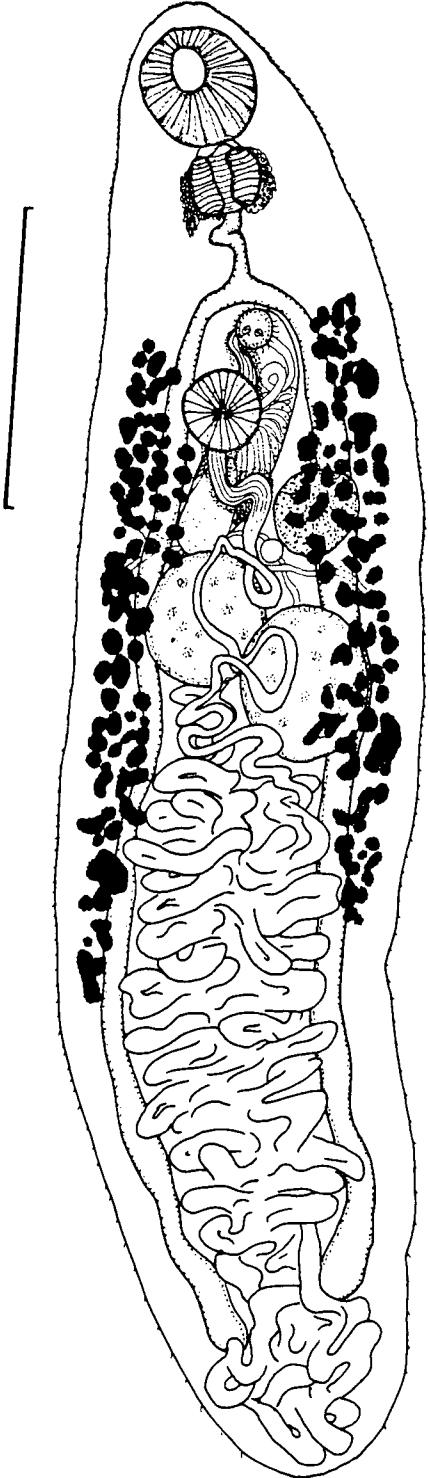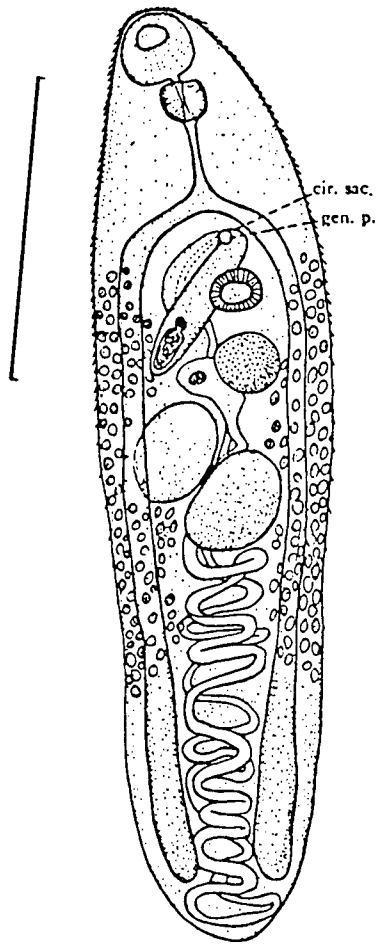
Figure 47 - Morphology of <u>Glypthelmins</u> <u>shastai</u>: Two

Specimen numbers are of those in the collection of the Dept. of Zoology, Univ. of Alberta. (a) Ventral view of the anterior of specimen no. 692, showing the medial glands (med. gl.) and their ducts (bar = 0.5 mm); (b) cirrus sac of specimen no. 715 (pc, prostatic cells; sv, seminal vesicle; ve, vasa efferentia; ga, genital atrium; bar = 0.5 mm); (c) ventral view of ootype region of specimen no. 7660 (MG, Mehlis' gland; LC, Laurer's canal; SR, seminal receptacle; O, ovary; VD, vitelline duct; bar = 0.2 mm); (d) ventral view of cirrus sac of specimen no. 715, showing metraterm (me) and associated gland cells (gc), uterus (ut), and ventral sucker (vs) (bar = 0.5 mm); (e) ventral view of specimen no. 690 (immature), showing I-shaped excretory vesicle (bar = 0.5 mm).
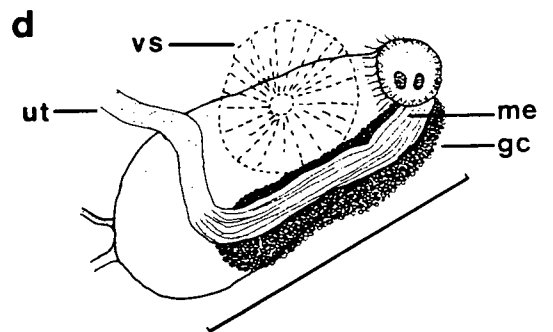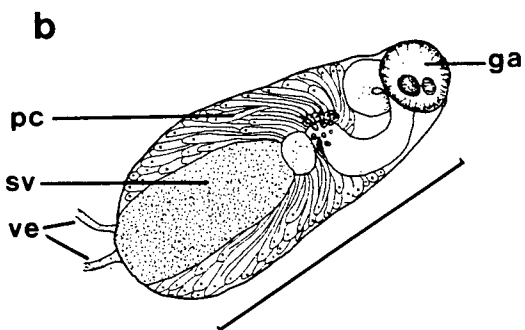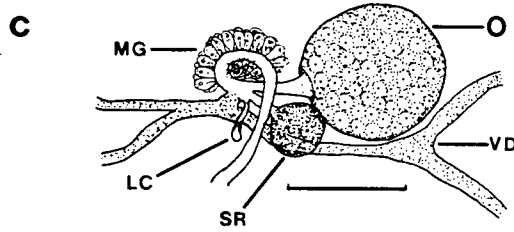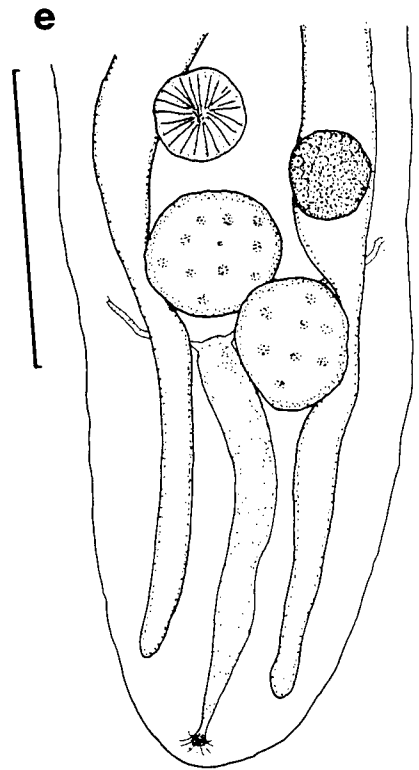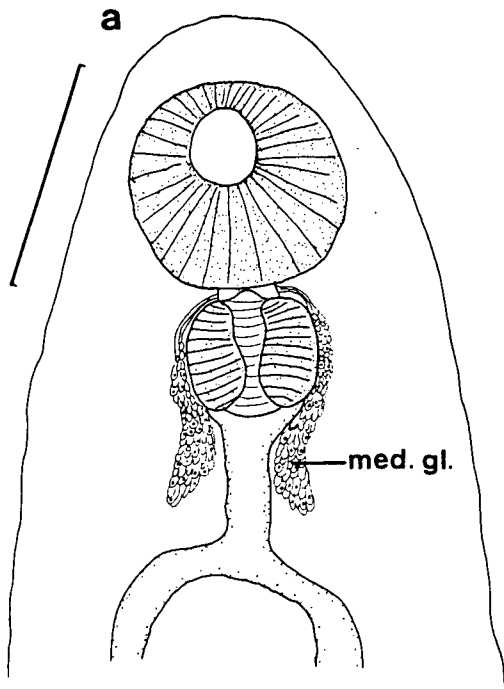
Figure 48 - Morphology of the Larval Stages of <u>Glypthelmins</u>
<u>californiensis</u>

(a) Ventral view of a cercaria, with the excretory system
illustrated on one side only (bar = 0.1 mm); (b) stylet (bar =
20 µm); (c) miracidium (bar = 20 µm); (d) ventral view of a
cercaria, showing the bipartite excretory vesicle (EV) and the
developing testes (T), ovary (O), and cirrus sac (dorsal to the
ventral sucker) (bar = 0.1 mm); (e) daughter sporocyst,
·containing cercariae, from a moribund snail (bar = 0.2 mm).
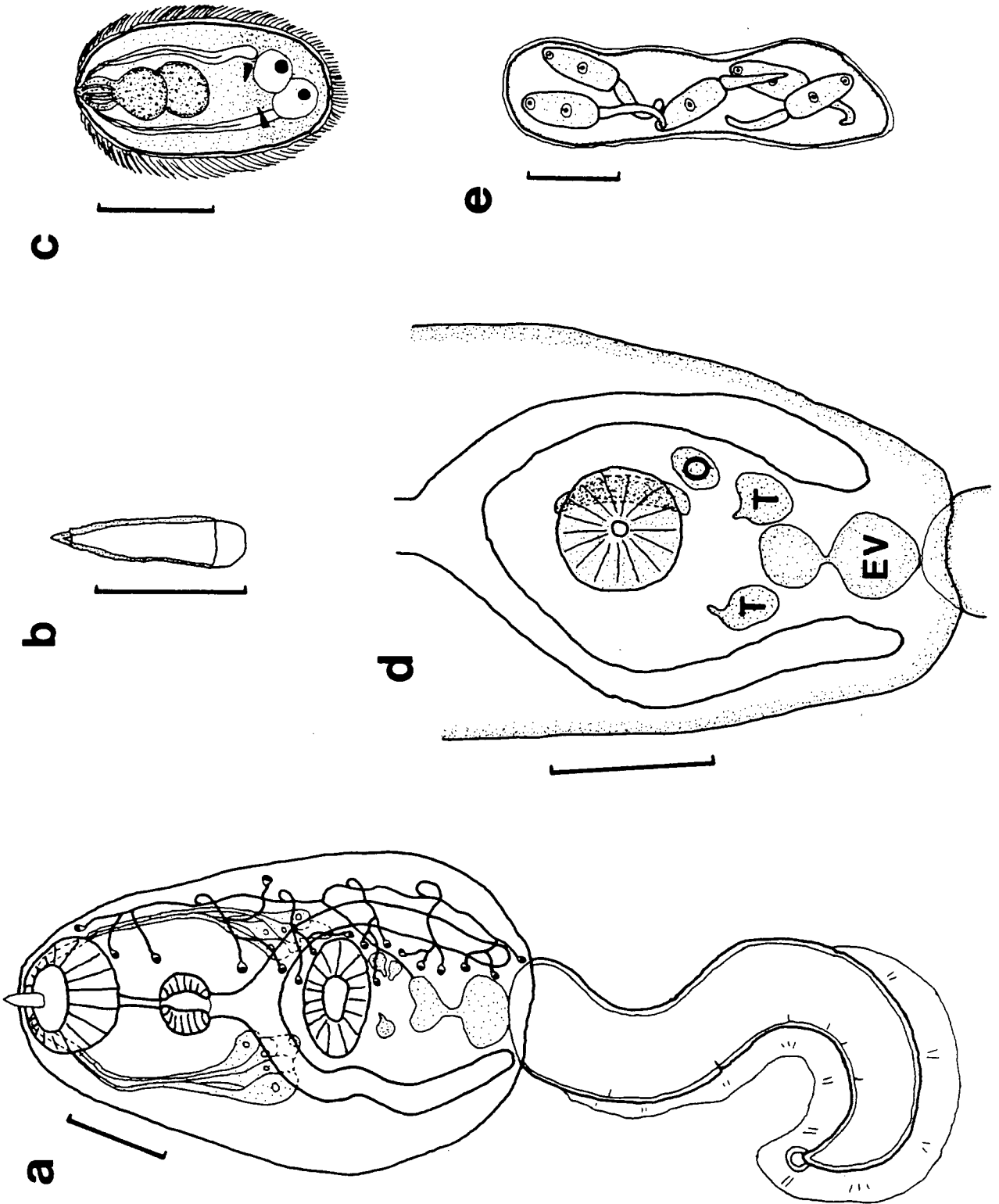
Figure 49 - Young Adult of <u>Glypthelmins</u> <u>californiensis</u>

A ventral view of a specimen of <u>G. californiensis</u> recovered from an adult of <u>Rana</u> <u>aurora</u> 20 days after exposing the frog to cercariae; (bar = 0.2 mm).
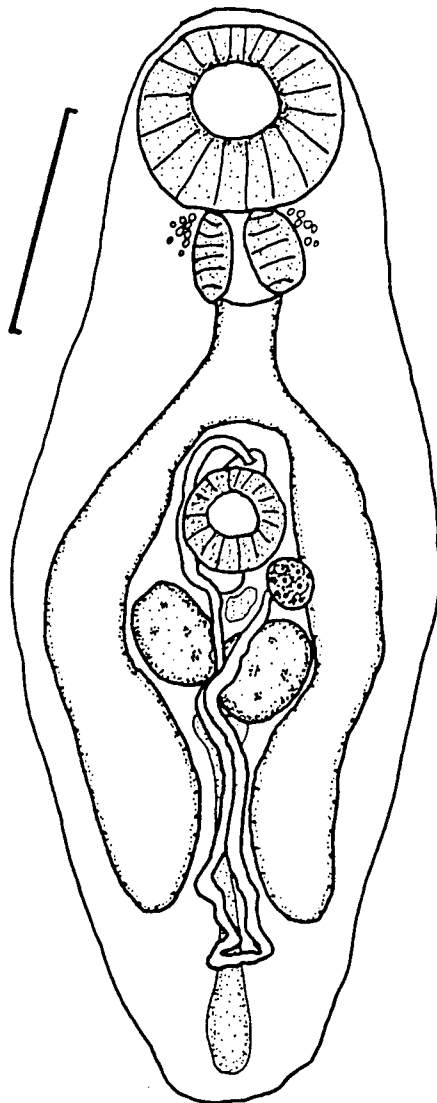
Figure 50 - Multistate Transformation Series

Series (a) and (b) are complex, (c) is linear, and (d) is basally bifurcating.

a)
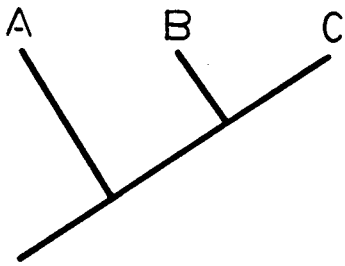
b)
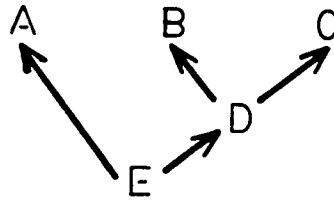
c)
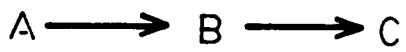
d)
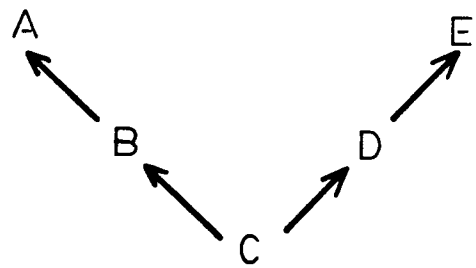
Figure 51 - Demonstration Tree for Multistate Coding
Methods

A contrived example; trees (a) and (b) are equivalent.

a)
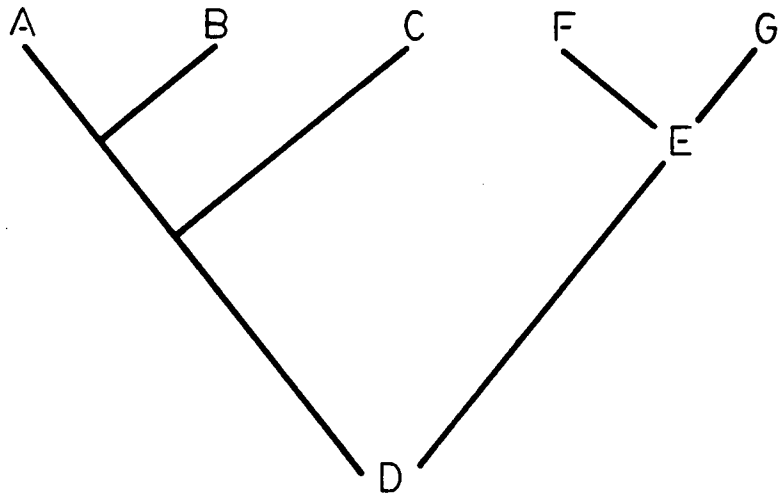
b)
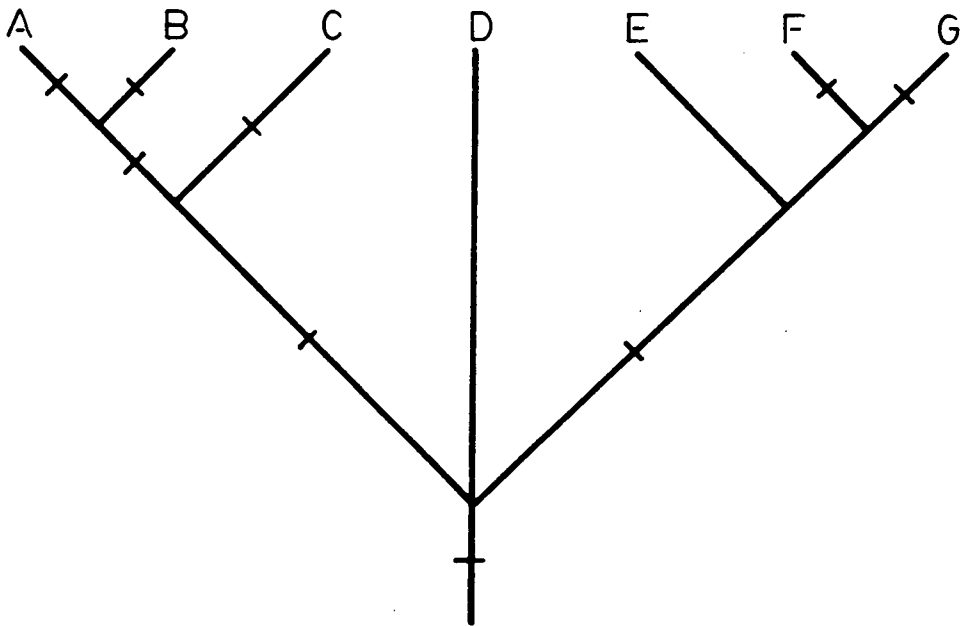
Figure 52 - Additive Binary Coding

(a) The initial tree; (b) the ABC matrix; (c) the reconstructed
polytomy; (d) the reconstructed tree, with all nine new binary
characters ; (e) the reconstructed tree, without the two
hypothetical characters, i and ii.

a)

b)
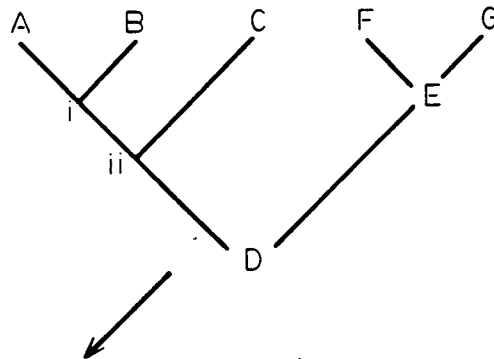
	1	2	3	4	5	6	7	8	9
	A	B	C	D	E	F	G	i	ii
A	1	0	0	1	0	0	0	1	1
B	0	1	0	1	0	0	0	1	1
C	0	0	1	1	0	0	0	0	1
D	0	0	0	1	0	0	0	0	0
E	0	0	0	1	1	0	0	0	0
F	0	0	0	1	1	1	0	0	0
G	0	0	0	1	1	0	1	0	0
i	0	0	0	1	0	0	0	1	1
ii	0	0	0	1	0	0	0	0	1

c)
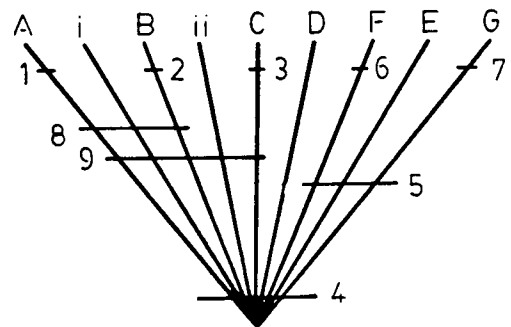
d)
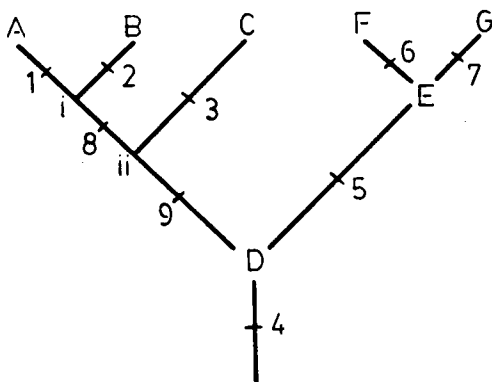
e)
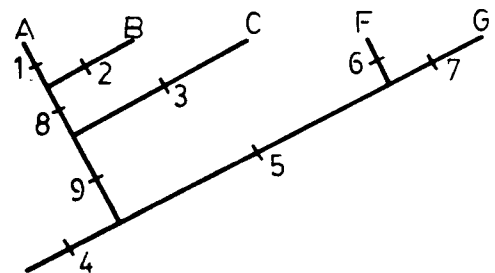
Figure 53 - Redundant Linear Coding

(a) The initial tree; (b) the RLC matrix; (c) the reconstructed polytomy; (d) the reconstructed tree.

a)
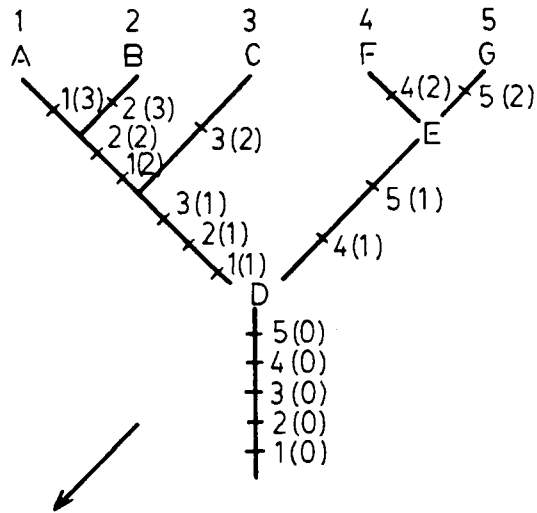
b)

	1	2	3	4	5
A	3	2	1	0	0
B	2	3	1	0	0
C	1	1	2	0	0
D	0	0	0	0	0
E	0	0	0	1	1
F	0	0	0	2	1
G	0	0	0	1	2

c)
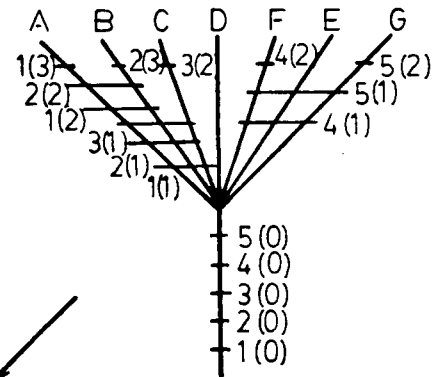
d)
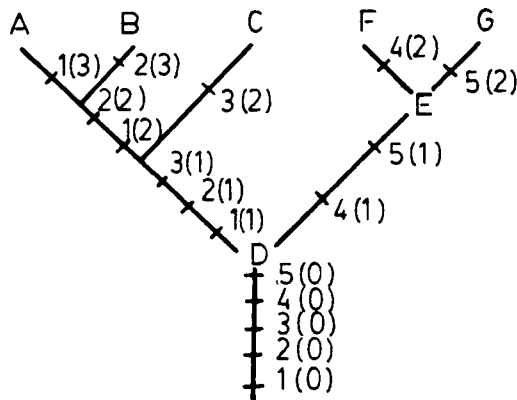
Figure 54 - Nonredundant Linear Coding

(a) The initial tree; (b) the NLC matrix; (c) the reconstructed polytomy; (d) the reconstructed tree; (e) an alternative coding for the left side of the tree.

a)

1   2   3   4   5
A   B   C   F   G
  1(3) 2(1)   4(2)
  1(2) 3(1)   5(1)
                  E
  1(1)   4(1)
        D
        5(0)
        4(0)
        3(0)
        2(0)
        1(0)
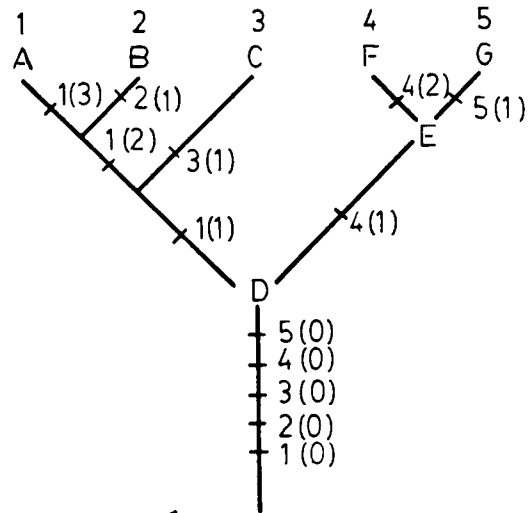
b)

	1	2	3	4	5
A	3	0	0	0	0
B	2	1	0	0	0
C	1	0	1	0	0
D	0	0	0	0	0
E	0	0	0	1	0
F	0	0	0	2	0
G	0	0	0	1	1

c)

A   B   C   D   F   E   G
1(3)   2(1) 3(1)   4(2)   5(1)
1(2)
1(1)   4(1)
        5(0)
        4(0)
        3(0)
        2(0)
        1(0)

d)

A   B   C   F   G
  1(3) 2(1)   4(2)
  1(2) 3(1)   5(1)
                    E
  1(1)   4(1)
        D
        5(0)
        4(0)
        3(0)
        2(0)
        1(0)

e)

A   B   C
3(1)   2(2)
              1(2)
  2(1)
              1(1)
        D
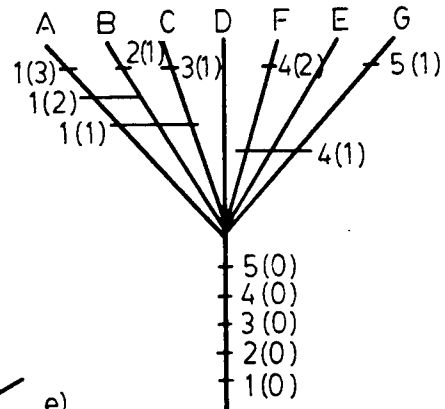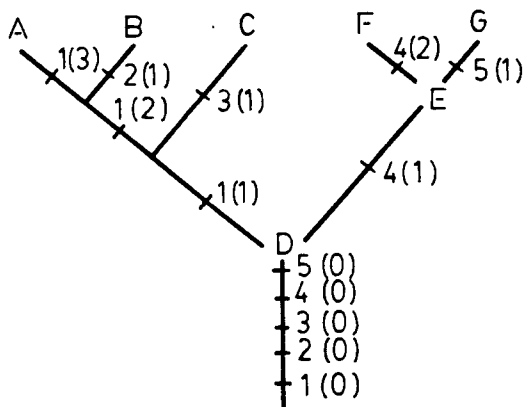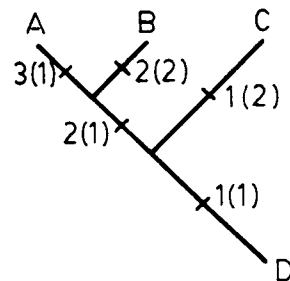
Figure 55 - Coding a Basally-Bifurcating Multistate Series

(a) The initial series; (b) coding with the ABC method; (c) coding with the RLC and NLC methods; (d) "internally rooting" a single linear code.

a)

A   E
B   D
C
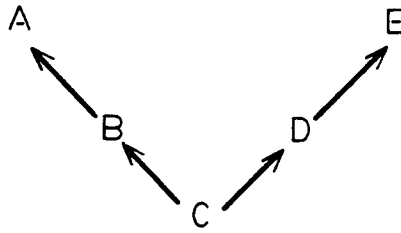
b)

	1	2	3	4	5
A	1	1	1	0	0
B	0	1	1	0	0
C	0	0	1	0	0
D	0	0	1	1	0
E	0	0	1	1	1

ABC →

A 1
E 5
B 2
D 4
C
3
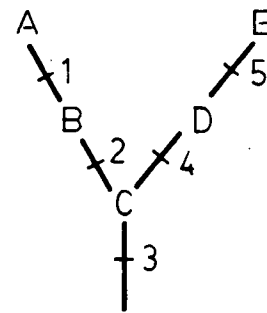
c)

	1	2
A	2	0
B	1	0
C	0	0
D	0	1
E	0	2

RLC
─────→
NLC

A 1(2)
E 2(2)
B
D
1(1) C 2(1)
2(0)
1(0)
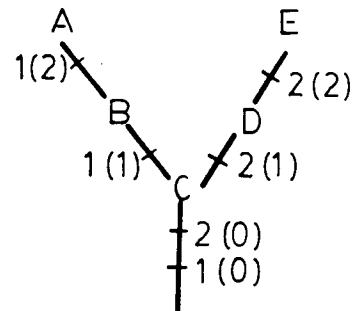
d)

*

	1
A	0
B	1
C	2
D	3
E	4

internal
rooting →

A 1(0)
E 1(4)
B
D
1(1) C 1(3)
1(2)
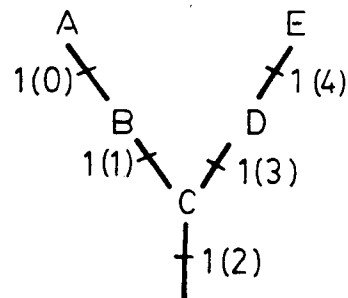
Figure 56 - Incorporating a Multistate Tree into a
Character Matrix

(a) The cladogram, supported by seven binary characters, with
the distribution of a four-state multistate character (i-iv);
(b) the character matrix for the cladogram; (c) the multistate
tree, coded with the NLC method; (d) the NLC matrix, from which
the appropriate horizontal codes are put into the character
matrix in (b); (e) the cladogram constructed from all nine
characters.

a)
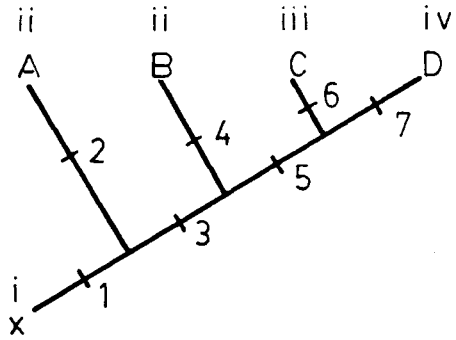
b)

	1	2	3	4	5	6	7	8	9	
x	0	0	0	0	0	0	0	0	0	(i)
A	1	1	0	0	0	0	0	1	0	(ii)
B	1	0	1	1	0	0	0	1	0	(ii)
C	1	0	1	0	1	1	0	2	0	(iii)
D	1	0	1	0	1	0	1	1	1	(iv)

c)
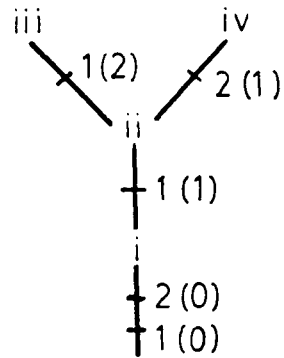
d)

	1	2
i	0	0
ii	1	0
iii	2	0
iv	1	1

e)
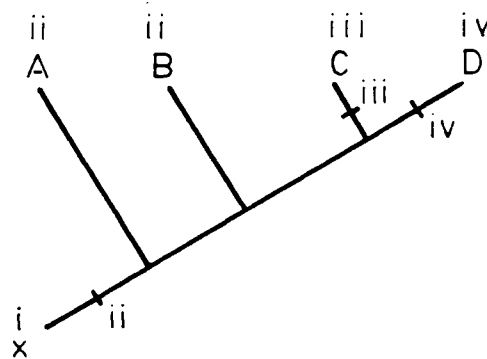
Figure 57 - Using Parasite Data to Infer Host Relationships

Figures (a) to (d) show the method of clustering hosts according
to shared parasite taxa, while (e) to (i) show the method of
using a parasite cladogram as a multistate character of the
hosts. (a) A presence/absence matrix for parasites (upper case
letters) in hosts (roman numerals); (b) the resulting host
cladogram; (c) another presence/absence matrix; (d) the
resulting host cladogram; (e) a cladogram for the parasite taxa
in (c), coded with the NLC method; (f) the NLC matrix for the
parasite cladogram; (g) the host matrix constructed by taking
the appropriate horizontal code from the NLC matrix in (f)
according to the information on parasite presence given in (c);
(h) the host cladogram constructed from the host matrix in (g);
(i) the parasite relationships implied by the host cladogram.
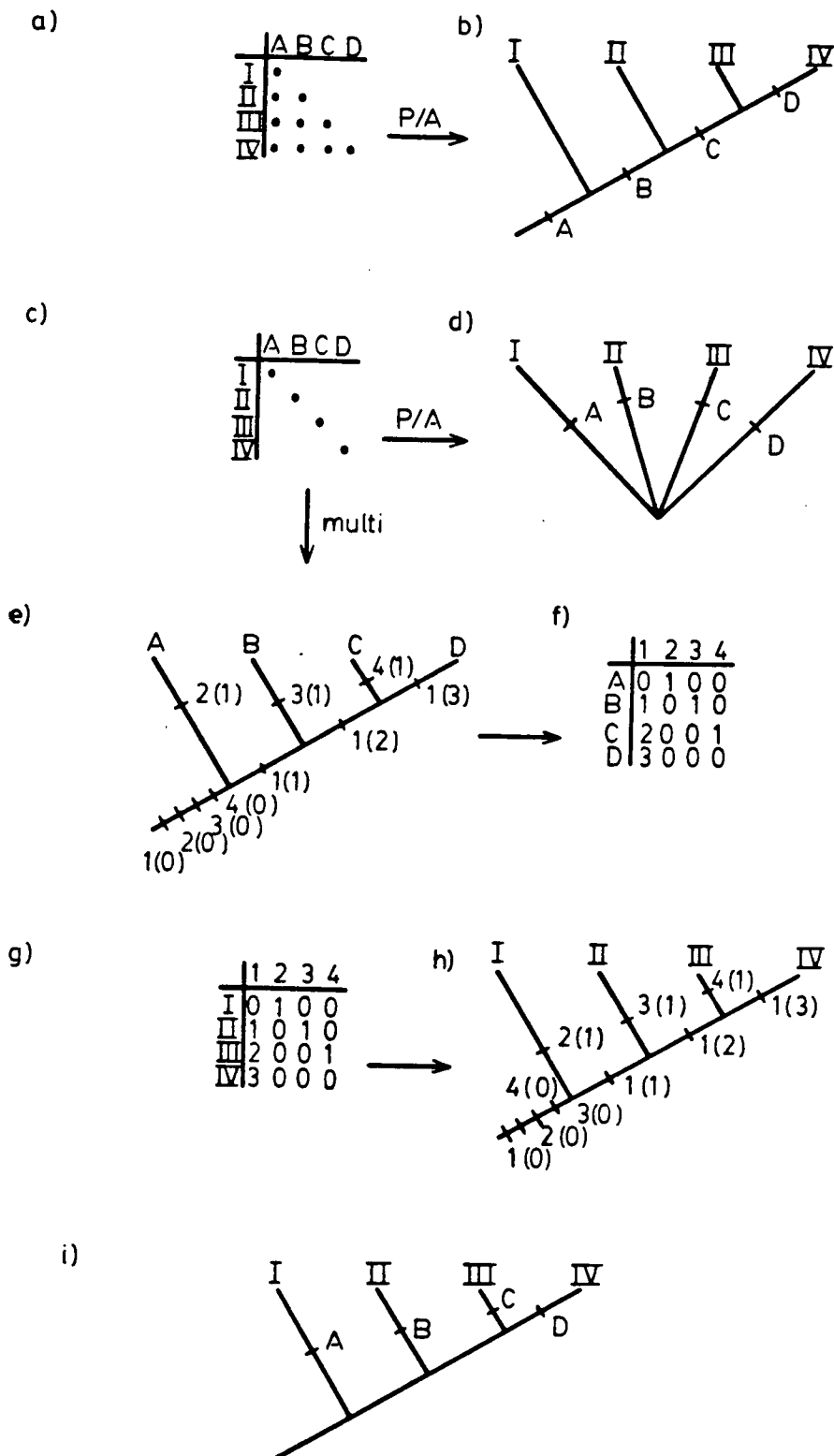
Figure 58 - Using Inclusive ORing to Deal with the
Occurrence of more than One Parasite Taxon in a Host Group

(a) The cladogram for parasite taxa A - D, showing their
occurrence in hosts I - IV; (b) the ABC matrix for the parasite
cladogram; (c) the expanded host matrix, constructed from the
ABC matrix - host IV receives the horizontal codes of the two
parasite taxa, C and D; (d) the compressed host matrix, produced
by the inclusive ORing of the two rows for host IV; (e) the host
cladogram constructed from the matrix in (d); (f) the parasite
relationships implied by the host cladogram - these are
congruent with those in the initial parasite cladogram in (a).

a)

I
A

II
B

IV
III
C

IV
D

E

F

G

$\xrightarrow{ABC}$
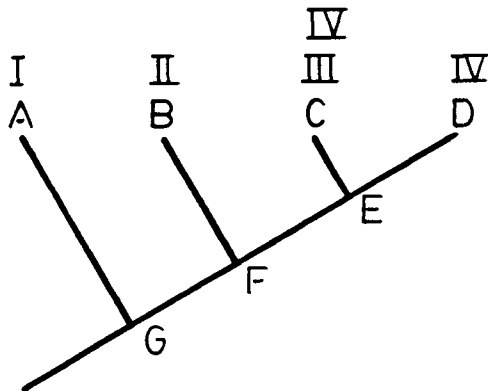
b)

	1	2	3	4	5	6	7
	A	B	C	D	E	F	G
A	1	0	0	0	0	0	1
B	0	1	0	0	0	1	1
C	0	0	1	0	1	1	1
D	0	0	0	1	1	1	1
E	0	0	0	0	1	1	1
F	0	0	0	0	0	1	1
G	0	0	0	0	0	0	1

c)

	1	2	3	4	5	6	7	
I	1	0	0	0	0	0	1	
II	0	1	0	0	0	1	1	
III	0	0	1	0	1	1	1	
IV	0	0	1	0	1	1	1	(C)
IV	0	0	0	1	1	1	1	(D)

$\xrightarrow{OR\ ing}$

d)

	1	2	3	4	5	6	7
I	1	0	0	0	0	0	1
II	0	1	0	0	0	1	1
III	0	0	1	0	1	1	1
IV	0	0	1	1	1	1	1

e)

I

II

III

IV

1

2

4

5

3

6

7

f)

I

II

III

IV

A

B

D

C

E

F

G
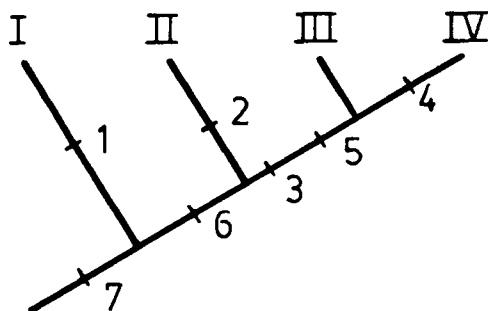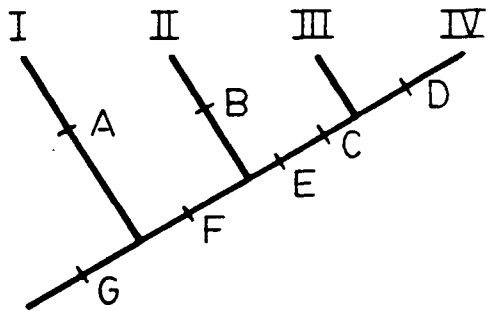
Figure 59 - The Limits of Inclusive ORing

(a) The cladogram for parasite taxa A - G, showing their occurrence in hosts I - VI; (b) the compressed host matrix produced by the inclusive ORing of the expanded host matrix (not shown) constructed from the ABC matrix (not shown) for the parasite cladogram in (a); (c) the host cladogram constructed from the compressed host matrix; (d) the parasite relationships implied by the host cladogram - these are incongruent with those in the initial parasite cladogram in (a). This is because, unlike the example illustrated in Figure 58, the parasite taxa that occur in more than one host group (viz., parasites C, D, and G in host III) are not each other's sister taxa.

264

a)
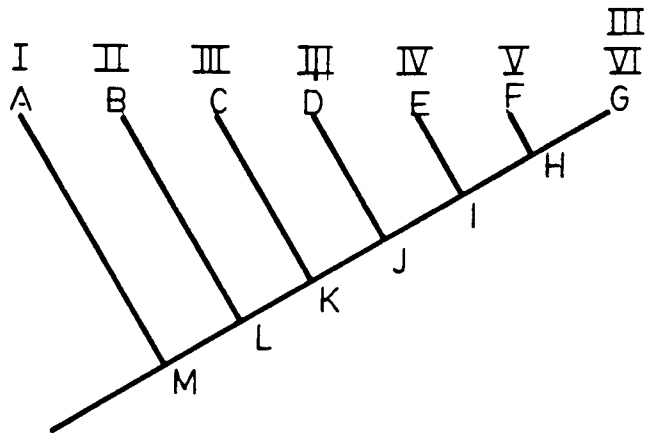
b)

	1	2	3	4	5	6	7	8	9	10	11	12	13
I	1	0	0	0	0	0	0	0	0	0	0	0	1
II	0	1	0	0	0	0	0	0	0	0	0	1	1
III	0	0	1	1	0	0	1	1	1	1	1	1	1
IV	0	0	0	0	1	0	0	0	1	1	1	1	1
V	0	0	0	0	0	1	0	1	1	1	1	1	1
VI	0	0	0	0	0	0	1	1	1	1	1	1	1

c)

d)

265
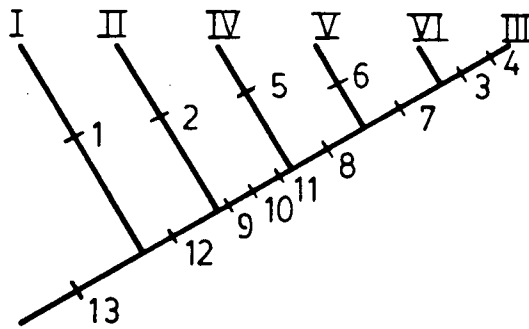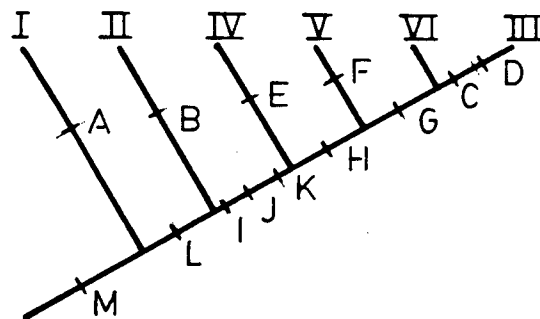
Figure 60 - The Inability of the Consistency Index to
Distinguish between Autapomorphies and Synapomorphies

Four trees with a common distribution of seven binary characters
(1 - 7), and different distributions of two other binary
characters (8 - 9); the Consistency Index for each of the trees
is the same. (From Brooks et al. 1987. Syst. Zool. 35:571.)
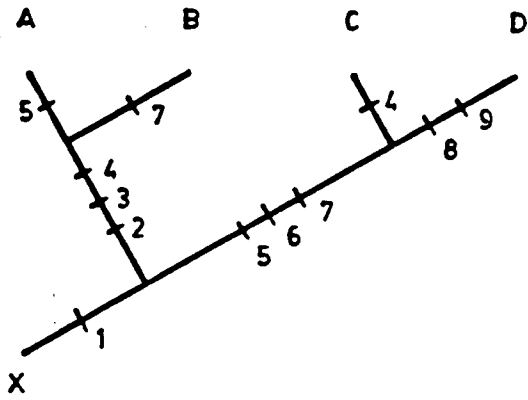
(a)
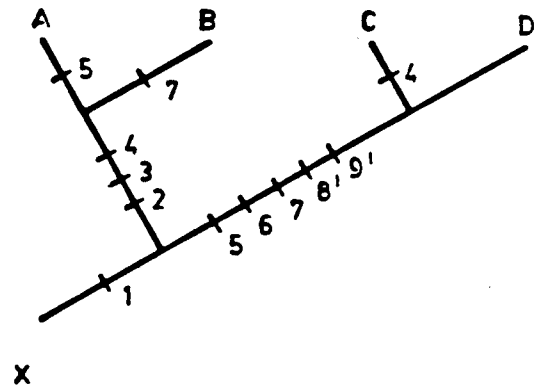
(b)
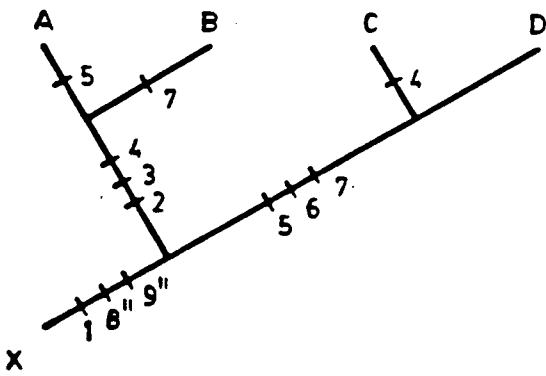
(c)
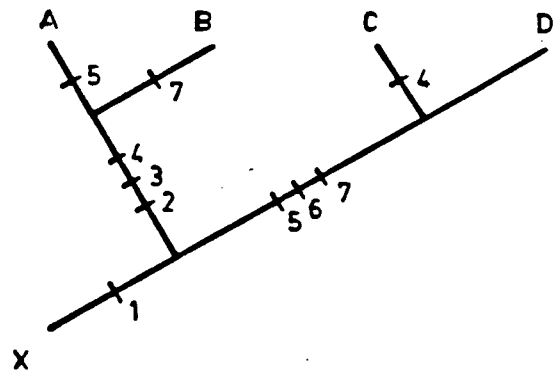
(d)

Figure 61 - Calculation of the F-Ratio

A matrix of phenetic distances (c) is constructed from the character matrix (a), then compared with a matrix of patristic distances (d) for a tree (b) inferred from the data. The sum of the matrix of the differences between the phenetic and patristic distance matrices is normalized by dividing it by the sum of the phenetic distance matrix. (From Brooks et al. 1987. Syst. Zool. 35:571.)

(a)

(b)

	1	2	3	4	5
X	0	0	0	0	0
A	1	0	0	0	1
B	1	1	0	0	0
C	1	1	1	1	0
D	1	1	1	1	1

	X	A	B	C	D
X					
A	2				
B	2	2			
C	4	4	2		
D	5	3	3	1	
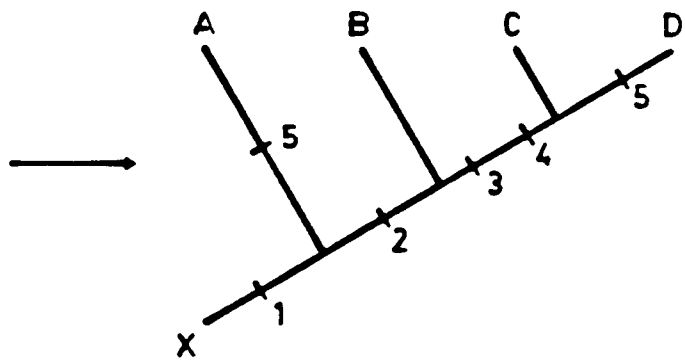
	X	A	B	C	D
X					
A	2				
B	2	2			
C	4	4	2		
D	5	⑤	3	1	

(c)

(d)

Figure 62 - The Sensitivity of the F-Ratio to Factors
Relevant to Unrooted, Rather than Rooted, Trees

Figures (b) - (d) present different distributions of character 4
on the tree in (a). When character 4 is placed as an
autapomorphy (b), an internal synapomorphy (c), and a basal
synapomorphy (d), the F-Ratio only distinguishes between cases
in which the character is on a terminal branch (b) and (d), and
a nonterminal (c) branch of the tree. (From Brooks et al.
1987. Syst. Zool. 35:571.)

(a)
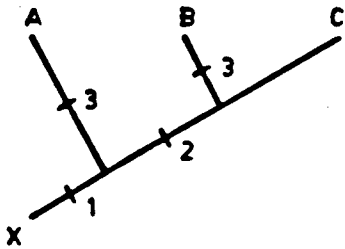
PHENETIC

	X	A	B	C
X				
A	2			
B	3	1		
C	2	2	1	

PATRISTIC

	X	A	B	C
X				
A	2			
B	3	③		
C	2	2	1	

(b)

PHENETIC

	X	A	B	C
X				
A	2			
B	3	1		
C	3	3	2	

PATRISTIC

	X	A	B	C
X				
A	2			
B	3	③		
C	3	3	2	

(c)

PHENETIC

	X	A	B	C
X				
A	2			
B	4	2		
C	3	3	1	

PATRISTIC

	X	A	B	C
X				
A	2			
B	4	④		
C	3	3	1	

(d)

PHENETIC

	X	A	B	C
X				
A	3			
B	4	1		
C	3	2	1	

PATRISTIC
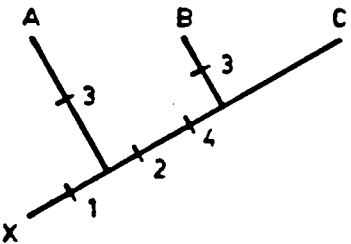
	X	A	B	C
X				
A	3			
B	4	③		
C	3	2	1	

Figure 63 - Agreement between the F-Ratio and the
Consistency Index

Tree (a) has the higher CI value and the lower F-Ratio
(character reversals are marked with an X). (From Brooks et al.
1987. Syst. Zool. 35:571.)

Figure 64 - Disagreement between the F-Ratio and the
Consistency Index

A demonstration that the shortest tree inferred from a data set
is not necessarily the tree with the lowest F-Ratio.   The tree
in (b) is shorter, and thus has the higher CI, while the tree in
(a) has the lower F-Ratio. (From Brooks et al. 1987. Syst.
Zool. 35:571.)

63
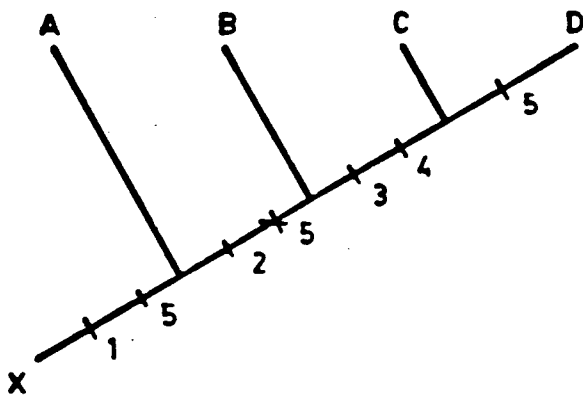
a
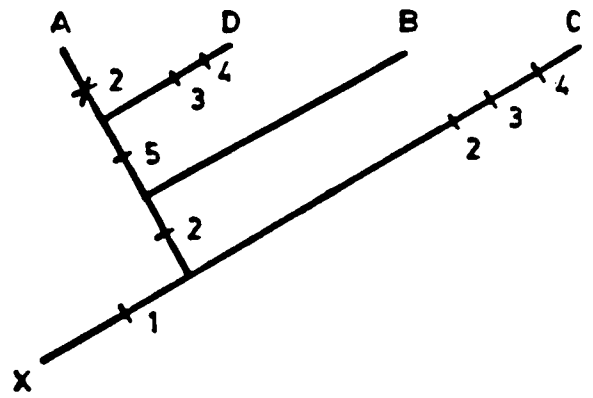
b

64
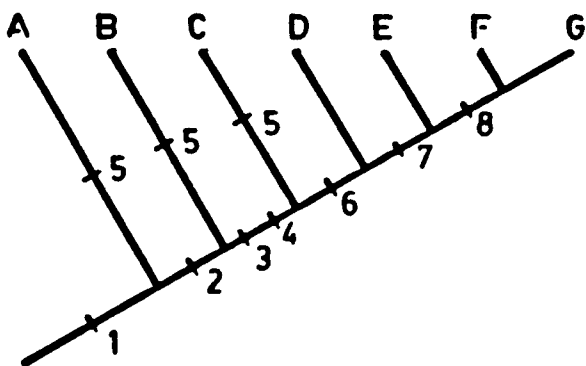
a
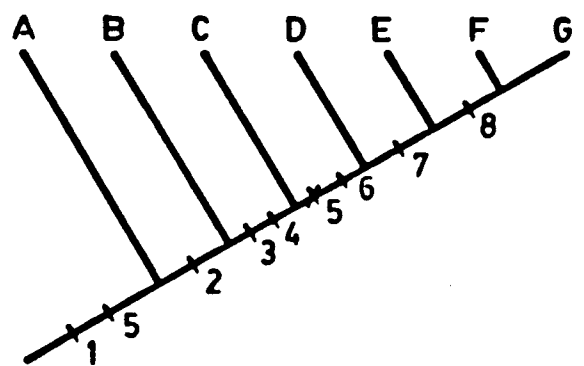
b

Figure 65 - A Demonstration that the F-Ratio is not Biased towards Parallelisms or Reversals when Comparing Trees of Equal Length

Three pairs of trees are presented. Each pair is of equal length. The trees that postulate parallelisms are (a), (c), and (e); the trees that postulate reversals (marked with an X) are (b), (d), and (f). The trees with the lower F-Ratios are (b), (c)=(d), and (e).

(a)

(b)

(c)

(d)

(e)

(f)

VS
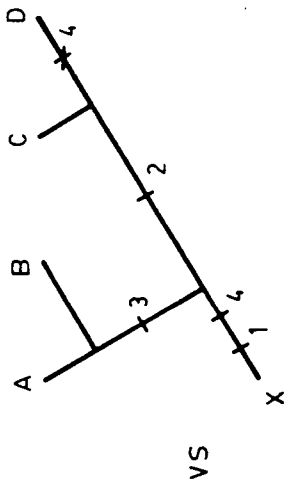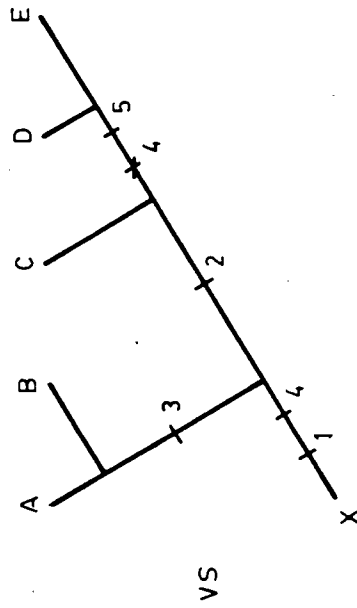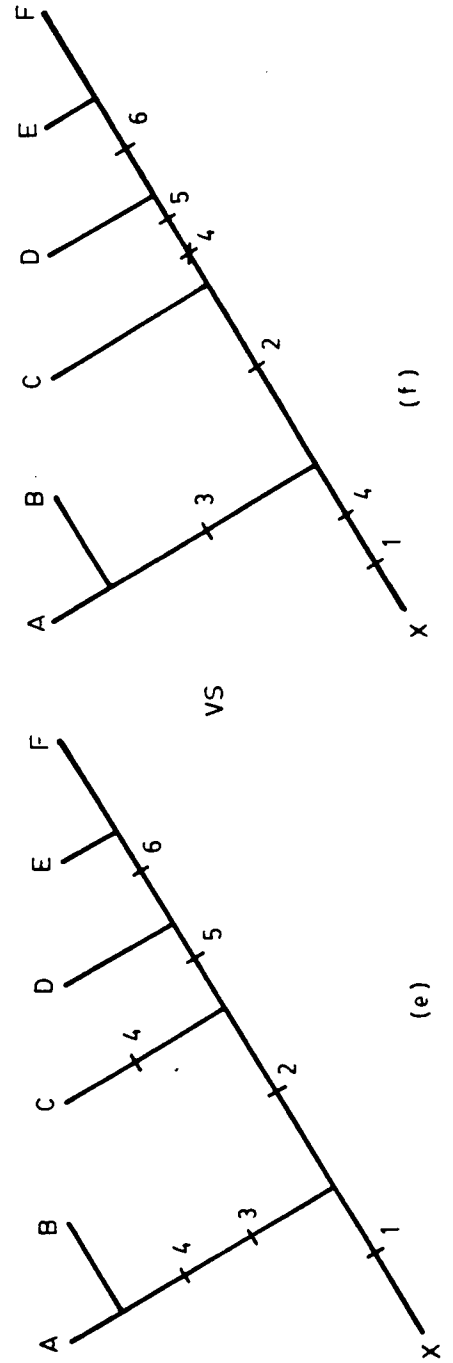
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APPENDIX <u>A</u> - COMPUTER ANALYSES WITH PAUP AND PHYSYS

This appendix contains (1) ALLTREES analyses from PAUP (with both Ordered and Unordered Analysis of multistate characters), (2) WAG.S analyses from PHYSYS, (3) the results of using the DIAGNOSE command of PHYSYS to assess how well the character data used in the present study fit the cladogram of Brooks (1977), and (4) the data matrices from Green (1986a,b) that were reanalyzed in Chapter IV. All runs were conducted on the Amdahl 5850 computer at the University of British Columbia, operating on the Michigan Terminal System. Parts of the printout have been edited for clarity.

$Log Output: Richard O'Grady, ZENO, Job#=6194, Host=G, 10:10:30 Sat Jan 17/87
#1 th.mx.unord

```
    1       !GLYPTHELMINS AND HAPLOMETRANA DATA
    1.1     !INITIAL RUN, WITH UNORDERED MULTISTATE CHARACTERS
    1.5     !UNORDERED ANALYSIS ON CHARACTERS 2,9,11,16,17, and 19
    1.6     *
    1.65    *CHARACTERS (SEE TEXT AND TABLE II FOR CHARACTER STATES):
    1.7     *(1)    shape of tegumental projections (2 states)
    1.8     *(2)    extent of tegumental projections (3 states)
    1.9     *(3)    oral sucker diam. / ventral sucker diam. (2 states)
    1.92    *(4)    penetration glands (2 states)
    1.94    *(5)    medial glands (2 states)
    1.96    *(6)    pharyngeal glands (2 states)
    1.98    *(7)    position of ovary (2 states)
    2       *(8)    position of Laurer's canal (2 states)
    2.02    *(9)    anterior extent of anterior vitelline field (3 states)
    2.04    *(10)   dorsal confluence of anterior vitelline field (2 states)
    2.06    *(11)   posterior extent of posterior vitelline field (4 states)
    2.08    *(12)   dorsal confluence of posterior vitelline field (2 states)
    2.1     *(13)   ventral confluence of both vitelline fields (2 states)
    2.12    *(14)   lateral extent of uterine loops (2 states)
    2.14    *(15)   anterior extent of uterine loops (2 states)
    2.16    *(16)   position of testes (3 states)
    2.18    *(17)   seminal vesicle (3 states)
    2.2     *(18)   cirrus sac length / forebody length (2 states)
    2.22    *(19)   egg length (3 states)
    2.24    *(20)   cercarial stylet (2 states)
    2.4     *(21)   shape of excretory vesicle (2 states)
    2.5     *
    3       param notu=9 nchar=21 outwidth=80 echo nolinks statrep
    3.3     otulab=right;
    4.2     symbols 0-1 A-C;
    5       data (21A2,2X,A8)
    6       0 0 0 0 ? 0 0 ? 0 0 0 0 0 0 0 0 0 0 0 0 0   outgroup
    7       0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 A A 0 B 1 1   hyloreus
    8       0 0 1 0 0 0 0 0 0 B 0 0 0 0 0 0 0 A 1 1   pennsylvaniensis
    9       0 A 0 0 0 0 1 1 0 0 A 0 0 1 1 0 0 0 A 0 ?   robustus
   10       0 0 0 0 1 0 0 0 0 0 0 0 1 1 1 0 B 1 B 0 1   shastai
   11       0 0 0 0 1 0 0 0 B 0 0 1 0 1 1 B B 1 B 0 1   intestinalis
   12       1 0 0 0 0 0 0 0 A 1 C 0 0 1 1 A B 0 B 0 1   californiensis
   13       1 0 0 0 0 1 0 0 0 1 B 0 0 1 1 A B 0 B 0 1   quieta
   14       1 B 0 1 0 0 0 0 1 B 1 0 1 1 0 B 0 A 0 1   facioi
   15       unordered 2 9 11 16 17 19
```

#$RUN PAUP
#Execution begins
 P A U P
 Phylogenetic Analysis Using Parsimony
 Illinois Natural History Survey
 10:11:05
 JAN 17, 1987
 Enter name of data file:
 th.mx.unord
 Enter name of output file:
 -unord
 GLYPTHELMINS AND HAPLOMETRANA DATA
 INITIAL RUN, WITH UNORDERED MULTISTATE CHARACTERS
 UNORDERED ANALYSIS ON CHARACTERS 2,9,11,16,17, and 19
 Character-state symbols read.
 Reading data matrix . . .

```
Data matrix stored.
Unordered characters set.
Entering interactive mode . . .
Enter command:
go/alltrees csposs chglist treeout=2;
********************
* Analysis No.   1 *
********************
Option settings:
     NOTU ...................        9
     NCHAR ...................       21
     User-tree(s) ............       NO
     HYPANC ..................        1
     ADDSEQ ..................      N/A
     HOLD ....................      N/A
     SWAP ....................      N/A
     MULPARS .................      N/A
     OPT .....................   FARRIS
     ROOT .................... ANCESTOR
     Weights applied .........       NO
     OUTWIDTH ................       80
     Missing data code .......        ?
     MAXTREE .................      N/A
The following characters are unordered:
       2    9   11   16   17   19
Exhaustive search of all possible topologies performed.
Computing lengths for all possible trees . . .
Exhaustive search completed.
Total number of trees examined = 135135.
    1 tree(s) found at    33.000 steps
```

Possible HTU character-state assignments

										Character											
Node	1	2	3	4	5	6	7	8	9	1 0	1 1	1 2	1 3	1 4	1 5	1 6	1 7	1 8	1 9	2 0	2 1
10	O	O	1	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	A B	1	1
11	O	O	O	O	1	O	O	O	O	O	O	O	O	O	1	1	O	B	1	B	O	1
12	1	O	O	O	O	O	O	O	O	1	B	O	O	1	1	A	B	O	B	O	1	
13	1	O	O	O	O	O	O	O	O	1	B	O	O	1	1	O	B	O	A B	O	1	
14	O	O	O	O	O	O	O	O	O	O	O	O	O	O	1	1	O	B	O	A B	O	1
15	O	O	O	O	O	O	O	O	O	O	O	O	O	O	1	1	O	O	O	A B	O	1
16	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	A B	O	1

```
Statistics for tree no.   1

     Length =    33.000
```

```
                                                              1.000
    9
                    O           A          12 ---> californ
                    O           B          11 ---> intestin
                                                              1.000
   10
                    O           1          14 ---> 13
                                                              1.000
   11
                    O           B          14 ---> 13
                    B           C          12 ---> californ
                    O           A          15 ---> robustus
                    O           B          10 ---> pennsylv
                                                              0.750
   12
                    O           1          13 ---> facioi
                    O           1          11 ---> intestin
                                                              0.500
   13
                    O           1          11 ---> shastai
                                                              1.000
   14
                    O           1          16 ---> 15
                                                              1.000
   15
                    O           1          16 ---> 15
                                                              1.000
   16
                    O           A          13 ---> 12
                    O           B          11 ---> intestin
                    O           A          10 ---> hyloreus
                                                              0.667
   17
                    O           B          15 ---> 14
                    O           A          10 ---> hyloreus
                                                              1.000
   18
                    O           1          14 ---> 11
                                                              1.000
   19
                    O           B     outgroup ---> 16
                    B           A          13 ---> facioi
                    B           A          15 ---> robustus
                    B           A          10 ---> pennsylv
                                                              0.500
   20
                    O           1          16 ---> 10
                                                              1.000
```

```
        21
                          O            1           outgroup ---> 16
                                                                              1.000
*** Analysis no. 1, completed.
Enter command:
mts

Returning to MTS.  Use $RESTART to continue.
#1 th.mx.ord

        1        !GLYPTHELMINS AND HAPLOMETRANA DATA
        2        !FINAL RUN, WITH MULTISTATE CHARACTERS ORDERED A POSTERIORI
        4        *
        5        *CHARACTERS (SEE TEXT AND TABLE III FOR CHARACTER STATES):
        6        *(1)   shape of tegumental projections
        7        *(2)   extent of tegumental projections (1st axis)
        7.2      *(3)   extent of tegumental projections (2nd axis)
        8        *(4)   oral sucker diameter / ventral sucker diameter
        9        *(5)   penetration glands
       10        *(6)   medial glands
       11        *(7)   pharyngeal glands
       12        *(8)   position of ovary
       13        *(9)   position of Laurer's canal
       14        *(10)  anterior extent of anterior vitelline field (1st axis)
       14.2      *(11)  anterior extent of anterior vitelline field (2nd axis)
       15        *(12) dorsal confluence of anterior vitelline field
       16        *(13) posterior extent of posterior vitelline field (1st axis)
       16.2      *(14) posterior extent of posterior vitelline field (2nd axis)
       17        *(15) dorsal confluence of posterior vitelline field
       18        *(16) ventral confluence of both vitelline fields
       19        *(17) lateral extent of uterine loops
       20        *(18) anterior extent of uterine loops
       21        *(19) position of testes (1st axis)
       21.2      *(20) position of testes (2nd axis)
       22        *(21) seminal vesicle (1st axis)
       22.2      *(22) seminal vesicle (2nd axis)
       23        *(23) cirrus sac length / forebody length
       24        *(24) egg length
       25        *(25) cercarial stylet
       26        *(26) shape of excretory vesicle
       26.2      *
       27        param notu=9 nchar=26 outwidth=80 echo nolinks statrep missing=9
       28        otulab=right;
       30        data (26I2,2X,A8)
       31         0 0.0 0 0 9 0 0 9 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0  outgroup
       32         0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 2 1 1  hyloreus
       33         0 0 0 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 1 1 1  pennsylvaniensis
       34         0 1 0 0 0 0 0 1 1 0 0 1 0 0 0 1 1 0 0 0 0 1 0 9  robustus
       35         0 0 0 0 0 1 0 0 0 0 0 0 0 0 1 1 1 0 0 0 1 1 2 0 1  shastai
       36         0 0 0 0 0 1 0 0 0 0 1 0 0 0 1 0 1 1 0 1 0 1 1 2 0 1  intestinalis
       37         1 0 0 0 0 0 0 0 0 1 0 1 0 2 0 0 1 1 1 0 0 1 0 2 0 1  californiensis
       38         1 0 0 0 0 0 1 0 0 0 0 1 0 1 0 0 1 1 1 0 0 1 0 2 0 1  quieta
       39         1 0 1 0 1 0 0 0 0 0 0 1 0 1 1 0 1 1 0 0 0 1 0 1 0 1  facioi
#$restart
 Enter command:
 newdata
 Enter name of data file:
 th.mx.ord
 Enter name of output file:
```

```
-ord
GLYPTHELMINS AND HAPLOMETRANA DATA
FINAL RUN, WITH MULTISTATE CHARACTERS ORDERED A POSTERIORI
Reading data matrix . . .
Data matrix stored.
Entering interactive mode . . .
Enter command:
go/alltrees treeout=2;
*********************
* Analysis No.  1 *
*********************
Option settings:

        NOTU ....................        9
        NCHAR ...................       26
        User-tree(s) ............       NO
        HYPANC ..................        1
        ADDSEQ ..................      N/A
        HOLD ....................      N/A
        SWAP ....................      N/A
        MULPARS .................      N/A
        OPT .....................   FARRIS
        ROOT ....................  ANCESTOR
        Weights applied .........       NO
        OUTWIDTH ................       80
        Missing data code .......        9
        MAXTREE .................      N/A

Exhaustive search of all possible topologies performed.
Computing lengths for all possible trees . . .
Exhaustive search completed.
Total number of trees examined = 135135.
    1 tree(s) found at    33.000 steps

Statistics for tree no.  1

        Length =    33.000
        Consistency index = 0.848

Tree no.  1 rooted using designated ancestor

*   1  outgroup
*
*                        ************************************************  2  hyloreus
*              *********10
*              *         ************************************************  3  pennsylvaniensis
***********16
        *         ************************************************  4  robustus
        *         *
        *********15          ********************  5  shastai
                 *         **********11
                 *         *         ********************  6  intestinalis
                 *********14
                         *                   ************  7  californiensis
                         *         **********12
                         *********13          ************  8  quieta
                                 *
                                 ********************  9  facioi

*** Analysis no. 1, completed.
```

```
Enter command:
end;
PAUP execution completed.
#Execution terminated
#1 th.mx.phy

      1       GLYPTHELMINS AND HAPLOMETRANA DATA, MULTIS ORDERED WITH PAUP
     26       026        009        001        9.0
     28       (26F2.0,2X,2A4)
     29       0 0 0 0 0 9 0 0 9 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0  outgroup
     30       0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 2 1 1  hyloreus
     31       0 0 0 1 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 1 1 1  pennsylvaniensis
     32       0 1 0 0 0 0 0 1 1 0 0 0 1 0 0 0 1 1 0 0 0 0 0 1 0 9  robustus
     33       0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 1 1 1 0 0 0 1 1 2 0 1  shastai
     34       0 0 0 0 0 1 0 0 0 0 1 0 0 0 1 0 1 1 0 1 0 1 1 2 0 1  intestinalis
     35       1 0 0 0 0 0 0 0 0 1 0 1 0 2 0 0 1 1 1 0 0 1 0 2 0 1  californiensis
     36       1 0 0 0 0 1 0 0 0 0 1 0 1 0 0 1 1 1 0 0 1 0 2 0 1  quieta
     37       1 0 1 0 1 0 0 0 0 0 1 0 1 1 0 1 1 0 0 0 1 0 1 0 1  facioi
#$RUN CLAD:PHYSYS
#Execution begins
>PPPPPP    HH     HH YY      YY  SSSSSS YY      YY  SSSSSS
>PPPPPPP   HH     HH YY    YY  SSSSSSS YY      YY  SSSSSSS
>PP     PP HH     HH  YY YY  SS         YY YY  SS
>PP     PP HHHHHHHH    YYY   SSSSS      YYY    SSSSS
>PPPPPPP   HHHHHHHH    YY    SSSSS      YY     SSSSS
>PPPPPP    HH     HH   YY         SS    YY          SS
>PP        HH     HH   YY         SS    YY          SS
>PP        HH     HH   YY    SSSSSSS    YY     SSSSSSS
>PP        HH     HH   YY    SSSSSS     YY     SSSSSS
>
> COPYRIGHT 1983 BY J. S. FARRIS & M. F. MICKEVICH
>                 ALL RIGHTS RESERVED
>/data.th.mx.phy;xread,input;wag.s,input,output;lfit;tp/
>XREAD         INPUT         INPUT
>GLYPTHELMINS AND HAPLOMETRANA DATA, MULTIS ORDERED WITH PAUP
>
>WAGNER        INPUT
>   1 TREES,   LENGTH=      33.000
>
>LFIT          OUTPUT
>
>TREE          1
>LENGTH        33.000
>C-INDEX       84.848
>F-RATIO        9.524
>
>TPLOT         OUTPUT
>
>TREE     1
>     2.000 californ----
>                       I
>     2.000       STEM0012----
>                       I    I
>     1.000 quieta++----    I
>                           I
>     3.000            STEM0013----
>                           I    I
>     3.000 facioi++--------    I
>                               I
>     1.000                STEM0014----
```

```
>                                    I    I
>     3.000 intestin--------     I    I
>                              I   I    I
>     3.000          STEMOO11----    I
>                              I         I
>     1.000 shastai+--------         I
>                                         I
>     2.000                  STEMOO15----
>                                    I    I
>     4.000 robustus--------------     I
>                                         I
>     3.000 hyloreus--------------     I
>                                    I    I
>     2.000                  STEMOO10----
>                                    I    I
>     1.000 pennsylv--------------     I
>                                         I
>    33.000                  STEMOO16----
>                                         I
>     2.000 outgroup--------------------
>
>$end
#Execution terminated
#1 th.mx.br

     1        GLYPTHELMINS MATRIX FOR MAPPING ONTO BROOKS 1977 CLADOGRAM
     2        026        008          9.0
     3        (26F2.0,2X,2A4)
     4        0 0 0 0 0 9 0 0 9 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0  outgroup
     5        0 0 0 1 0 0 0 0 0 0 0 0 1 0 0 0 0 0 1 0 1 0 0 2 1 1  hyloreus
     6        0 0 0 1 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 1 1 1  pennsylvaniensis
     7        0 1 0 0 0 0 0 1 1 0 0 0 0 0 0 1 1 0 0 0 0 0 1 0 9  robustus
   7.5        1 0 1 0 1 0 0 0 0 0 0 1 0 1 1 0 1 1 0 0 1 0 1 0 1  facioi
     8        0 0 0 0 0 1 0 0 0 1 0 0 1 1 1 0 0 0 1 1 2 0 1  shastai
    10        1 0 0 0 0 0 0 0 1 0 1 0 2 0 0 1 1 1 0 1 0 2 0 1  californiensis
    11        1 0 0 0 0 0 1 0 0 0 0 1 0 1 0 0 1 1 1 0 1 0 2 0 1  quieta
#1 th.tr.br

     1        1977 BROOKS CLADOGRAM FOR GLYPTHELMINS
     2        008
     3        outgroup  hyloreus  pennsylv  robustus  facioi    shastai    califor
              n  quieta
     4        015
     5          15    9   9   13   12   11   10   10   14   11   12   13   14   15
#$RUN CLAD:PHYSYS
#Execution begins
>PPPPPP    HH    HH YY      YY  SSSSSS YY     YY  SSSSSS
>PPPPPPP   HH    HH  YY    YY  SSSSSSS YY    YY  SSSSSSS
>PP   PP HH    HH   YY YY  SS           YY YY  SS
>PP     PP HHHHHHHH    YYY    SSSSS       YYY     SSSSS
>PPPPPPP HHHHHHHH    YY    SSSSS       YY     SSSSS
>PPPPPP    HH    HH  YY         SS  YY         SS
>PP        HH    HH  YY         SS  YY         SS
>PP        HH    HH  YY  SSSSSSS    YY  SSSSSSS
>PP        HH    HH  YY  SSSSS      YY  SSSSS
>
```

```
>/data.th.mx.br;xread.matrix;data.th.tr.br;tread.tree;tp.tree;
>/diag.tree.matrix.output;lfit.output/
```

```
>XREAD          MATRIX        INPUT
>GLYPTHELMINS MATRIX FOR MAPPING ONTO BROOKS 1977 CLADOGRAM
>TREAD          TREE          INPUT
>1977 BROOKS CLADOGRAM FOR GLYPTHELMINS
>TREE      1:
>
>TPLOT          TREE
>
>TREE      1
>californ----
>          I
>     STEM0010----
>          I   I
>quieta++----   I
>              I
>         STEM0011----
>              I   I
>shasta!+--------   I
>                  I
>             STEM0012----
>                  I   I
>facio!++------------   I
>                      I
>                 STEM0013----
>                      I   I
>robustus----------------   I
>                          I
>                     STEM0014----
>                          I   I
>pennsylv----------------   I   I
>                          I   I   I
>                     STEM0009----   I
>                          I       I
>hyloreus----------------       I
>                              I
>                         STEM0015----
>                              I
>outgroup----------------------
>
>
>LFIT          OUTPUT
>
>TREE          1
>LENGTH        33.000
>C-INDEX       78.788
>F-RATIO       12.644
>$end
#Execution terminated
```

```
!allozyme data for the R. boylii group (Green, 1986a)
!chars 4 5 6 17 19 22 25 31 32 41 43 deleted as uninformative
param notu=11 nchar=47 otulab=right outwidth=80 echo statrep;
data (47I1,1X,A8)
00000000000000000000000000000000000000000000000 outgroup
10000133543220130400100100215200122035110004011 Ra.er
10000133442220020200100100005200123034110003011 Ra.me
00000133333220010201000100004200211034110003011 Ra.br
01000134333220010300100100004200211034210003011 Ra.ti
00200136333220010200000100004200211034110003011 Ra.du
1000013533232101010100002000116200111034120101021 Rc.ca
000001434623210100000002001003000000201140000200 Rp.id
100001434400010100000002000123000122200140001200 Rp.or
10000113452320030000001000212200132003130202011 Rb.bo
10000142222220040000002000002200124024140003100 Rm.mu
unordered 7-12 16 18 23 27-29 33-35 37-40 42 44-46


!karyological data for the R. boylii group (Green, 1986b)
param notu=5 nchar=13 outwidth=80 echo statrep otulab=right;
data (13I1,1X,A8)
0111001000000 Ra
0010110001002 Rc
1010000001101 Rp
2000100002011 Rb
2000100113001 Rm
unordered 1 3 7 9 10 11 12 13
```

APPENDIX <u>B</u> – HOST AND DISTRIBUTION DATA FOR <u>GLYPTHELMINS</u> <u>QUIETA</u>

(Literature citations are listed as they appear in the Index
Catalogue of Medical and Veterinary Zoology, Oryx Press,
Phoenix, Arizona, U.S.A.)

BUFONIDAE

<u>Bufo</u> <u>americanus</u> Holbrook

    Presque Isle, Maine (Bouchard, 1951)

<u>B.</u> <u>microscaphus</u> Cope

    Utah (Parry and Grundmann, 1965)

<u>B.</u> <u>woodhousii</u> Girard

    Nebraska (Brooks, 1976)
    Utah (Parry and Grundmann, 1965)

HYLIDAE

<u>Acris</u> <u>crepitans</u> Baird

    Iowa (Ulmer, 1970)

<u>Hyla</u> <u>crucifer</u> Weid (= <u>H.</u> <u>pickeringii</u> Kennicott)

    Eastern Canada (Stafford, 1905)
    Western Massachusetts (Rankin, 1945)
    Athens, Clarke Co., Georgia (Byrd and Maples, 1963)
    Ann Arbor, Michigan (Najarian, 1955)

<u>Pseudacris</u> <u>nigrita</u> (LeConte)

    Athens, Clarke Co., Georgia (Byrd and Maples, 1963)

<u>P.</u> <u>triseriata</u> Weid

    Iowa (Ulmer, 1970)

RANIDAE

<u>Rana</u> <u>blairi</u> Meacham <u>et</u> <u>al.</u>

    Nebraska (Brooks, 1976)

<u>R.</u> <u>catesbeiana</u> Shaw

Eastern Canada (Stafford, 1905)
Ile Perrot, Quebec (Rau et al., 1978)
Gaspe Penninsula, Quebec (Rankin, 1944)
Urbana, Illinois (Miller, 1930)
Illinois (Leigh, 1937)
Ohio (Odlaug, 1954)
Ann Arbor, Michigan (Najarian, 1955)
Indiana (Lank, 1971)
Wisconsin (Williams and Taft, 1980)
Houston and Huntsville, Texas (Harwood, 1932)
East Texas (Hollis, 1972)
Cleveland Co., Oklahoma (Trowbridge and Hefley, 1934)
Oklahoma (Brooks, 1979)
Arkansas (Rosen and Manis, 1976)
Beaufort Co., N. Carolina (Brandt, 1936)
Florida (Manter, 1938)
Louisiana (Bennett, 1938)
Athens, Clarke Co., Georgia (Parker, 1941)
Amherst, Massachusetts (Rankin, 1944)
North Carolina (Rankin, 1944)
Virginia (Campbell, 1968)
Seattle, Washington (Rankin, 1944)
Iowa (Ulmer, 1970)
Burke, Chatham, Taliaferro, Oconee, and Screven Co.,
            Georgia (Sullivan, 1976)
Terrebonne and East Baton Co., Louisiana (Sullivan, 1976)
Oktibbeha Co., Misssissippi (Sullivan, 1976)
Mississippi (Brooks, 1979)
Nye Co., Nevada (Babero and Golling, 1974)
Havana, Cuba (Odening (1968)

R.   clamitans Latreille

Western Massachusetts (Rankin, 1945)
Urbana, Illinois (Miller, 1930)
Ann Arbor, Michigan (Najarian, 1955)
Virginia (Campbell, 1968)
Presque Isle, Maine (Bouchard, 1951)
De Kalb and Oglethorpe Co., Georgia (Sullivan, 1976)
Warren Co., New Jersey (Sullivan, 1976)
Connecticut (Brooks, 1976)
Louisiana (Brooks, 1979)
Wisconsin (Williams and Taft, 1980)
Ile Perrot, Quebec (Rau et al., 1978)

R.   palustris Le Conte

Presque Isle, Maine (Bouchard, 1951)

R.   pipiens Schreber (= R. virescens Garman)

Eastern Canada (Stafford, 1905)
Franklin Co., Ohio (Sullivan, 1976)
Urbana, Illinois (Miller, 1930)

Illinois (Leigh, 1937)
Iowa (Ulmer, 1970)
Alamance Co., N. Carolina (Sullivan, 1976)
Franklin Co., Tennessee (Sullivan, 1976)
Arkansas (Rosen and Manis, 1976)
West Virginia (Brooks, 1979)
Mountrail Co., N. Dakota (present study)
Utah (Parry and Grundmann, 1965)

R. septentrionalis Baird

Presque Isle, Maine (Bouchard, 1951)

R. sphenocephala Cope

Cleveland Co., Oklahoma (Trowbridge and Hefley, 1934)
Houston and Huntsville, Texas (Harwood, 1932)

R. utricularia Harlan

Louisiana (Brooks, 1979)
Mississippi (Brooks, 1979)

APPENDIX C - COLLECTION SITES OF ANURANS

Rana pipiens: 9 km E. of Sprague, Neb. / White Earth R., Mountrail Co., 85 km E of Williston, N. Dak. / Elk Point, Union Co., S. Dak.

R. aurora: Bonsall Crk., Duncan, Vancouver Island, B.C. / Little Campbell R., approximately 9 km E of White Rock, B.C. / Ponds at 232nd and 0 ave., Langley, B.C. / Hicks and Moss L., Sasquatch Park, 20 km NE of Chilliwack, B.C.

R. pretiosa: Little Muddy Pond, Manning Park, 65 km E of Hope, B.C. / Okanagan Falls Prov. Campground, Okanagan Falls, B.C. / Wilgress L., 15 km E of Greenwood, B.C. / Champion L., Champion Lakes Park, 25 km NE of Trail, B.C. / Creston, B.C. / Loon L., 25 km S of Elko, B.C. / Pelican Crk., Yellowstone National Park, Wyoming

R. catesbeiana: Little Campbell R., 9 km E of White Rock, B.C. / Ponds at 232nd and 0 ave., Langley, B.C. / Osoyoos Lake, Osoyoos, B.C. / Parks Crk., 25 km NW of Weed, Siskiyou Co., Calif. / Thousand Springs, 10 km NW of Glenburn, Shasta Co., Calif. / Glenburn, Calif. / McArthur, Shasta Co., Calif

R. cascadae: Lily Pad L., 16 km W of Callahan, Siskiyou Co., Calif. / Kangaroo L., 16 km W of Callahan, Siskiyou Co., Calif. / Dead Fall L., 20 km W of Weed, Siskiyou Co., Calif. / Little and Big Bear Flats, 30 km N of Burney, Shasta Co., Calif.

Bufo boreas: Little Muddy Pond, Manning Park, 65 km E of Hope, B.C. / Kings Crk., Lassen Volcanic National Park, Calif. / Medicine Lake, 75 km N of Burney, Shasta Co., Calif. / Little and Big Bear Flats, 30 km N of Burney, Calif. / Brown Rd., Glenburn, Shasta Co., Calif.

Hyla regilla: Ponds at 232nd and 0 ave., Langley, B.C. / Penticton, B.C. / Christina L. 25 km E of Grand Forks, B.C.

APPENDIX D - REDESCRIPTION OF <u>GLYPTHELMINS SHASTAI</u>,
SYNONYMIZATION OF HAPLOMETRANA <u>WITH GLYPTHELMINS</u>, AND
REDESCRIPTION OF <u>G. INTESTINALIS</u> N. COMB.

REDESCRIPTION OF <u>GLYPTHELMINS SHASTAI</u>

This species has not been reported since Ingles (1936)
described it from <u>Bufo boreas</u> collected in the area of Glenburn,
Shasta County, California. In the present study, I examined 19
adults of <u>B. boreas</u> from the type locality during 1985-1986, but
I did not find any intestinal digenean parasites. I obtained
additional specimens of <u>G. shastai</u> through the courtesy of Dr.
John C. Holmes, Department of Zoology, University of Alberta,
Edmonton, Canada. These worms had been collected from <u>B. boreas</u>
in two localities in southwestern Canada during the 1960s: Gorge
Creek, Alberta (approximately 60km southwest of Calgary), and
Nelson, British Columbia. [1] The examination of 24 specimens from
this collection, as well as the re-examination of the holotype,
has allowed a redescription of the species. In addition to
providing a larger set of observations and measurements, it is
necessary to make some corrections and augmentations to the
original description. All specimens examined were wholemounts
stained with hematoxylins or acetocarmine and mounted in balsam.

<u>Glypthelmins shastai</u> Ingles, 1936
(Figs 10, 17, 46, 47)

<u>DESCRIPTION</u> (Fig. 46a; measurements given as ranges and
(means), based upon the holotype, and 24 fixed specimens from
the small intestine of adults of <u>Bufo boreas</u> in southwestern
Canada): Plagiorchioidea: body rounded at both ends; 1.62 - 5.87
(4.14) mm long; 0.60 - 1.15 (1.12) mm wide; tegument spinose
(Fig. 10); spines flattened, tapering with ends rounded, non-
overlapping, extending to posterior end of body. Oral sucker
subterminal, 0.25 - 0.39 (0.32) mm long, 0.25 - 0.40 (0.32) mm
wide. Ventral sucker medial, in second fifth of body, 0.16 -
0.27 (0.22) mm long, 0.0.16 - 0.27 (0.22) mm wide. Prepharynx
short, receiving ducts of medial glands dorso-laterally; medial
glands at level of pharynx and esophagus (Fig. 47a). Pharynx
0.14 - 0.26 (0.18) mm long, 0.14 - 0.27 (0.21)mm wide.
Esophagus bifurcating mid-way between pharynx and ventral
sucker; intestinal ceca extending to near posterior end of body.
Genital pore medial, mid-way between cecal bifurcation and
ventral sucker. Cirrus sac 0.46 - 0.92 (0.68) mm long, more

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[1] Collectors: W.R. Turner, M. Aleksiuk, L Graham, and E.
Huebner.

than one-half length of forebody, containing straight,
bipartite, internal seminal vesicle (Fig. 47b). Vas deferens
present in some adults, absent in others, presence not
correlated with size. Testes paired, intercecal, oblique,
spherical to oval with smooth edges, in middle fifth of body;
anterior testis 0.21 - 0.45 (0.36) mm long, 0.34 - 0.41
(0.35) mm wide; posterior testis 0.35 - 0.52 (0.42) mm long,
0.25 0.44 (0.35) mm wide. Ovary sinistral, pretesticular,
postacetabular, spherical to oval with smooth edges, 0.20 - 0.31
(0.26) mm long, 0.19 - 0.31 (0.25) mm wide; seminal receptacle
spherical, immediately postero-medial to ovary; Laurer's canal
originating in ootype region between seminal receptacle and
common vitelline duct (Fig. 47c); uterus extending posteriad to
end of body, in numerous intercecal and post-testicular
transverse loops, filling body posteriad to intestinal ceca;
metraterm muscular, ventral to cirrus sac, shorter than cirrus
sac, surrounded by gland cells (Fig. 47d). Vitellaria
follicular, lying dorsal, lateral, and ventral to ceca,
extending along sides of body from level of cecal bifurcation to
posterior third of hindbody, often ventro-medially confluent, or
nearly so, at level of ootype region (Fig. 17). Excretory
bladder I-shaped, extending to just posteriad to level of testes
(Fig. 46e). Eggs 41 - 50 (44) μm long.

HOST: _Bufo boreas_ Baird and Girard.
SITE OF INFECTION: Small intestine.
TYPE LOCALITY: Glenburn, Shasta County, California.
OTHER LOCALITIES : Nelson, British Columbia; Gorge Creek,
          Alberta.
SPECIMENS : Holotype, U.S. Helm. Coll. No. 8925; additional
          specimens from the Dept. of Zoology, University of
          Alberta. Specimens from this collection have been
          deposited (HWML no. 23661) at the Harold W. Manter
          Laboratory, University of Nebraska State Museum, 529-W
          Nebraska Hall, University of Nebraska, Lincoln, NE
          68588-0514. Additional specimens from this collection
          will be deposited at the National Museum of Natural
          Sciences, Ottawa, Ontario, Canada.

REMARKS: The following characters were not included in the
description by Ingles (1936) (see Fig. 46b): the bipartite
condition of the seminal vesicle, the position of the Laurer's
canal, and the shape of the excretory vesicle. Through the
examination of additional specimens, the vitellaria are found to
extend from the level of the cecal bifurcation to the posterior
third of the hindbody; there is also frequently a ventro-medial
confluence of the vitellaria at the level of the ootype region.
The range and (mean) lengths for the eggs in the holotype are 41
- 46 (43) μm. The values for the specimens from southwestern
Canada are 42 - 50 (45) μm. (Differences between the two sets
of measurements are not significant at the 0.05 level.) Ingles
(1936) reported a range of 31 - 32 μm for the length of the eggs
in the holotype. The following two observations are also not in

accord with those given in the original description: the presence of small glands (herein termed medial glands, after Leigh, 1946) lateral to the pharynx and esophagus, and the location of the metraterm ventral to the cirrus sac (Ingles reported the location to be dorsal, but a re-examination of the holotype shows it to be ventral).

Cheng (1959) cited G. shastai as one of the species included in a proposed re-establishment of Margeana Cort, 1919. Byrd and Maples (1963) argued against the re-establishment of this genus. I concur. Rankin (1944) included G. shastai in a synonymization of a number of glyptheminths with G. quieta. This opinion was based primarily upon the distribution of the vitellaria. Although a small number of specimens of G. quieta have vitellaria with a ventro-medial confluence and an extension to the middle of the hindbody, these two characters are regularly observed only in G. shastai. The two species also differ with respect to: (1) the presence in G. quieta of large pharyngeal glands in addition to the smaller, more anterior, medial glands that are also present in G. shastai; (2) a scaled tegument in G. quieta, and a spined tegument in G. shastai; (3) the presence in G. shastai of a cirrus sac that is more than one-half the length of the forebody; and (4) symmetrical testes in G. quieta, and oblique testes in G. shastai.

Nasir and Diaz (1970) synonymized G. shastai with G. linguatula (Rudolphi, 1819) Travassos, 1924 [= Choledocystus l. (Rudolphi, 1819) Byrd and Maples, 1963]. I do not accept this because (1) G. linguatula has a Y-shaped excretory vesicle, bifurcating anterior to the level of the testes, while that of G. shastai is I-shaped, bifurcating posterior to the level of the testes, and (2) only G. linguatula has extracecal and pretesticular loops of the uterus. As noted by Sullivan (1976), the holotype of G. shastai is not a fully mature specimen. This limited the conclusions that could be drawn from Ingles' (1936) description.

SYNONYMIZATION OF HAPLOMETRANA WITH GLYPTHELMINS
AND REDESCRIPTION OF GLYPTHELMINS INTESTINALIS N. COMB.

The results of the phylogenetic analysis presented in
Chapter III support the synonymization of Haplometrana with
Glypthelmins, Haplometrana being the junior subjective synonym.
Haplometrana intestinalis shares a number of traits with the
species of Glypthelmins studied, in particular, with its
postulated sister species, G. shastai. Its exclusion from
Glypthelmins would render that genus paraphyletic.

Glypthelmins intestinalis (Lucker, 1931) n. comb.
(Figs 10, 12, 17, 18, 35)

SYNONYMS: Haplometrana intestinalis Lucker, 1931
                 H. utahensis Olsen, 1937

DESCRIPTION (Fig. 35a; measurements given as ranges and
(means), based upon the holotypes and paratypes of
H. intestinalis and H. utahensis, and 20 fixed specimens of
H. intestinalis collected from R. pretiosa in southern British
Columbia): Plagiorchioidea: body rounded at both ends; 2.70 -
5.28 (4.03) mm long, 0.55 - 0.67 (0.62) mm wide. Tegument
spinose (Fig. 10); spines flattened, tapering with ends
rounded, in non-overlapping rows to posterior end of body. Oral
sucker subterminal, 0.27 - 0.35 (0.29) mm long, 0.25 - 0.38
(0.29) mm wide. Ventral sucker medial, in second fifth of body,
0.16 - 0.23 (0.18) mm long, 0.13 - 0.23 (0.18) mm wide.
Prepharynx short, receiving ducts of medial glands dorso-
laterally; medial glands at level of pharynx and esophagus (Fig.
12). Pharynx 0.10 - 0.14 (0.12) mm long, 0.11 - 0.14 (0.12)mm
wide. Esophagus bifurcating mid-way between pharynx and ventral
sucker; intestinal ceca extending to posterior end of body.
Genital pore medial, immediately anterior to ventral sucker;
cirrus sac 0.35 - 0.76 (0.45) mm long, more than one-half length
of forebody, containing straight, bipartite, internal seminal
vesicle. Vas deferens present in older adults, absent in
younger adults. Testes paired, intercecal, spherical to oval,
with smooth edges, in middle fifth of body, usually tandem,
occasionally oblique; anterior testis 0.23 - 0.32 (0.28) mm
long, 0.19 - 0.26 (0.23) mm wide; posterior testis 0.25 - 0.35
(0.29) mm long, 0.21 - 0.28 (0.24) mm wide. Ovary sinistral,
pretesticular, postacetabular, spherical to oval, with smooth
edges, 0.17 - 0.21 (0.19) mm long, 0.14 - 0.19 (0.17) mm wide;
seminal receptacle spherical, immediately postero-medial to
ovary; Laurer's canal originating in ootype region between
seminal receptacle and common vitelline duct; uterus extending
posteriad to end of body, in numerous intercecal and post-
testicular transverse loops, filling body posteriad to
intestinal ceca; metraterm muscular, ventral to cirrus sac,
shorter than cirrus sac, surrounded by gland cells. Vitellaria
follicular, lying dorsal, lateral, and ventral to ceca,
extending along sides of body from level of ovary to posterior

third of hindbody, dorso-medially confluent posterior to testes, emptying into single vitelline duct on each side (Figs 17, 18). Excretory bladder I-shaped, extending to just posterior to level of testes; flame cell pattern 2(3+3+3)(3+3+3) = 36. Eggs 45-53 (50) μm long.

HOST : <u>Rana</u>   <u>pretiosa</u>   (natural   infections),   <u>R.</u> <u>cascadae</u> (laboratory[1]   infections),   <u>Bufo</u>   <u>boreas</u> (laboratory[2] and natural[3]   infections).

SITE OF INFECTION: Small intestine
TYPE LOCALITY: Bothell, King County, Washington State
SPECIMENS: USNM Helm. Coll. No. 29903, holotype; no. 29904, paratypes. Nos. 9025-9026, holotype and paratype, respectively, of <u>H.</u> <u>utahensis</u>. Additional specimens collected from <u>R.</u> <u>pretiosa</u> in southern British Columbia.

<u>REMARKS</u>: This genus has been monotypic since Waitz (1959) synonymized <u>H.</u> <u>utahensis</u> Olsen, 1937 with <u>H.</u> <u>intestinalis</u> Lucker, 1931. The character states cited by Waitz as justification for a synonymy are supported by the present redescription. These are: the length/width ratio, the shape of the ventral sucker, a bipartite seminal vesicle, tandem testes, and the position of the vasa efferentia, vas deferens, and Laurer's canal. Additional characters are reported herein that were observed from the examination of living and fixed specimens collected from <u>R.</u> <u>pretiosa</u> in southern British Columbia. These consist of: (a) medial glands at the level of the pharynx and esophagus, with ducts entering the prepharynx dorso-laterally; (b) the absence of a vas deferens in young adults; and (c) the absence of the anterior vitelline fields and their associated ducts.

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[1] [2] Present study.

[3] Waitz (1959).

EMENDED DIAGNOSIS OF GLYPTHELMINS

In conjunction with the synonymization of Haplometrana and Glypthelmins, the generic diagnosis of Glypthelmins must be emended so as to include species with tandem paired testes. The diagnosis includes the results of the present study and those of Brooks (1977). Emendations are underlined.

Glypthelmins Stafford, 1905, emend. from Sullivan (1976)

SYNONYMS: Margeana Cort, 1919
Haplometrana Lucker, 1931
Choledocystus Pereira and Cuoculo, 1941
Rauschiella Babero, 1951
Reynoldstrema Cheng, 1959
Repandum Byrd and Maples, 1963

DIAGNOSIS: Plagiorchioidea: Body elongate, cylindrical to subcylindrical. Tegument spined or scaled. Oral sucker subterminal. Ventral sucker medial, in anterior half of body. Pharynx well-developed. Esophagus present. Cecal bifurcation midway between pharynx and ventral sucker; ceca long, reaching to posterior quarter of body. Testes postacetabular, symmetrical, oblique, or tandem in position. Cirrus pouch elongate, usually overlapping ventral sucker. Ovary pretesticular, close to ventral sucker, sinistral or dextral; seminal receptacle present; Laurer's canal present, originating in ootype region between seminal receptacle and common vitelline duct, or distal to common vitelline duct. Uterus transversely coiled, reaching to posterior extremity of body; uterine coils intercecal or extracecal, pretesticular or not; metraterm muscular or not, surrounded by numerous gland cells. Genital pore medial, between ventral sucker and cecal bifurcation. Vitellaria follicular, in lateral fields of body, lying dorsal, lateral and ventral to ceca, may intrude mediad, dorsally or ventrally. Longitudinal extent of vitellaria variable, ranging from level of pharynx to posterior extremity of body. Excretory vesicle I-shaped or Y-shaped. Parasitic in intestine, rarely gall bladder, of anurans.

TYPE SPECIES: Glypthelmins quieta (Stafford, 1900), Stafford, 1905.

APPENDIX E - THE LIFE CYCLE OF GLYPTHELMINS CALIFORNIENSIS

From 1983 to 1986, I conducted surveys of the populations of snails in the ponds and streams in Langley, British Columbia, where specimens of R. aurora infected with G. californiensis had been caught. Five species of snails were examined (identifications based on Clarke, 1981): Physa gyrina Say (70 specimens), P. propinqua Tyron (238 specimens), P. lordi Baird (24 specimens), Stagnicola elodes (Say) [= Lymnaea palustris (Muller)] (193 specimens), and Pseudosuccinea columella (Say) (46 specimens). This last species is indigenous to eastern North America and has been introduced to the western part of the continent (Clarke, 1981). The only cercariae obtained from these field studies were echinostome cercariae and furcocercous cercariae. Accordingly, attempts were made to infect snails with the eggs of G. californiensis in the laboratory. The results provided information on the life cycle, miracidia, daughter sporocysts, and cercariae of this species.

MATERIALS AND METHODS

Uninfected snail populations were established by raising second generation stocks of Physa gyrina, P. propinqua, Stagnicola elodes, and Pseudosuccinea columella in tanks of dechlorinated water, containing boiled lettuce, stems of Elodea, and crushed snail shells. Snails were fed overnight on eggs of G. californiensis that had been teased out of mature worms and had been allowed to sit in a mixture of filtered pond water and the host frog's feces for at least a week. In addition to the four species of snails reared in the laboratory, miracidial hatching behavior was also studied in specimens of the planorbid snail, Gyraulus circumstriatus Tryon, collected from the same areas of water. The developmental stages obtained were examined as living specimens, stained with Neutral Red or Nile Blue vital stains, and immobilized with slight coverslip pressure. Miracidia and sporocysts were placed in 0.3% saline, while cercariae were placed in filtered pond water. Drawings from light microscope examinations were made with the aid of a drawing tube.

RESULTS

EGG AND MIRACIDIUM (Fig. 48c): Eggs range in length from 42 to 50 μm, with a mean length of 45 μm. They are light brown in color, operculated, and contain a fully developed miracidium when either oviposited or removed from the distal part of the uterus. In all of the species of physid, lymnaeid, and planorbid snails examined, it was found that after an overnight feeding of eggs, followed by the collection of the snails' feces over the next 12 hours, miracidia had emerged from approximately 30% of the eggs in the feces. Eggs kept in filtered pond water

for up to four months at 15°C still showed this hatching activity.

Morphological examinations were done on miracidia obtained from the gut contents of specimens of P. propinqua one hour after feeding eggs to the snails. The miracidia range in length from 40 to 47 μm, with a mean of 44 μm. The surface is ciliated. An examination for epidermal plates was not conducted, but the plates' presence is suggested by the interuption of the ciliary pattern in the anterior and posterior regions of the miracidium. At the anterior end of the miracidium, which lies at the operculated end of the egg, there is a longitudinally striated apical papilla that can be protruded. Immediately posterior to the papilla are two large, irregularly rounded, penetration glands, the contents of which are granular. Lateral to these glands are two longer glands. These are spindle-shaped and run from the anterior region of the apical papilla to the posterior region of the miracidium. The contents of these glands are relatively more granular when the miracidia are within the eggs than when they are recovered from the intestine of the snail, suggesting that the glandular secretions are associated with hatching. At the posterior of the miracidium there are two large nucleated germinal cells and a single pair of flame cells.

SPOROCYSTS (Fig. 48e): Development beyond the miracidial stage occurred only in P. gyrina and P. propinqua. The mortality of infected snails was very high, reaching approximately 90% within the first week of infection. The sporocysts examined were obtained by crushing moribund snails that were still shedding cercariae. For this reason, observations on intramolluscan development are incomplete, in that it was not possible to look for two sporocyst generations or their site of development in the host. The sporocysts from which cercariae emerge lie loose in the digestive gland of the snail. They are elongate, ranging in length from 0.5 to 1.5 mm (Fig. 48e), containing 4 to 10 cercariae. There is no birth pore evident, and the cercariae escape through ruptures in the body wall of the sporocyst. This wall gives the appearance of having two layers, the inner one of which is the thicker. The poor condition of the sporocysts precluded further observations.

CERCARIA (Figs 48a,b,d): Specimens of P. gyrina and P. propinqua began shedding cercariae at 50 days after infection. No phototropism or periodicity was observed. The cercariae are active swimmers and are usually found near the surface of the water in the collecting vessel.

DESCRIPTION: Distomate xiphidiocercaria. Body 0.47 - 0.51 (0.49) mm long, 0.26 - 0.32 (0.30) mm wide at ventral sucker. Tail 0.45 - 0.49 (0.48) mm long, with extensive dorso-ventral finfold; ventral portion of finfold running nearly entire length of tail, dorsal portion running along posterior third of tail. Narrow scales present on tegument to posterior of body. Oral sucker subterminal, bearing stylet. Stylet clear and rounded at base, tapering to a point, with slight shouldering; 24 - 26 (25) μm long (Fig. 48b). Ventral sucker medial, postequatorial. Prepharynx present. Pharynx muscular, approximately one-half width of oral and ventral suckers. Cecal bifurcation immediately anterior to ventral sucker; ceca terminating at posterior end of body. Genital primordia differentiated into testes, ovary, and cirrus sac (Fig. 48d). Testes symmetrical, antero-lateral to anterior chamber of excretory vesicle. Penetration glands composed of at least five glands on each side, occupying lateral areas of body from posterior border of pharynx to anterior border of ventral sucker, emptying at oral sucker near base of stylet. Excretory bladder dumbbell-shaped, contractile. Flame cell pattern = $2[(3+3+3)(3+3+3)] = 36$.

When placed in a dish of pond water with pithed adults of R. aurora, the cercariae continue to swim about actively. When a cercaria contacts the frog, it stops swimming, crawls over the surface of the skin for a short distance, and begins to penetrate the epithelium, losing its tail in the process. Once under the epithelium, the cercaria balls up and begins rolling actions. The elapsed time from contact with the frog to balling up beneath the epithelium is approximately 15 minutes. Contact with the frog does not inevitably lead to penetration. Some cercariae crawl over the skin for up to ten minutes, and then swim away. Penetration behavior with respect to tadpoles of R. aurora was not examined.

DEVELOPMENT IN RANA AURORA (Fig. 49): Attempts to raise uninfected R. aurora to adulthood in the laboratory were unsuccessful. Frogs were therefore collected from areas where no parasitism by G. californiensis had been encountered. These areas were the streams associated with Hicks and Moss Lakes, Sasquatch Provincial Park, British Columbia (approximately 20 km NE of Chilliwack). Frogs were either kept separate after exposure to cercaria in the laboratory, or were fed portions of the skin of another adult of R. aurora in which cercariae had encysted.

At the end of the twenty-day period during which these infections were studied, both groups of frogs contained young adults of G. californiensis in the anterior region of the small intestine (Fig. 49). These worms are approximately 1 mm long. All of the major organ systems are developed. Medial glands are evident in the region of the prepharynx and pharynx. The uterus extends posteriad beyond the ends of the intestinal ceca in a single, intercecal loop. A lack of infected frogs precluded the study of development beyond twenty days after infection.

DISCUSSION

My incomplete study of the intramolluscan development stages of G. californiensis prevents a full comparison to life history studies of G. quieta, by Leigh (1946), Rankin (1944), and Schell (1962a), and of H. intestinalis, by Schell (1961). In both of these species, the mother and daughter sporocysts possess two walls, the outer of which is derived from the basement membrane of the snail intestine (Schell, 1961, 1962a). As noted by Schell (1962b), although this surrounding layer of host origin, termed a paletot, occurs in the sporocyst generations of many plagiorchioids (Biehringer, 1885; Leuckart, 1863, 1886-1901; Cort and Olivier, 1943; Cort and Ameel, 1944; Cort et al., 1952), it does not always form from the same source. Schell (1962a) noted that the similarities in the host response that produces the paletots in G. quieta and H. intestinalis appear to result from the depth to which the miracidia penetrate the wall of the host' intestine. On the basis of this character and similarities in cercarial and sporocyst morphology, Schell (1962a) considered G. quieta and H. intestinalis to be more closely related to one another than they are to Telorchis bonnerensis Waitz, 1960, another plagiorchioid parasite of amphibians that develops in physid snails (Schell, 1962b). Furthermore, I infer from Schell's discussion that, based upon the data of Cheng (1961), he included G. pennsylvaniensis as a close relative of G. quieta and H. intestinalis. Sullivan and Byrd's (1970) work on G. pennsylvaniensis offers limited evidence that supports this inclusion.

In light of these studies, and in light of the additional observation by Schell (1962a) that the mother sporocysts of H. intestinalis develop in the wall of the stomach, cecum, and foregut of the snail, while those of G. quieta develop in the wall of the midgut, it is unfortunate that my work on G. californiensis could not examine these same properties. Limited observations on the daughter sporocysts show that their size and shape are similar to those of G. quieta (as reported by Leigh, 1946; Rankin, 1944; Schell, 1962a), H. intestinalis (as reported by Schell, 1961), G. pennsylvaniensis (as reported by Cheng, 1961; Sullivan and Byrd, 1970), and T. bonnerensis (as reported by Schell, 1962b). A second layer is apparent, but because of the poor condition of the sporocysts, it could not be determined whether this was a paletot or the result of delamination of the inner layer.

The cercaria of G. californiensis infects the anuran host in the same manner as do the cercariae of G. quieta and H. intestinalis, in that they all penetrate and encyst in the skin of the adult frog, which infects itself upon eating the shed skin. The morphology of the cercaria of G. californiensis places it in the Cercariae Ornatae group. From surveys of physid snails in Illinois, Miller (1936) reported Cercaria mesotyphla Miller, 1935 as another member of this group, and

concluded that it was the cercaria of G. quieta. The studies of Leigh (1946) and Rankin (1944) confirmed this. In addition to the characters described by Miller (1936), Leigh (1946) also observed medial glands, pharyngeal glands, and recognizably differentiated gonads in the cercariae of G. quieta. I observed these same characters in the cercariae of this species that I obtained during the present study (Chapter IV, LIFE HISTORY DATA). It was also observed that, although the difference is not as marked as in the adults, the tegumental projections are more scale-like than spine-like (i.e., more rectangular than tapering).

The cercaria of G. californiensis differs from that of G. quieta only by its lack of medial and pharyngeal glands. Although the former are present in the adults of both species, I could not observe them in the available specimens of G. californiensis. All of the other characters observed in the cercaria of the two species are the same. These cercariae differ from the cercaria of H. intestinalis with respect to (a) their symmetrical testes, rather than the oblique to tandem testes of the latter species (Olsen, 1937), and (b) a scaled, rather than spined, tegument. In my observations of the cercariae of H. intestinalis (Chapter IV, LIFE HISTORY DATA), I found that the tail fold is not strictly ventral, as described by Olsen (1937), but that it also has a shorter, shallower, dorsal portion that often lies against the surface of the tail.

APPENDIX F - CODING MULTISTATE CHARACTERS

This is a technical note with three parts. The first
demonstrates four multistate character coding methods, the
second compares their properties, and the third examines some of
their uses in phylogenetic studies. This last part is primarily
concerned with the use of parasite data to infer host
relationships. A multistate character is herein considered to
be any set of more than two organic or inorganic states that
have, through some process, transformed from one into the other.
The order of transformation of these states will describe a
multistate tree. This definition is broad enough to include
phenomena such as organic character evolution, changes in
ecological properties, host-parasite coevolution, and
biogeographic events.

Whatever type of transformation is involved, there are two
aspects to dealing with multistate characters. The first is
that of determining the order of transformation. In
evolutionary studies, the transformations have not been
observed, and so their order must be inferred. This note is not
primarily concerned with these methods of inference. The use of
parsimony analysis and outgroup comparisons in such procedures
has been addressed by Mickevich (1982), Fitch (1971), Swofford's
PAUP program, and by Swofford and Maddison (in review) (see
Chapter III, THE INFERENCE OF PHYLOGENETIC RELATIONSHIPS). If
one wants to construct a multistate tree by other criteria, such
as excluded transformations, functional restrictions, etc., it
is necessary to justify that tree's differences from a tree
constructed with parsimony techniques that place no restrictions
on the order of character transformation.

The second aspect of dealing with multistate characters,
and that with which this note is concerned, is that regardless
of how the order of character state transformations in a tree is
determined, the tree's shape must be unambiguously represented
when using that tree in further phylogenetic studies. The goal
is always the same: to represent the multistate tree so that the
relationships among its states can be used to study the
relationships among the entities (taxa, land masses, etc.)
possessing those states.

CODING MULTISTATE CHARACTERS

The first method is Additive Binary Coding (ABC), developed
by Farris et al. (1970). I refer to the second method as
Redundant Linear Coding (RLC), and to the third method as
Nonredundant Linear Coding (NLC). The RLC method was explained
to me by Mary Mickevich (pers. comm., 1983), and the NLC method
by David Swofford (pers. comm., 1984). The fourth method,
internal rooting, has specialized applications for coding

basally dichotomous multistate series.

A multistate tree can have various configurations. There may be observed states at only the terminal branches (Fig. 50a), as occurs when a parasite phylogeny is used as a character of the hosts (see below). Or, a tree may have observed states at the nodes as well (Fig. 50b), as occurs with a transformation series for character evolution, ontogenetic stages, or land mass break-up. The tree shape of this second type of multistate character may be complex (Fig. 50b), linear (Fig. 50c), or basally dichotomous (Fig. 50d). I will demonstrate the coding procedures with the contrived tree in Figure 51a. This tree has unspecified nodal states on its left side, and specified nodal states on its right side. For each of the three main coding methods, this tree will be coded, a data matrix will be made, and the tree will be reconstructed from the matrix in order to demonstrate the retention of the topological information. When examining these reconstructions it should be noted that the trees in Figures 51a and 51b are equivalent: a state collapses to the node beneath when that state is not characterized by an autapomorphy.

Additive Binary Coding - Every node on the tree is labelled. In the example in Figure 52a, there are nine states to be connected: five terminal branches and four internal nodes. The tree is represented in a matrix (Fig. 52b) with nine columns of binary characters. The matrix values are determined by assigning a "1" code to every state lying along the minimum path between each state and the base of the tree. For example, the minimum path from A to D passes through i and ii, and so only these four states receive a "1" in the code entered horizontally in the matrix. When reconstructing a tree from the matrix, with no reference to the original tree in Figure 52a, one first builds a polytomy for all nine states onto which the character distributions are mapped (Fig. 52c). This produces the original tree (Fig. 52d).

Redundant Linear Coding - Each terminal branch on the tree becomes the terminal state in a linear transformation series constructed as the minimum path from the base of the tree. Unspecified nodal values are not labelled, and nonterminal branches become intermediate transformation states. Every transformation series starts as a "0" state at the bottom of the tree and changes at every nonterminal branch as it passes up the tree to the terminal state (Fig. 53a). The data matrix in the example (Fig. 53b) has five multistate characters, one for each terminal branch. The matrix values are entered horizontally, and are determined by assigning the most derived state of every character passed through. For example, the minimum path from A to the base of the tree contains states 1, 2, and 3 of character 1, states 1 and 2 of character 2, state 1 of character 3, and state 0 of characters 4 and 5. The code for A is thus 32100. The tree constructed from the matrix begins as a polytomy (Fig. 53c), and finishes as the original tree (Fig. 53d).

Nonredundant Linear Coding - This method is similar to the RLC method, except that not all of the transformation series change states at every nonterminal branch they pass through. There are major and minor axes. In the tree in Figure 54a, for example, there is a four state series (character 1) running from the base of the tree to A. This is the major axis. B is part of a minor axis, and is assigned state 1 of character 2, rather than state 3 as it was with the RLC method. It should be noted that the major axis is arbitrarily set, and need not even be the longest multistate series. For example, the left side of the tree could be coded as in Figure 54e. There appear to be no reasons for preferring one major axis over another. The matrix in the example has five multistate characters, one for each terminal branch (Fig. 54b). As in the RLC method, the values are determined by assigning the most derived state of every character passed through. For example, the minimum path from A to the base of the tree contains state 3 of character 1, and state 0 of the other four characters. The code for A is thus 30000. Figure 54c shows the polytomous tree constructed from the matrix, and Figure 54d shows the original tree.

Internal Rooting - This is an additional coding method that can be used when a transformation series contains no unspecified nodal values and consists of a single basal bifurcation (Fig. 55a). This tree could be coded with the ABC (Fig. 55b), RLC (Fig. 55c), or NLC (Fig. 55c) methods (the latter two give the same matrix). Or, it can be assigned a single linear multistate character that starts at one terminus of the tree, runs through the base, and ends at the other terminus (Fig. 55d). This series is rooted internally by specifying the basal state as the outgroup in the data matrix (C in this example). The diverging numerical values of the coding will describe the basal bifurcation.

COMPARISONS

All of the coding methods described above are capable of unambiguously representing a multistate tree. This is not a trivial capability, for it is possible to code a multistate tree so that its Consistency Index (Kluge and Farris, 1969; Farris et al., 1970) is less than 1.0. This can occur when the properties of the character states, which may have already been utilized in constructing the multistate tree, are allowed to influence the coding of the representation of the tree. Glen and Brooks (1985) give an example in which a basally bifurcating multistate tree might be inadvertently coded with a linear code because of a numerical progression in the character states. The ABC, RLC, and NLC methods can represent trees of various configurations. In its specialized application, the internal rooting method has the advantage of occupying a single matrix column, compared with two columns in the RLC and NLC methods, and three or more columns in the ABC method. A disadvantage is that (1) it produces a plesiomorphic state with other than the standard "0" code, which could be confusing in an otherwise standardly coded

matrix of other characters, and (2) one arm of the transformation series will have a code with values that decrease (2-1-0 in Fig. 55) even though no reversals are being postulated. This would, for example, preclude its use with a Camin-Sokal parsimony tree-building algorithm.

There are three things to note about the ABC method. (1) It assigns a single character state change to each branch, (2) only the codes for the terminal states (A, B, C, F, and G in Fig. 52) are needed to reconstruct the topology of the tree (Fig. 5e), and (3) the number of new binary characters created is the number of terminal states plus the number of nodes to be connected. Because of the third property, ABC matrices for all but the simplest trees can be quite large. Because of the second property, some of this size comes from what might be unnecessary codings of internal nodes, depending on whether or not the nodal states are observed (e.g., in Fig. 52, E is observed, while i and ii are not).

Two things to note about the RLC method are that (1) it assigns more than one character state change to some nonterminal branches, and (2) the matrix is smaller than that produced with the ABC method: there are only as many new multistate characters as there are terminal branches on the tree. The RLC method can be seen as a methodological precursor to the NLC method. However, its redundant coding can produce trees with areas of misleadingly high synapomorphic support. That is, internal branches receive another instance of character support every time they are included in the minimum path between a terminal branch and the base of the tree. It is thus the NLC method that appears to be preferable. It avoids the unnecessary node labelling and the resulting large matrix size of the ABC method, and it avoids the redundant branch coding and the resulting unjustified weighting of the RLC method.

SOME USES OF MULTISTATE CODING METHODS

Intrinsic Characters - I refer here to an organism's properties that are manifested during its ontogenetic development, so as to make a distinction with its parasites, which will be discussed next. If such an intrinsic character is distributed among the study taxa in a binary manner, then its order of transformation is established when it is polarized. If it occurs in a multistate manner, then its order is not established with the determination of the plesiomorphic state. A phylogenetic analysis will often contain both binary and multistate characters. An investigator may want to (1) see the effect of using a multistate tree suggested by functional considerations, previous studies, etc.; or (2) he may want to produce a multistate tree with parsimony techniques such as Farris Optimization (Farris, 1970), Transformation Series Analysis (Mickevich, 1982), and Unordered Character Analysis (Swofford, PAUP, version 2.4, 1985), and then incorporate that tree into the character matrix for the taxa studied.

Figure 56a shows a cladogram for five taxa produced by Wagner parsimony analysis of seven binary characters. These are represented in the first seven columns of the data matrix in Figure 56b. There is also a multistate character (i - iv) distributed among the taxa as shown in Figure 56a. Figure 56c shows a possible tree, determined by whatever means, for that character. The tree has been coded with the NLC method, which gives the multistate matrix in Figure 56d. This information is then put into the character matrix in Figure 56b by using the multistate matrix as a source from which the appropriate horizontal code is taken, depending on which state a particular taxon possesses (i.e., the entire multistate matrix is not necessarily used). Figure 56e is the cladogram constructed from all nine characters. With either the ABC or NLC method, the codings for the multistate tree will have no greater weight in the cladogram than will the codings for the other characters.

Parasites as Characters of Their Hosts - There are a number of operations involved in assessing the degree of host-parasite coevolution, and the reader should refer to Brooks (1979a, 1980, 1981a, and 1985) for a fuller discussion. Applications can be found in Brooks and Mitter (1984), Collette and Russo (1985), Cressey et al. (1983), Glen and Brooks (1986), and Mitter and Brooks (1983). One can compare phylogenetic trees constructed from the intrinsic organic characters of hosts and parasites, but this necessitates the designation of either the host tree or the parasite tree as the standard of comparison (i.e., as the more likely of the two to be correct).

Alternatively, based upon the aforementioned homologous and homoplasious nature of host-parasite associations, one can treat the phylogenetic tree for the parasites like a multistate character tree of the hosts. Hosts are assigned the multistate code associated with the parasite they harbor, and a host phylogeny is generated. This tree can then be compared to host phylogenies produced using other parasite data (i.e., looking for consilience with other taxonomic groups of parasites), or it can be compared to a phylogeny produced through an analysis of intrinsic organic characters of the hosts themselves. In all of these approaches, which are complementary and not mutually exclusive, the goal is to distinguish homologous from homoplasious parasite associations, and to use the former for information on host relationships. In this note I will demonstrate the representation of a parasite phylogeny as a multistate tree in light of my earlier comments on coding methods. Brooks (1981a) showed the use of the ABC method, and I feel that further explication would be helpful. I will also discuss coding procedures for the occurrence of more than one parasite taxon per host.

I begin by contrasting the multistate approach with another method of inferring host relationships from parasite data. This groups hosts on the basis of a common presence or absence of a

parasite (see Glen and Brooks, 1986, for additional discussion).
If overall similarity criteria are used, the result has the
usual shortcomings of using phenetic analysis in evolutionary
studies (see Ernst and Ernst, 1980, and the reply of Brooks,
1981b). Provided that either the presence or absence of the
parasite is taken to be plesiomorphic, one can instead cluster
by special similarity. As noted by Glen and Brooks (1986), this
plesiomorphy can be set as simply an a priori assumption, or by
reference to whether or not the outgroup of the hosts possesses
the parasite (this would, of course, necessitate the elimination
of ambiguity about the state of the outgroup node). While it is
true that this third approach introduces an accountability to
evidence that is missing from the other two, it requires a
previous phylogenetic analysis to have established an outgroup
for the hosts. It thus does not rely exclusively on parasite
data to infer host relationships.

Figures 57a-d show the limits of presence/absence analysis.
In Figure 57a, there has been retention of the ancestral
parasites after each speciation event with the hosts. The
presence of a parasite taxon (capital letters) in a host taxon
(roman numerals) is indicated with a dot. By treating each
parasite as an independent binary character, and by considering
the presence of the parasite to be apomorphic, the host
phylogeny in Figure 57b is produced. But if there has been no
such retention, as in Figure 57c, then presence/absence analysis
will produce an unresolved tree with nothing but autapomorphies.
There are, of course, intermediate degrees of retention that
would give partial resolution.

Alternatively, evolutionary relationships can be taken into
consideration by first doing a phylogenetic analysis of the
parasites, and then treating that cladogram as a multistate
character tree of the hosts. Such a tree is constructed with a
source of information that is not available in standard
multistate analyses, namely, the cladistic analysis of the
parasites themselves. The characters have characters, so to
speak, that can be used to infer their relationships. Because
of this property, a parsimony analysis of the order of
transformation of the multistate characters can be done
intrinsically, without having to refer to a tree topology
determined by a preceding analysis of binary characters at that
level. At the same time, it must be noted that this additional
source of information introduces an additional source of error.
Incorrect inference of host relationships can be made because
(1) the parasite cladogram is correct but there is homoplasious
distribution of the parasites among the hosts, (2) the parasite
cladogram is incorrect despite strictly homologous distribution
of the parasites among the hosts, or (3) the parasite cladogram
is incorrect and there is homoplasious distribution of the
parasites among the hosts.

Figures 57e-i illustrate the multistate approach with
parasite data. Assume that a phylogenetic analysis of the four

parasite taxa in Figure 57c produces the cladogram in Figure
57e. This can be represented with the NLC matrix in Figure 57f.
Using this matrix as the source of the horizontal code for each
host taxon, the host matrix in Figure 57g is produced. This
matrix gives the host relationships in Figure 57h, for which the
corresponding parasite relationships are shown in Figure 57i.
These latter relationships are the same as those in Figure 57e.
Thus, the information in the transformation series is retained
in the tree that is constructed with that series as its only
source of information.

<u>Inclusive ORing</u> - Presence/absence analysis relies upon there
being a hierarchy of multiple occurrences of a parasite taxon in
different host groups to produce a resolved host tree (Fig.
57a). Multistate analysis is most straightforward when there
are no multiple occurrences of any sort. This latter condition,
in which the host simultaneously possesses more than one state
in the multistate tree, creates a coding situation not usually
encountered with standard multistate data of intrinsic organic
characters (although it might arise with serial homologues, for
example). There is currently one method that has been applied
in such a situation. Known as inclusive ORing (see, e.g., Copi,
1968, p. 216), it has been used with the ABC method implicitly
by Brooks (1981a: Figs 20-21) and Glen and Brooks (1986), and
explicitly by Cressey <u>et</u> <u>al</u>. (1983), and Collette and Russo
(1985). Consider Figure 58a, a parasite phylogeny with the
hosts (roman numerals) indicated. Host IV has the sister
parasite taxa C and D. I use the ABC method for the tree (Fig.
58b), for clarity.

As Cressey <u>et</u> <u>al</u>. (1983) note, when inclusively ORing more
than one set of characters (the horizontal codes for parasites C
and D in this example), a character state is said to be present
in the union of the sets so long as it is present in at least
one of the sets. (In formal logic, an inclusive OR statement
uses the word "or" in its weak sense, as an AND/OR statement.
This is in contradistinction to exclusive ORing, in which
something can be one state or another, but not both.) Thus, the
two horizontal codes assigned to host IV in the host matrix in
Figure 58c can be compressed into the single code shown in the
host matrix in Figure 58d. Either one or two occurrences of the
"1" code in a column result in the union being set to "1". The
compressed matrix produces the tree in Figure 58e, with the
implied parasite relationships in Figure 58f. Parasite C is
inferred to have evolved in the same speciation event in which
the common ancestor of hosts III and IV evolved, and then to
have been retained by hosts III and IV (i.e., like an ancestral
trait) when parasite D evolved with host IV. (See Brooks,
1981a, pp. 240-242 for comments on inferring such ancestor-
descendant relationships.)

There are, however, data configurations in which inclusive
ORing will distort phylogenetic information and thus lead to
incorrect inferences of host relationships. This occurs because

the most apomorphic code is always given precedence in determining the compressed code, no matter what the distribution or number of the more plesiomorphic codes might be. The logical basis for this decision is that only the apomorphic codes are considered to represent real properties of organisms ("true" statements) that can be used as grouping criteria. The plesiomorphic codes are considered to represent the absence of a property ("not" statements), which, clearly, cannot be used to group. Thus, with the ABC method, a "1" code takes precedence over any number of "0" codes. And, with the RLC and NLC methods, the most derived state of a multistate series takes precedence over all preceding states.

This procedure does not create any problems when a parasite is shared by sister host taxa, as in Figure 58, because it produces a synapomorphy for that clade (character 5, parasite C, in Figs 58e-f). Problems arise, however, when a host group possesses parasites that are phylogenetically distant from one another, as would occur when there has been symplesiomorphic retention of some parasites and host-switching of others. In such a case, inclusive ORing will lose the kinship information contained in the distributions of the more plesiomorphic codes. If, for example, the colonizing parasite taxa are more derived (i.e., a "1" code) than the coevolved parasite taxa, then the coevolutionary pattern, and thus the data for a correct inference of host phylogeny, will be lost.

This is demonstrated in Figure 59a, in which parasite taxa C, D, and G are present in host III. The "1" code that G, the more derived parasite group, receives in the ABC matrix (not shown) overides the "0" codes that the more plesiomorphic parasite taxa (C and D) receive. The compressed host matrix (Fig. 59b) gives the phylogeny in Figure 59c, in which taxon III has been misplaced. (I say that it is misplaced because there are two characters, C and D, placing it between II and IV, and one character, G, placing it with VI. This occurs whether C and D are paraphyletic or monophyletic.) The parasite relationships implied by this tree (Fig. 59d) are inconsistent with those in the initial cladogram in Figure 59a. (For example, G is placed as the ancestor of C and D.) Restating this in a context broader than that of host-parasite coevolution: the pattern of relationships among the group, "capital letters", which here was used as the only source of information on the relationships of the group, "roman numerals", is not retrievable from the inferred roman numeral cladogram. The data have been distorted by the method with which they were handled.

CONCLUSIONS

The purpose of this study has been to explicate the usage of some coding methods for multistate characters, and to examine their utility in certain types of multistate character analysis. My concerns come primarily from the perspective of studying host-parasite relationships. A major part of such studies is

examining the host relationships that are indicated by a parasite phylogeny. This necessitates the use of a coding method that does not distort the parasite tree (as can inclusive ORing) or bias the results (as can the RLC method). For these reasons, we find the ABC and NLC methods to be preferable. The ABC method produces coding which is perhaps a little easier to follow (hence its use in Figures 58 and 59), while the NLC method produces a smaller and more efficient code.

APPENDIX G - SOME PROPERTIES OF THE CONSISTENCY INDEX AND THE F-
                                                        RATIO

CONSISTENCY INDEX

The Consistency Index (CI) (Kluge and Farris, 1969) is
calculated as the sum of the ranges of all characters in the
data set (i.e., the minimum tree length required to explain the
data) divided by the number of evolutionary changes, or steps,
postulated on the tree (i.e., the tree length). A value of 1.0
indicates a perfect fit of the tree to the data. [1] A data set of
four binary characters, say, would have a total range of four
steps [(0 - 1) x 4]. If a tree inferred from those data posited
five steps, its CI would be 4/5 = 0.80.

The CI does not take into account the distribution of
characters on a tree or in a data set. The range of a binary
character, for example, is counted as "one" in a data set
whether the derived state is present in one or many taxa, and
its appearance on the tree is counted as "one" whether it is
shared or unique. Because of this, synapomorphies are not
distinguished from autapomorphies. Figures 60a-c show three
trees, constructed from three data sets with the same first
seven binary characters, and with different distributions of two
other binary characters. All three trees have a CI of 0.75
(9/12), even though characters 8 and 9 are autapomorphic in 60a,
internally synapomorphic in 60b, and basally synapomorphic in
60c. Consider two systematists working on the ABCD clade, one
of whom has constructed tree 60a, the other of whom has
constructed tree 60c. A comparison of CI values alone will not
indicate that one person has found two autapomorphies, thus
corroborating the monophyly of that taxon, but adding no support
to the proposed genealogical groupings, while the other has
found two synapomorphies corroborating the monophyly of the
entire clade.

The CI can be increased by the addition of autapomorphies.
The tree in Figure 60d presents the first seven characters used
in Figures 60a-c. Its CI is 0.70 (7/10). The addition of
characters 8 and 9 as autapomorphies in Figure 60a increases the
CI to 0.75. The discovery of one additional autapomorphy for
each of the taxa would further increase the CI to 0.81 (13/16).
This apparent increase in optimality occurs although the degree
of synapomorphic support for the postulated genealogy remains
the same. The sensitivity of the CI to autapomorphies could be
eliminated by modifying the measure so as to not count any non-

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[1] CI values are often reported as percentages.

homoplasious characters on terminal branches. Some systematists have begun to report both modified and unmodified CI values with their results (e.g., Fink, 1985). The phylogenetic trees presented in this thesis (Chapter III) are reported with both values. [1]

So long as the number of steps of character evolution are equal, the CI will not distinguish between parallelisms and reversals. Character 5 in Figure 60d, for example, is explained as a parallelism evolving in two steps: on the terminal branch of taxon A, and on the nonterminal branch bearing taxa C and D. It could also be explained as a reversal in two steps: appearing at the base of the tree, and reversing in taxon B. The CI thus offers no way of choosing among equal-length trees with alternative interpretations of homoplasious character evolution. Of course, if a postulate of parallelism requires more steps than one of reversal, or vice versa, the CI will distinguish the shorter tree. For this reason, there will not be equal CI values for postulates of reversal and convergence in a homoplasious character in polyphyletic taxa (e.g., if in Figure 60d there were a character common to Taxa A and D).

F-RATIO

The F-Ratio is derived from the f-statistic of Farris (1972), and is a comparison of Manhattan distance matrices constructed from the original data set and an inferred tree. Differences in F-Ratio values can be used to choose between trees of equal CI. Figure 61 demonstrates the calculation. From the character matrix (Fig. 61a), a matrix of the phenetic distances between each pair of taxa is constructed (Fig. 61c). These values are the fixed, observed distances between taxa. For any tree inferred from the character matrix (e.g., 61b), a matrix of patristic distances between pairs of taxa is constructed (Fig. 61d). These patristic values are the variable, postulated distances between taxa. If the tree fits the data perfectly, its postulated patristic distances will be the same as the observed phenetic distances. If there is homoplasy, the patristic distance will exceed the phenetic distance.

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[1] This modification of the CI requires a distinction between different types of autapomorphic characters, that is, characters that appear on the terminal branches of a tree. As homoplasies, they may appear one (a single reversal) or more times (paralellisms, convergences, and multiple reversals) on the entire tree. As homologues, they appear only once. In order to give a truer measure of the support for a tree, a modified CI should eliminate only the non-homoplasious autapomorphies. No homoplasy, be it on terminal or nonterminal branches, should be eliminated.

The sum of the difference matrix for the phenetic and patristic distance matrices is the f-statistic. This is normalized with division by the sum of the phenetic distance matrix, giving the F-Ratio. [1] For the example in Figure 61, homoplasy in character 5 results in an f-statistic of 2 (an extra 2 steps in the patristic distance between taxa A and D), and an F-Ratio of 7.14 [(2/28) x 100]. As with a CI less than 1, an F-Ratio greater than 0 indicates a less-than-perfect fit of the tree to the data.

Unlike the CI, the F-Ratio is sensitive to the distribution of characters among taxa, but only under certain conditions. It can distinguish between equal-length postulates of parallelism and reversal, as well as between autapomorphies and internal synapomorphies. It cannot, however, distinguish between autapomorphies and basal synapomorphies. This is because in its pairwise comparison of taxa it treats relationships as undirected, rather than directed, trees. It thus discriminates between characters on terminal and nonterminal branches, rather than between synapomorphies and autapomorphies. Basal synapomorphies are treated in the same manner as autapomorphies in patristic distance calculations because both occur on a terminal branch of the tree. These two types of character distribution will give different matrices, but the f-statistic and the F-Ratio will be the same.

This property is demonstrated in Figure 62. The F-Ratio of tree 62a is 18.18 (2/11: extra steps in the AB distance). The value drops to 14.29 (2/14) when a fourth character is added as an autapomorphy (Fig. 62b) (thus showing that, as with the CI, the F-Ratio can be improved by the addition of such characters), and to 13.33 (2/15) when it is added as an internal synapomorphy (Fig. 62c). When it is added as a basal synapomorphy (Fig. 62d), however, the F-Ratio is once more 14.29 (2/14). The value for the internal synapomorphy is lower not because of a change in the amount of departure of the patristic distance from the phenetic distance (the f-statistics of all the trees are 2), but because of an increase in the denominator of the F-Ratio, that is, the sum of the phenetic distance matrix.

The F-Ratio cannot be used as the only criterion for choosing the prefered tree, for the tree with the lowest F-Ratio is not necessarily the shortest tree. This has also been noted by Swofford, in the documentation for PAUP (version 2.4, 1985),

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[1] The phylogenetics computer program PHYSYS (developed by J.S. Farris and M.F. Mickevich) gives the normalized value multiplied by 100 as the F-Ratio, while the phylogenetics program PAUP (developed by D. L. Swofford) simply gives the normalized value.

and in Swofford and Maddison (in review). Compare the trees in Figures 63a and 63b. The first is from Figure 61, but with character 5 interpreted as a reversal rather than as a parallelism. This adds a step to the tree and decreases its CI from 0.83 (5/6) to 0.71 (5/7). The F-Ratio increases from 7.14 to 28.57 (8/28: extra steps in the XB, XC, XD, and AD distances). In Figure 63b, a different tree for the same data has an even lower CI, of 0.56 (5/9), and an even higher F-Ratio, of 42.86 (12/28: extra steps in the XA, AC, BC, and CD, distances). In this case, then, both measures indicate a poorer fit to the data. But this agreement of CI and F-Ratio assessments does not always hold. Consider the trees in Figures 64a and 64b. The first postulates a parallelism in character 5, giving a CI of 0.80 (8/10). This tree is longer than the tree in Figure 64b, which postulates a reversal, giving a CI of 0.89 (8/9). The F-Ratios, however, rank the trees in the opposite order. The F-Ratio for 64a, the parallelism, is 5.61 (6/107: extra steps in the AB, AC, and BC distances), while the value for 64b, the reversal, is 7.48 (8/107: extra steps in the XD, XE, XF, and XG distances). Assessing trees on the basis of their F-Ratios alone would result in choosing a longer tree.

When choosing among equal-length shortest trees, the F-Ratio is not biased towards parallelism or reversal in a homoplasious character. Returning to character 5 in Figure 60d, the F-Ratio for the parallelism postulate is 30.00 (12/40: extra steps in the AC, AD, BC, BD distances), while for the reversal (character 5 appearing on the bottom branch, then being lost in taxon B) it is 25.00 (10/40: extra steps in the XB, AC, BC, and BD distances). The F-Ratio prefers the reversal tree. It is possible, however, to have trees with F-Ratios that prefer parallelisms over reversals, or that do not distinguish between either. In Figure 65a, a parallelism in character 4 gives an F-Ratio of 18.18 (4/22: extra steps in the AC and BC distances), while a reversal (Fig. 65b) gives a better value, of 9.09 (2/22: extra steps in the XD distance). In Figures 65c and 65d, taxon E has been added to the analysis, and the F-Ratios for parallelism and reversal in character 4 are equal, at 10.26 (4/39: extra steps in either the AC and BC, or the XD and XE distances). Finally, in Figures 65e and 65f, taxon F has been added, and the F-Ratio for a parallelism (Fig. 65e) is 6.45 (4/62: extra steps in the AC and BC distances) while the reversal tree (Fig. 65f) gives a poorer value, of 9.68 (6/62: extra steps in the XD, XE, and XF distances). The F-Ratio has this property because in the pairwise comparison of taxa, the more taxa there are on either side of a homoplasious character, the more times will that step appear in the patristic distance matrix, and the more will it contribute to departures from the phenetic distance matrix.

As with the CI, the F-Ratio can be improved by the addition of autapomorphies to an analysis. From Figure 61, the tree in Figure 61b has an F-Ratio of 7.14 (2/28). Adding one autapomorphy to taxon B reduces it to 6.25 (2/32); adding

another reduces it to 5.56 (2/36). This will also occur with the addition of basal synapomorphies, since they are also on terminal branches of the undirected tree. In addition, the F-Ratio can be improved by adding taxa to a tree. If a taxon with the characters of taxon C is added to the tree in Figure 61b, the F-Ratio decreases to 5.13 (2/39). If another taxon with the same characters is added, the F-Ratio decreases further to 4.00 (2/50). Because of the measure's distinction between terminal and nonterminal characters, the same improved fit can be produced by adding taxa identical to the outgroup. This creates more pairwise comparisons to be made, and increases the sum of the phenetic distance matrix.