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Phylogenetics of Lymnaeidae (Mollusca: Gastropoda) with Emphasis on the Evolution of Susceptibility to Fascioloides magna Infection

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Phylogenetics of Lymnaeidae (Mollusca: Gastropoda) with Emphasis on the Evolution of Susceptibility to *Fascioloides magna* Infection

(TITLE)

BY

Sarah Renee Joyce

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Abstract

Snails in Lymnaeidae serve as intermediate hosts in the transmission of many trematode species, including *Fascioloides magna* that is responsible for disease and death in domestic livestock in North America. Previous classifications of lymnaeid snails have relied primarily on morphological characters that exhibit high levels of homoplasy; thereby, impeding a sound assessment of relationships in this group. The present study provides a phylogenetic hypothesis for lymnaeid snails employing sequences from the internal transcribed spacer (ITS) region of nrDNA, and addresses the evolution of susceptibility to *Fascioloides* infection in lymnaeid snails. The final data set, comprising ten species of lymnaeid snails and one species of Physidae, included 1368 characters, of which 327 were parsimony-informative. Three major clades were recovered in neighbor-joining analyses that consisted of individuals of *Stagnicola caperata*, *Fossaria* s.s., and *Stagnicola* s.s. + *F. Bakerlymnea* sp. *Stagnicola caperata*, the main host of *F. magna* in Minnesota, revealed a closer alliance to *Fossaria* spp. than to other species of *Stagnicola*, suggesting that its placement in the stagnicoline sub-genus *Hinkleyia* is suspect. Members of *Fossaria* s.s., that have tricuspid first lateral teeth in the radula, were monophyletic to the exclusion of *F. Bakerlymnea*, a well-supported member of the stagnicoline clade. Therefore, our estimate of lymnaeid phylogeny supports the taxonomic scheme proposed by Baker (1911) that suggests members of *Bakerlymnea* be classified as stagnicolines based on their shared bicuspid dentition. Although a stagnicoline clade was strongly supported, there was low resolution of species within the clade. Log-determinent distances between species of *Stagnicola* s.s. were less than those observed between individuals of *Stagnicola caperata*, indicating that a region with higher

rates of evolution is necessary to determine relationships in this group. Susceptibility to *Fascioloides magna* infection is widespread in North American lymnaeid snails based on experimental infections. However, an examination of naturally infected intermediate hosts suggests that host status may be due to high exposure rates that result from close interactions between intermediate and definitive hosts.

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As buds give rise by growth to fresh buds,
and these, if vigorous, branch out and overtop
on all sides many a feebler branch, so by generation
I believe it has been with the great Tree of Life,
which fills with its dead and broken branches
the crust of the earth, and covers the surface with
its ever branching and beautiful ramifications.

Charles Darwin
On the Origin of Species

Introduction

Lymnaeidae (Rafinesque, 1815) is a group of primarily aquatic snails that are cosmopolitan in distribution, occurring in a variety of ecological habitats. The greatest diversity of lymnaeid snails is found in the extensive freshwater ecosystems of the northern United States and central Canada (Burch, 1982). Taxonomically, this group is quite complex with between 57 to 100 species historically recognized in North America alone (e.g., Baker, 1911; Hubendick, 1951; Clarke, 1973; Burch, 1982). Clearly, classification of this group remains controversial despite considerable efforts. In addition, lymnaeid snails serve as intermediate hosts for more than 70 trematode species world-wide (Brown, 1978). Therefore, significance in investigating this group ranges from taxonomic uncertainty to their role in transmitting parasitic disease.

On the American continents, lymnaeid snails serve as intermediate hosts for fasciolid trematodes, a group of major medical and economic concern. *Fasciola hepatica*, the causative agent of fascioliasis in humans and other mammals, is emerging as a medical concern in parts of Central and South America, as well as the Caribbean (Mas-Coma et al., 1999a, 1999b). In North America, *F. hepatica* and *Fascioloides magna*, the giant liver fluke, are causative agents of animal diseases in both wild and

domesticated mammals. The definitive host of *F. magna* in North America is white-tailed deer, *Odocoileus virginianus* (Cheatum, 1951; Griffiths, 1962; Dutson et al., 1967; Pursglove et al., 1977; Addison et al., 1988); however, domestic livestock sympatric with deer are at risk to *F. magna* infection (Figure 1). Infection in cattle has resulted in economic loss due to chronic disease and condemnation of livers at slaughter. In sheep infection often results in death (Foreyt and Todd, 1976a, b). *Fascioloides magna* has a disjunct range in North America (Figure 2), with isolated populations reported from the Pacific Northwest, Rocky Mountains, Gulf Coast, and Great Lakes regions (Cheatum, 1951; Griffiths, 1962; Knapp and Shaw, 1963; Dutson et al., 1967; Behrand et al., 1973; Pursglove et al., 1977; Foreyt, 1981; Schillhorn Van Veen, 1987; Knapp et al., 1992). This is likely due to the requirement for suitable snail intermediate hosts and cervid definitive hosts to complete the life cycle (Foreyt, 1990). *Stagnicola caperata*, *S. elodes* (also called *S. palustris*), *Fossaria parva*, *F. modicella*, and *F. bulimoides* are the only documented natural hosts of *Fascioloides magna* in North America (Sinitsin, 1930, 1933; Swales, 1935; Griffiths, 1959; Laursen, 1993). However, *Stagnicola catascopium*, *S. exilis*, *Lymnea stagnalis*, *Pseudosuccinea columella*, *F. obrussa rustica*, and *F. ferruginea* have been infected in laboratory studies (Krull, 1933a, 1933b, 1934; Dutson et al., 1967; Griffiths, 1973;). Due to the reliance of *F. magna* on lymnaeid snails for transmission, confidence in lymnaeid taxonomy is imperative for correct identification of intermediate hosts, and a robust phylogeny is necessary to evaluate host-parasite relationships.

Previous classifications of lymnaeid snails have defined species based upon shell morphology, reproductive morphology, and radular teeth formulas, along with some

emphasis on ecology. However, identification and classification has been compromised by morphological and anatomical similarity between species of some genera (Bargues et al., 2001), while other species exhibit high intraspecific variation in the presence of different environmental conditions (Burch, 1982). Therefore, there is little consensus among the available taxonomic keys. Different taxonomic schemes have recognized a single genus (Walter, 1968), two genera (Hubendick, 1951; Clarke, 1973), and up to seven genera (Baker, 1911; Burch, 1965, 1982) in the family. Proposed genera include *Lymnea*, *Bulimnea*, *Stagnicola*, *Fossaria*, *Radix*, *Psuedosuccinea*, and *Acella* (Burch, 1982). Use of sub-groups within genera, of which many are considered artificial (Hubendick, 1951; Walter, 1968), has contributed to the taxonomic confusion by putting increased weight on characters like shell morphology that may exhibit extreme homoplasy. Studies of immunology (Burch, 1968; Burch and Lindsay, 1968), karyology (Burch, 1965; Inaba, 1969), cross-breeding (Burch and Ayers, 1973), and enzyme electrophoresis (Rudolph and Burch, 1989) have either provided low systematic resolution or disagreed with results from morphological examinations (Bargues et al., 2001). Morphological examinations of reproductive organs have been useful in differentiating some lymnaeid species, and may be more reliable than other morphological characters for phylogeny reconstruction in this group (Hubendick, 1978; Jackiewicz, 1988; Glöer and Meier-Brook, 1998).

Molecular data have been useful in evaluating relationships of European (Bargues et al., 1997,2001; Bargues and Mas-Coma, 1997) and a limited number of North American (Remigio and Blair, 1997a, 1997b; Remigio, in press) lymnaeid snails. Sequences from 18S nrDNA have provided initial insights into lymnaeid relationships

(Bargues et al., 1997; Bargues and Mas-Coma, 1997). However, this region is highly conserved, and may not be appropriate for species-level differentiation. Previous analyses of 16S mtDNA (Remigio and Blair, 1997b; Remigio, in press) and the internal transcribed spacer (ITS) from nrDNA (Remigio and Blair, 1997a; Bargues et al., 2001) have shown promise in resolving relationships among lymnaeid genera. For example, sequences from 16S mtDNA revealed a paraphyletic relationship among stagnicoline snails, and produced a topology that disputed previous hypotheses regarding the evolution of chromosome number in Lymnaeidae (Remigio and Blair, 1997b). In addition, parsimony analyses of ITS sequences revealed that *Stagnicola caperata* did not cluster with three additional species of *Stagnicola* s.s. (*S. catascopium*, *S. emarginata*, and *S. elodes*), thus suggesting this taxon may deserve recognition as a distinct genus (Remigio and Blair, 1997a). *Stagnicola caperata* has previously been classified as a stagnicoline based on size, radular dentition, and, to a limited extent, shell morphology (Burch, 1982). However, differences between *S. caperata* and other stagnicolines do exist, most notably observed in the smaller size and pronounced periostracal ridges on shells of *S. caperata*. In addition, *S. caperata* and species of *Fossaria* serve as the main natural intermediate hosts for *F. magna* in the United States, which might indicate a close alliance between these two groups. Determining the taxonomic position of *Stagnicola caperata* will help to develop hypotheses regarding host susceptibility in Lymnaeidae.

Phylogenetic models have provided interesting insights into the evolution of symbioses among a diverse array of organisms (Moran et al., 1995; Herre et al., 1996; Clark et al., 2000), including parasites and their hosts (Hafner and Page, 1995; Baker et al., 1998; Hugot, 1998; DeJong et al., 2001; Xiao et al., 2001; Refardt et al., 2002).

Molecular data, in particular, allow for robust analyses of host-parasite relationships by providing independent estimates of phylogeny for both the host and parasite lineage(s) (Page et al., 1998), while also reducing the phylogenetic noise that results from morphological characters highly influenced by the association (Downes, 1990). In addition, studies that have used molecular, morphological, and parasitiological data in concert (e.g., Hugot, 1998) have been more successful in resolving host phylogeny.

In the absence of a robust host phylogeny, associations between host and parasite can be useful in evaluating relationships. Xiao et al. (2001) used *Hexamita* infections of fish in the family Xenocyprinae as a character for phylogenetic analysis, along with segments from the mitochondrial genome, and found that host phylogeny based on parasite fauna was congruent with that based on mtDNA sequences. Snail-trematode interactions show equal promise for revealing patterns of parallel evolution due to high host specificity exhibited by most digenean trematodes for their molluscan host (Roberts and Janovy, 1996). For instance, mapping patterns of susceptibility to trematode infection on snail phylogeny has been used in the development of hypotheses regarding host and parasite biogeography, as well as predicting new potential host species due to their genetic similarity to known host species (DeJong et al., 2001). Similar patterns have been found between lymnaeid snails and fasciolid trematodes (Bargues and Mas-Coma, 1997), but these studies have not included North American snails or *Fascioloides magna*. I hypothesize that inclusion of North American lymnaeid snails in the phylogeny, in addition to mapping *Fascioloides* susceptibility, will provide additional insights into the evolution of fasciolid susceptibility in lymnaeid snails, allowing for inference of the ancestral character states and the origins of susceptibility.

My goals are to estimate phylogeny for lymnaeid snails in enzootic fluke regions of Minnesota using internal transcribed spacer sequences, and use this phylogenetic hypothesis as an evolutionary framework to examine the phylogenetic distribution of susceptibility to *Fascioloides magna* infection in lymnaeid snails. In addition, the evolution of radular dentition will be explored on this framework, as it is typically used to define species of *Fossaria*.

Methods

Sampling of *Fascioloides magna* and Lymnaeid Snails

Fascioloides magna adults and eggs were collected from white-tailed deer in November of 1999, 2000, and 2001 during fire-arm hunts supervised by the MnDNR at St. Croix State Park, MN. *Fascioloides magna* adults were collected by slicing the liver in approximately 0.25 inch intervals, and removing worms from cysts within the organ. Adult worms were deposited into warm, 0.9% saline solution or distilled water for several hours to shed eggs. Eggs were concentrated by sedimentation, washed several times with distilled water, and incubated at room temperature until fully embryonated (~3 weeks). They were then kept at 4°C to suspend development, so that small aliquots of eggs could be hatched as needed for infection studies.

Lymnaeid snails were collected from eight sites in six counties in Minnesota. Wetland habitats (*sensu* Eggers and Reed, 1987) sampled were emphemeral ponds/ditches, marshes, prairie potholes, lakes, and streams. Specimens either were kept alive in containers of water packed on ice or were fixed in 100% ETOH in the field. Snails were identified based on shell morphology and radular dentition using available taxonomic keys (e.g., Clarke, 1973; Burch, 1982). Eight putative species from three genera within Lymnaeidae were identified (Table 1): one from *Lymnea* (*stagnalis*); four from *Stagnicola* (*caperata*, *catascopium*, *exilis*, and *elodes*); and three from *Fossaria* (*Bakerlymnea sp.*, *obrussa rustica*, and *parva*). Snail colonies were established in separate 10-gallon aquaria for each of five species of lymnaeid snails (*S. caperata*, *S. exilis*, *S. elodes*, *S. stagnalis* and *S. catascopium*) for use in infection studies. A species from the genus *Physa*, a proposed sister group to Lymnaeidae (Hubendick, 1947, 1978),

was collected for use as an outgroup, and sequences for five additional lymnaeid snails were retrieved from GenBank to compliment taxon sampling (Table 1).

Infection Study

Individuals of *L. stagnalis*, *S. exilis*, and two populations of *S. caperata* were exposed to 3-6 miracidia in a 48-well plate for 3 hours in modification of Foreyt and Todd (1978) and Laursen (1993). An infection tank was established for each species where snails were housed and fed frozen lettuce *ad libidum* for 40 days to allow for larval amplification. On day 40 post-infection, snails were crushed to determine infection status. Individual species were coded as susceptible to infection if any individual of that species contained redia or cercaria, or susceptibility based on experimental infection was known from previous studies.

Phylogenetic Study

Whole DNA was extracted from either frozen or preserved foot tissue using a HotSHOT protocol (Truett et al, 2000) modified for snails (J.A.T. Morgan, pers. comm.). A small amount of tissue (~ 3 mg) from each snail was dissected and pulverized in a 1.5 ml tube using sterile micro-grinders with 150 μL of an alkaline lysis reagent (25 mM NaOH and 0.2 mM disodium EDTA at pH 12). Ground samples were incubated at 95°C for one hour, then cooled to 25°C prior to the addition of 150 μL of neutralizing reagent (40 mM Tris-HCl at pH 5). Presence of whole DNA was verified by electrophoreses on a 1% agarose gel followed by staining with ethidium bromide. The complete ITS region (ITS1- 5.8S - ITS2) of the nrDNA was amplified using the primers BD1 and BD2 of Degnan et al. (1990). Individual reactions contained 1 μL of template DNA in a 100 μL reaction mixture with sterile double-distilled water, 0.2 mM of dNTP's, 10X PCR buffer,

5% DMSO, 0.25 μM primer concentrations, and one-half unit of TAQ polymerase. Amplification used 40 cycles of 94°C for 45 seconds, 48°C for one minute, and 72°C for four minutes. PCR products were purified using QIAquick® PCR purification kit following manufacturers instructions (QIAGEN Inc., Valencia, CA) and were resuspended in 35-50 μL sterile double-distilled water. DNA fragments were sequenced using external primers BD1 and BD2 (Degnan et al., 1990), internal primers ER1 and ER2 (Remigio and Blair, 1997a), and a Dye Terminator Cycle Sequencing Kit (Beckman-Coulter, Fullerton, CA), and visualized on a Beckman-Coulter CEQ 2000XL automated sequencer. Sequences were edited with Sequencher (Gene Codes, Ann Arbor, MI), and edited contigs were initially aligned using ClustalX (Thompson et al., 1997) followed by visual adjustment. Distance and Parsimony analyses were conducted using PAUP* (Swofford, 1998) on a G-4 Macintosh (400 mHz). The data set was analyzed using 1000 replications of RANDOM TAXON addition with NNI branch-swapping, which generated a pool of "starting" trees, the shortest of which were subsequently used for more rigorous analyses employing TBR branch-swapping. The relative support for the clades recovered by these analyses was assessed using fast bootstrapping (Mort et al, 2000). Susceptibility to infection was coded for terminal taxa, and phylogenetic distribution of susceptibility was examined using MacClade (Maddison and Maddison, 1992).

Results

Sequence data

Sequences for sixteen individuals, comprising 11 species and four genera, were included in the final data set (Appendix 1). The final alignment included 1368 sites, with 759 constant and 609 variable characters, of which 327 were parsimony-informative. Mean base frequencies for individual nucleotides were 17% (A), 31% (C), 29% (G), and 23% (T), with a G + C content of 60%. No significant shifts in nucleotide usage were observed across taxa based on a chi-square test ($x^2 = 25.6$, $df = 45$, $P > 0.99$). Evolutionary distances computed using a log-determinant model (Lockhart, 1994) ranged from 0.08 to 54% among ingroup taxa, with smallest distances observed among stagnicoline snails, excluding *S. caperata* (Table 2). Pairwise sequence comparisons revealed 27.8 transitions and 38.1 transversions on average, and a mean transition to transversion ratio of 0.73:1, suggesting standard models of evolution would apply (Swofford et al., 1996). The average proportion of sites differing between sequences was 0.167.

Lymnaeid Phylogenetics

Parsimony analyses of the aligned data set recovered 250 minimum-length trees of 900 steps (Figure 3). Homoplasy appeared to be low as assessed by the consistency index (0.8778) and retention index (0.8503). Variation among tree topologies was limited to the clade representing *Stagnicola* spp., in which low sequence divergence was observed; mean sequence divergence between *Stagnicola elodes*, *S. catascopium*, *S. exilis*, and *S. emarginata* was 0.00571, based on pairwise sequence comparisons. Strict consensus analyses of the minimum length trees recovered the same three major clades as

parsimony analyses (Figure 3) with strong bootstrap support: 1) individuals of *S. caperata* (100%); 2) *Stagnicola* spp. + *F. (Bakerlymnea)* sp. (100%); and, 3) *F. o. rustica* + *F. parva* (100%). Placement of *Fossaria truncatula* and *Lymnea stagnalis* in the consensus tree were suspect in light of previous hypotheses of lymnaeid taxonomy and phylogeny (Burch, 1982; Remigio and Blair, 1997; Remigio, in press). In addition, this topology did not show high support for relationships among *Stagnicola* s.s. An examination of branch-lengths (Figure 3) suggested long-branch attraction could be a factor, possibly causing parsimony analyses to be misleading (Felsenstein, 1978). Greater sequence divergence was observed for *S. caperata, F. truncatula, F. o. rustica, F. parva*, and *L. stagnalis* in contrast to nominal taxa in *Stagnicola* (i.e., *S. catascopium, S. elodes, S. exilis*, and *S. emarginata*). These unequal rates of change among ingroup taxa can cause tree topology to be incongruent with phylogenetic relationships (Felsenstein, 1978; Hendy and Penny, 1989).

Additional analyses were conducted to test topology from parsimony analyses using methods less prone to long-branch phenomena. One method used was neighbor-joining analyses with log-determinent (Lockhart, 1994) distances; this method is better suited for long-branch phenomena since it operates under a wider set of models and is robust to base changes across taxa (Hillis et al., 1996). The resulting topology (Figure 4) resolved three distinct clades with high bootstrap support: 1) *Stagnicola* spp. + *F. (Bakerlymnea)* sp. (100%); 2) individuals *of S. caperata* (99%); and, 3) *Fossaria* spp. (81%). Neighbor-joining analyses also resolved a close alliance between *S. caperata* and species of *Fossaria*, as opposed to other stagnicolines, but support for this relationship was low (< 50%). Constraining the topology from neighbor-joining analyses, employing

a log-detertiment model, in parsimony analyses produced a tree with 13 additional steps, which is less than a 2% difference from the initial tree produced by parsimony analyses. Therefore, tree topology produced by neighbor-joining analyses was not appreciably different than the maximum parsimony tree, and was accepted as the best estimate of relationships among lymnaeid snails from Minnesota.

Traits for susceptibility based on experimental and natural infections, along with radular dentition, were coded to examine their phylogenetic distribution (Table 3) using topology from neighbor-joining analyses. Snails susceptible to *Fascioloides magna* infection, as determined from infection studies in our laboratory and from a review of literature, as well as species that are known intermediate hosts of *F. magna* were coded with a value of one. Radular dentition, determined from slide mounts of the organ, was also included (Table 3) since it has been used to define groups within the genus *Fossaria* (Baker, 1911; Burch, 1982).

Discussion

Phylogenetics of North American Lymnaeid Snails

Taxonomic certainty and a robust phylogenetic hypothesis for lymnaeid snails are imperative for correctly identifying hosts of *Fascioloides magna* and interpreting host-parasite relationships in the group, respectively. The present study indicates that DNA sequences of the internal transcribed spacer region will resolve relationships between genera in Lymnaeidae, as first proposed by Remigio and Blair (1997a). Clades comprising *Stagnicola caperata*, *Fossaria s.s*, and *Stagnicola* s.s. + *F. Bakerlymnea* sp. were strongly supported in both parsimony and distance analyses. However, until additional sequences from the ITS region are included to overcome unequal rates of change along different branches, distance methods that use multiple-hit corrections are more reliable for assessing relationships in this group (Hendy and Penny, 1989; Hillis et al., 1996). Particularly, distance methods employing a log-determinent model (Lockhart, 1994; Steel, 1994), which operates under a wider set of models and is robust to changes in base composition across taxa, will be most successful (Hillis et al., 1996).

Neighbor-joining analyses found a closer alliance between *Stagnicola caperata*, the primary intermediate host of *F. magna* in Minnesota, and members of the genus *Fossaria*, in contrast to other members of *Stagnicola*. Although support for this relationship was low (<50%), a recent study employing sequences from the 16S mtDNA region revealed a similar alliance between *S. caperata* and fossariad snails (Remigio, in press). This relationship disputes previous taxonomic schemes (Baker, 1928; Burch, 1982) which place *S. caperata* in the stagnicoline subgenus *Hinkleyia*. Also, *S. caperata* exhibits extreme differences in shell morphology from other members of *Stagnicola* (e.g.,

smaller size and pronounced periostracal ridges); therefore, all available evidence suggests that its placement in the subgenus *Hinkleyia* is discordant with both morphological and molecular data.

Species of *Fossaria* have rarely been included in phylogenetic studies, mostly due to the difficulty in locating and identifying members of this group. The present study includes more species of *Fossaria* than have previously been evaluated. *Fossaria obrussa rustica*, *F. parva*, and *F. truncatula* formed a well-supported clade (81%) and a strong relationship was supported between *F. o. rustica* and *F. parva* (100%) in neighbor-joining analyses. *Fossaria Bakerlymnea sp.* was excluded from the clade of other species of *Fossaria*, and instead clustered with the stagnicoline clade. Exclusion of *F. Bakerlymnea sp.* from the *Fossaria* clade is also supported by differences in radular dentition (see below). More than 40 species of *Fossaria* have been described, but it is likely that many are synonyms (Burch, 1982). Extensive sampling of *Fossaria* species is needed to resolve, with confidence, relationships among members of the genus, and to evaluate taxonomy in this group effectively. Furthermore, continued use of molecular data in *Fossaria* may provide markers useful for the identification of cryptic species in the group, as this method has been successful in other snail (Jones et al., 1997, 1999) and invertebrate taxa (Walton et al., 1999).

Species from *Stagnicola* s.s. (e.g., *S. elodes*, *S. emarginata*, *S. catascopium*, and *S. exilis*) form a well-supported clade (100%), but relationships within the clade are largely unresolved. In fact, the average percent nucleotide difference from pairwise sequence comparisons (Table 2) were higher between individuals of *S. caperata* (3%)

than those observed between species comprising the *Stagnicola* s.s. clade (0.6%). One clade within *Stagnicola*, consisting of *S. exilis*, *S. catascopium* (from Minnesota), and *F. Bakerlymnea* sp., was moderately supported (74%); but, seems unreliable due to low sequence divergence. Unlike other genera in Lymnaeidae, particularly *Fossaria*, members of *Stagnicola* s.s. have distinctive shell characteristics that have allowed more reliable identification of taxa. Previous descriptions of *Stagnicola* s.s. have circumscribed species into two groups, *elodes* and *emarginata/catascopium* (Burch, 1982). Members of the *elodes* group, including *S. exilis*, typically have narrow, elongated spires and inhabit stagnant waters such as ditches and marshes. Species in the *emarginata/catascopium* group have more globose body whorls with shorter spires and are found in open and/or flowing water systems (e.g., lakes and streams). However, shell morphology among stagnicoline snails is greatly influenced by environmental conditions (Burch, 1968; Burch and Lindsay, 1973; Patterson and Burch, 1978), which may explain the discrepancies seen between highly diverse shell morphology and low sequence divergence. Failure of both ITS nrDNA (this study) and 16S mtDNA sequences (Remigio and Blair, 1997b; Remigio, in press) to resolve relationships in *Stagnicola* robustly indicates that additional data are needed to determine species relationships. If this group has undergone recent speciation events, then molecular studies that employ even more rapidly evolving markers, and maybe hybridization studies, will be necessary to define *Stagnicola* species. Also, studies that examine the effects of limnological variables on shell morphology may be necessary to determine how environmental factors are influencing putative taxa.

The phylogenetic position of *Lymnea stagnalis* was unresolved in neighbor-joining analyses, reflecting the high sequence divergence between *L. stagnalis* and other North American lymnaeids (e.g., 21.5 % on average from pairwise sequence comparisons). Other studies employing molecular data (Barques et al., 2001; Remigio, in press) consistently place N. American *L. stagnalis* as a sister to European *Stagnicola* species, suggesting a recent introduction from Eurasia (Remigio and Blair, 1997b; Remigio, in press). Like stagnicoline snails, *L. stagnalis* exhibits environmental variations in shell morphology that have resulted in assignment of sub-species names. With at least two type species recognized by both morphological and molecular data (Clarke, 1973; Burch, 1982; Remigio, in press), molecular studies employing population-level markers will be necessary to evaluate relationships among the various conspecifics.

Fossariad Snails and the Evolution of Radular Dentition

Historically, taxonomists have recognized members of *Fossaria* based on their small size and marked absence of the shell characteristics used to define other taxa. Burch (1982) identified two subgenera within *Fossaria*, based upon differences in radular dentition. Species of *Fossaria* s.s. (e.g., *F. obrussa rustica*, *F. parva*, and *F. truncatula*) have tricuspid teeth in the first lateral position, whereas *F. Bakerlymnea* spp. (e.g., *F. bulimoides*, *F. cubensis*, and *F. dalli*) are bicuspid. Bicuspid radular dentition is a trait shared between the *Bakerlymnea* group and *Stagnicola* species, causing Baker (1928) to classify bicuspid fossariad snails as the subgenus *Nasonia* in *Stagnicola*. Internal transcribed spacer sequences support the relationship proposed by Baker, since *F. Bakerlymnea* sp. was well-resolved within the stagnicoline clade (Figure 4). An examination of the phylogenetic distribution of radular dentition suggests a single origin

for tricuspid, first lateral teeth that is limited to *Fossaria* s.s. (Figure 5). Based on our estimate of phylogeny, radular dentition may provide more reliable phylogenetic signal than shell morphology or ecology in this group. Noteworthy is that sequences from 16S mtDNA (Remigio, in press) do not agree with our results, instead suggesting that bicuspid and tricuspid *Fossaria* are congeners in accordance with Burch (1982). Both the neighbor-joining tree presented here and the 16S phylogeny proposed by Remigio (in press) included only one species from the *Bakerlymnea* group. Therefore, it is not yet clear whether the *Bakerlymnea* group is monophyletic. Inclusion of additional species from *Bakerlymnea* is required to test the monophyly of this taxon and ascertain its position in the family. In addition, using a combination of molecular markers may be necessary to elucidate the patterns of evolution of radular dentition; since ITS nrDNA and 16S mtDNA have very different rates and modes of evolution (Hillis and Dixon, 1991) that may cause them to continue to be counteractive.

Evolution of Susceptibility to *Fascioloides* Infection

Experimental infections show that susceptibility to *F. magna* infection is widespread among the taxa sampled in this study, as all lymneaid species tested show some degree of susceptibility to infection. *Stagnicola emarginata* has not been tested, therefore, no conclusions can be made regarding its susceptibility. *Fossaria Bakerlymnea* sp. is not coded because it is not identified to species. However, it is likely that both of these species would be susceptible to *F. magna* infection if tested, especially *F. Bakerlymnea* sp., since members of this group include *Fossaria bulimoides* and *F. cubensis* that are intermediate hosts found naturally infected in the United States.

Only a subset of species experimentally susceptible to *F. magna* infection serve as natural intermediate hosts (Figure 6). These include *S. caperata*, *S. elodes*, and *F. parva* in the United States, as well as *F. truncatula* in Europe. Each of these species is commonly found in ephemeral waters, such as woodland ponds and ditches, where deer may be more likely to forage. Therefore, host status among lymnaeid snails may be a function of high exposure rates that result from close interactions between intermediate and definitive hosts in endemic areas. Studies of cervid foraging behavior lend support to different feeding strategies in enzootic and fluke-free regions of Minnesota (Laursen, 1993), providing one explanation for increased contact between intermediate and definitive hosts. The northeast region of Minnesota that is endemic to *F. magna* typically has low sodium concentrations in terrestrial vegetation and aquatic systems (Botkin et al., 1973; Helgensen et al., 1973; Jordan et al., 1973; Olcott et al., 1978). Deer in these areas make increased use of sodium-rich mineral licks, such as seeps or small springs (Weeks and Kirkpatrick, 1976; Fraser and Hristienko, 1981). These ephemeral sites are also suitable habitat for intermediate host species; therefore, frequent use by deer would increase contact between intermediate and definitive hosts, and would help perpetuate the fluke (Laursen, 1993).

Snails that were infected under laboratory conditions may be susceptible to infection as a result of shared ancestry (i.e., genetic similarity), but not found as natural hosts due to differences in habitat use. Susceptible species, such as *S. catascopium* are typically found in large lakes and streams, while *Stagnicola exilis* and *Lymnea stagnalis* are commonly found in marshes and prairie potholes. Larger aquatic habitats with deeper water could make snail location by *F. magna* larvae more difficult, reducing the number

of infected intermediate hosts. This may prevent infection in numbers required for continued transmission. In addition, a case can be made for moderate levels of resistance in some snail taxa. *Stagnicola exilis* and *L. stagnalis* are generally considered poor intermediate hosts, as they are only readily infected at juvenile life stages (Campbell and Todd, 1955a, 1955b; Griffiths, 1973; Wu and Kingscote, 1953, 1954). This is not typical of natural intermediate hosts, which remain susceptible throughout their lifetime. It is not clear whether this change in susceptibility from juvenile to adult snail is due to physiological differences or development of resistance prompted by historic exposure. Either way, it is obvious that genetic and ecological variables need to be evaluated in concert to better understand host susceptibility.

Literature Cited

Addison, E.M., Hoeve, J., Joachim, D.G., and McLachlin, D.J. 1988. *Fascioloides magna* (Trematoda) and *Taenia hydatigena* (Cestoda) from white-tailed deer. *Can. J. Zool.* 66: 1359-1364.

Baker, F.C. 1911. Lymnaeidae of North and Middle America, recent and fossil. *Chicago Acad of Sci.* Spec. Publ. No. 3, p i-xvi + 1-539, 58 pls.

Baker, F.C. 1928. The freshwater mollusca of Wisconsin. 1. Gastropoda. *Wisconsin Geol. Nat. Hist. Surv. Bull.* 70: 1-507.

Baker, M.D., Vossbrinck, C.R., Becnel, J.J., and Andreadis, T.G. 1998. Phylogeny of *Amblyospora* (Microsporidia: Amblyosporidae) and related genera based on small subunit ribosomal DNA data: A possible example of host parasite cospeciation. *J. Invert. Path.* 71: 199-206.

Bargues, M.D., and Mas-Coma, S. 1997. Phylogenetic Analysis of Lymnaeid Snails Based On 18S rDNA Sequences. *Mol. Biol. Evol.* 14(5): 569-577.

Bargues, M.D., Mangold, A.J., Munoz-Antoli, C., Pointier, J., and Mas-Coma, S. 1997. SSU rDNA Characterization of Lymnaeid Snails Transmitting Human Fascioliasis in South and Central America. *J. Parasito.* 83(6): 1086-1092.

Bargues, M.D., Vigo, M., Horak, P., Dvorak, J., Patzner, R.A., Pointier, J.P., Jackiewicz, M., Meier-Brook, C., and Mas-Coma, S. 2001. European Lymnaeidae (Mollusca: Gastropoda), intermediate hosts of trematodiases, based on nuclear ribosomal DNA ITS-2 sequences. *Infection, Genetics and Evolution.* 1: 85-107.

Behrend, D.F., Mattfeld, G.F., and Willey, J.E. 1973. Incidence of liver flukes in a sample of white-tailed deer from the Adirondacks. *New York Fish and Game Journal* 20: 158-161.

Botkin, D.B., Jordan, P.A., Dominski, A.S., Lowendorf, H.S., and Hutchinson, G.E. 1973. Sodium dynamics in a northern ecosystem. *Proc. Nat. Acad. Sci.* 70: 2745-2748.

Brown, D.S. 1978. Pulmonate molluscs as intermediate hosts for digenetic trematodes. In: *Pulmonates. Systematics, Evolution, and Ecology*, Vol. 2A (V. Fretter and J. Peake, eds). Academic Press, London. pp. 287-333.

Burch, J.B. 1965. Chromosome numbers and systematics in euthyneuran snails. In: *Proceedings of the First European Malacologia Congress* 215-41.

Burch, J.B. 1968. Morphology vs. immunology. Response. *Am. Malacol. Union* 34: 25-8.

Burch, J. B. 1982. North American Freshwater Snails. *Trans. Poets Soc.* 1(4): 217-365.

Burch, J.B., and Ayers, P.A. 1973. Breeding experiments with *Stagnicola elodes* and *Stagnicola emarginata*. *Malacol. Rev.* 6: 51-2.

Burch, J.B., and Lindsay, G.K. 1968. An immunological approach to lymnaeid systematics. *Am. Malacol. Union* 34: 22-3.

Burch, J.B., and Lindsay, G.K. 1973. Taxonomic groupings in the Lymnaeidae. *Am. Malacol. Union* 38: 15.

Campbell, W.C., and Todd, A.C. 1955a. In vitro metamorphosis of the miracidium of *Fascioloides magna* (Bassi, 1875), Ward, 1917. *Trans. Amer. Micro. Soc.* 74: 225-228.

Campbell, W.C., and Todd, A.C. 1955b. Behavior of miracidium of *Fascioloides magna* (Bassi, 1875), Ward, 1917 in the presence of a snail host. *Trans. Amer. Micro. Soc.* 74: 342-347.

Cheatum, E.L. 1951. Disease in relation to winter mortality in deer in New York. *J. Wild. Man.* 15: 216-220.

Clark, M.A., Moran, N.A., Baumann, P., and Wernegreen, J.J. 2000. Cospeciation between bacterial endosymbionts (*Buchnera*) and a recent radiation of aphids (*Uroleucon*) and pitfalls of testing for phylogenetic congruence. Evolution 54: 517-525.

Clarke, A.H. 1973. Canadian Freshwater Mollusks (Lymnaeidae). *Malacologia* 13-14: 1-509.

Darwin, C. 1859. *On the origin of species by means of natural selection.* London, England: John Murray.

Degnan, B.M., Yan, J., Hawkins, C.J., and Lavin, M.F. 1990. rRNA genes from the lower chordate *Herdmania momus*: structural similarity with higher eukaryotes. *Nucleic Acids Res.* 18: 7063-7070.

DeJong, R.J., Morgan, J.A.T., Paraense, W.L., Pointier, J.P., Amarista, M., Ayeh-Kumi, P.F.K., Babiker, A., Barbosa, C.S., Brémond, P., Canese, A.P., Pereira de Souza, C., Dominguez, C., File, S., Gutierrez, A., Incani, R.N., Kawano, T., Kazibwe, F., Kpikpi, J., Lwambo, N.J.S., Mimpfoundi, R., Njiokou, F., Poda, J.N., Sene, M.,

Velásquez, L.E., Yong, M., Adema, C.M., Hofkin, B.V., Mkoji, G.M., and Loker, E.S. 2001. Evolutionary relationships and biogeography of *Biomphalaria* (Gastropoda: Planorbidae) with implications regarding its role as host of the human bloodfluke, *Schistosoma mansoni*. *Mol. Biol. Evol.* 18(12): 2225-2239.

Downes, B.J. 1990. Host-induced morphology in mites: Implications for host parasite coevolution. *Syst. Zool.* 39: 162-168.

Dutson, V.J., Shaw, J.N., and Knapp, S.E. 1967. Epizootiologic factors of *Fascioloides magna* (Trematoda) in Oregon and southern Washington. *Amer. J. Vet. Res.* 28: 853-860.

Eggers, S.D., and Reed, D.M. 1987. *Wetland plants and plant communities of Minnesota and Wisconsin*. Report of the U.S. Army Corps of Engineers, St. Paul District. 201 pp.

Felsenstein, J. 1978. Cases in which parsimony and compatibility methods will be positively misleading. *Syst. Biol.* 27: 27-33.

Foreyt, W.J. 1981. Trematodes and Cestodes. *In*: W. R. Davidson (Ed.), Diseases and parasites of the white-tailed deer. Tall Timbers Research Station, Misc. Pub. No. 7, pp. 237-265.

Foreyt, W.J. 1990. Domestic sheep as a rare definitive host of the large american liver fluke *Fascioloides magna*. *J. Parasitol.* 76: 736-739.

Foreyt, W.J., and Todd, A.C. 1976a. Development of the large american liver fluke, *Fascioloides magna*, in white-tailed deer, cattle, and sheep. *J. Parasitol.* 62: 26-32.

Foreyt, W.J., and Todd, A.C. 1976b. Liver flukes in cattle. *Med. Small Animal Clinician*. 71: 816-822.

Foreyt, W.J., and Todd, A.C. 1978. Experimental infection of lymnaeid snails in Wisconsin with miracidia of *Fascioloides magna* and *Fasciola hepatica*. *J. Parasitol*. 64: 1132-1134.

Fraser, D., and Hristienko, H. 1981. Activity of moose and white-tailed deer at mineral springs. *Can. J. Zool.* 59: 1991-2000.

Glöer, P., Meier-Brook, C. 1998. Süsswassermollusken. In: *Bestimmungsschlussel für die Bundesrepublik Deutschland*, 12th Edition. Deutscher Jugendbund für Naturbeobachtung, Hamburg, Germany.

Griffiths, H.J. 1959. *Stagnicola (Hinkleyia) caperata* (Say), a natural intermediate host for *Fascioloides magna* (Bassi, 1975), in Minnesota. *J. Parasitol*. 45: 146.

Griffiths, H.J. 1962. Fascioloidiasis of cattle, sheep, and deer in northern Minnesota. *J. Amer. Vet. Med. Assoc*. 140: 342-347.

Griffiths, H.J. 1973. *Galba modicella* and *Lymnea stagnalis* as experimental Intermediate hosts for *Fascioloides magna* in Minnesota. *J. Parasitol*. 59: 121.

Hafner, M.S., and Page, R.D.M. 1995. Molecular phylogenies and host-parasite cospeciation: Gophers and lice as a model system. *Phil. Trans. R. Soc. Lond. B* 349: 77-83.

Helgesen, J.O., Lindholm, G.F., Broussard, W.L., and Ericson, D.W. 1973. Water resources of the Kettle River watershed, East-central Minnesota. *Hydrologic Investigations Atlas HA-437*. U.S. Geological Survey.

Hendy, M.D., and Penny, D. 1989. A framework for the quantitative study of evolutionary trees. *Syst. Zool.* 38: 297-309.

Herre, E.A., Machado, C.A., Bermingham, E., Nason, J.D., Windsor, D.M., McCafferty, S.S., VanHouten, W., and Bachmann, K. 1996. Molecular phylogenies of figs and their pollinator wasps. *J. Biogeogr.* 23: 521-530.

Hillis, D.M., and Dixon, M.T. 1991. Ribosomal DNA: Molecular evolution and phylogenetic inference. *Quart. Rev. Biol.* 66(4): 411-431.

Hillis, D.M., Moritz, C., and Mable, B.K. 1996. *Molecular Systematics.* 2nd ed. Sinauer Associates, Sunderland, Massachusetts.

Hubendick, B. 1947. Phylogenetic relations between higher limnic Basommatophora. *Zoologiska Bidrag fran Uppsala.* 25: 141-164.

Hubendick, B. 1951. Recent Lymnaeidae, their variation, morphology, taxonomy, nomenclature, and distribution. *Kungl. Svenska Vetenskapsakademiens Handl., Fijarde Ser.* 3(1): 1-223, 5 pls.

Hubendick, B. 1978. Systematics and comparative morphology of the Basommatophora. In: Fretter, V., Peake, J. (Eds.), *Pulmonates*, Vol. 2A, 1-47. Academic Press, London.

Hugot, J.P. 1998. Phylogeny of neotropical monkeys: The interplay of morphological, molecular, and parasitological data. *Mol. Phyl. Evol.* 9(3): 408-413.

Inaba, A. 1969. Cytotaxonomic studies of lymnaeid snails. *Malacologia* 7: 143-169.

Jackiewicz, M. 1988. The penis as a valuable diagnostic feature in lower taxonomic units of the family Lymnaeidae (Gastropoda, Pulmonata). *Malakol. Abh. Mus. Tierkd. Dresden* 13: 23-6.

Jones, C.S., Noble, L.R., Lockyer, A.E., Brown, D.S., and Rollinson, D. 1997. Species-specific primers discriminate intermediate hosts of schistosomes: unambiguous PCR diagnosis of *Bulinus forskalii* group taxa (Gastropoda: Planorbidae). *Molecular Ecology* 6: 843-49.

Jones, C.S., Noble, L.R., Ouma, J., Kariuki, H.C., Mimpfoundi, R., Brown, D.S., and Rollinson, D. 1999. Molecular identification of schistosome intermediate hosts: case studies of *Bulinus forskalii* group species (Gastropoda: Planorbidae) from central and east Africa. *Biological Journal of the Linnean Society* 68(1/2): 215-240.

Jordan, P.A., Botkin, D.B., Dominski, A.S., Lowendorf, H.S., and Belovsky, G.E. 1973. Sodium as a critical nutrient for moose on Isle Royale. *Proc. N. Amer. Moose Conf. Workshop* 9: 13-42.

Knapp, S.E., Dunkel, A.M., Han, K., and Zimmerman, L.A. 1992. Epizootiology of fascioliasis in Montana. *Vet. Parasitol.* 42: 241-246.

Knapp, S.E., and Shaw, J.N. 1963. Occurance of *Fascioloides magna* (Bassi) in Oregon cattle and deer. *J. Parasitol.* 49: 39.

Krull, W.H. 1933a. New snail hosts for *Fasciola magna* (Bassi, 1975) Stiles, 1894. *J. Parasitol.* 20: 107-108.

Krull, W.H. 1933b. A new intermediate host for *Fascioloides magna* (Bassi, 1875) Ward, 1917. *Science* 78: 508-509.

Krull, W.H. 1934. The intermediate hosts of *Fasciola hepatica* and *Fascioloides magna* in the United States. *N. Amer. Vet.* 15: 13-17.

Lausen, J.R. 1993. *Fascioloides magna* in Minnesota: Regional ecology of the fluke and serodiagnosis in cattle. Ph.D. Dissertation: University of Minnesota, Minneapolis, MN.

Lockhart, P.J., Steel, M.A., Hendy, M.D., and Penny, D. 1994. Reconstructing evolutionary trees under a more realistic model of sequence evolution. *Mol. Biol. Evol.* 11: 605-612.

Maddison, W.P., and Maddison, D.R. 1992. *MacClade: Analysis of Phylogeny and Character Evolution*. Sinauer Associates, Inc., Sunderland, MA.

Mas-Coma, S., Bargues, M.D., and Esteban, J.G. 1999a. Human fasciolosis. *In*: Dalton, J.P. (Ed.), *Fasciolosis*. CAB International Publishing, Wallingford, UK, pp. 411-434.

Mas-Coma, S., Esteban, J.G., and Bargues, M.D. 1999b. Epidemiology of human fascioliasis: a review and proposed new classification. *Bull. WHO* 77: 340-346.

Mas-Coma, S., Funatsu, I.R., and Bargues, M.D. 2001. Fasciola hepatica and lymnaeid snails occurring at very high altitude in South America. *Parasitology* 123(7): 115-127.

Moran, N.A., Von Dohlen, C.D., and Baumann, P. 1995. Faster evolutionary rates in endosymbiotic bacteria than in cospeciating insect hosts. *J. Mol. Evol.* 41: 727-731.

Mort, M.E., Soltis, P.S., Soltis, D.E., and Mabry, M.L. 2000. Comparison of three methods for estimating internal support on phylogenetic trees. *Syst. Biol.* 49(1): 160-171.

Olcott, P.G., Ericson, D.W., Felsheim, P.E., and Broussard, W.L. 1978. Water resources of the Lake Superior watershed, northeastern Minnesota. Hydrological Investigations Atlas HA-582. U.S. Geological Survey.

Page, R.D.M., Lee, P.L.M., Becher, S.A., Griffiths, R., and Clayton, D.H. 1998. A different tempo of mitochondrial DNA evolution in birds and their parasitic lice. *Mol. Phyl. Evol.* 9(2): 276-293.

Patterson, C.M., and Burch, J.B. 1978. Chromosomes of pulmonate molluscs. In: Gretter, V., Peake, J. (Eds.), *Pulmonates. Systematics, Evolution and Ecology*, Vol. 2A. Academic Press, London, 171-217.

Pursglove, S.R., Prestwood, A.K., Ridgeway, T.R., and Hayes, F.A. 1977. *Fascioloides magna* infection in white-tailed deer of southeastern United States. *J. Amer. Vet. Med. Assoc.* 171: 936-938.

Rafinesque, C.S. 1815. *Analyse de la nature, ou tableau de l'univers et des corps organises*. Palerme. pp. 136-139

Refardt, D., Canning, E.U., Mathis, A., Cheney, S.A.; Lafranchi-Tristem, N.J., and Ebert, D. 2002. Small subunit ribosomal DNA phylogeny of microsporidia that infect *Daphnia* (Crustacea: Cladocera). *Parasitology* 124(4): 381-389.

Remigio, E.A. 2002. Molecular phylogenetic relationships in the aquatic snail genus *Lymnea*, the intermediate host of the causative agent of fascioliasis: insights from broader taxon sampling. *Mol. Biol. Evol., in press.*

Remigio, E.A., and Blair, D. 1997a. Relationships Among Problematic North American Stagnicoline Snails (Pulmonata: Lymnaeidae) Reinvestigated Using Nuclear Ribosomal DNA Internal Transcribed Spacer Sequences. *Can. J. Zoology* 75: 1540-1545.

Remigio, E.A., and Blair, D. 1997b. Molecular Systematics of the Freshwater Snail Family Lymnaeidae (Pulmonata: Basommatophora) Utilising Mitochondrial Ribosomal DNA Sequences. *J. Moll. Stud.* 63: 173-185.

Roberts, L.S., and Janovy, J. 1996. *Foundations in Parasitology*. 5th edition. W. C. Brown: Dubuque, IA

Rudolph, P.H., and Burch, J.B. 1989. Electrophorectic analysis of enzymes in three species of *Stagnicola* (Pulmonata: Lymnaeidae). *J. Med. Appl. Malacol.* 1: 57-64.

Schillhorn Van Veen, T.W. 1987. Prevalence of *Fascioloides magna* in cattle and deer in Michigan. *J. Amer. Vet. Med. Assoc.* 191: 547-48.

Sinitsin, D.F. 1930. A note on the life history of the large american fluke, *Fasciola magna* (Bassi). *Science* N.S. 72: 273-774.

Sinitsin, D.F. 1933. Studien uber die phylogenie der trematoden VI. The life histories of some american liver flukes. *Zeit. Fur Parasitol.* 6: 170-191.

Steel, M.A. 1994. Recovering a tree from the Markov leaf colourations it generates under a Markov model. *Appl. Math. Lett.* 7: 19-23.

Stromberg, B.E., Schlotthauer, J.C., Karns, P.D. 1983. Current status of *Fascioloides magna* in Minnesota. *Minnesota Veterinarian* 23(1): 8-13.

Swales, W.E. 1935. The life cycle of *Fascioloides magna* (Bassi, 1975), Ward, 1917, the large liver fluke of ruminants, in Canada. *Can. J. Res.* 12: 177-215.

Swiderski, D.L. 1990. Analysis of lymnaeacean relationships using phylogenetic systematics. *Malcologia* 31(2): 249-257.

Swofford, D.L. 1998. *PAUP* 4.0: Phylogenetic Analysis Using Parsimony (and other methods)* BetaVers. 4.0. Sinauer Associates, Sunderland.

Swofford, D.L., Olsen, G.J., Waddell, P.J., and Hillis, D.M. 1996. Phylogenetic Inference. In: *Molecular Systematics*, 2nd ed. (D.M. Hillis, C. Moritz, and B.K. Mable, eds), 407-514. Sinauer, Massachusetts.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.J. 1997. The ClustalX-Windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.* 25: 4876-4882.

Truett, G.E., Heeger, P., Mynatt, R.L., Truett, A.A., Walker, J.A., Warman, M.L. 2000. Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and Tris (HotSHOT). *Biotechniques* 29(1): 52-54.

Walter, H.J. 1968. Evolution, taxonomic revolution, and zoogeography of the Lymnaeidae. *The American Malacological Union, Inc., Annual Reports*: 18-20.

Walton, C., Sharpe, R.G., Pritchard, S.J., Thelwell, N.J., and Butlin, R.K. 1999. Molecular identification of mosquito species. *Biological Journal of the Linnean Society* 68(1/2): 241-256.

Weeks, A.J., and Kirkpatrick, C.M. 1976. Adaptations of white-tailed deer to naturally occurring sodium deficiencies. *J. Wild. Manag.* 40: 610-625.

Wu, L.Y., and Kingscote, A.A. 1953. A note on *Lymnea stagnalis* (L.) as a snail host for *Fascioloides magna* (Bassi, 1975) (Trematoda). *J. Parasitol.* 39: 568.

Wu, L.Y., and Kingscote, A.A. 1954. Further study on *Lymnea stagnalis* (L.) as a snail host for *Fascioloides magna* (Bassi, 1975) (Trematoda). *J. Parasitol.* 40: 90-93.

Xiao, W., Zhang, Y., and Liu, H. 2001. Molecular systematics of Xenocyprinae (Teleostei: Cyprinidae): Taxonomy, biogeography, and coevolution of a special group restricted to east Asia. *Mol. Phylogenet. .Evol.* 18(2): 163-173.

Table 1: Lymnaeid snails sampled for infection and phylogenetic studies, with reference to sample location.

Species Sampled	Locality	Country	Genbank Accession Number
Lymnea stagnalis	Douglas County, MN	USA	*
Fossaria obrussa rustica	Kanabec County, MN	USA	*
Fossaria parva	Crow Wing County, MN	USA	*
Fossaria truncatula	Minho	Portugal	AJ243018 (Mas-Coma et al., 2001); AJ243017 (Barges et al., 2001)
Fossaria (Bakerlymnea) sp.	Pine County, MN	USA	*
Stagnicola caperata 1	Mille Lacs County, MN	USA	*
Stagnicola caperata 2	Mille Lacs County, MN	USA	*
Stagnicola caperata 3	Manitoba	Canada	AF013139 (Remigio and Blair, 1997a)
Stagnicola elodes 1	Pine County, MN	USA	*
Stagnicola elodes 2	Traverse County, MN	USA	*
Stagnicola elodes 3	Ann Arbor, MI	USA	AF013138 (Remigio and Blair, 1997a)
Stagnicola exilis	Mille Lacs County, MN	USA	*
Stagnicola catascopium 1	Crow Wing County, MN	USA	*
Stagnicola catascopium 2	Ann Arbor, MI	USA	AF013143 (Remigio and Blair, 1997a)
Stagnicola emarginata	Ann Arbor, MI	USA	AF013141 (Remigio and Blair, 1997a)
Physa sp.	Kanabec County, MN	USA	*

* Genbank accession number available from authors

Table 2: Evolutionary distances according to logdeterminent/paralinear model (shown below the diagonal), and percent nucleotide difference (shown above the diagonal) from pairwise comparison of partial ITS nrDNA sequences of lymnaeid snails sampled.

		LSTA	FRUS	FPAR	SCAP1	SCAP2	SCAP3	FTRU	SELO3	SELO1	SEXI	SCAT1	SCAT2	SEMO	SELO2	FBAK	PHYS
Lymnea stagnalis	LSTA	_	33	33	21	20	20	22	26	18	18	18	18	18	18	18	30
Fossaria obrussa rustica	FRUS	0.46845	_	2	32	32	35	29	29	28	29	29	28	28	28	29	39
Fossaria parva	FPAR	0.48552	0.01970	_	33	33	36	31	29	29	30	29	29	29	29	29	41
Stagnicola caperata 1	SCAP1	0.25185	0.45957	0.48674	_	1	4	17	13	12	12	12	12	12	12	12	27
Stagnicola caperata 2	SCAP2	0.24433	0.45502	0.48237	0.00579	_	4	17	13	12	12	12	12	12	12	12	27
Stagnicola caperata 3	SCAP3	0.26836	0.51530	0.54553	0.04595	0.04171	_	16	15	14	14	14	14	14	14	14	28
Fossaria truncatula	FTRU	0.34235	0.38571	0.42515	0.20501	0.19970	0.18913	_	20	20	20	20	20	20	19	20	36
Stagnicola elodes 3	SELO3	0.21986	0.38698	0.40448	0.15127	0.14965	0.17697	0.26007	_	0	1	1	1	1	1	1	27
Stagnicola elodes 1	SELO1	0.21723	0.38109	0.39834	0.14516	0.14353	0.17064	0.25063	0.00519	_	1	0	0	0	0	0	27
Stagnicola exilis	SEXI	0.21772	0.39460	0.41210	0.14731	0.14569	0.17277	0.25665	0.01497	0.00963	_	0	1	1	1	1	27
Stagnicola catascopium 1	SCAT1	0.21012	0.38936	0.40690	0.14468	0.14308	0.17035	0.25482	0.00944	0.00415	0.00552	_	1	0	1	0	27
Stagnicola catascopium 2	SCAT2	0.21595	0.37939	0.39661	0.14631	0.14467	0.17177	0.25093	0.00783	0.00262	0.01229	0.00681	_	0	1	1	27
Stagnicola emarginata	SEMA	0.21871	0.38463	0.40198	0.14646	0.14483	0.17199	0.25296	0.00628	0.00107	0.01071	0.00523	0.00369	_	0	1	27
Stagnicola elodes 2	SELO2	0.21995	0.38113	0.39838	0.14509	0.14345	0.17051	0.24691	0.00801	0.00281	0.01153	0.00594	0.00546	0.00391	_	1	27
Fossaria (Bakerlymnea) sp.	FBAK	0.21498	0.38783	0.40525	0.14521	0.14361	0.17068	0.25262	0.01024	0.00498	0.00635	0.00088	0.00586	0.00605	0.00785	_	27
Physa sp.	PHYS	0.40696	0.60025	0.64156	0.34587	0.34477	0.36667	0.52372	0.34741	0.34842	0.34757	0.34677	0.34765	0.34715	0.34782	0.34470	_

Table 3: Codes used to evaluate character evolution in lymnaeid taxa. Susceptible species and known intermediate hosts were coded with a value of one. Radular dentition was coded as a zero for bicuspid, one for tricuspid, and two for a multi-cuspid first lateral tooth in the radula. Species were coded with a dash if no information was available for a particular trait.

	Experimental Host [c,d,e,f,g]	Natural Host [h,i,j]	Radular dentition [a,b]
Lymnea stagnalis	1	0	0
Fossaria obrussa rustica	1	0	1
Fossaria parva	1	1	1
Stagnicola caperata 1	1	1	0
Stagnicola caperata 2	1	1	0
Stagnicola caperata 3	1	1	0
Fossaria truncatula	1	1	1
Stagnicola elodes 3	1	1	0
Stagnicola elodes 1	1	1	0
Stagnicola exilis	1	0	0
Stagnicola catascopium 1	1	0	0
Stagnicola catascopium 2	1	0	0
Stagnicola emarginata	?	0	0
Stagnicola elodes 2	1	1	0
Fossaria (Bakerlymnea) sp.	?	0	0
Physa sp.	0	0	2

References: a) Burch, 1982; b) Clarke, 1973; c) Dutson et al., 1967; d) Griffiths, 1959; e) Griffiths, 1973; f) Krull, 1933a; g) Krull, 1933b; h) Sinitson, 1930; I) Sinitson, 1933; and, j) Swales, 1935.

Figure 1: Life cycle of *Fascioloides magna* courtesy of Stromberg et al. (1983) that describes each stage in the development of the parasite: a) eggs are shed in feces of infected deer, b) miracidia hatch from eggs in an aquatic environment, c) miracidia penetrate snails and undergoes larval amplification, d) cercaria emerge from snail, and, e) encyst to become metacercaria, a stage infective to deer and domestic livestock.

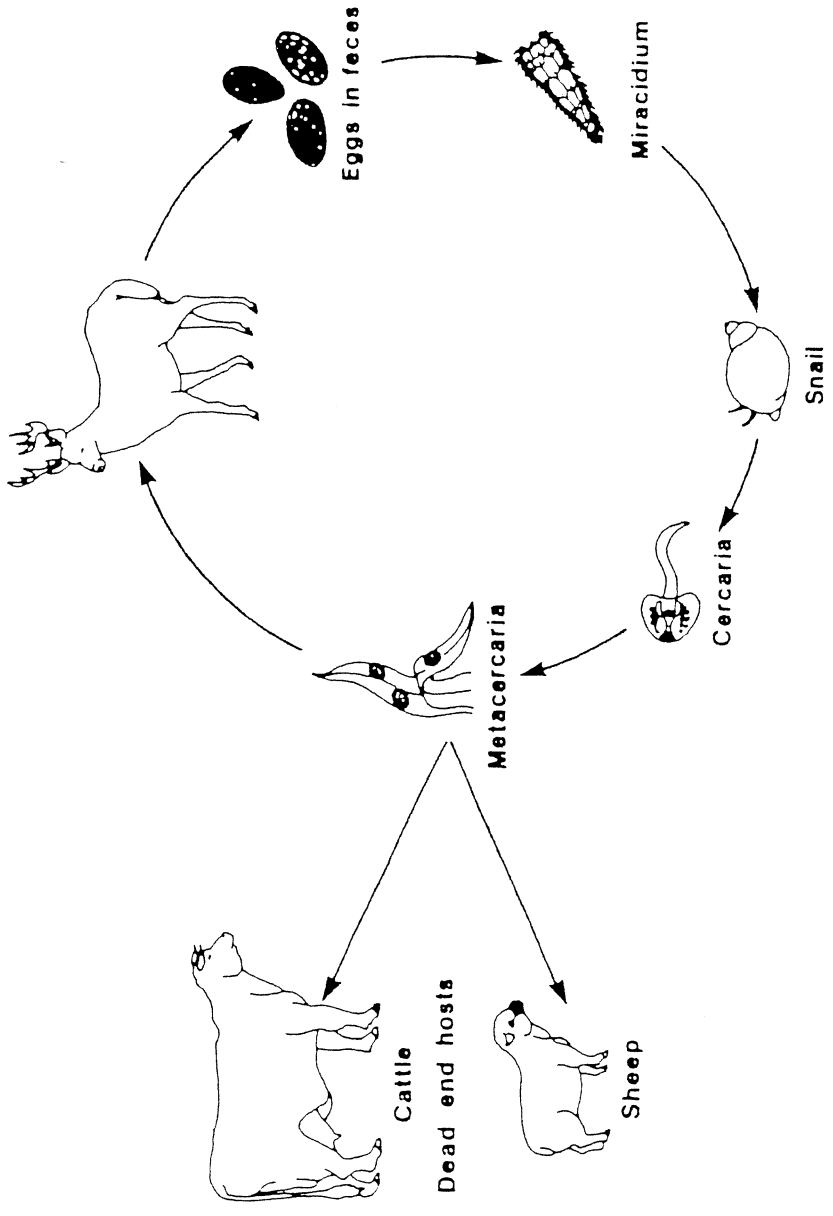
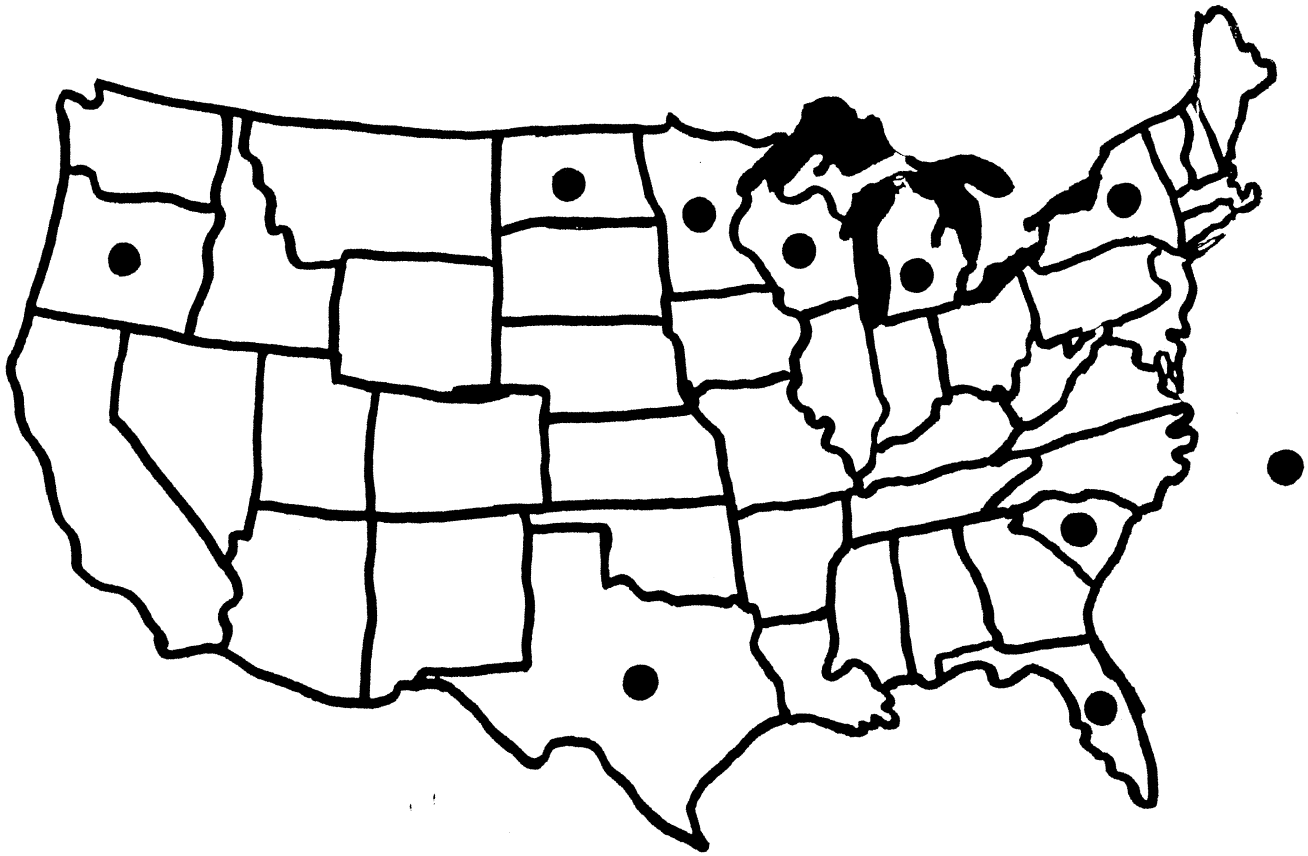

Eggs in feces
Miracidium
Snail
Cercaria
Metacercaria
Cattle
Dead end hosts
Sheep

Figure 2: Map adapted from Griffiths (1962) documenting the occurrence of *Fascioloides magna* in white-tailed deer in the United States.



● Documented occurrence of *Fascioloides magna* in deer

Figure 3: One of 250 minimum-length trees produced by parsimony analyses employing a heuristic search (100 replicates) and a tree-bisection-reconnection branch-swapping algorithm. Trees were 900 steps, with a consistency index of 0.8778 and a retention index of 0.8503. Bootstrap values (500 replicates) are shown above branches, while branch-length values are below branches.

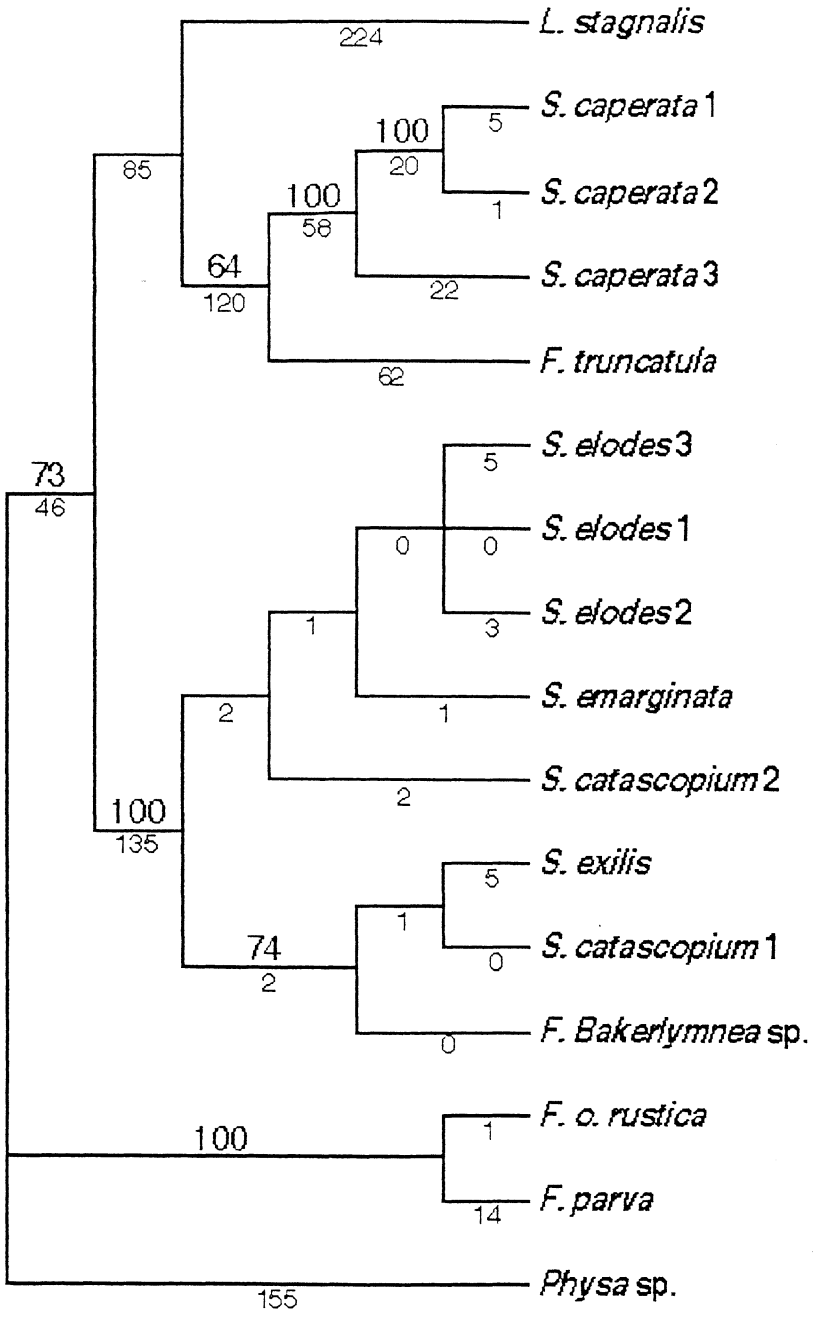

L. stagnalis
224
S. caperata 1
5
100
20
S. caperata 2
1
100
58
S. caperata 3
22
64
120
F. truncatula
62
85
S. elodes 3
5
73
46
S. elodes 1
0
0
S. elodes 2
3
1
S. emarginata
1
2
S. catascopium 2
2
100
135
S. exilis
5
1
S. catascopium 1
0
74
2
F. Bakerlymnea sp.
0
F. o. rustica
1
100
F. parva
14
Physa sp.
155

Figure 4: Neighbor-joining dendrogram inferred using the log-determinent/paralinear model, with bootstrap values (500 replicates) shown above branches.

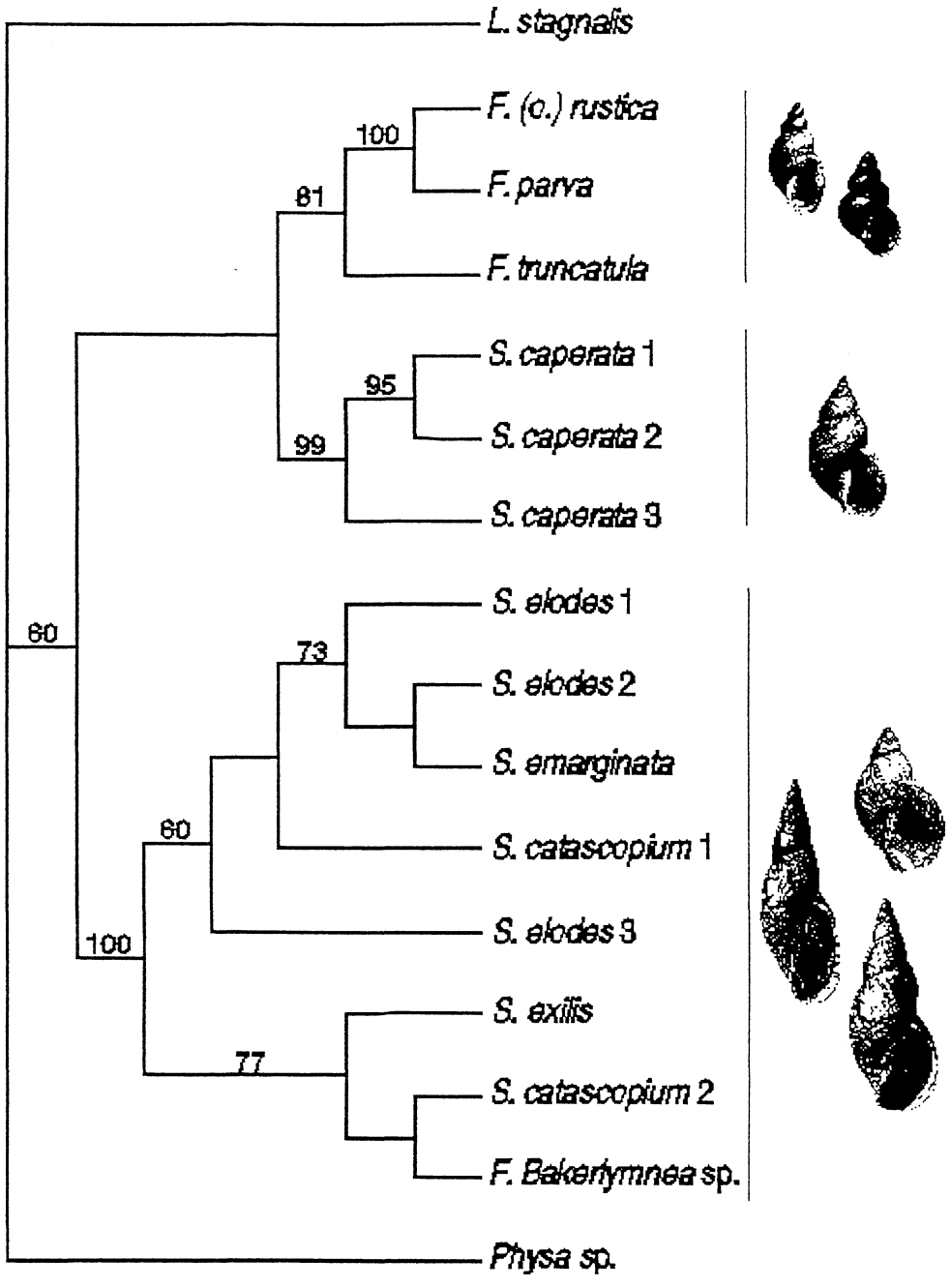

L. stagnalis
F. (c.) rustica
F. parva
F. truncatula
S. caperata 1
S. caperata 2
S. caperata 3
S. elodes 1
S. elodes 2
S. emarginata
S. catascopium 1
S. elodes 3
S. exilis
S. catascopium 2
F. Bakerilymnea sp.
Physa sp.
100
81
95
99
60
73
60
100
77

Figure 5: Topology from neighbor-joining analyses examining the evolution of radular dentition. Tricuspid lymnaeid snails are located on dashed branches.

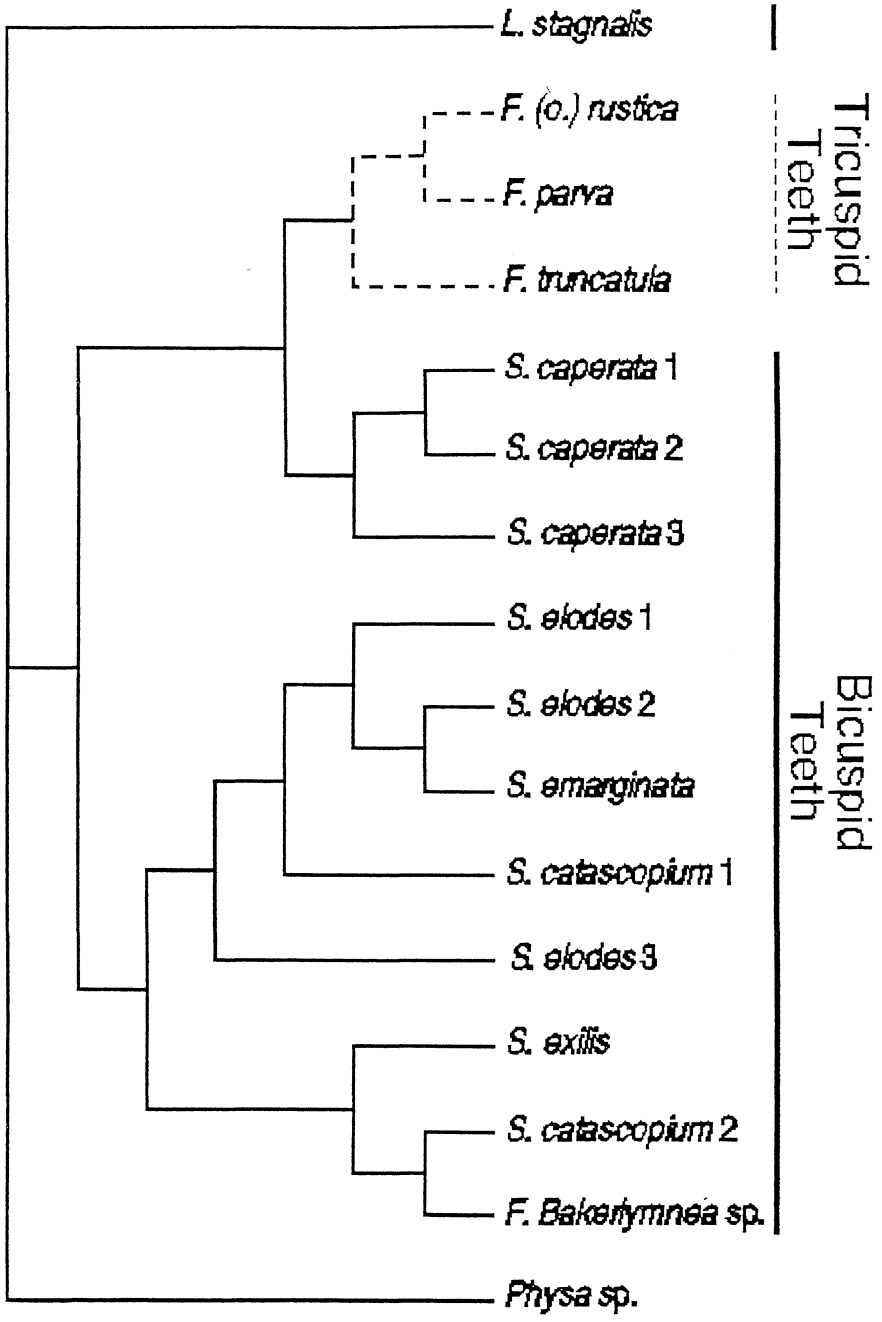
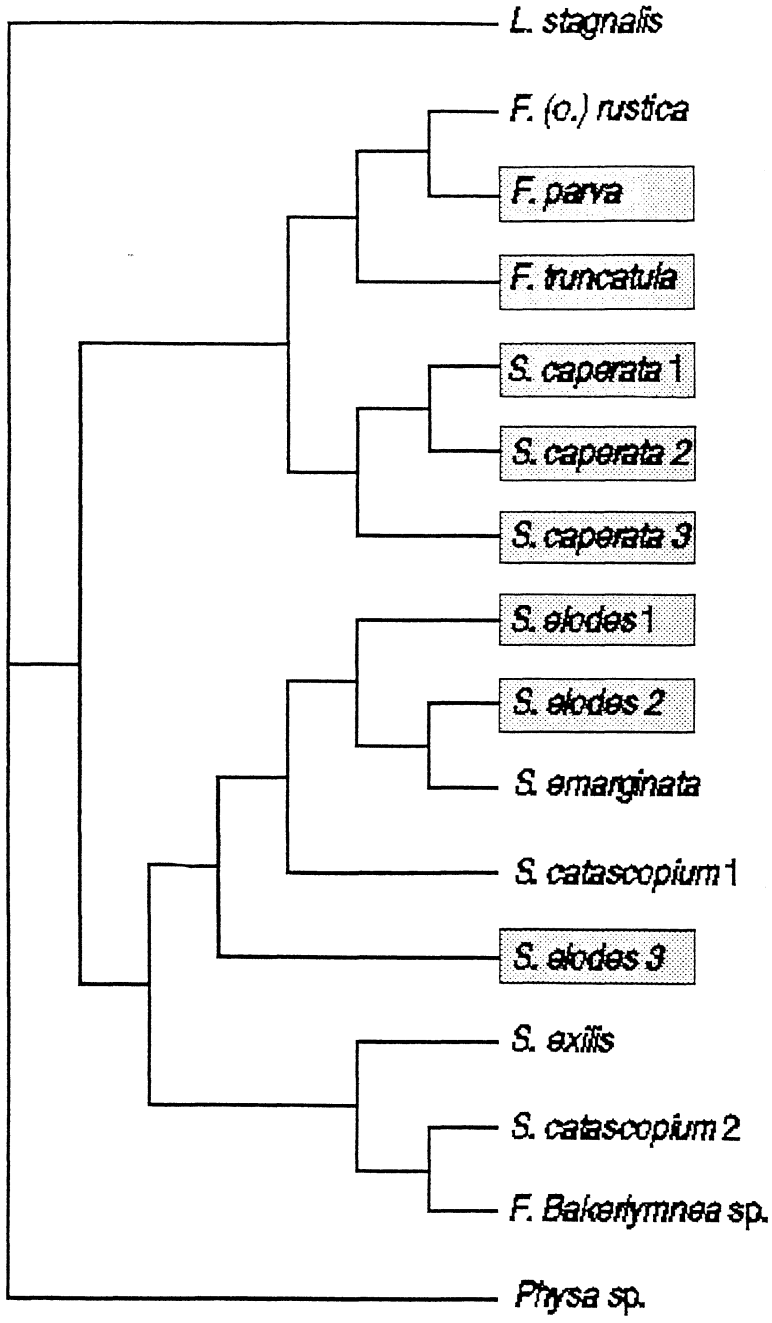

L. stagnalis
F. (o.) rustica
F. parva
F. truncatula
Tricuspid Teeth
S. caperata 1
S. caperata 2
S. caperata 3
S. elodes 1
S. elodes 2
S. emarginata
S. catascopium 1
S. elodes 3
S. exilis
S. catascopium 2
F. Bakerlymnea sp.
Bicuspid Teeth
Physa sp.

Figure 6: Topology from neighbor-joining analyses coded for known intermediate hosts of *Fascioloides magna*. Host species are shown in boxes.


L. stagnalis
F. (o.) rustica
F. parva
F. truncatula
S. caperata 1
S. caperata 2
S. caperata 3
S. elodes 1
S. elodes 2
S. emarginata
S. catascopium 1
S. elodes 3
S. exilis
S. catascopium 2
F. Bakerlymnea sp.
Physa sp.

Appendix 1: Aligned data set of internal transcribed spacer sequences for lymnaeid snails sampled that was used in parsimony and distance analyses.

```
#NEXUS

[Created by Se-Al]
BEGIN DATA;
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    FORMAT MISSING=? GAP=- DATATYPE=DNA;
    MATRIX


L. stagnalis        ATT--TTGGTCAGTCTC-AGTCTCAGTCAGTANCTCCATG-TCCTT--GG-TCCCGCGGCAGGCGTG-CATGAAGCGCTGTC
F. o. rustica       AGTTGCTCCTCGTCTCGTCACTTGTCAGTCAGTCAGTCAGTCTCGTTGGGGCCCCGCGGCAGGCAACGCATGAAGCGCTGTC
F. parva            AGTTGCTCCTCGTCTCGTCACTTGTCAGTCAGTCAGTCAGTCTCGTTGGGGCCCCGCGGCAGGCAACGCATGAAGCGCTGTC
S. caperata 1       AGAAAAGGGAAGAAAAC-ATCCTAACTCAGTCAN-GTCT--CAGCTC-GG-CCCCGCGACAGACGCG-CATGAAGCGCCGTC
S. caperata 2       AGAAAAGGGAAGAAAAC-ATCCTAAGTCAGTCA--GTCT--CAGTT--GGCCCCCGCGACAGACGCG-CATGAAGCGCCGTC
S. caperata 3       agaaaagggaagaaaac-atcctaagtcagtca--gtct--cagtt--gg-cccegcgacagacgcg-catgaagcgccgtc
F. truncatula       aaccgagcgttgactactttgttgtctcagtca--gtcagtcagt-cagg-cccegcggcaggcacg-catgaagcgctgtc
S. elodes 3         agcagctactgtgtttttcgtctcagtcagtctctgtccgctctct--gg-cccegtggtcggcacg-catgaagcgtcgcc
S. elodes 1         AGCAGCTACTGTGTTTTTCGTCTCAGTCAGTCTCTGTCCGCTCTCT--GG-CCCCGTGGTCGGCACG-CATGAAGCGTCGCC
S. exilis           NGACGCACCTGTGTTTT-CGTCTCAGTCAGTCTCTGTCCGCTCTCT--GG-CCCCGTGGTCGGCACG-CATGAAGCGTCGCC
S. catascopium 1    AGCAGCTACTGTGTTTT-CGTCTCAGTCAGTCTCTGTCCGCTCTCT--GG-CCCCGTGGTCGGCACG-CATGAAGCGTCGCC
S. catascopium 2    agcagctactgtgtttt-cgtctcagtcagtctctgtccgctcct--gg-cccegtggtcggcacg-catgaagcgtcgcc
S. emarginata       agcagctactgtgtttt-cgtctcagtcagtctctgtccgctctct--gg-cccegtggtcggcacg-catgaagcgtcgcc
S. elodes 2         AGCAGCCACTGTGTTTTTCGTCTCAGTCAGTCTCTGTCCGTCTCT--GG-CCCCGTGGTCGGCACG-CATGAAGCGTCGCC
F. b. bulimoides    AGCAGCTACTGTGTTTT-CGTCTCAGTCAGTCTCTGTCCGCTCTCT--GG-CCCCGTGGTCGGCACG-CATGAAGCGTCGCC
Physa sp.           ??CCATGAACAATGAAACGTATCAAAACGTCTCGTCCGATGTCTGCCCGGTCGGGACTT--GGC--CGCACGAAGCGCGGTC


L. stagnalis        ----GCGGGGC-TGTGAACGC-----TATGTCTTT-CGGGGTACCTAT---CACTGTNNTCGATGCGACCCACGGTGACGGC
F. o. rustica       GACAGCGGGGCCTGCTATGTGTCCGCTTCGTCTTT-CGGGGTACCTAC---TACTGTCCTCGATGCGACCCACGGTGACGGC
F. parva            GACAGCGGGGCCTGCTATGTGTCCGCTTCGTCTTT-CGGGGTACCTAC---TACTGTCCTCGATGCGACCCACGGTGACGGC
S. caperata 1       ----TCGGGGC-TGTGACCGC------ATGTCTTTGCGGGGTACCTATACTTACTGTCCTCGATGCGACCCACGGTGGCGGC
S. caperata 2       ----GCGGGGC-TGTGACCGC------ATGTCTTT-CGGGGTACCTAT-CTTACTGTCCTCGATGCGACCCACGGTGGCGGC
S. caperata 3       ----tcgggge-tgtgaccgc------atgtcttt-cggggtacctat-cttactgtcctcgatgcgacccacggtggcggc
F. truncatula       ----gcgggge-tgtgtccgc-----ttcgtcttt-cggggtacctat---tactgtcctcgatgcgacccacggtgacggc
S. elodes 3         ----gcgggge-tgcgaccac------atgtcttt-cggggtacctat-cacactgtcctcgatgcgacccacggtgacggc
S. elodes 1         ----GCGGGGC-TGCGACCAC------ATGTCTTT-CGGGGTACCTAT-CACACTGTCCTCGATGCGACCCACGGTGACGGC
S. exilis           ----GCGGGGC-TGCGACCAC------ATGTCTTT-CGGGGTACCTAT-CACACTGTCCTCGATGCGACCCACGGTGACGGC
S. catascopium 1    ----GCGGGGC-TGCGACCAC------ATGTCTTT-CGGGGTACCTAT-CACACTGTCCTCGATGCGACCCACGGTGACGGC
S. catascopium 2    ----gcgggge-tgcgaccac------atgtcttt-cggggtacctat-cacactgtcctcgatgcgacccacggtgacggc
S. emarginata       ----gcgggge-tgcgaccac------atgtcttt-cggggtacctat-cacactgtcctcgatgcgacccacggtgacggc
S. elodes 2         ----GCGGGGC-TGCGACCAC------ATGTCTTT-CGGGGTACCTAT-CACACTGTCCTCGATGCGACCCACGGTGACGGC
F. b. bulimoides    ----GCGGGGC-TGCGACCAC------ATGTCTTT-CGGGGTACCTAT-CACACTGTCCTCGATGCGACCCACGGTGACGGC
Physa sp.           TCGATCGGCTC--G--ACCGCTCCCC----TGTTT-CGGGGTACCTAG--TGCATGTCCTCGATGCGACCCACGGTGACGGC


L. stagnalis        TTAGAGCCCG-TGTGCTCGCCGGG-TCGC---GAC------GGTTCAAAGAGTGGCCGGCCTCACG----------------
F. o. rustica       TTAGAG-CCAGTGTGCTCGCCGGG-TCGC---GAC------GGTTCAAAGAGTGGCCTGC-TC-GGCCTC--CTCGGCCTCC
F. parva            TTAGAG-CCAGTGTGCTCGCCGGG-TCGC---GAC------GGTTCAAAGAGTGGCCTGC-TC-GGCCTC--CTCGGCCTCC
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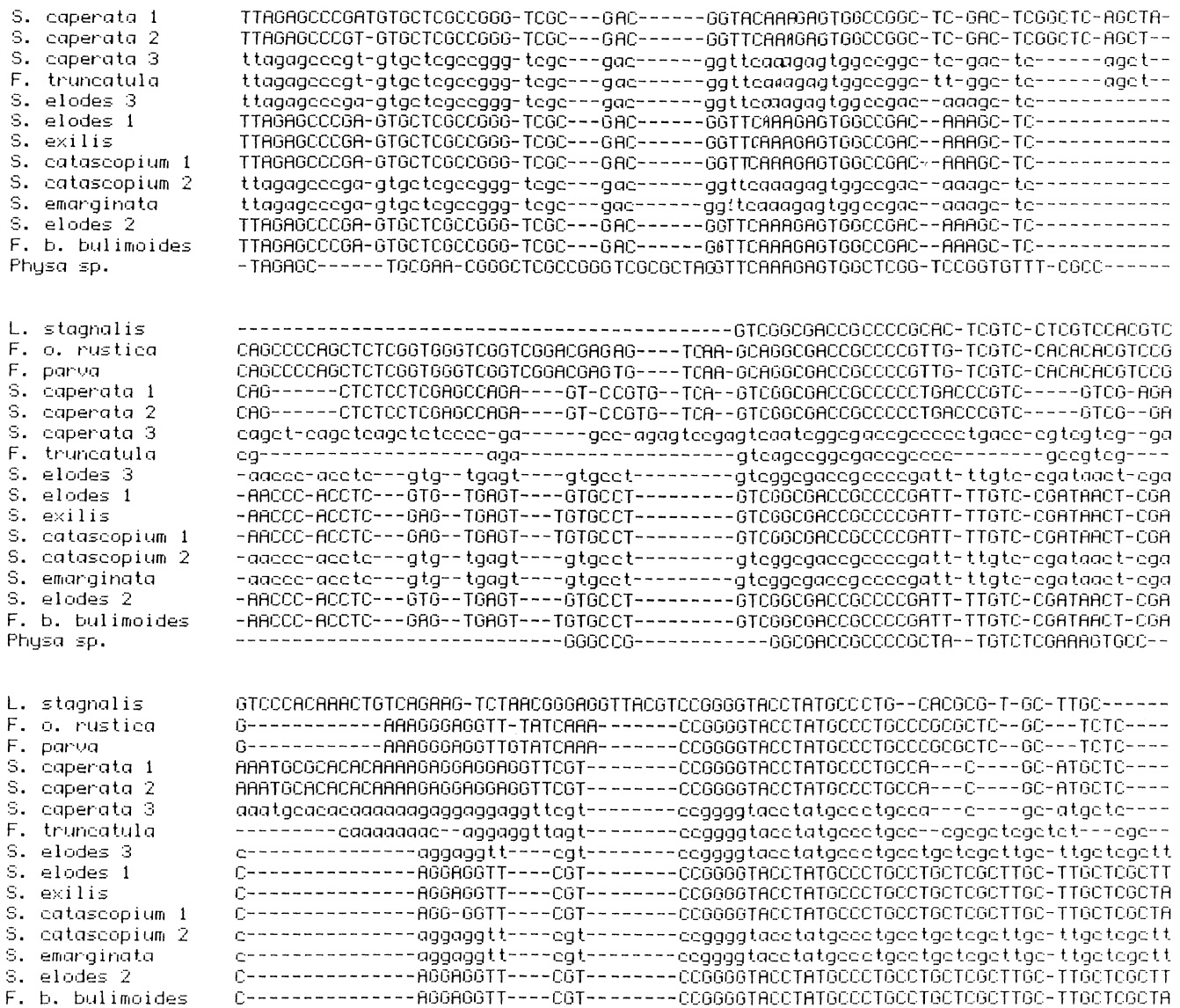
S. caperata 1
S. caperata 2
S. caperata 3
F. truncatula
S. elodes 3
S. elodes 1
S. exilis
S. catascopium 1
S. catascopium 2
S. emarginata
S. elodes 2
F. b. bulimoides
Physa sp.
L. stagnalis
F. o. rustica
F. parva
S. caperata 1
S. caperata 2
S. caperata 3
F. truncatula
S. elodes 3
S. elodes 1
S. exilis
S. catascopium 1
S. catascopium 2
S. emarginata
S. elodes 2
F. b. bulimoides
Physa sp.
L. stagnalis
F. o. rustica
F. parva
S. caperata 1
S. caperata 2
S. caperata 3
F. truncatula
S. elodes 3
S. elodes 1
S. exilis
S. catascopium 1
S. catascopium 2
S. emarginata
S. elodes 2
F. b. bulimoides

```
Physa sp.           --------------------GGT---------------CAAGGGGTACCTGCGTCTCGTCCCGCGAGGGA-----------


L. stagnalis        -----------ATGCAGGGTGGTAGCTCCAGCTTAGCTAGCTCGTC-ACTT-TGGCCGCGAGGTTCAAAGAGTC-GGCC-GT
F. o. rustica       GCGCTGGCAGCA---GGGT-GGTAGCTCCATCA-----AGCTCGAT--CTT-TGGCCGCGAGGTTCAAAGAGAC-GACC-GT
F. parva            GCGCTGGCAGCAT--GGGT-GGTAGCTCCATCA-----AGCTCGAT--CTT-TGGCCGCGAGGTTCTAAAGAGA-CGACCGT
S. caperata 1       GCG-TGCCTGCA---GGGC-GGTAGCTCCAGCA--------TCGTTTACTTATGGCCGCGAGGTTCAAAGAGTC-GACC-GC
S. caperata 2       GCG-TGCCTGCA---GGGC-GGTAGCTCCAGC---------TCGTTTACTT-TGGCCGCGAGGTTCAAAGAGTC-GACC-GC
S. caperata 3       gcg-tgcctgca---gggc-ggtagctccagc---------tcgtttactt-tggccgcgaggttcaaagagtc-gacc-gc
F. truncatula       gcc--g---gca---aggc-ggtagctccagc---------tcgct-a-tt-tggccgcgaggttcaaagagac-gacc-gt
S. elodes 3         gcc-tgcctgca---gggt-ggtagctccagc---------tcgtt--ctt-tggccgcgaggttcaaagagtc-ggcc-gt
S. elodes 1         GCC-TGCCTGCA---GGGT-GGTAGCTCCAGC---------TCGTT--CTT-TGGCCGCGAGGTTCAAAGAGTC-GGCC-GT
S. exilis           GCC-TGCCTGCA---GGGT-GGTAGCTCCAGC---------TCGTT--CTT-TGGCCGCGAGGTTCAAAGAGTC-GGCC-GT
S. catascopium 1    GCC-TGCCTGCA---GGGT-GGTAGCTCCAGC---------TCGTT--CTT-TGGCCGCGAGGTTCAAAGAGTC-GGCC-GT
S. catascopium 2    gcc-tgcctgca---gggt-ggtagctccagc---------tcgtt--ctt-tggccgcgaggttcaaagagtc-ggcc-gt
S. emarginata       gcc-tgcctgca---gagt-ggtagctccagc---------tcgtt--ctt-tggccgcgaggttcaaagagtc-ggcc-gt
S. elodes 2         GCC-TGCCTGCA---GGGT-GGTAGCTCCAGC---------TCGTT--CTT-TGGCCGCGAGGTTCAAAGAGTC-GGCC-GT
F. b. bulimoides    GCC-TGCCTGCA---GGGT-GGTAGCTCCAGC---------TCGTT--CTT-TGGCCGCGAGGTTCAAAGAGTC-GGCC-GT
Physa sp.           --------------TGGGGCGGGAGCTCCAGCTTCCATAGCA-----------GGCCGCGAGGTTCAAAGAGTCCGACCCGC


L. stagnalis        -GCCATTCCC-GCTCTC---GATGGCACCG-GTCGCCGCCCCGGGCCTCCCACAA------TTTTTTCCTTTA-CGAAA---
F. o. rustica       TGCCC--ACTT---------------GTCG-GTCGCCGCCCCGGGCCTCCTTAAA------TTT?????????????????
F. parva            TGCCC--ACTT---------------GTCGAGTCGCCGCCCCGGGCCTCCTTAAA------TTT?????????????????
S. caperata 1       -GCCA-TACATGCTCTC---GTTGGCA-CG-GTCGCCGCCCCGGGCCTCCTAAAA------TTT---CATTTA-CCAAAAAA
S. caperata 2       -GCCA-TACATGCTCTC---GTTGGCA-CG-GTCGCCGCCCCGGGCCTCCTAAAA------TTT---CATTTA-CCAAAAAA
S. caperata 3       -gcca-tacatgctctc---gtgggca-cg-gtcgccgccccgggcctcctaaaa------ttt---cattta-ccagaa--
F. truncatula       -gccttaacttgctctctccgtgggcaacg-gtcgccgccccgggcctcctaaaa------ttt---cattta---------
S. elodes 3         -gccac-acttgctctc---gatggcttcg-gtcgccgccccgggcctcctacaa------ttt---cattt-ccaaa---
S. elodes 1         -GCCAC-ACTTGCTCTC---GATGGCTTCG-GTCGCCGCCCCGGGCCTCCTAAAA------TTT---CCTTTTTCCAAA---
S. exilis           -GCCAC-ACTTGCTCTC---GATGGCTTCG-GTCGCCGCCCCGGGCCTCCTAAAA------TTT---CCTTTTTCCAAA---
S. catascopium 1    -GCCAC-ACTTGCTCTC---GATGGCTTCG-GTCGCCGCCCCGGGCCTCCTAAAA------TTT---CCTTTTTCCAAA---
S. catascopium 2    -gccat-acttgctctc---gatggcttcg-gtcgccgccccgggcctcctaaaa------ttt---cattttccaaa---
S. emarginata       -gccac-acttgctctc---gatggcttcg-gtcgccgccccgggcctcctaaaa------ttt---cattttccaaa---
S. elodes 2         -GCCAC-ACTTGCTCTC---GATGGCTTCG-GTCGCCGCCCCGGGCCTCCTAAAA------TTT---CCTTTT-CCAAA---
F. b. bulimoides    -GCCAT-ACTTGCTCTC---GATGGCTTCG-GTCGCCGCCCCGGGCCTCCTAAAA------TTT---CCTTTTTCCAAA---
Physa sp.           ----------------------TCGCGTCG----GCCGCCCCCTTGTCCTCACGTTACATTTTTT---CCATTTT--GAACGC


L. stagnalis        CACAAAA----TTAATTTCAAAAACGC-CGATT-AGG-TC-----AGGC---CACGAACACGACCAG------TCGGCTGGT
F. o. rustica       ?????????????????????????????????????????????????????????????????????????????
F. parva            ?????????????????????????????????????????????????????????????????????????????
S. caperata 1       CA-AAAA-----TA-TTTTTT-AATCAATCGACTCGG-TCG----TGGC--ATACGA-TC--GG---------TCG---TGT
S. caperata 2       CA-AAAA-----TA-TTTTTT-AATCAATCGACTCGG-TCG----TGGC--ATACGA-TC--GG---------TCG---TGT
S. caperata 3       ca-aaat-----ta-ttttttaatcaatcgactcgg-tcg----tggc--atacga-tc--gg---------tcg---tgt
F. truncatula       ataaaacgaaatta-ttttttaaaaatgtgtg-tcggctcgatcgtggc--acac----------------------------
S. elodes 3         ct-aaaataaa-atattt-----atcgttgaaa--------------gc--agtcgactcgaggcaatcgagttcggtctgt
S. elodes 1         CT-AAAAGAAA-TA-TTT-----ATCGTTGAAA--------------GC--AGTCGACTCGAGGCAATCGAGTTCGGTCTGT
```

```
S. exilis          CT-AAAAGAAA-TA-TTT-----ATCGTTGAAA----------GC--AGTCGACTCGAGGCAATCGAGTTCGGTCTGT
S. catascopium 1   CT-AAAAGAAA-TA-TTT-----ATCGTTGAAA----------GC--AGTCGACTCGAGGCAATCGAGTTCGG-----
S. catascopium 2   ct-aaaagaaa-ta-ttt-----atcgttgaaa----------gc--agtcgactcgaggcaatcgagttcggtctgt
S. emarginata      ct-aaaagaaa-ta-ttt-----atcgttgaaa----------gc--agtcgactcgaggcaatcgagttcggtctgt
S. elodes 2        CT-AAAAGAAA-TA-TTT-----ATCGTTGAAA----------GC--AGTCGACTCGAGGCAATCGAGTTCGGTCTGT
F. b. bulimoides   CT-AAAAGAAA-TA-TTT-----ATCGTTGAAA----------GC--AGTCGACTCGAGGCAATCGAGTTCGGTCTGT
Physa sp.          ATTGTGCA-------TTTTTTCACTTTTACGAA-CGGCTGTC-----GGACAGGAGCGCCG----------CGGTC---

L. stagnalis       GCAAG-------T-CAAATAAAAAAAGTCTATAACTTTGAGCGGTGGATCACTCGGCTCGTGCGTCGATGAAGAGCGCAGCC
F. o. rustica      ??????????????????????????????????????????????????????????????????????????????
F. parva           ??????????????????????????????????????????????????????????????????????????????
S. caperata 1      C-GAAAA-----TACAAA------AAGTCTATAACTTTGAGCGGTGGATCACTCGGCTCGTGCGTCGATGAAGAGCGCAGCC
S. caperata 2      C-GAAAA-----TACAAA------AAGTCTATAACTTTGAGCGGTGGATCACTCGGCTCGTGCGTCGATGAAGAGCGCAGCC
S. caperata 3      c-gaaaa-----tacaaa------aagtctataactttgagcggtggatcactcggctcgtgcgtcgatgaagagcgcagcc
F. truncatula      --gaaaaa------caaaa--caaaagt-cttat??????????????????????????????????????????????
S. elodes 3        cggaa-------tacaaa----aaaagtttataactttgagcggtggatcactcggctcgtgcgtcgatgaagagcgcagcc
S. elodes 1        CGGAA-------TACAAA--AAAAAAGTTTATAACTTTGAGCGGTGGATCACTCGGCTCGTGCGTCGATGAAGAGCGCAGCC
S. exilis          CGGAA-------TACAAA---AAAAAGTTTATAACTTTGAGCGGTGGATCACTCGGCTCGTGCGTCGATGAAGAGCGCAGCC
S. catascopium 1   ---AA-------TACAAA---AAAAAGTTTATAACTTTGAGCGGTGGATCACTCGGCTCGTGCGTCGATGAAGAGCGCAGCC
S. catascopium 2   cggaa-------tacaaa--aaaaaagtttataactttgagcggtggatcactcggctcgtgcgtcgatgaagagcgcagcc
S. emarginata      cggaa-------tacaaa--aaaaaagtttataactttgagcggtggatcactcggctcgtgcgtcgatgaagagcgcagcc
S. elodes 2        CAGAA-------TACAAA----AAAAGTTTATAACTTTGAGCGGTGGATCACTCGGCTCGTGCGTCGATGAAGAGCGCAGCC
F. b. bulimoides   CGGAA-------TACAAA-AAAAAAAGTTTATAACTTTGAGCGGTGGATCACTCGGCTCGTGCGTCGATGAAGAGCGCAGCC
Physa sp.          --GAAAAAAAAATCCAAA-----------TACAACTTTGGGCGGTGGATCACTCGGCTCGTGCGTCGATGAAGAGCGCAGCC

L. stagnalis       AGCTGCGTGAATTAATGTGAATTGCAGAACACATTGAACATCGATATCTTGAACGCATATGGCGGCCTCGGGTCAATCCCGG
F. o. rustica      ??????????????????????????????????????????????????????????????????????????????
F. parva           ??????????????????????????????????????????????????????????????????????????????
S. caperata 1      AGCTGCGTGAATTAATGTGAATTGCAGAACACATTGAACATCGATATCTTGAACGCATATGGCGGCCTCGGGTCAATCCCGG
S. caperata 2      AGCTGCGTGAATTAATGTGAATTGCAGAACACATTGAACATCGATATCTTGAACGCATATGGCGGCCTCGGGTCAATCCCGG
S. caperata 3      agctgcgtgaattaatgtgaattgcagaacacattgaacatcgatatcttgaacgcatatggcggcctcgggtcaatcccgg
F. truncatula      ??????????????????????????????????????????????????????????????????????????????
S. elodes 3        agctgcgtgaattaatgtgaattgcagaacacattgaacatcgatatcttgaacgcatatggcggcctcgggtcaatcccgg
S. elodes 1        AGCTGCGTGAATTAATGTGAATTGCAGAACACATTGAACATCGATATCTTGAACGCATATGGCGGCCTCGGGTCAATCCCGG
S. exilis          AGCTGCGTGAATTAATGTGAATTGCAGAACACATTGAACATCGATATCTTGAACGCATATGGCGGCCTCGGGTCAATCCCGG
S. catascopium 1   AGCTGCGTGAATTAATGTGAATTGCAGAACACATTGAACATCGATATCTTGAACGCATATGGCGGCCTCGGGTCAATCCCGG
S. catascopium 2   agctgcgtgaattaatgtgaattgcagaacacattgaacatcgatatcttgaacgcatatggcggcctcgggtcaatcccgg
S. emarginata      agctgcgtgaattaatgtgaattgcagaacacattgaacatcgatatcttgaacgcatatggcggcctcgggtcaatcccgg
S. elodes 2        AGCTGCGTGAATTAATGTGAATTGCAGAACACATTGAACATCGATATCTTGAACGCATATGGCGGCCTCGGGTCAATCCCGG
F. b. bulimoides   AGCTGCGTGAATTAATGTGAATTGCAGAACACATTGAACATCGATATCTTGAACGCATATGGCGGCCTCGGGTCAATCCCGG
Physa sp.          AGCTGCGTGAATTAATGTGAATTGCAGAACACATTGAACATCGATATCTTGAACGCACATGGCGGCCTCGGGTCCATCCCGG

L. stagnalis       GGCCACGCCCGTCTGAGGGTCGGCTAGTCACAA-GCAATCGTGTCCCGTTTGC---TCTCACGAAACCGG-AGCCTTTTCTC
F. o. rustica      ??????????????????????????????????????????????????????????????????????????????
F. parva           ??????????????????????????????????????????????????????????????????????????????
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S. caperata 1       GGCCACGCCCGTCTGAGGGTCGGCTAGTCACAAAGCAATCGTGTCC----TGTAGCTCTCGCAAAACTGG-AGCCGTCTC-C
S. caperata 2       GGCCACGCCCGTCTGAGGGTCGGCTAGTCACAAAGCAATCGTGTCC----TGTAGCTCTCGCAAAACTGG-AGCCGTCTC-C
S. caperata 3       ggccacgcccgtctgagggtcggctagtcacaaagcaatcgtgtcc----tgtagctctcgcaaaactgg-agccgtctc-c
F. truncatula       ??????????????????????gctagtcacaaagcattcgtgtcc---ttgcagctctcgcaaaaaccgaagcctt----g
S. elodes 3         ggccacgcccgtctgagggtcggctagtcacaaagcaatcgtgtcc---ttgcagctctcgcaggaccgg-agcctt----c
S. elodes 1         GGCCACGCCCGTCTGAGGGTCGGCTAGTCACAAAGCAATCGTGTCC---TTGCAGCTCTCGCAGGACCGG-AGCCTT----C
S. exilis           GGCCACGCCCGTCTGAGGGTCGGCTAGTCACAAAGCAATCGTGTCC---TTGCAGCTCTCGCAGGACCGG-AGCCTT----C
S. catascopium 1    GGCCACGCCCGTCTGAGGGTCGGCTAGTCACAAAGCAATCGTGTCC---TTGCAGCTCTCGCAGGACCGG-AGCCTT----C
S. catascopium 2    ggccacgcccgtctgagggtcggctagtcacaaagcaatcgtgtcc---ttgcagctctcgcaggaccgg-agcctt----c
S. emarginata       ggccacgcccgtctgagggtcggctagtcacaaagcaatcgtgtcc---ttgcagctctcgcaggaccgg-agcctt----c
S. elodes 2         GGCCACGCCCGTCTGAGGGTCGGCTAGTCACAAAGCAATCGTGTCC---TTGCAGCTCTCGCAGGACCGG-AGCCTT----C
F. b. bulimoides    GGCCACGCCCGTCTGAGGGTCGGCTAGTCACAAAGCAATCGTGTCC---TTGCAGCTCTCGCAGGACCGG-AGCCTT----C
Physa sp.           GGCCACGTCCGTCTGAGGGTCGGCGACT---AAATCAATCGAGCTCGTCTGTTTTTCCACG----------------------

L. stagnalis        GG--------TTCCTCCCATCAACCACCCACC--GCTCGT---TGGGTGGAAGGAAGAGGGGGGGGGGGAC----------TG
F. o. rustica       ?GCCGTGGCCTTGCGGCGGCGCCAGTCTCTCCTCAACGCTCGAT-GGTGGGGGG------------AACACAACGCGCTGTT
F. parva            ?GCCGTGGCCTTGCGGCGGGCGCAGTCTCTCCTCA-CGCTCGAT-GGTGGGGGG-----------GAACA-AACGCGCTGTT
S. caperata 1       CCCCCTGGCA-------CACACCGTCTCCGACTTGCTCGT---T-GGAGACGCGACGCT-GGGG-GAGTA------------
S. caperata 2       CCCCCTGGCA-------CACACCGTCTCCGACTTGCTCGT---T-GGAGACGCGACGCT-GGGG-GAGTA------------
S. caperata 3       ccc--tggca-------cacaccgtctccgacttgctcgt---t-ggagacgcgacgct-ggggggagta------------
F. truncatula       cggcgtgagctct----cacgctg-ctcggc---gatggt---t-ggatacgc----------------------------
S. elodes 3         cgccgtggactctcattcacagcgcctccgacttgctcgtcg---gggt-------gcttgggg-gaccc---------gtc
S. elodes 1         CGCCGTGGACTCTCATTCACAGCGCCTCCGACTTGCTCGTCG---GGGT-------GCTTGGGG-GACCC---------GTC
S. exilis           CGCCGTGGACTCTTATTCACAGCGCCTCCGACTTGCTCGTCG---GGGGG------GCTTGGGG-GACCC---------GTC
S. catascopium 1    CGCCGTGGACTCTTATTCACAGCGCCTCCGACTTGCTCGTCG---GGGGG------GCTTGGGG-GACCC---------GTC
S. catascopium 2    cgccgtggactctcattcacagcgcctccgacttgctcgtcg---gggt-------gcttgggg-gaccc---------gtc
S. emarginata       cgccgtggactctcattcacagcgcctccgacttgctcgtcg---gggt-------gcttgggg-gaccc---------gtc
S. elodes 2         CGCCGTGGACTCTCATTCACAGCGCCTCCGACTTGCTCGTCG---GGGT-------GCTTGGGG-GACCC---------GTC
F. b. bulimoides    CGCCGTGGACTCTTATTCACAGCGCCTCCGACTTGCTCGTCG---GGGGG------GCTTGGGG-GACCC---------GTC
Physa sp.           ------------------------------------------------------------ACAC----------TC

L. stagnalis        AATCGG-CACCGGCTGGACACGCTCTGGACCTTCGCGG-----TTTGC----GCTGTC--GCTGCACTATTCGT--------
F. o. rustica       GCTCGG-CGATGGCTGGATACGCCTTGGACCCTCGCGG---CCTTAGCCGTTGCTGCCTTGCTTTTTTTTTGGCTCTCGGCA
F. parva            GCTCGG-CGATGGCTGGATACGCCTTGGACCCTCGCGG---CCTTAGCCGTCGCTGCCTTGCTTTTTTTTTGGCTCTCGGCA
S. caperata 1       ----GG-CACCGGTTGGACACGCCCTGGACCCTCGCGG---CCT-ATACCGTCGTCGCTGCCGCTAGCCCTTGCGGTTGGTG
S. caperata 2       ----GG-CACCGGTTGGACACGCCCTGGACCCTCGCGG---CCT-ATACCGTCGTCGCTGCCGCTAGCCCTTGCGGTTGGTG
S. caperata 3       ----gg-caccggttggacacgccctggaccctcgcgg---cct-ataccgtcgtcgccgccgccttccctggtggttggtg
F. truncatula       ----------------------cctggaccctcgcgg---cca-aagctgtcgttgc-----------ctgctcg------
S. elodes 3         gttcgg-caccggtcggacacgccctggaccctcgcgg---cct-acaccgtcgctgctt----------------------
S. elodes 1         GTTCGG-CACCGGTCGGACACGCCCTGGACCCTCGCGG---CCT-ACACCGTCGCTGCTT----------------------
S. exilis           GTTCGG-CACCGGTCGGACACGCCCTGGACCCTCGCGG---CCT-ACACCGTCGCTGCTT----------------------
S. catascopium 1    GTTCGG-CACCGGTCGGACACGCCCTGGACCCTCGCGG---CCT-ACACCGTCGCTGCTT----------------------
S. catascopium 2    gttcgg-caccggtcggacacgccctggaccctcgcgg---cct-acaccgtcgctgctt----------------------
S. emarginata       gttcgg-caccggtcggacacgccctggaccctcgcgg---cct-acaccgtcgctgctt----------------------
S. elodesm 2        GTTCGG-CACCGGTCGGACACGCCCTGGACCCTCGCGG---CCT-ACACCGTCGCTGCTT----------------------
F. b. bulimoides    GTTCGG-CACCGGTCGGACACGCCCTGGACCCTCGCGG---CCT-ACACCGTCGCTGCTT----------------------
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Physa sp.           GTCGGGCTGGAAAGCGGGCTCGCCCTGGACCGTCGAGGTTTCCT--------CGCTCC----------------------AC

L. stagnalis        GCGGTGATGGCAAC----GGCCCCGTGGTCTTAAGT-ACAAACCGCGCCGTTGTCCGTT-CATCTCGTAACGTCTT-CGACG
F. o. rustica       GCGGCGACGGTGAC----GGCCCCGTGGTCTTAAGTGC--AAGCCGC--GCCG-----ATGTCCGTTTCACCTCGTAACGTC
F. parva            GCGGCGACGGTGAC----GGCCCCGTGGTCTTAAGTGC--AAGCCGC--GCCG-----ATGTCCGTTTCACCTCGTAACGTC
S. caperata 1       GCGGTGATAGTGGTGG-TGGCCCCGTGGTCTTAAGC-GCAAGCCGCGCCGTTGTCCGTT-CATCTCGTAACGTCTT-CGACG
S. caperata 2       GCGGTGATAGTGGTGG-TGGCCCCGTGGTCTTAAGC-GCAAGCCGCGCCGTTGTCCGTT-CATCTCGTAACGTCTT-CGACG
S. caperata 3       gcggtgatagtggtgggtggccccgtggtcttaagc-gcaagccgcgccgttgtccgtt-catctcgtaacgtctt-cgacg
F. truncatula       gcggcgacggtgac----ggtcccgtggtcttaagc-gcaagccgcgccgttgtccgtt-catctcgtaacgtctt-cgacg
S. elodes 3         gcggtgacagtggt----ggccccgtggtcttaagc-gcaagccgcgccgttgtccgtt-catctcgtaacgtctt-cgacg
S. elodes 1         GCGGTGACAGTGGT----GGCCCCGTGGTCTTAAGC-GCAAGCCGCGCCGTTGTCCGTT-CATCTCGTAACGTCTT-CGACG
S. exilis           GCGGTGACAGTGGT----GGCCCCGTGGTCTTAAGC-GCAAGCCGCGCCGTTGTCCGTT-CATCTCGTAACGTCTT-CGACG
S. catascopium 1    GCGGTGACAGTGGT----GGCCCCGTGGTCTTAAGC-GCAAGCCGCGCCGTTGTCCGTT-CATCTCGTAACGTCTT-CGACG
S. catascopium 2    gcggtgacagtggt----ggccccgtggtcttaagc-gcaagccgcgccgttgtccgtt-catctcgtaacgtctt-cgacg
S. emarginata       gcggtgacagtggt----ggccccgtggtcttaagc-gcaagccgcgccgttgtccgtt-catctcgtaacgtctt-cgacg
S. elodes 2         GCGGTGACAGTGGT----GGCCCCGTGGTCTTAAGC-GCAAGCCGCGCCGTTGTCCGTT-CATCTCGTAACGTCTT-CGACG
F. b. bulimoides    GCGGTGACAGTGGT----GGCCCCGTGGTCTTAAGC-GCAAGCCGCGCCGTTGTCCGTT-CATCTCGTAACGTCTT-CGACG
Physa sp.           TCGGGCGG------GGAT--CCCCGTGGTTTCAAGT-CCAAGCTGCGCCGTCGTCCT----ATGTCCT---------CGATG

L. stagnalis        CTGCCCTGCTCATGGCGGC---CTGTCCGTTTTCTCTA-------CCGCC----AGGCAGGACCCGGCTCG-CTACTTGATT
F. o. rustica       TT-CGACGCTGCCCTGCTCTTGGTGGCGGCCTGTCCGTTTTGTCTCCGCC-GCCAGGCAGGACCCGGCTCG----CTTTATA
F. parva            TTTCGACGCTGCCCTGCTCTTGGTGGCGGCCTGTCCGTTTTGTCTCCGCC-GCCAGGCAGGACCCGGCTCG----CTTTATA
S. caperata 1       CTGCCCTGCTCTTGGCGGCGGTCTGTCCATTTTCTCTA-------CCGCC----AGGCAGGACCCGGCTCG----CTAATC-
S. caperata 2       CTGCCCTGCTCTTGGCGGCGGTCTGTCCATTTTCTCTA-------CCGCC----AGGCAGGACCCGGCTCG----CTAATC-
S. caperata 3       ctgccctgctcttggcggtggtctgtccattttctcta-------ccgcc----aggcaggaccccggctcg----ctactc-
F. truncatula       ctgccctgctcttggcggc---ctgtccgttttctcta-------ccgcc----aggcaggaccccggctcg-cttactttat
S. elodes 3         ctgccctgctcttggcggc---ctgtccattttctcta-------ccgcc----aggcaggaccccggctcg-ctactttat-
S. elodes 1         CTGCCCTGCTCTTGGCGGC---CTGTCCATTTTCTCTA-------CCGCC----AGGCAGGACCCGGCTCGGCTACTTTAT-
S. exilis           CTGCCCTGCTCTTGGCGGC---CTGTCCATTTTCTCTA-------CCGCC----AGGCAGGACCCGGCTCG-CTACTTTAT-
S. catascopium 1    CTGCCCTGCTCTTGGCGGC---CTGTCCATTTTCTCTA-------CCGCC----AGGCAGGACCCGGCTCG-CTACTTTAT-
S. catascopium 2    ctgccctgctcttggcggc---ctgtccattttctcta-------ccgcc----aggcaggaccccggctcg-ctactttat-
S. emarginata       ctgccctgctcttggcggc---ctgtccattttctcta-------ccgcc----aggcaggaccccggctcg-ctactttat-
S. elodes 2         CTGCCCTGCTCTTGGCGGC---CTGTCCATTTTCTCTA-------CCGCC----AGGCAGGACCCGGCTCG-CTACTTTAT-
F. b. bulimoides    CTGCCCTGCTCTTGGCGGC---CTGTCCATTTTCTCTA-------CCGCC----AGGCAGGACCCGGCTCG-CTACTTTAT-
Physa sp.           CTGC-------TTGCGCGG--------------TTTCA-------CCGCCTGGCAGGAC----TCGGCTCGTGTA???????

L. stagnalis        AATTTCTGGAGGCGAT-CACGGGCCTGCA-GTCCTCATGGCACATCG-TCC---TTT--GCC--------------------
F. o. rustica       -------CTCGGCGTA-CTCGGGCCTGCAAGTCC--ATGGCA--TCCCAGCAGCTCGT-GGGTGGAGAGCGAACGAACACCG
F. parva            -------CTCGGCGTA-CTCGGGCCTGCAAGTCC--ATGGCA--TCCCAGCAGCTCGT-GGGTGGAGAGCGAACGAACACCG
S. caperata 1       -----------GCGAT-CTCGGGCCTGCA-GTCC--ATGGCG--TTACCGC---TCT-AGGGTGGAGA--------------
S. caperata 2       -----------GCGAT-CTCGGGCCTGCA-GTCC--ATGGCG--TTACCGC---TCT-AGGGTGGAGA--------------
S. caperata 3       -----------gcggt-ctcgggcctgca-gtcc--atggcg--ttattgc---tct-agggtggagt--------------
F. truncatula       ttattatcgt-ggcgttctcgggcctgca-gtcc--atggca--tcgcagc---tcgt-gggtggaga--------------
S. elodes 3         ggt--------gcgat-cacgggcctgca-gtcc--atggca--tcgctgc---tct-agggcggaga--------------
S. elodes 1         GGT--------GCGAT-CACGGGCCTGCA-GTCC--ATGGCA--TCGCTGC---TCT-AGGGTGGAGA--------------
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S. exilis           GGT--------GCGAT-CACGGGCCTGCA-GTCC--ATGGCA--TCGCTGC---TCT-AGGGTGGAGA--------------
S. catascopium 1    GGT--------GCGAT-CACGGGCCTGCA-GTCC--ATGGCA--TCGCTGC---TCT-AGGGTGGAGA--------------
S. catascopium 2    ggt--------gcgac-cacgggcctgca-gtcc--atggca--tcgctgc---tct-agggtggaga--------------
S. emarginata       ggt--------gcgat-cacgggcctgca-gtcc--atggca--tcgctgc---tct-agggtggaga--------------
S. elodes 2         GGT--------GCGAT-CACGGGCCTGCA-GTCC--ATGGCA--TCGCTGC---TCT-AGGGTGGAGA--------------
F. b. bulimoides    GGT--------GCGAT-CACGGGCCTGCA-GTCC--ATGGCA--TCGCTGC---TCT-AGGGTGGAGA--------------
Physa sp.           ??????????????????????????????????????????????????????????????????????????????

L. stagnalis        -TCTGCCAGC-TCGTTCATTGGTGGT-----AGGCGGGGGA???????????????
F. o. rustica       ACCGAAGAACGTAA--C----AACGAGTGTGTTGTCGGTCGGCGCCTGTC---CAC
F. parva            ACCGAAGAACTTAA--C----AACGA-TGTGTTGTCGGTCGGCGCCTGTC---CAC
S. caperata 1       ATGAGGGCTC-TCTTTCGAGGAC-GA-----TACCTGATCGGCGCCAGCCTTTCAC
S. caperata 2       ATGAGGGCTC-TCTTTCGAGGAC-GA-----TACCTGATCGGCGCCAGCCTTTCAC
S. caperata 3       ttgagggctt-tctatcgaggac-ga-----tacctgatcggcgccagcctttcac
F. truncatula       acaaggggct-cta------agacgc-----tacgtggtcggcgcccgtcgttgaa
S. elodes 3         atcggggctc-tat--cgaggaccga-----tacctgatcggcgcccgtctgtcac
S. elodes 1         ATCGGGGCTC-TAT--CGAGGACCGA-----TACCTGATCGGCGCCCGTCTGTCAC
S. exilis           ATCGGGGCTC-TAT--CGAGGACCGA-----AACCTGATCGGCGCCCGTCTGTCAC
S. catascopium 1    ATCGGGGCTC-TAT--CGAGGACCGA-----TACCTGATCGGCGCCCGTCTGTCAC
S. catascopium 2    atcggggctc-tat--cgaggaccga-----tacctgatcggcgcccgtctgtcac
S. emarginata       atcggggctc-tat--cgaggaccga-----tacctgatcggcgcccgtctgtcac
S. elodes 2         ATCGGGGCTC-TAT--CGAGGACCGA-----TACCTGATCGGCGCCCGTCTGTCAC
F. b. bulimoides    ATCGGGGCTC-TAT--CGAGGACCGA-----TACCTGATCGGCGCCCGTCTGTCAC
Physa sp.           ??????????????????????????????????????????????????????

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END;
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