Journal of Helminthology (2005) **79,** 257–267 DOI: 10.1079/JOH2005297

Reviewing lymnaeid vectors of fascioliasis by ribosomal DNA sequence analyses

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Abstract

Snails of the family Lymnaeidae are of great parasitological importance due to the numerous helminth species they transmit, mainly trematodiases (such as fascioliasis) of considerable medical and veterinary impact. The present knowledge of the genetics and host–parasite relationships of this gastropod group is far from adequate. Fascioliasis is caused by two species, *Fasciola hepatica* and *F. gigantica*, which, as in the case of other trematodes, show a marked snail host specificity. Many lymnaeid species involved in fascioliasis transmission still show a confused systematic-taxonomic status. The need for tools to distinguish and characterize species and populations of lymnaeids is evident and the present review concerns new molecular tools developed in recent years using nuclear ribosomal DNA sequences. The small subunit or 18S gene and the internal transcribed spacers ITS-2 and ITS-1 are analysed and evaluated as markers for taxon differentiation and relationships within the Lymnaeidae from genus and species levels to subspecies and population levels. rDNA sequence differences and genetic distances, and their value for reconstructing phylogenetic trees using different methods are considered. Nuclear rDNA sequences are appropriate tools on which to base a review of the systematics and taxonomy of the family Lymnaeidae, without excluding other valuable snail characteristics already available. A reconstruction of the lymnaeid system towards a more natural classification will undoubtedly be helpful in understanding parasite transmission and epidemiological features as well the dispersion of an emerging-reemerging disease such as fascioliasis. Nomenclature for nuclear rDNA genotyping in lymnaeids includes the main rDNA sequence regions able to furnish important information on interspecific differentiation and grouping as well as intraspecific variability of lymnaeid species. The composite haplotype code includes the rDNA markers arranged in order according to their well-known usefulness, in its turn related to their respective, more or less rapid evolutionary ratios, to distinguish between different taxonomic levels, from supraspecific taxa to the species level and up to the population level.

Introduction

Snails of the family Lymnaeidae are of great parasitological importance, because of their capacity to act as intermediate hosts for numerous trematode parasites, including those of medical and veterinary impact such as *Fasciola hepatica* and *F. gigantica* (Malek, 1980; Boray, 1982; Chen & Mott, 1990; Mas-Coma *et al.*, 1999a,b, 2000; Mas-Coma, 2004a,b). In recent years, the interest in lymnaeids has markedly increased due to the detection of human fascioliasis endemics, ranging from low to very high prevalences and intensities and with estimates of up to 17 million people infected (Mas-Coma *et al.*, 1999a,b, 2000, 2001, 2003; Mas-Coma, 2004a,b).

At the first intermediate host level, trematodes show a marked snail host specificity, from usually oioxenous (one digenean species/one snail species) or stenoxenous

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(one digenean species/a few, closely related snail species) to less frequently oligoxenous (one digenean species/numerous, family-, subfamily- or tribe-related snail species) (Wright, 1973; Adema & Loker, 1997; Kalbe *et al.*, 1997). Variability in the susceptibility of a concrete snail species to infection by a concrete digenean species has recently been shown to be related to differences between snail populations and also between individuals among a concrete snail population (Rollinson & Southgate, 1985; Adema & Loker, 1997). Differences in compatibility between a trematode species and different geographic populations of the same snail host species are already known, including *Fasciola* (Boray, 1969, 1978). Among lymnaeids, there are pronounced differences in susceptibility between snail populations that occur in close proximity to one another (Perez-Reyes *et al.*, 1985), and some snail populations even show a total lack of susceptibility or resistance (Kendall & Parfitt, 1959; Gutierrez *et al.*, 2003).

The present knowledge of the classification of this gastropod group, as well as their host–parasite interrelationships, is inadequate (Bargues *et al.*, 2001). Both aspects are crucial when taking into account the importance of snail-trematode specificity in the epidemiology and control of distomatoses. The aim of the present paper is to briefly review the molecular tools developed in recent years in relation to nuclear ribosomal DNA sequences, which have provided valuable information for clarifying the classification of species, subspecies and populations, as well as to establish higher taxons within the familiy Lymnaeidae.

Fasciolid–lymnaeid specificity

Fasciola hepatica is present in five continents. In Europe, *Galba truncatula* is its preferred snail host, although other European lymnaeids, i.e. *Omphiscola glabra* and *Lymnaea (Stagnicola) palustris*, have also been found to be infected under special natural conditions. In the laboratory, *O. glabra*, *L. (S.) palustris* and *L. (S.) fuscus*, and even *L. (L.) stagnalis*, *Radix peregra* and *Myxas glutinosa* can become heavily infected if infection by miracidia takes place during the first few days of the snail's life, although a high mortality level is obtained (Mas-Coma & Bargues, 1997; Bargues *et al.*, 2001). Natural infections with *F. hepatica* have also been reported in Polish populations of *Catascopia occulta* and *L. (S.) p. turricula* (Bargues *et al.*, 2003). In other continents, other main or obligatory intermediate snail hosts of *F. hepatica* are *G. truncatula* and *Pseudosuccinea columella* in Africa, *Fossaria humilis*, *F. bulimoides* and *F. cubensis* in North America, *F. cubensis* and *P. columella* in Central America, *F. viatrix* (= *F. viator*), *L. diaphana*, *F. cubensis* and *G. truncatula* in South America, *G. truncatula* and *Austropeplea ollula* (= *A. viridis*) in Asia, *L. tomentosa* in Australia, *L. tomentosa*, *P. columella* and *G. truncatula* in New Zealand, and *A. ollula* in Hawaii, Papua New Guinea, the Philippines and Japan. Alternate or facultative host species cited in other continents are *P. columella* in North and South America, *P. columella* and *A. ollula* in Australia, and *Radix gedrosiana* in Iran (Boray, 1982; Malek, 1985; Mas-Coma & Bargues, 1997).

Fasciola gigantica is mainly distributed in Africa and Asia. Less important endemic areas of *F. gigantica* are the southern parts of Europe, Turkey, the Near East, and some southern states of the old USSR, particularly Armenia, and sporadically in North America. Principal or obligatory intermediate snail hosts mentioned for *F. gigantica* are *R. natalensis* in Africa, *R. auricularia* sspp. in the Near East, Middle East, Far East and southern states of the old USSR, *F. cubensis* in the North American gulf coast, *R. rufescens* in Asia and the Indian subcontinent, *R. rubiginosa* in the Far East and Malaysia, *R. swinhoei* in South East Asia and the Philippines, and *A. ollula* in Hawaii and Japan. Alternate or facultative host species are *G. truncatula* in Africa, *R. peregra* in the Near East, Middle East, and southern states of the old USSR, *P. columella* in the North American gulf coast, and *A. ollula* in the Far East (Boray, 1982; Mas-Coma & Bargues, 1997). *Radix caillaudi* (= junior synonym of *R. natalensis*) in Egypt, *R. gedrosiana* in Iran, *R. euphratica* in Iraq, *R. luteola* in Nepal, and *R. bactriana*, *R. tenera* and *R. subdisjuncta* in Turkmenia were more recently added (Mas-Coma & Bargues, 1997).

Many of these lymnaeid species involved in fascioliasis transmission still show a confused systematic-taxonomic status. At the lymnaeid species level, problems are encountered due to the interspecific morphological and anatomic uniformity which numerous species show, making classification difficult or impossible (Oviedo *et al.*, 1995). Moreover, intraspecific variation of shell shape is particularly well marked within lymnaeids according to environmental conditions (Burch, 1968a; Burch & Lindsay, 1973b), although a genetic component in shell shape has been demostrated at least in some lymnaeid populations (Samadi *et al.*, 2000). In Europe, there are many classification problems, mainly concerned with species of the 'stagnicola' and 'radix' type groups (Glöer & Meier-Brook, 1998). Similar confusion arises at the species level in other continents, specially with species of the 'fossaria' group in the Americas, the 'natalensis' group in Africa, or the 'stagnicola' and 'radix' groups in Asia.

At the supraspecific (genus, subgenus) level, the confusion is even more evident, including from specialists considering numerous genera and subgenera in the Lymnaeidae (e.g. Malek, 1985) up to authors who only accept the large genus *Lymnaea* Lamarck, 1799 *sensu lato*, following the old classification of Hubendick (1951, 1978). About 1800 species and 34 genera of lymnaeids have been previously recorded (Hubendick, 1951; Te, 1976), with classifications recognizing a single genus (Walter, 1968), two genera (Hubendick, 1951; Jackiewicz, 1998), or more than two genera (Zilch, 1959–1960; Burch, 1965, 1980, 1982a,b; Malek, 1985; Jackiewicz, 1993; Glöer & Meier-Brook, 1998). Although not always followed, the multigeneric scheme of Burch (1965, 1980, 1982a) has served as a convenient means for species-group identification (Burch & Lindsay, 1973a; Burch, 1982a).

Tools for characterizing lymnaeids

Within the Lymnaeidae, several approaches have been used to evaluate taxonomic relationships: morphology

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(Hubendick, 1951; Walter, 1968; Burch, 1982a,b, 1988), palaeontology (Zilch, 1959–1960; Inaba, 1969), karyology (Burch, 1965; Inaba, 1969), experimental cross-breeding (Burch & Ayers, 1973), enzyme electrophoresis (Rudolph & Burch, 1989), and immunology (Burch, 1968b; Burch & Lindsay, 1968). However, a consensus has not yet been reached, due to inadequate systematic resolution, as with chromosome numbers (Patterson & Burch, 1978), or the disagreement of results of morphological studies on the shell, radula, and prostate gland with those from karyological and biochemical methods (see reviews by Davis, 1978 and Patterson & Burch, 1978), suggesting that morphological homoplasy is common among lymnaeids. On the other hand, reproductive tract characteristics have occasionally been useful for lower taxonomic unit distinction between closely related lymnaeid species (i.e. Jackiewicz, 1988, 1989; Glöer & Meier-Brook, 1998).

Some genetic and molecular techniques have proved to be useful tools for studies on lymnaeids. Isoenzyme electrophoresis and DNA microsatellites are useful at the population level and have shown that a large range of situations can be found within the lymnaeids, from heterogeneous, polymorphic populations (Rudolph & Burch, 1989; Jarne & Delay, 1990a; Coutellec-Vreto *et al.*, 1994) to completely homogeneous, monomorphic populations (Jabbour-Zahab *et al.*, 1997; Trouve *et al.*, 2000; Meunier *et al.*, 2001), a phenomenon related to both selfing and crossing capacities of these freshwater snails (Jarne & Delay, 1990b; Jarne *et al.*, 1993). Randomly amplified polymorphic DNA (RAPD) analysis was not, however, sufficient conclusive when applied to lymnaeids (Rybska *et al.*, 2000) despite having been used on other gastropod groups; Backeljau *et al.* (1995) have already emphasized that results of RAPD studies should be used with great caution in taxonomic analyses.

DNA sequencing of well known markers has proved to be the best tool to date. Some studies have been made with mitochondrial DNA markers. Sequence analyses of the large subunit (16S) mitochondrial ribosomal DNA have indicated differences between several lymnaeid species and provided more information on their phylogenetic relationships (Remigio & Blair, 1997a; Remigio, 2002). However, mitochondrial DNA sequences have not been used to further our knowledge of lymnaeid systematics and taxonomy.

Most of the information used to clarify systematic and taxonomic aspects and population genetic characterization of lymnaeids in recent years has been furnished by nuclear ribosomal DNA sequences. rRNA molecules provide a good opportunity to examine the patterns of nucleotide sequence change (Wheeler & Honeycutt, 1988). Different ribosomal genes (28S, 5.8S and 18S) have different rates of evolution and hence have been extensively used in phylogenetic analyses. 18S rRNA genes evolve slower than 28S rRNA genes and are thus used to construct deeper phylogenies. The efficacy of small ribosomal RNA sequences for resolving evolutionary relationships among taxa has been well demonstrated, and a large body of sequences representing diverse organisms has been compiled (De Rijk *et al.*, 1992) and used extensively in phylogenetic studies, even for molluscs (Winnepenninckx *et al.*, 1994).

Differences in nucleotide sequences in the two internal transcribed spacers (ITS-1 and ITS-2) are useful for resolving affiliations of closely related taxa that have diverged relatively recently (<50 million years ago). There is, moreover, much experience already available on the usefulness of ITS sequences as excellent markers for species distinction and hybridization in many groups of organisms (Mas-Coma, 1999). Two additional aspects of ITSs are of interest. Firstly, the sequencing of only one of the two spacers is usually sufficient, as a balanced G + C content between ITS-1 and ITS-2 sequences is a shared feature of all eukaryotic taxa (Torres *et al.*, 1990) due to the apparent coevolution of the spacers. This structural conservatism of the ITS regions has been ascribed to their function in ribosomal RNA maturation (Gonzalez *et al.*, 1990). Secondly, ITSs usually present microsatellite sequences (Almeyda-Artigas *et al.*, 2000a,b). Microsatellites are tandemly repeated sequences whose units of repetition are usually between one and five base pairs. They may be classified into three families (pure, compound and interrupted repeats) and di-, tri- and tetranucleotide repeats are the types that are mostly found (Jarne & Lagoda, 1996). Neither the origin of microsatellites, nor their mutation model evolution and function, if any, are fully understood (Jarne *et al.*, 1998), but a recent large bibliography proves that microsatellite alleles exhibit extreme intraspecific variability, neutrality, Mendelian inheritance, codominance and high mutation rates and are thus excellent polymorphic molecular markers for the differentiation of populations within a given species (see reviews by Jarne & Lagoda, 1996; Roos *et al.*, 1998).

Ribosomal DNA markers in lymnaeids

Small subunit or 18S rRNA gene

The sequencing of the whole 18S rRNA gene of several species of Lymnaeidae showed that this gene has a length between 1843 and 1860 bp: (i) European species: *L. (L.) stagnalis* 1849 bp; *L. (S.) palustris* 1851 bp; *O. glabra* 1849 bp; *G. truncatula* 1843 bp; *R. auricularia* 1850 bp; and *R. balthica* (= *R. peregra*; = *R. ovata*) 1852 bp; (ii) American species: *L. (Bakerilymnaea) cubensis* 1860 bp. The larger number of nucleotides in the 18S rDNA sequence of *L. cubensis* was tentatively related to the more ancient palaeogeographic origin of this species. There were no significant differences in nucleotide composition between species, the average G + C content being 51.5%. The degree of genetic variability among all those molluscan species, over the entire 18S rDNA, ranges between 0.22% and 12.01%. The average substitution rate was 0.054 per site, with an average transition rate of 0.037 and an average of transversion rate of 0.017 (Bargues & Mas-Coma, 1997; Bargues *et al.*, 1997).

The 18S rDNA sequence is able to differentiate between species belonging to different genera and subgenera, and sometimes even between species of the same genus. It is, however, not useful when comparing populations of the same species, even in cases far removed from one another, as with *G. truncatula* from Europe and Bolivia or *L. cubensis* from Mexico and the Caribbean Guadeloupe island (Bargues *et al.*, 1997).

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When aligning the 18S rDNA sequences of different lymnaeid species, modified positions appear scattered throughout the whole sequence, but about half of them appear concentrated in a concrete region extending between positions 232 and 266. When locating the positions showing nucleotide differences in the 18S rRNA secondary structure, the latter group of modified positions is entirely included in helix E10-1 of the variable region V2. Interestingly, the helix E10-1 showed four possible nucleotide sequences allowing us to distinguish between species groups: (i) a first sequence common to *L. (L.) stagnalis*, *L. (S.) palustris* and *O. glabra*; (ii) a sequence identical in *R. auricularia* and *R. balthica*; (iii) a sequence specific for *G. truncatula*; (iv) a sequence specific for *L. cubensis*. Within one of these groups the stagnicoline and *Omphiscola* species or the *Radix* species could be differentiated on the basis of nucleotide differences detected in scattered positions 72–74 and 157–158, respectively (Bargues & Mas-Coma, 1997; Bargues *et al.*, 1997).

Results obtained by Bargues & Mas-Coma (1997) and Bargues *et al.* (1997), suggested that 18S rDNA sequence may be a good marker for species distinction and species grouping within the Lymnaeidae. The small region of helix E10-1 in V2, together with the phylogenetic cladograms allow species groupings, thus distinguishing supraspecific entities and suggesting the usefulness of the 18S rDNA as a marker for the definitive, supraspecific taxonomic reorganization of the Lymnaeidae.

Phylogenetic analyses using the entire 18S rDNA sequence furnished trees in which three branches were emphasized with high bootstrap values: (i) the *L. (L.) stagnalis*–*L. (S.) palustris*–*O. glabra* branch (including the *L. (L.) stagnalis*–*L. (S.) palustris* grouping); (ii) the *R. auricularia*–*R. balthica* branch; and (iii) the *G. truncatula*–*L. (B.) cubensis* branch (fig. 1A). Bootstrap values were only relatively low for the branch supporting *G. truncatula* and *L. (B.) cubensis* species.

In phylogenetic trees obtained by Bargues & Mas-Coma (1997) and Bargues *et al.* (1997), there is an evolutionary parallelism of *F. gigantica* with the *R. auricularia*–*R. peregra* branch and of *F. hepatica* with the *G. truncatula*–*L. cubensis* branch. Consequently, these results also showed an applied parasitological interest of the 18S rRNA gene in the distinction between groups of transmitter and non-transmitter lymnaeid species.

However, this chapter on 18S rDNA cannot be closed without emphasizing the results obtained by Stothard *et al.* (2000). By sequencing and analysing a partial sequence including variable regions V1 and V2, these authors were able to detect nucleotide variation within and between populations of *R. natalensis* from Madagascar and South Africa. Up to nine nucleotide positions were found to vary within the V1 and V2 regions. Levels of intraspecific divergence of the V1 and V2 were not appreciably different (1%) from interspecific divergence when compared with other lymnaeid species and would therefore question the validity of the 18S rDNA marker for lymnaeid taxonomy and phylogeny. However, further studies of DNA divergence within and between African, Madagascan and Far Eastern populations of *R. natalensis* as well as the related superspecies *R. auricularia* are needed, as already noted by Stothard *et al.* (2000).

Second internal transcribed spacer ITS-2 of the rDNA

The nuclear rDNA ITS-2 has been the most used molecular marker in lymnaeid studies to date. A total of 72 populations of lymnaeid species and subspecies from Europe, Morocco, Bolivia and the USA were sequenced by Bargues *et al.* (2001, 2003) and Mas-Coma *et al.* (2001). Those species belonged to the genus/subgenus taxa *Lymnaea*, *Stagnicola*, *Omphiscola*, *Radix*, *Hinkleyia*, *Catascopia* and *Galba*, thus offering sufficient materials to appropriately evaluate the usefulness of the ITS-2 as marker for lymnaeids. The length of the ITS-2 sequences varied between 370 and 491 bp and the nucleotide compositions appeared uniformly G + C biased: 55.0–61.5%, with a mean of 58.5% (table 1).

Three different groupings could be distinguished according to their ITS-2 length: (i) *Radix* and *Galba* groups including 370–406 bp lengths; (ii) European *C. occulta* and American stagnicolines of the genera *Catascopia* (*C. catascopium*, *C. elodes* and *C. emarginata*) and *Hinkleyia* (*H. caperata*), in which the sequences are 434–450 bp long; (iii) *Lymnaea* s. str., European *Stagnicola*, and *Omphiscola* with 468–491 bp lengths (Bargues *et al.*, 2001, 2003; Remigio & Blair, 1997b). The existence of three lymnaeid groups according to ITS-2 lengths, with *Galba* included in that presenting the shortest sequences, is worth noting. The oldest lymnaeid fossil known is *Galba* from the Jurassic (Zilch, 1959–1960), which suggests that a shorter ITS-2 would be the plesiomorphic condition and that an increase in ITS-2 length occurred during lymnaeid evolution. This agrees with the general pattern known in ITSs of eukaryotes. In this way, *Radix* and *Galba* may be considered the oldest taxa, *Lymnaea* s. str., European *Stagnicola* and *Omphiscola* the most recent, and European *C. occulta* and American *Catascopia* and *Hinkleyia* the intermediate taxa. This hypothesis fully agrees with the only previously published phylogeny of lymnaeids proposed by Inaba (1969) based on palaeontological data, chromosome numbers and radular dentition.

When comparing different sequences in the alignments, several populations originally classified as belonging to different species showed identical ITS-2 sequences, and other populations originally classified as pertaining to the same species presented different ITS-2 sequences. Sometimes sequence differences were very few, suggesting intraspecific variability. But occasionally differences detected among populations classified as pertaining to the same species were numerous, and sufficient to consider that different species might be involved. Moreover, the number of sequence differences between species sometimes appeared lower than that between populations of the same species (Bargues *et al.*, 2001, 2003). This clearly indicates both the classification problems and systematic-taxonomic confusion present in Lymnaeidae.

The analysis of the ITS-2 sequence alignments showed a conserved central region flanked by two variable lateral regions corresponding to the 5′ and 3′ ends of the ITS-2 sequence. Moreover, several interesting microsatellites were found. Polymorphic microsatellites presenting a different number of repeats, related to different sequence lengths between populations among a given species or proximal species group, were found in *R. auricularia* and

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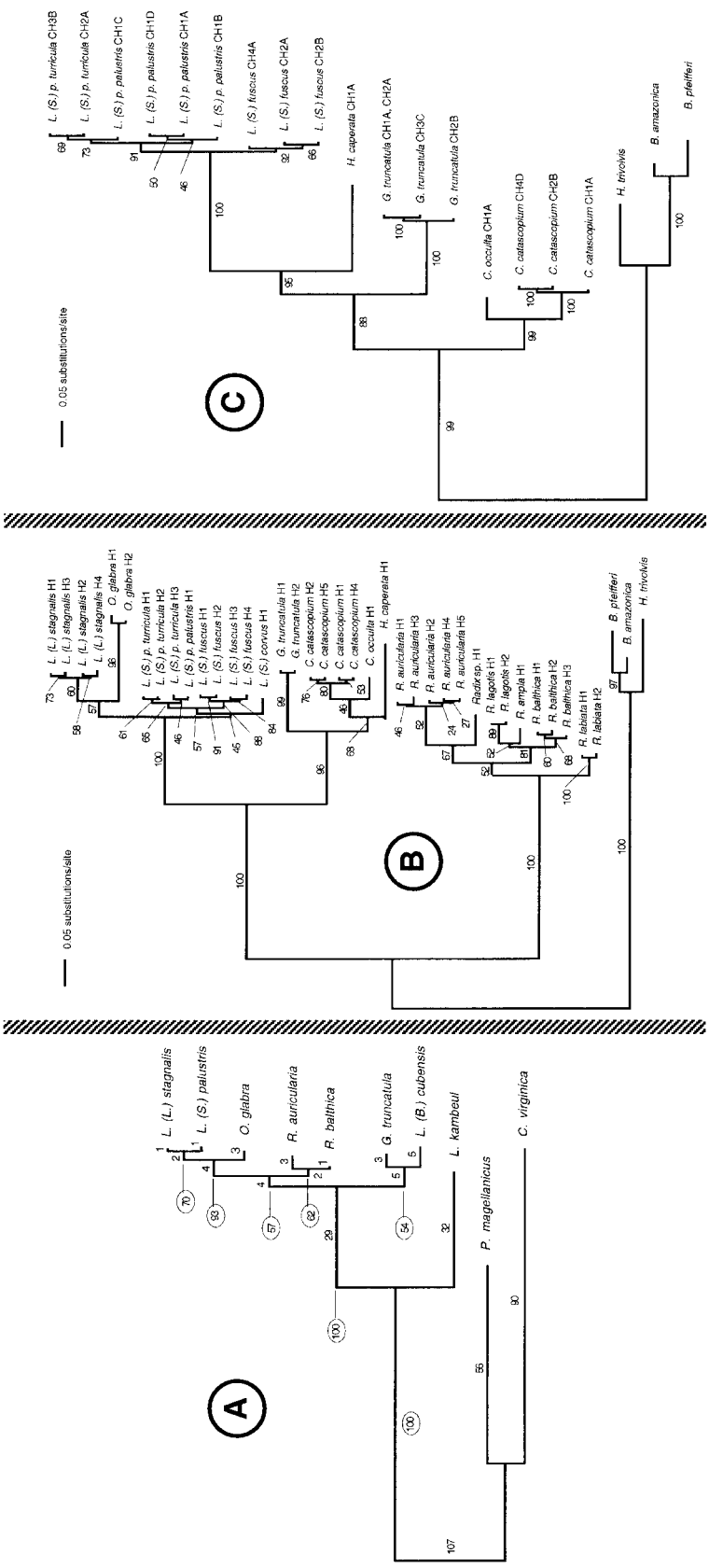

Fig. 1. Phylogenetic trees of the lymnaeid species. (A) 18S rDNA neighbour-joining tree based on Kimura's distance, using molecular evolutionary genetics analysis; the species *Limicolaria kambeul* (Stylommatophora) was included in the ingroup; *Crassostrea virginica* and *Placopecten magellanicus* (Bivalvia: Basommatophora) were used as outgroups; branch lengths are proportional to the scale given in nucleotide substitutions per site; circled numbers correspond to bootstrap percent values based on 1000 replications. (B) rDNA ITS-2 tree derived from the maximum likelihood (ML HKY85) model; all lymnaeid species included are Palaearctic excepting *Catascopia catascopium* and *Hinkleyia caperata* which are Nearctic. (C) rDNA ITS-1 tree derived from the maximum likelihood (ML HKY85 + I) model. ML trees (B, C) obtained using three planorbids, *B. pfeifferi*, *B. amazonica* and *H. trivolvis* as the outgroup; scale bars indicate the number of substitutions per sequence position; numbers represent the percentage of 1000 puzzling replicates. For lymnaeid haplotype codes in B and composite haplotype codes in C see tables 1 and 2, respectively.

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Table 1. ITS-2 haplotypes of European, American and African lymnaeid species, including country of origin, nucleotide length of the ITS-2 sequence and corresponding GenBank accession nos.

Species-haplotype code	Country	ITS-2 length (bp)	GenBank accession no.
Lymnaea (Lymnaea) stagnalis			
Ls-H1	Germany	489	AJ319614
Ls-H2	France	490	AJ319615
Ls-H3	France	491	AJ319616
Ls-H4	Italy, France, Germany	490	AJ319617
Lymnaea (Stagnicola) palustris palustris			
Sp-H1	France, Germany, The Netherlands, Denmark	473	AJ319620
Lymnaea (Stagnicola) palustris turricola			
St-H1	Austria	473	AJ319618
St-H2	Austria	473	AJ319619
St-H3	Poland	473	AJ457043
Lymnaea (Stagnicola) fuscus			
Sf-H1	France	472	AJ319621
Sf-H2	Germany	472	AJ319622
Sf-H3	Austria, France	468	AJ319623
Sf-H4	Spain	468	AJ319624
Lymnaea (Stagnicola) corvus			
Sc-H1	Austria	484	AJ319625
Omphiscola glabra			
Og-H1	Germany	481	AJ319626
Og-H2	France	481	AJ319627
Radix auricularia			
Ra-H1	Czech Republic, Austria, UK	401	AJ319628
Ra-H2	Czech Republic	403	AJ319629
Ra-H3	France	406	AJ319630
Ra-H4	France	404	AJ319631
Ra-H5	France	402	AJ319632
Radix balthica (= *R. peregra*; = *R. ovata*)			
Rb-H1	France, The Netherlands, Iceland	395	AJ319633
Rb-H2	France	395	AJ319634
Rb-H3	France	395	AJ319635
Radix labiata (= *R. peregra* sensu Ehrmann, 1933)			
Rl-H1	Czech Republic, Turkey	370	AJ319636
Rl-H2	Germany	370	AJ319637
Radix lagotis			
Rla-H1	Czech Republic	378	AJ319638
Rla-H2	Austria	378	AJ319639
Radix ampla			
Ram-H1	Austria	387	AJ319640
Radix sp.			
Rsp-H1	Turkey	373	AJ319641
Galba truncatula			
Gt-H1	Spain, Portugal, Switzerland, Morocco	401	AJ243017
Gt-H2	Spain, Portugal, France, The Netherlands	401	AJ296271
Gt-H3	Bolivia	401	AJ272051
Catascopia catascopium (= *C. emarginata*; = *C. elodes*)			
Cc-H1	USA (Michigan) (= *S. catascopium* of Remigio & Blair, 1997b)	442	AF013143
Cc-H2	USA (= *S. emarginata* of Remigio & Blair, 1997b)	448	AF013142
Cc-H3	USA (= *S. emarginata* of Remigio & Blair, 1997b)	448	AF013141
Cc-H4	USA (= *S. elodes* of Remigio & Blair, 1997b)	448	AF013138
Cc-H5	USA (Wisconsin)	449	AJ319642
Catascopia occulta			
Co-H1	Poland	448	AJ457042
Hinkleyia caperata			
Hc-H1	Canada (= *S.(H.) caperata* of Remigio & Blair, 1997b)	435	AF013140
Hc-H2	Canada (= *S.(H.) caperata* of Remigio & Blair, 1997b)	434	AF013139

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in the American stagnicolines. Two microsatellites found in the conserved central region are worth noting, as they provide a means of differentiating *Radix* from other lymnaeid species (Bargues *et al.*, 2001).

Maximum-parsimony (MP), neighbour-joining (NJ) and maximum likelihood (ML) analyses (fig. 1B) yielded similar trees and showed the main branches supported by very high bootstrap and puzzle values (all higher than 90, most of them of 100). The ingroup taxa, representing all lymnaeids studied, appeared divided into two big clades, one for *Radix* species and another for all other genera. In this second clade, two branches were well defined: one branch for the European lymnaeids including *Lymnaea (Lymnaea)*, *Lymnaea (Stagnicola)* and *Omphiscola*, and another branch comprising the European *Galba* and *C. occulta* together with the North American stagnicolines (Bargues *et al.*, 2001, 2003). European stagnicolines always appearing in a clade different from that of North American ones, and the great nucleotide differences and genetic distances between them suggested that North American and European stagnicolines do not belong to the same supraspecific taxon, despite shell morphology and visceral anatomy similarities which may be homoplasic. Exceptions were the unexpected sequence similitudes and phylogenetic link between the Eurasian *C. occulta* and North American stagnicolines, facts which justified the erection of the new genus *Catascopia* (Meier-Brook & Bargues, 2002; Bargues *et al.*, 2003).

The results of sequence comparisons and MP, distance by NJ and ML analyses, indicate that the ITS-2 spacer is a useful marker for resolving supraspecific, specific and population relationships within the Lymnaeidae. Analyses of genetic distances and sequence differences between distinct populations and taxa studied distinguished the upper limit to be expected within a single species and showed that different sister species can be expected to be at rDNA ITS-2 sequence level. Hence, the rDNA ITS-2 proved to be an excellent marker for systematic and taxonomic purposes, as well as to compare different populations among a species. Therefore, the species studied were systematically revisited, several synonymies were proposed, and the taxonomic validity and relationships of genera and subgenera involved were established. Additionally, up to 34 different ITS-2 haplotypes were distinguished and distributed as follows: four in *L. (L.) stagnalis*, one in *L. (S.) palustris palustris*, three in *L. (S.) palustris turricola*, four in *L. (S.) fuscus* (= *S. vulnerata*); one in *L. (S.) corvus*, two in *Omphiscola glabra*, five in *R. auricularia*, three in *R. balthica* (= *R. peregra*; = *R. ovata*), two in *R. labiata* (= *R. peregra* sensu Ehrmann, 1933; = *R. alpicola*), two in *R. lagotis*, one in *R. ampla*, one in *Radix* sp., three in *Galba truncatula*, one in *Catascopia catascopium* and one in *C. occulta* (Bargues *et al.*, 2001, 2003).

The information which the ITS-2 marker provides is of an applied nature concerning the molluscan host specificity of different trematode species. The phylogenetic trees inferred from the ITS-2 sequences are able to differentiate between lymnaeids transmitting and those non-transmitting fasciolids, as well as between those transmitting *F. hepatica* and those transmitting *F. gigantica*, as in the case of trees inferred from 18S rDNA sequences (Bargues & Mas-Coma, 1997; Bargues *et al.*, 1997). *Fasciola* specificity is linked to the two oldest genera which, moreover, cluster together in the phylogenetic trees, suggesting an origin of the *Fasciola* ancestors related to the origin of this branch.

Another species from which the ITS-2 sequence has been obtained is *P. columella* which originated from Central America, the Caribbean and the southern part of North America, and is currently present in South America, Europe, Africa, Australia, New Zealand and even Tahiti (Mas-Coma *et al.*, 2003). The ITS-2 sequence of *P. columella* proved to be identical in many American states far removed one another, suggesting a recent geographical spread probably related to humans (Vigo *et al.*, 2000). Interestingly, populations of this species from Cuba, showing resistence to infection by *F. hepatica*, presented a mutation in the ITS-2 sequence. The ITS-2 thus becomes a genetic marker for differentiating between resistant and susceptible *P. columella* populations (Gutierrez *et al.*, 2003).

First internal transcribed spacer ITS-1 of the rDNA

In lymnaeids, the nuclear rDNA ITS-1 has been less used than the ITS-1. A total of 13 complete rDNA ITS-1 sequences were obtained by Mas-Coma *et al.* (2001) and Bargues *et al.* (2005), from populations of *L. (S.) p. palustris* (four haplotypes), *L. (S.) p. turricola* (two haplotypes), *L. (S.) fuscus* (three haplotypes), *G. truncatula* (three haplotypes), and *C. occulta* (one haplotype) (table 2). Sequences were analysed and compared with those of North American stagnicolines of the genera *Catascopia* and *Hinkleyia* (Remigio & Blair, 1997b). Their length ranged between 529 and 562 bp, and their GC content between 55.9% and 59.0% (average 58.6%).

Although not so many populations have been analysed, it is worth noting that each population studied showed a different sequence, which was not the case in studies on the ITS-2 of lymnaeids (Bargues *et al.*, 2001, 2003). This, together with a slightly higher percentage of nucleotide differences, suggests that ITS-1 may evolve somewhat faster than ITS-2 in Lymnaeidae. Consequently, the ITS-1 may offer a valuable marker for taxon differentiation and relationships within the Lymnaeidae not only at genus and species levels, but also at the subspecies and population levels, as already verified in the case of ITS-2. However, ITS-1 sequence and microsatellites may offer more precise information at the population level than ITS-2.

At species and subspecies levels, results obtained with ITS-1 by Bargues *et al.* (2005) fully confirmed the results and conclusions previously reached by the ITS-2 sequence studies (Bargues *et al.*, 2001, 2003). In interspecific comparisons, a high number of nucleotide differences appeared, the majority of which were indels, with substitutions being very few between lymnaeids of the subgenus *Stagnicola*. However, this was not the case between the three *Stagnicola* and *C. occulta*, the three comparisons showing a number of substitutions higher than that of indels.

Sequence repeats detected in the first spacer were microsatellites, being responsible for marked differences in the ITS-1 length and providing more information for species and population differentiation, e.g. microsatellite repeats allow *C. occulta* from Europe to be distinguished from the American *Catascopia* species.

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Table 2. ITS-1 haplotypes of European, American and African lymnaeid species, including country of origin, nucleotide length of the ITS-1 sequence and corresponding GenBank accession nos.

Species-haplotype code	Country	ITS-1 length (bp)	GenBank accession no.
Lymnaea (Stagnicola) palustris palustris			
Spp-CH1A	Denmark	536	AJ626849
Spp-CH1B	France	537	AJ626850
Spp-CH1C	Germany	549	AJ626851
Spp-CH1D	The Netherlands	538	AJ626852
Lymnaea (Stagnicola) palustris turricula			
Spp-CH2A	Austria	555	AJ626853
Spp-CH3B	Poland	551	AJ626854
Lymnaea (Stagnicola) fuscus			
Sf-CH2A	Germany	535	AJ626855
Sf-CH2B	Germany	545	AJ626856
Sf-CH4C	Spain	529	AJ626857
Catascopia catascopium			
Cc-CH1A	USA (Michigan) (= *S. catascopium* of Remigio & Blair, 1997b)	542	AF013143
Cc-CH2B	USA (= *S. emarginata* of Remigio & Blair, 1997b)	542	AF013142
Cc-CH3C	USA (= *S. emarginata* of Remigio & Blair, 1997b)	542	AF013141
Cc-CH4D	USA (= *S. elodes* of Remigio & Blair, 1997b)	541	AF013138
Catascopia occulta			
Co-CH1A	Poland	562	AJ626858
Hinkleyia caperata			
Hc-CH1A	Canada (= *S.(H.) caperata* of Remigio & Blair, 1997b)	586	AF013140
Hc-CH2B	Canada (= *S.(H.) caperata* of Remigio & Blair, 1997b)	586	AF013139
Galba truncatula			
Gt-CH1A	Spain, Portugal, Switzerland	504	AJ243017
Gt-CH2A	Spain, Portugal, France, The Netherlands	504	AJ243017
Gt-CH2B	Morocco	504	AJ296270
Gt-CH3C	Bolivia	504	AJ272052

Composite haplotype (CH) code include number and letter according to ITS-2 and ITS-1, respectively.

Lymnaea (S.) p. palustris and *L. (S.) p. turricola* haplotypes are so close one another that specific differentiation is not possible. The scarce genetic distances in the ITS-1 between both agree with those known in organisms able to crossbreed and give rise to viable hybrid forms (Mas-Coma, 1999). In *L. (S.) fuscus*, the relatively numerous differences detected in the ITS-1 agree with results obtained with ITS-2 sequences, where nucleotide divergences and genetic distances between haplotypes appear to be the highest recorded within a concrete lymnaeid species, suggesting that this species may probably follow a process of geographic differentiation developing in Europe at present (Bargues *et al.*, 2001).

Concerning *G. truncatula*, ITS sequence analyses of the lymnaeid morph I (= *L. viatrix* of Ueno *et al.*, 1975) and morph II (= *L. cubensis* of Ueno *et al.*, 1975) proved that there is only one species in the Northern Bolivian Altiplano. Moreover, the very few differences between the ITS sequences of the Bolivian lymnaeids and *G. truncatula* populations from Europe and Morocco indicate that the snail species involved in the transmission of human and animal fascioliasis in this Andean endemic area is *G. truncatula* (Mas-Coma *et al.*, 2001). However, nucleotide differences in both ITS-1 and ITS-2 sequences allow us to differentiate the Altiplano population from other European and African populations studied (Bargues *et al.*, 2001, 2003; Mas-Coma *et al.*, 2001).

Within *P. columella*, similarly to the case of ITS-2, a mutation in the ITS-1 allows us to differentiate between populations susceptible and resistant to *F. hepatica* miracidial infection (Gutierrez *et al.*, 2003).

At the genus level, results of both genetic distances and phylogenetic analyses again support the conclusions previously made between European and American stagnicolines by means of the ITS-2 sequence and phylogenetic analyses. MP, NJ and ML analyses yielded similar phylogenetic trees (fig. 1C), and showed branches supported by very high bootstrap and puzzle values. The ingroup taxa, representing all lymnaeids studied, show three well-defined branches: (i) a branch for the European *Stagnicola*, including the American stagnicoline species *H. caperata* as a basal or sister group (except in MP); (ii) a branch grouping the haplotypes of *G. truncatula;* (iii) a branch comprising the European *C. occulta* together with the North American stagnicolines species of the genus *Catascopia* (Bargues *et al.*, 2005).

Lymnaeid rDNA haplotype code nomenclature

Summing up, nuclear rDNA sequences offer appropriate tools on which to base a systematic and taxonomic review of the family Lymnaeidae, without forgetting the other valuable snail characteristics already available.

https://doi.org/10.1079/JOH2005297 Published online by Cambridge University Press

A reconstruction of the lymnaeid system towards a more natural classification will undoubtedly be useful in furthering our understanding of the epidemiology of an emerging-reemerging disease such as fascioliasis.

To facilitate the way forward, Bargues *et al.* (2005) have introduced a new nomenclature for nuclear ribosomal DNA genotyping. It considers the main rDNA sequence regions to provide important information on interspecific differentiation and groupings as well as the intraspecific variability of lymnaeid species. The code follows an order relating to the potential well-known information capacity of different rDNA markers with more or less rapid evolutionary ratios, for gradually distinguishing different levels, from supraspecific taxa to the species level and up to the population level. For instance, the composite haplotype (CH) code CH2Badb will refer to haplotype H2 according to the ITS-2, haplotype HB after ITS-1, and haplotypes Ha, Hd and Hb after the first, second and third variable domains D1, D2 and D3 of the 28S gene, respectively. Other variable domains of the 28S gene can successively be added in this way, as necessary. This short and simple nomenclature is expected to be very useful in future genetic studies on lymnaeids.

Acknowledgements

This work was supported by funding from the STD Program of EC (DG XII) (Contract No. TS3-CT94-0294), Brussels, EU, the Project PDP B2/181/125 of the WHO of Geneva, Switzerland, the Project No. BOS2002-01978 of the DGICYT of the Spanish Ministry of Science and Technology, Madrid, by the Red de Investigación de Centros de Enfermedades Tropicales – RICET (Project No. C03/04 of the Programme of Redes Temáticas de Investigación Cooperativa, FIS) and Project No. PI030545 of FIS, Spanish Ministry of Health, Madrid.

References

Adema, C.M. & Loker, E.S. (1997) Specificity and immunobiology of larval digenean–snail associations. pp. 229–253 *in* Fried, B. & Graczyk, T.K. (*Eds*) *Advances in trematode biology.* Boca Raton, Florida, CRC Press.

Almeyda-Artigas, R.J., Bargues, M.D. & Mas-Coma, S. (2000a) ITS-2 rDNA sequencing of *Gnathostoma* species (Nematoda) and elucidation of the species causing human gnathostomiasis in the Americas. *Journal of Parasitology* **86**, 537–544.

Almeyda-Artigas, R.J., Bargues, M.D. & Mas-Coma, S. (2000b) rDNA of *Gnathostoma* species (Nematoda): ITS-2 microsatellites and 5.8S gene secondary structure. *Research and Reviews in Parasitology* **60**, 51–56.

Backeljau, T., De Bruyn, L., De Wolf, H., Jordaens, K., Van Dongen, S., Vehagen, R. & Winnepenncxk, B. (1995) Random amplified polymorphic DNA (RAPD) and parsimony methods. *Cladistics* **11**, 119–130.

Bargues, M.D. & Mas-Coma, S. (1997) Phylogenetic analysis of lymnaeid snails based on 18S rDNA sequences. *Molecular Biology and Evolution* **14**, 569–577.

Bargues, M.D., Mangold, A.J., Muñoz-Antoli, C., Pointier, J.P. & Mas-Coma, S. (1997) SSU rDNA characterization of lymnaeid snails transmitting human fascioliasis in South and Central America. *Journal of Parasitology* **83**, 1086–1092.

Bargues, M.D., Vigo, M., Horak, P., Dvorak, J., Paztner, R.A., Pointier, J.P., Jackiewicz, M., Meier-Brook, C. & 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.

Bargues, M.D., Horak, P., Paztner, R.A., Pointier, J.P., Jackiewicz, M., Meier-Brook, C. & Mas-Coma, S. (2003) Insights into the relationships of Palaearctic and Nearctic lymnaeids (Mollusca: Gastropoda) by rDNA ITS-2 sequencing and phylogeny of stagnicoline intermediate host species of *Fasciola hepatica*. *Parasite* **10**, 243–255.

Bargues, M.D., Artigas, P., Jackiewicz, M., Pointier, J.P. & Mas-Coma, S. (2005) Ribosomal DNA ITS-1 sequence analysis of European stagnicoline Lymnaeidae (Gastropoda) *in* Glöer, P. & Falkner, G. (*Eds*) *Beiträge zur Süβwasser-Malakologie* – Festschrift für Claus Meier-Brook und Hans D. Boeters, *Heldia (Münchner Malakologische Mitteilungen)* **6**, 57–68.

Boray, J.C. (1969) Experimental fascioliasis in Australia. *Advances in Parasitology* **7**, 95–210.

Boray, J.C. (1978) The potential impact of exotic *Lymnaea* spp. on fascioliasis in Australia. *Veterinary Parasitology* **4**, 127–141.

Boray, J.C. (1982) Fascioliasis. pp. 71–88 *in* Hillyer, G.V. & Hopla, C.E. (*Eds*) *Handbook series in zoonoses, Section C, Parasitic zoonoses.* Vol. III. Boca Raton, Florida, CRC Press.

Burch, J.B. (1965) Chromosome numbers and systematics in euthyneuran snails. *Proceedings of the First European Malacological Congress, Abstracts Book* **1**, 215–241.

Burch, J.B. (1968a) *Erinna newcombi* of Hawaii and *Limnaea onychia* of Japan. *Malacological Review* **1**, 15–30.

Burch, J.B. (1968b) Morphology vs. immunology. Response. *Bulletin of the American Malacological Union* **34**, 25–28.

Burch, J.B. (1980) A guide to the freshwater snails of the Philippines. *Malacological Review* **13**, 121–143.

Burch, J.B. (1982a) North American freshwater snails. Identification keys, generic synonymy, supplemental notes, glossary, references, index. *Walkerana, Transactions of the POETS Society* **1**, 216–365.

Burch, J.B. (1982b) Freshwater snails (Mollusca: Gastropoda) of North America (North of Mexico). Environmental monitoring and support laboratory office of research and development, US Environmental Protection Agency, Cincinnati, Ohio, 249 pp.

Burch, J.B. (1988) North American freshwater snails. Introduction, systematics, nomenclature, identification, morphology, habitats, distribution. *Walkerana, Transactions of the POETS Society* **2**, 1–80.

Burch, J.B. & Ayers, P.A. (1973) Breeding experiments with *Stagnicola elodes* and *Stagnicola emarginata*. *Malacological Review* **6**, 51–52.

Burch, J.B. & Lindsay, G.K. (1968) An immunological approach to lymnaeid systematics. *Bulletin of the American Malacological Union* **34**, 22–23.

Burch, J.B. & Lindsay, G.K. (1973a) Taxonomic groupings in the Lymnaeidae. *Bulletin of the American Malacological Union* **38**, 15.

Burch, J.B. & Lindsay, G.K. (1973b) Apparent hybrids from a laboratory culture of *Lymnaea stagnalis* and *Bulimnea megasoma*. *Malacological Review* **6**, 61–62.

Coutellec-Vreto, M.A., Guiller, A. & Daguzan, J. (1994) Allozyme variation in some populations of the freshwater snails *Lymnaea peregra*, *L. auricularia* and *L. stagnalis* (Gastropoda: Pulmonata). *Journal of Molluscan Studies* **60**, 393–403.

Chen, M.G. & Mott, K.E. (1990) Progress in assessment of morbidity due to *Fasciola hepatica* infection: a review of recent literature. *Tropical Diseases Bulletin* **87**, R1–R38.

Davis, G.M. (1978) Experimental methods in molluscan systematics. pp. 99–169 *in* Fretter, V. & Peake, J. (*Eds*) *Pulmonates,* Vol. 2A. *Systematics, evolution and ecology,* London, Academic Press.

De Rijk, P., Neefs, J.M., Van De Peer, Y. & De Wachter, R. (1992) Compilation of small ribosomal subunit RNA sequences. *Nucleic Acids Research* **20**, 2075–2089.

Glöer, P. & Meier-Brook, C. (1998) *Süsswassermollusken. Ein Bestimmungsschlüssel für die Bundesrepublik Deutschland*. (11th and 12th edns) Deutscher Jugendbund für Naturbeobachtung, Hamburg, Germany.

Gonzalez, I.L., Chambers, C., Gorski, J.L., Stambolian, D., Schmickel, R.D. & Sylvester, J.E. (1990) Sequence and structure correlation of human ribosomal transcribed spacers. *Journal of Molecular Biology* **212**, 27–35.

Gutierrez, A., Pointier, J.P., Fraga, J., Jobet, E., Modat, S., Perez, R.T., Yong, M., Sanchez, J., Loker, E.S. & Theron, A. (2003) *Fasciola hepatica*: identification of molecular markers for resistant and susceptible *Pseudosuccinea columella* snail hosts. *Experimental Parasitology* **105**, 211–218.

Hubendick, B. (1951) Recent Lymnaeidae. Their variation, morphology, taxonomy, nomenclature, and distribution. *Kungliga Svenska Vetenskapsakademiens Handlingar, Fjärde Serien* **3**, 1–223 + 5 pl.

Hubendick, B. (1978) Systematics and comparative morphology of the Basommatophora. pp. 1–47 *in* Fretter, V. & Peake, J. (*Eds*) *Pulmonates,* Vol. 2A. *Systematics, evolution and ecology,* London, Academic Press.

Inaba, A. (1969) Cytotaxonomic studies of lymnaeid snails. *Malacologia* **7**, 143–168.

Jabbour-Zahab, R., Pointier, J.P., Jourdane, J., Jarne, P., Oviedo, J.A., Bargues, M.D., Mas-Coma, S., Angles, R., Perera, G., Balzan, C., Khallaayoune, K. & Renaud, F. (1997) Phylogeography and genetic divergence of some lymnaeid snails, intermediate hosts of human and animal fascioliasis, with special reference to lymnaeids from the Bolivian Altiplano. *Acta Tropica* **64**, 191–203.

Jackiewicz, M. (1988) The penis as a valuable diagnostic feature in lower taxonomic units of the family Lymnaeidae (Gastropoda, Pulmonata). *Malakologische Abhandlungen* **13**, 23–26.

Jackiewicz, M. (1989) Innenstruktur der Prostata und des Praeputiums bei *Omphiscola glabra* (O.F. Müll.) (Gastropoda, Basommatophora: Lymnaeidae). *Malakologische Abhandlungen* **14**, 7–13.

Jackiewicz, M. (1993) Phylogeny and relationships within the European species of the family Lymnaeidae. *Folia Malacologica* **5**, 61–95.

Jackiewicz, M. (1998) European species of the family Lymnaeidae (Gastropoda: Pulmonata: Basommatophora). *Genus* **9**, 1–93.

Jarne, P. & Delay, B. (1990a) Population genetics of *Lymnaea peregra* (Müller) (Gastropoda: Pulmonata) in Lake Geneva. *Journal of Molluscan Studies* **56**, 317–322.

Jarne, P. & Delay, B. (1990b) Inbreeding depression and self-fertilization in *Lymnaea peregra* (Gastropoda: Pulmonata). *Heredity* **64**, 169–175.

Jarne, P. & Lagoda, P.J.L. (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution* **11**, 424–429.

Jarne, P., Vianey-Liaud, M. & Delay, B. (1993) Selfing and crossing in hermaphrodite freshwater gastropods (Basommatophora): where, when and why. *Biological Journal of the Linnean Society* **49**, 99–125.

Jarne, P., David, P. & Viard, F. (1998) Microsatellites, transposable elements and the X chromosome. *Molecular Biology and Evolution* **15**, 28–34.

Kalbe, M., Haberl, B. & Haas, W. (1997) Miracidial host-finding in *Fasciola hepatica* and *Trichobilharzia ocellata* is stimulated by species-specific glycoconjugates released from the host snails. *Parasitology Research* **83**, 806–812.

Kendall, S.B. & Parfitt, J.W. (1959) Studies on the susceptibility of some species of *Lymnaea* to infection with *Fasciola gigantica* and *F. hepatica*. *Annals of Tropical Medicine and Parasitology* **53**, 220–227.

Malek, E.A. (1980) *Snail-transmitted parasitic diseases*, Vol. 1 and 2. Boca Raton, Florida, CRC Press.

Malek, E.A. (1985) Snail hosts of schistosomiasis and other snail-transmitted diseases in tropical America: a manual. *Pan American Health Organization, Scientific Publication* **478**, 1–325.

Mas-Coma, S. (1999) Los espaciadores transcritos internos (ITSs) del ADN ribosomal como marcadores en sistemática, ecología, evolución y filogenia de parásitos y vectores. *XIV Congreso Latinoamericano de Parasitología (FLAP), Acapulco, México, Libro de Resúmenes* **1**, 5–6.

Mas-Coma, S. (2004a) Human fascioliasis. pp. 305–322 *in* Cotruvo, J.A., Dufour, A., Rees, G., Bartram, J., Carr, R., Cliver, D.O., Craun, G.F., Fayer, R. & Gannon, V.P.J. (*Eds*) *Waterborne zoonoses: identification, causes and control*. World Health Organization (WHO) London, IWA Publishing.

Mas-Coma, S. (2004b) Human fascioliasis: epidemiological patterns in human endemic areas of South America, Africa and Asia. *Southeast Asian Journal of Tropical Medicine and Public Health* **35**, 1–11.

Mas-Coma, S. & Bargues, M.D. (1997) Human liver flukes: a review. *Research and Reviews in Parasitology* **57**, 145–218.

Mas-Coma, S., Bargues, M.D. & Esteban, J.G. (1999a) Human fasciolosis. pp. 411–434 *in* Dalton, J.P. (*Ed.*) *Fasciolosis*. Wallingford, Oxon, CAB International.

Mas-Coma, S., Esteban, J.G. & Bargues, M.D. (1999b) Epidemiology of human fascioliasis: a review and proposed new classification. *Bulletin of the World Health Organization* **77**, 340–346.

Mas-Coma, S., Bargues, M.D., Marty, A.M. & Neafie, R.C. (2000) Hepatic trematodiases. pp. 69–92 *in* Meyers, W.M., Neafie, R.C., Marty, A.M. & Wear, D.J. (*Eds*) *Pathology of infectious diseases.* Vol. 1. *Helminthiases*, Washington DC, Armed Forces Institute of Pathology and American Registry of Pathology.

Mas-Coma, S., Funatsu, I.R. & Bargues, M.D. (2001) *Fasciola hepatica* and lymnaeid snails occurring at very high altitude in South America. *Parasitology* **123**, S115–S127.

Mas-Coma, S., Bargues, M.D., Valero, M.A. & Fuentes, M.V. (2003) Adaptation capacities of *Fasciola hepatica* and their relationships with human fascioliasis: from below sea level up to the very high altitude. pp. 81–123 *in* Combes, C. & Jourdane, J. (*Eds*) *Taxonomy, ecology and evolution of metazoan parasites.* Vol. 2. Perpignan, Presses Universitaires de Perpignan.

Meier-Brook, C. & Bargues, M.D. (2002) *Catascopia*, a new genus for three Nearctic and one Palaearctic stagnicoline species (Gastropoda: Lymnaeidae). *Folia Malacologica* **10**, 83–84.

Meunier, C., Tirard, C., Hurtrez-Bousses, S., Durand, P., Bargues, M.D., Mas-Coma, S., Pointier, J.P., Jourdane, J. & Renaud, F. (2001) Lack of molluscan host diversity and the transmission of an emerging parasitic disease in Bolivia. *Molecular Ecology* **10**, 1333–1340.

Oviedo, J.A., Bargues, M.D. & Mas-Coma, S. (1995) Lymnaeid snails in the human fascioliasis high endemic zone of the Northern Bolivian Altiplano. *Research and Reviews in Parasitology* **55**, 35–43.

Patterson, C.M. & Burch, J.B. (1978) Chromosomes of pulmonate molluscs. pp. 171–217 *in* Fretter, V. & Peake, J. (*Eds*) *Pulmonates,* Vol. 2A. *Systematics, evolution and ecology.* London, Academic Press.

Perez-Reyes, R., Jimenez-Nava, J.J. & Varela-Ramirez, A. (1985) Fascioliasis en el Estado de Chihuahua. México. I. Susceptibilidad de *Fossaria modicella* (Say, 1825), huésped intermediario local. *Revista Latino-Americana de Microbiología* **27**, 367–372.

Remigio, E.A. (2002) Molecular phylogenetic relationships in the aquatic snail genus *Lymnaea*, the intermediate host of the causative agent of fascioliasis: insights from broader taxon sampling. *Parasitology Research* **88**, 687–696.

Remigio, E.A. & Blair, D. (1997a) Molecular systematics of the freshwater snail family Lymnaeidae (Pulmonata: Basommatophora) utilising mitochondrial ribosomal DNA sequences. *Journal of Molluscan Studies* **63**, 173–185.

Remigio, E.A. & Blair, D. (1997b) Relationships among problematic North American stagnicoline snails (Pulmonata: Lymnaeidae) reinvestigated using nuclear ribosomal DNA internal transcribed spacer sequences. *Canadian Journal of Zoology* **75**, 1540–1545.

Rollinson, D. & Southgate, V.R. (1985) Schistosome and snail populations: genetic variability and parasite transmission. pp. 91–109 *in* Rollinson, D. & Anderson, R.M. (*Eds*) *Ecology and genetics of host–parasite interactions*, London, Academic Press.

Roos, M.H., Hoekstra, R., Plas, M.E., Otsen, M. & Lenstra, J.A. (1998) Polymorphic DNA markers in the genome of parasitic nematodes. *Journal of Helminthology* **72**, 291–294.

Rudolph, P.H. & Burch, J.B. (1989) Electrophorectic analysis of enzymes in three species of *Stagnicola* (Pulmonata: Lymnaeidae). *Journal of Medical and Applied Malacology* **1**, 57–64.

Rybska, E., Pacak, A., Szweykowska-Kulinska, Z. & Lesicki, A. (2000) Taxonomy of European Lymnaeidae (Gastropoda: Pulmonata) in studies with the use of molecular biology techniques. I. Preliminary view on the subgenus *Stagnicola* Leach, 1830 on the basis of RAPD analysis. *Folia Malacologica* **8**, 277–284.

Samadi, S., Roumegoux, A., Bargues, M.D., Mas-Coma, S., Yong, M. & Pointier, J.P. (2000) Morphological studies of lymnaeid snails from the human fascioliasis endemic zone of Bolivia. *Journal of Molluscan Studies* **66**, 31–44.

Stothard, J.R., Bremond, P., Andriamaro, L., Loxton, N.J., Sellin, B., Sellin, E. & Rollinson, D. (2000) Molecular characterization of the freshwater snail *Lymnaea natalensis* (Gastropoda: Lymnaeidae) on Madagascar with an observation of an unusual polymorphism in ribosomal small subunit genes. *Journal of Zoology* **252**, 303–315.

Te, G.A. (1976) A summary of pulmonate distribution information contained in Zilch's 1959–1960 monograph: Gastropoda, Teil 2, Euthyneura. *Malacological Review* **9**, 39–53.

Torres, R.A., Ganal, M. & Hembleben, V. (1990) GC balance in the internal transcribed spacers ITS1 and ITS2 of nuclear ribosomal RNA genes. *Journal of Molecular Evolution* **30**, 170–181.

Trouve, S., Degen, L., Meunier, C., Tirard, C., Hurtrez-Bousses, S., Durand, P., Guegan, J.F., Goudet, J. & Renaud, F. (2000) Microsatellites in the hermaphroditic snail, *Lymnaea truncatula*, intermediate host of the liver fluke, *Fasciola hepatica*. *Molecular Ecolology* **9**, 1662–1663.

Ueno, H., Arandia, R., Morales, G. & Medina, G. (1975) Fascioliasis of livestock and snail host for *Fasciola* in the Altiplano region of Bolivia. *National Institute of Animal Health Quarterly* **15**, 61–67.

Vigo, M., Bargues, M.D., Yong, M., Arenas, J.A., Naquira, C., Paraense, W.L., Pointier, J.P. & Mas-Coma, S. (2000) Molecular characterisation of a snail species transmitting fascioliasis, *Lymnaea columella* (Gastropoda: Lymnaeidae), from Cuba, Guadeloupe, Venezuela, Peru and Brazil. *VX International Congress for Tropical Medicine and Malaria, Cartagena, Colombia, Abstracts Book* **2**, 96.

Walter, H.J. (1968) Evolution, taxonomic revolution, and zoogeography of the Lymnaeidae. *Bulletin of the American Malacological Union* **34**, 18–20.

Wheeler, W.C. & Honeycutt, R.L. (1988) Paired sequence difference in ribosomal RNAs: evolutionary and phylogenetic implications. *Molecular Biology and Evolution* **51**, 90–96.

Winnepenninckx, B., Backeljau, T. & De Wachter, R. (1994) Small ribosomal subunit RNA and the phylogeny of Mollusca. *Nautilus* **2**, 98–110.

Wright, C.A. (1973) *Flukes and snails*. New York, Macmillan.

Zilch, A. (1959–1960) Gastropoda (Euthyneura). pp. 91–102 *in* Schindewolf, O. (*Ed.*) *Handbuch der Paleozoologie*. Berlin, Borntraeger.

(Accepted 23 May 2005)