SNAIL-*SCHISTOSOMA, PARAGONIMUS* INTERACTIONS IN CHINA: POPULATION ECOLOGY, GENETIC DIVERSITY, COEVOLUTION AND EMERGING DISEASES

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ABSTRACT

This paper focuses on two snail-borne helminths in China infecting man, *Schistosoma* and *Paragonimus,* but primarily on *Schistosoma* (Asian caenogastropod-transmitted). Of concern are 1) the direction and timing of the evolution of the snail family Pomatiopsidae and the pattern of coevolution of *Schistosoma* and *Paragonimus* with defined clades within the Pomatiopsidae, 2) the question of monophyly of the Pomatiopsidae and its two subfamilies Pomatiopsinae and Triculinae, 3) the amount of genetic diversity within *Oncomelania* throughout China as revealed by allozymes and DNA sequences, 4) *Oncomelania* ecology and genetics with regard to different modes of transmission of *Schistosoma japonicum,* 5) the implications of the Three Gorges dam across the Yangtze River on emerging diseases.

Allozyme and COI gene sequence data confirm the monophyly of the Pomatiopsidae with its two subfamilies. The timing and direction of evolution of the Pomatiopsidae in Asia are congruent with area cladograms based on geological-paleontological events and evolving river systems (Yangtze, Mekong, Red) from about the end of the Miocene. The two subfamilies are highly divergent on the basis of morphology and ecology but less so on the basis of molecular genetics. The Pomatiopsidae are in a clade distinctly divergent from the Hydrobiidae, a family that has been used in China to classify the Pomatiopsinae and Triculinae. Figs. 2 and 3 show the relationships of the two parasite genera with the morphology-based clades of those caenogastropod families transmitting *Schistosoma* and *Paragonimus* throughout the world (excluding Africa for which too little is known). Two caenogastropod superfamilies are involved (Ceritheacea and Rissoacea); Only *Paragonimus* has evolved with taxa of the Thiaridae, Pleuroceridae, Hydrobiidae; both parasite genera have evolved with various pomatiopsid taxa of both subfamilies. While all data available (reviewed in Davis, 1980, 1992) show that *Schistosoma* is tightly linked genetically in a coevolved system with its snail host, such a close linked genetic coevolved system operating at the population to genus level in *Paragonimus* is in doubt. *Erhaia* (Pomatiopsinae) and *Tricula* (Triculinae) were found in sympatry in Fujian Province, both reported transmitting putative *Paragonimus skrjabini*. *P. skrjabini* has been reported from at least 22 species of pomatiopsine and triculine snails in China. The evidence suggests that species of *Paragonimus* can switch hosts between different snail subfamilies and genera. The higher classification of *Erhaia* is in doubt. Should *Erhaia* be found not to be a pomatiopsid snail, then host switching can occur between some families (at least of the Rissoacea).

Based on COI and allozyme data there are three geographically isolated subspecies of *Oncomelania* on the mainland of China: *O. h robertsoni* in Yunnan and Sichuan, *O. h. tangi* in Fujian Province, *O. h. hupensis* throughout the Yangtze drainage below the Three Gorges of the Yangtze River as well as parts of Guangxi and Zhejiang Provinces. Within *O. h. hupensis* there is considerable genetic diversity. Nei's minimum genetic distance among populations is 0.204 ± 0.085. We conclude, on the basis of allozyme data from Miao River populations of *O. h. hupensis* in Hubei Province, that ribbed and smooth-shelled populations (shell with varix; shell growth with the same allometry) are the same species. Ribbing is found in populations affected by annual floods, especially the annual flooding of the Yangtze River. Smooth-shelled populations are upstream, above the effects of flooding. Thus, smooth-shelled *O. fausti* and *O. h. guangxiensis,* nominal taxa used by some authors, are synonyms of *O. h. hupensis.*

Using allozyme population genetics and COI gene sequence data we have found that there is considerable genetic instability in what one would initially presume to be a population. During

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flooding, snails are swept from flood plains and islands, float down the Yangtze, and are deposited in diverse locations or are swept into canals and become deposited along canals. These aggregates of snails derived from various places do not exhibit Hardy-Weinberg equilibrium for polymorphic loci. Haplotypes of the COI gene are shown to be most useful for demonstrating genetic instability. Data are presented from six "populations" from around Dong Ting Lake of Hunan Province. Sequence data were obtained from 10 individuals taken from each site. In a stable population, one expects 0 to 0.5% nucleotide differences within a population (0 – 3 nucleotide differences), or one to two haplotypes per 10 individuals. One such population was found at low elevation beyond effects of flooding. The other sites were around the edge of the lake and are flooded annually. Snails from these lowland localities had 6 to 10 haplotypes per 10 individuals (3.5 to 3.9% polymorphic sites), i.e. they were genetically unstable aggregates. The diversity of haplotypes enables us to map gene flow and patterns of intermixing of snails among localities.

Four different modes of transmission of *Schistosoma japonicum* are discussed. Differences are based on genetically differentiated subspecies, whether or not the populations are affected by annual flooding, life expectancy, population genetic stability, biogeography, and whether or not cattle play a dominant role in transmission of the disease. The implications of the Three Gorges Dam across the Yangtze River is discussed both in terms of the potential for snail transport into the vast reservoir but also of the impact on Poyang Lake, the largest lake in China and a major endemic area for schistosomiasis. A remote sensing image is used in conjunction with GIS technology to discuss snail-schistosome interactions and epidemiology on Poyang Lake marshlands.

Key words: *Oncomelania, Schistosoma,* schistosomiasis, *Paragonimus,* China, allozymes, DNA sequences, COI gene, population genetics, genetic diversity, coevolution, remote sensing, GIS, Three Gorges Dam, Yangtze River, Poyang Lake, evolution, ecology, disease transmission, emerging diseases.

INTRODUCTION

The Tropical Medical Research Center (TMRC) was established in 1996 at the Institute of Parasitic Diseases of the Chinese Academy of Preventive Medicine, Shanghai, P. R. China. Funded by the United States National Institutes of Health, there are four objectives: (1) to study the genetic diversity of three helminths infecting man; *Schistosoma, Paragonimus,* and hookworms; (2) to attempt to uncover emerging helminthic diseases by the study of genetic diversity throughout the southern provinces of China with emphasis on the riparian provinces of the Yangtze River; (3) to assess the impact of genetic diversity on human pathogenicity as well as on vaccine and drug research; (4) to assess the impact of the Three Gorges Dam on known and potentially emerging diseases.

By establishing a modern biotechnology laboratory in the TMRC and having open access to field sites throughout southern China, it became possible to address a number of questions that are fundamental to objectives of the TMRC. This paper focuses on the two snail-borne helminths, *Schistosoma* and *Paragonimus,* but primarily on *Schistosoma.* Studies are based (1) on basic principles of population genetics, and evolution; (2) on the patterns and processes of evolution and coevolution; (3) on the timing of evolution; and (4) on the direction of evolution. Additionally, we have focused on the ecological factors affecting population genetics, evolution/coevolution, disease transmission and emerging diseases. A fundamental hypothesis continues to be: as snail populations diverge genetically in time and space, so must *Schistosoma* transmitted by these populations. This has proven to be the case in the Mekong River, Malaysia, and northern Thailand (Davis, 1980, 1992; Davis & Greer, 1980; Davis et al., 1976; Greer et al., 1997; Voge et al. 1978). What about *Schistosoma* in China? Does the same hold true for *Paragonimus?*

Major questions are: (1) How much genetic diversity is there among populations of *Oncomelania* (transmitting *Schistosoma japonicum*) throughout China? (2) If there are substantial differences, would one find parallel genetic differences among the schistosomes they transmit as called for in the hypothesis above? (3) Is *S. japonicum* the only schistosome infecting man in China? (4) Is there a triculine-borne schistosome infecting man in China? (5) What are the evolved relationships between triculine and pomatiopsine snails? Are they monophyletic? This is an important question as species of both lineages transmit schistosomes infecting man and other mammals. (*Schistosoma mekongi* in the Mekong River of Laos infects man and is transmitted by a triculine species.) (6) Are there substantial ecological differences among populations of Chinese *Oncomelania* and if so, are these reflected in genetic differences and differences in mode of transmission of *S. japonicum* such

that the differences must be taken into consideration in any epidemiological model for control of schistosomiasis? (7) What effect will the Three Gorges Dam have on the genetic diversity of *Oncomelania, Schistosoma* and the modes of disease transmission? (8) How many triculine and pomatiopsine species in China transmit *Paragonimus?* As numerous snail species of these subfamilies are implicated in the transmission of *Paragonimus* (Davis et al., 1994a), are there likewise numerous species of *Paragonimus* in China?

ANALYSIS AND SYNTHESIS

Direction and Timing of Evolution and Co-evolution: Phylogeny and Relevant Vectors

Figure 1 is derived from Davis (1979, 1992); it shows the area cladogram of evolving river drainage systems, the morphology-based phylogeny of the relevant taxa, and the timing of evolution. The direction of evolution is down evolving river systems from northern Burma and western Yunnan, China. *Oncomelania* (Pomatiopsinae) evolved as amphibious snails, while the Triculinae radiate as aquatic snails. However, *Erhaia,* currently classified in

the Pomatiopsinae, is found in an arc from northern India (Davis & Rao, 1997) into southern China (Davis & Kang, 1995); it is an aquatic genus. Note that *Schistosoma* infecting man is transmitted by genera of the two subfamilies, and that the triculine genus *Neotricula* is found not only in the Mekong River but also in southern China.

Figures 2 and 3 show the phylogeny of the superfamilies and families involved in the transmission of *Paragonimus* (human lung fluke) and *Schistosoma* (human blood fluke) infecting man in Asia. Our studies of *Schistosoma* are restricted to caenogastropod snails (snails with gill and operculum; separate sexes), thus excluding Asian schistosomes transmitted by pulmonate snails (snails with lung and no operculum; hermaphroditic), which do not infect man (also derived from the Indian Plate). On each branch of the trees a S or P is placed indicating the ability of taxa pertaining to those sections of the clades to transmit *Schistosoma* (S) or *Paragonimus* (P).

The result is an historical map of the evolution and coevolution of these genera with relevant snail lineages. We learn from these trees the following:

(1) *Paragonimus* evolved with two snail lineages, the Ceritheacea and Rissoacea, while
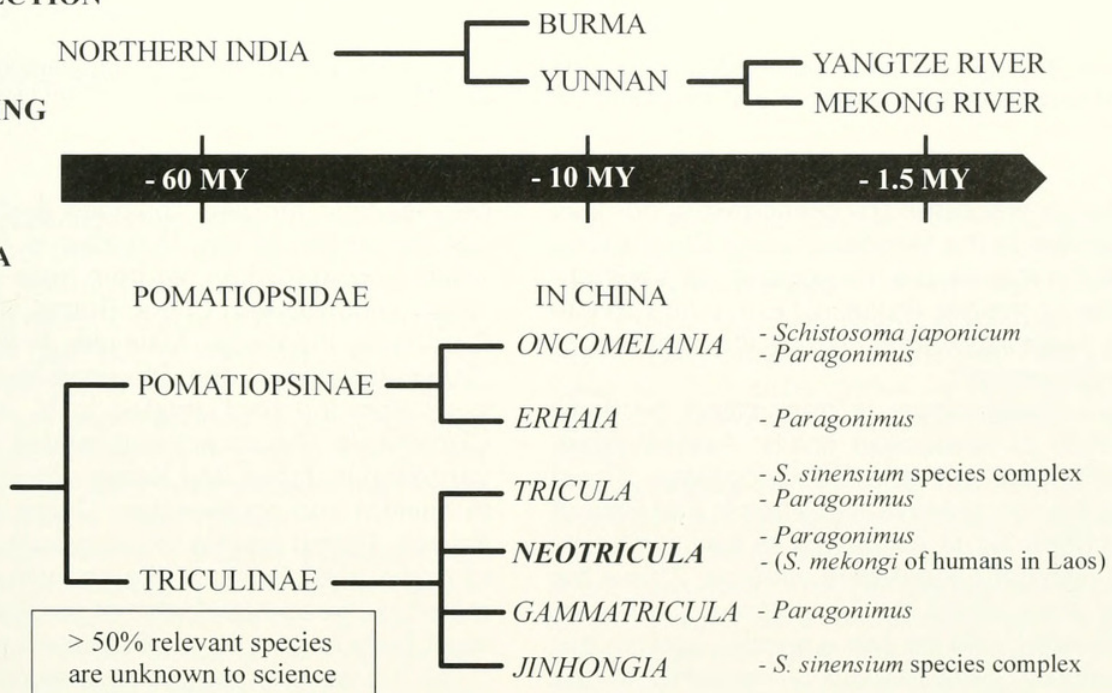

FIG. 1. Direction and timing of evolution of Asian Pomatiopsidae and relevant Asian river systems. The phylogeny of the relevant Pomatiopsidae is shown.
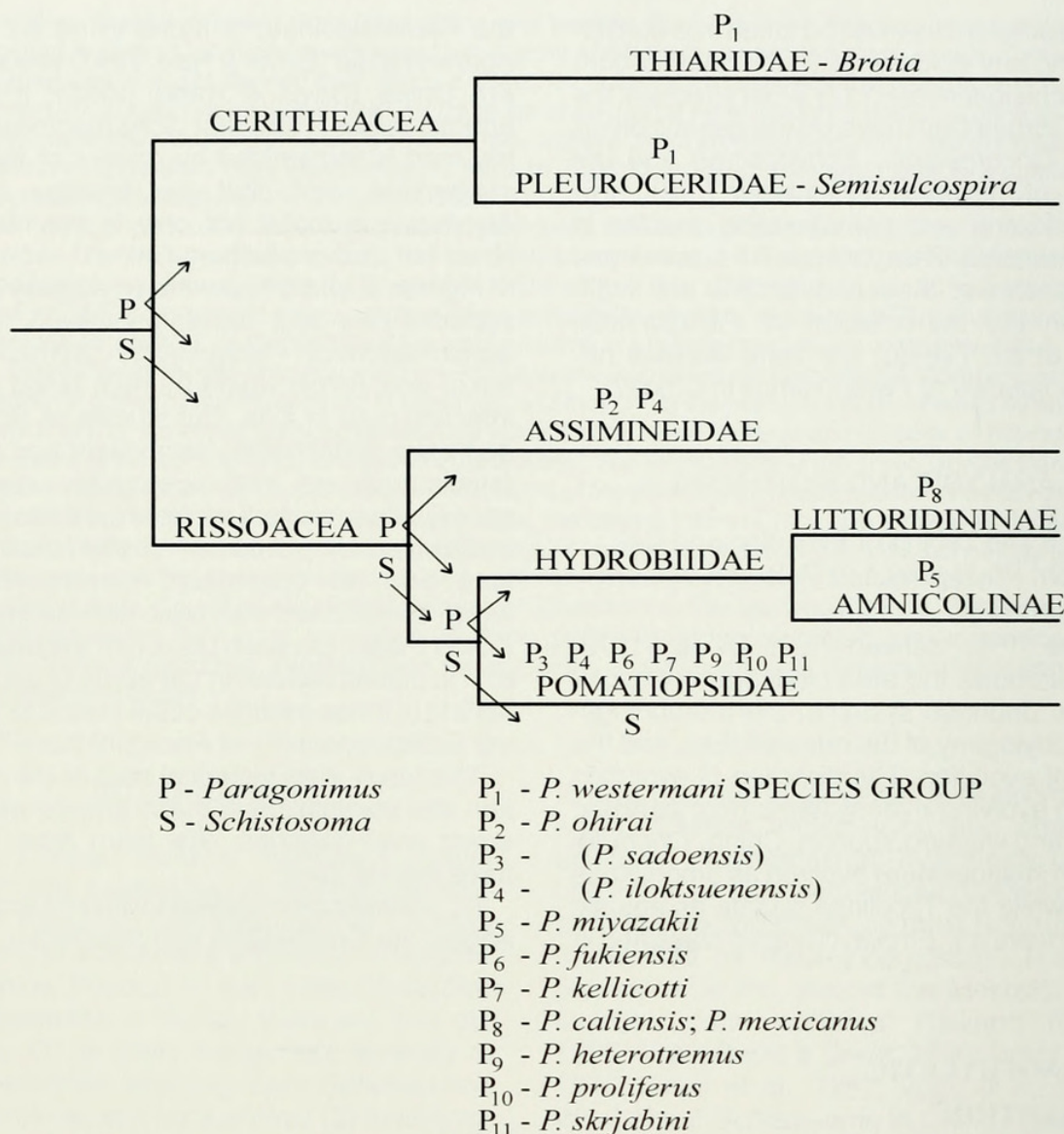
P_1
THIARIDAE - *Brotia*

CERITHEACEA

P_1
PLEUROCERIDAE - *Semisulcospira*

P
S

P_2 P_4
ASSIMINEIDAE

P_8
LITTORIDININAE

RISSOACEA P

HYDROBIIDAE

P_5
AMNICOLINAE

P
S

P_3 P_4 P_6 P_7 P_9 P_{10} P_{11}
POMATIOPSIDAE

S

P - *Paragonimus* P_1 - *P. westermani* SPECIES GROUP
S - *Schistosoma* P_2 - *P. ohirai*
                          P_3 -   (*P. sadoensis*)
                          P_4 -   (*P. iloktsuenensis*)
                          P_5 - *P. miyazakii*
                          P_6 - *P. fukiensis*
                          P_7 - *P. kellicotti*
                          P_8 - *P. caliensis*; *P. mexicanus*
                          P_9 - *P. heterotremus*
                          P_{10} - *P. proliferus*
                          P_{11} - *P. skrjabini*

FIG. 2. Phylogeny of those caenogastropod superfamilies and families involved in the transmission of both *Paragonimus* and *Schistosoma,* and the pattern of co-evolution within those clades.

Schistosoma carried by caenogastropods was restricted to the Rissoacea. The Ceritheacea and Rissoacea are recognized as separate clades in the late Paleozoic Era, over 240 million years ago, long before the breakup of Gondwanaland.

(2) *Paragonimus* is transmitted by three families of rissoacean snails: Assimineidae, Hydrobiidae *s.l.,* and Pomatiopsidae. There may be one possible exception in that Blair et al. (1999) list *P. siamensis* as transmitted by the viviparid *Filopaludina martensi.* This is the only case where the Viviparidae have been implicated, and we see a need to confirm this possibility. *Schistosoma* is restricted to the Pomatiopsidae.

(3) Considering *Paragonimus,* the *P. westermani* species complex is restricted to the ceritheacean families Thiaridae and Pleuroceridae. *Brotia* of the Thiaridae is of Gondwanian origin (distributed from India into S.E. Asia – southwestern China, Burma, Thailand, Cambodia, Indonesia, Malaysia, Philippines). *Semisulcospira* of the Pleuroceridae is derived from the east, related to N. American *Goniobasis, Pleurocera* and related taxa, diversifying in Japan and Korea and spreading to eastern and southeastern China including Taiwan. Based on this biogeographic pattern of origin and dispersal, *Paragonimus,* transmitted by these two clades of ceritheaceans, most likely is comprised of different species.

(4) The world's greatest species complexity involving *Paragonimus* is found in China and is reviewed by Davis et al. (1994a), and most recently by Blair et al. (1999). There are pos-
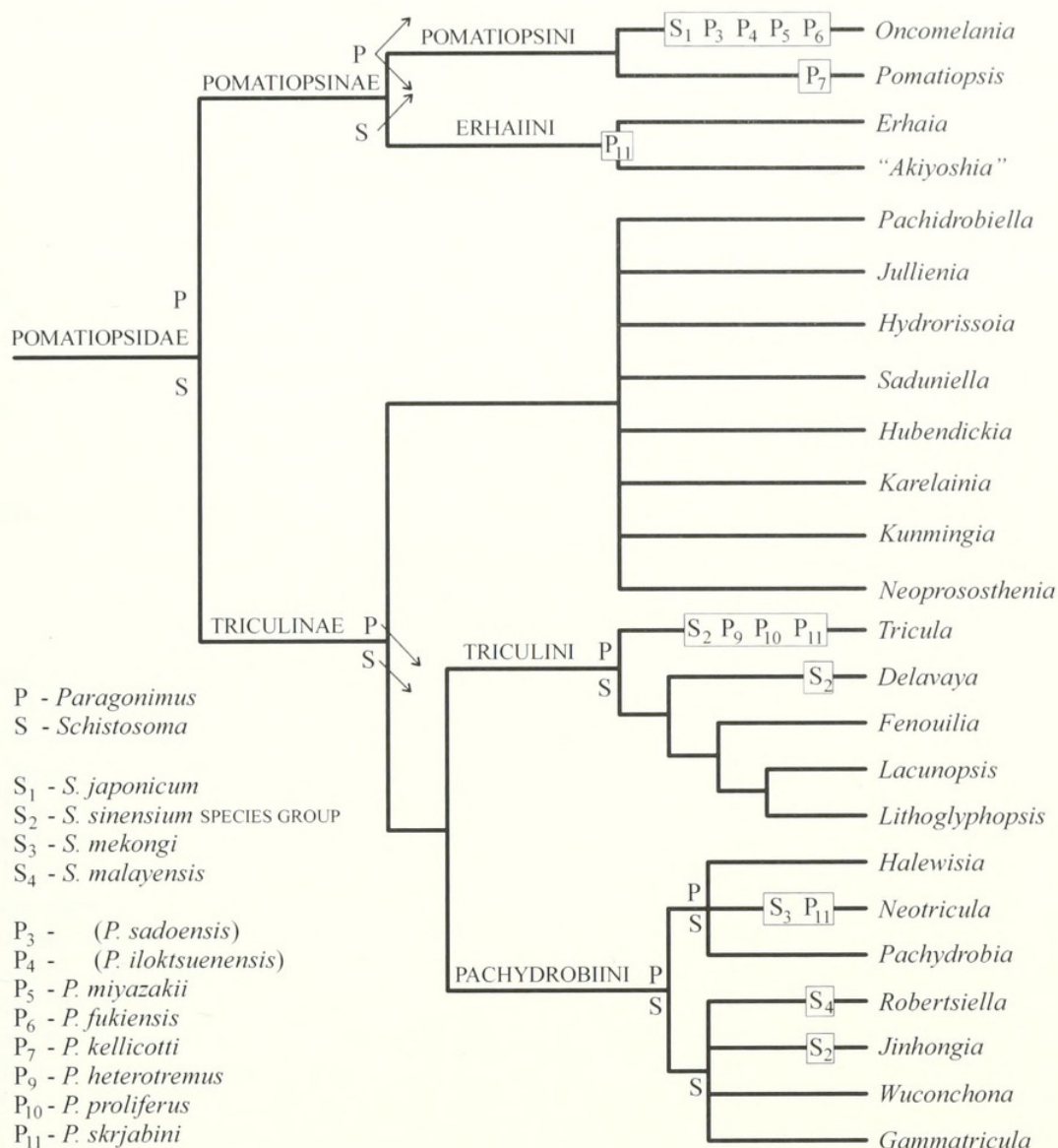
FIG. 3. Continuation of Fig. 2 with emphasis on the family Pomatiopsidae.

sibly 18 species (excluding numerous synonyms), but the exact number of species has yet to be determined on the basis of combining sound morphological and molecular data. This is an objective of the TMRC. One "species", *P. skrjabini*, deserves particular attention as it has been reported to be transmitted by some 22 species belonging to four genera of two families, and three tribes of the Pomatiopsidae (Davis et al., 1994a).

(5) *Oncomelania* transmits *Paragonimus*! There are two species of *Oncomelania* (Davis, 1979, 1980, 1992; Davis et al., 1994a). *Oncomelania minima* of Japan, a fully aquatic species, transmits *P. ohirai;* it does not transmit *Schistosoma. Oncomelania hupensis chiui* of Taiwan, more aquatic than amphibious, also transmits *P. ohirai,* but not *Schistosoma* in na-

ture. However, this subspecies can transmit all known geographic strains of *S. japonicum* in laboratory challenges. *Oncomelania h. tangi* from Fujian Province, China, is (or was, parasite now presumed extinct) the snail host for *P. fukiensis.* The sister genus to *Oncomelania, Pomatiopsis* in eastern U.S.A. transmits *P. kellicotti.*

Considering *Schistosoma* coevolution: (6) There are two species complexes of *Schistosoma* transmitted by the Pomatiopsidae that infect mammals. The complexes are recognized by adult worm morphology and, especially, egg morphology. Davis & Greer (1980) described the *S. japonicum* complex of three species, all infecting man with *S. japonicum* transmitted by *Oncomelania* of the Pomatiopsinae. *Schistosoma mekongi* (Mekong

River, Laos) and *S. malayensis* (Malaysia), are transmitted by two different genera of two different tribes of the Triculinae. Davis (1992) proposed a *S. sinensium* species complex in China and Thailand of at lease three species, none of which, at this time, infect man. They are transmitted by three genera of two tribes of the Triculinae.

Specificity of Coevolution: All evidence to date indicates that schistosomes have coevolved through time with ever increasing specificity, so that today the snail-parasite interaction is species, regional and often population specific (Davis, 1992). While this is apparently true of schistosomes coevolving with pomatiopsine snails, the situation is not at all clear for *Paragonimus*. But then, there is much that is unknown about *Paragonimus* species boundaries, definitions, phylogenetic relationships, and parasite-snail interactions.

While there does seem to be a clear coevolved association between the *P. westermani* species complex and ceritheacean snails, there appears to be board spectrum association between some species of *Paragonimus* and snails of different families of the Rissoacea. For example, *P. ohirai* is transmitted by both *Oncomelania* of the Pomatiopsidae (*O. minima* of Sado Island, Japan; and *Assiminea* of the Assimineidae in Japan and China). *Paragonimus fukiensis,* considered by some as *P. iloktsuensis* (a synonym of *P. ohirai;* Blair et al., 1999) of Fujian Province, China, is transmitted by *O. hupensis tangi. Paragonimus heterotremus* of China, Thailand, Laos, and Vietnam has been found in nature in *Tricula* (Pomatiopsidae) and reported from *Assiminea* sp. (Assimineidae) (Blair et al., 1999); it has been passed experimentally in various subspecies of *Oncomelania hupensis,* and *Neotricula aperta* (different subfamilies of Pomatiopsidae).

Finally, the taxonomic diversity and numbers of snail hosts of *P. skrjabini* suggest that, while there may indeed be more than one species of *Paragomimus* involved, this species may readily parasitize diverse, if not all, triculine taxa. On a recent field trip to Fujian Province, we collected, along with the parasitologists of the Fujian Institute of Parasitic Diseases, two species of minute rissoacean snails close to the headwaters of a small mountain stream. At the very top of the stream we collected a species of *Erhaia.* Some 100 m down stream we collected a species of *Tricula.* The Fujian IPD parasitologists said that they had screened thousands of these two

snails in the past and obtained the same species of *Paragonimus* from both species of snail, that is, *P. skrjabini.* As the snails are essentially sympatric, the implication is that species of *Paragonimus* can switch hosts between different subfamilies of the Pomatiopsidae.

There is a problem concerning the classification of *Erhaia.* Davis et al. (1985), Davis & Kang (1995), and Davis (1992) classified the genus in the Pomatiopsidae on the basis of overall anatomy. Species now classified as *Erhaia* had previously been considered by Chinese authors as *Bythinella* or *Pseudobythinella* of the Hydrobiidae. The above authors pointed out that the Chinese snails lacked the male reproductive system anatomy of *Bythinella,* a European genus belonging to the Hydrobiidae: Amnicolinae. The genus name *Pseudobythinella,* as applied to Chinese taxa, was preoccupied by the same generic name for a fossil Hydrobiidae of England. The problem of classification involves three morphological characters and character-states found in *Erhaia* that do not conform to overall Pomatiopsidae character-states: (1) the shape of the central tooth of *Erhaia* is trapezoidal while that of all other pomatiopsids is rectangular; (2) the shell has the characteristic shape of European *Bythinella,* a shape not found in all other pomatiopsids; (3) the spermathecal duct is so closely fused to the pallial oviduct as to seem indistinguishable from the pallial oviduct, while in all other pomatiopsids the spermathecal duct is seen to be clearly distinct from the pallial oviduct and separated from the latter. As *Erhaia* exists in an arc from northeastern India through Yunnan, China, down along the Yangtze River drainage into Hunan and Hubei with a species in Fujian Province, and as the genus is clearly not *Bythinella,* Davis et al. (1985), Davis (1992), and Davis & Kang (1995) considered the above three morphological character-states to be convergent on the Hydrobiidae: Amnicolinae.

To settle issue of familial status, the TMRC has initiated molecular genetic studies of *Erhaia.* Does *Erhaia* belong in the Pomatiopsidae, in the Amnicolinae, or is it unrelated to either?

The question arises: Is the important factor for *Paragonimus* transmission one of ecology rather than phylogeny? *Paragonimus ohirai* invades different rissoacean snail species in two different environments; *Oncomelania* in freshwater, *Assiminea* in brackish water. A second question then arises: if both a thiarid

or pleurocerid snail were sympatric with a rissoacean snail along with appropriate crabs, would both transmit *Paragonimus* in that system? Does such sympatry occur in Asia, South and Central America? Davis (1982), in a review of historic and biogeographic factors involved in the evolution and radiation of all freshwater snail groups, noted that ceritheacean and rissoacean snail radiations in freshwater are generally biogeographically exclusive of each other. Where one finds populations of ceritheacean snails, one generally does not find populations of rissoacean snails in sympatry. This phenomenon requires close examination through field work and verification. For example, is the pleurocerid *Semisulcospira libertina,* host for *P. westermani* in Japan, sympatric with *Oncomelania minima* on Sado island? (4) It is noteworthy that rissoacean families Stenothyridae and Bithyniidae, commonly found from the Ryuku Islands through southern China, are not implicated in the transmission of *Paragonimus.* We have found *Stenothyra* sympatric with triculine taxa. One would like to determine if *Stenothyra* can be found in sympatry with either a

ceritheacean or rissoacean in a habitat where *Paragonimus* is transmitted. The ecological requirements of the Bithyniidae perhaps remove them from any potential taxon hopping by *Paragonimus.*

The Question of Hydrobiidae in China and Monophyly of the Pomatiopsidae

Chinese workers have long considered *Oncomelania* to belong in the family Hydrobiidae (Liu et al., 1997; Liu, 1979; Kang, 1981, 1998a, b). On the basis of comparative anatomy of rissoacean snails from China, there is no evidence for the family Hydrobiidae in China (unless *Erhaia* is found to belong to the Amnicolinae, and molecular data indicate the Amnicolinae belongs within the family Hydrobiidae). Based on sequencing data for the mitochondrial gene cytochrome c oxidase subunit I (COI), it is clear that the Pomatiopsidae, with *Oncomelania* and *Tricula,* belong to a family apart from the Hydrobiidae *s.l.* (Fig. 4, adopted from Davis et al., 1998).

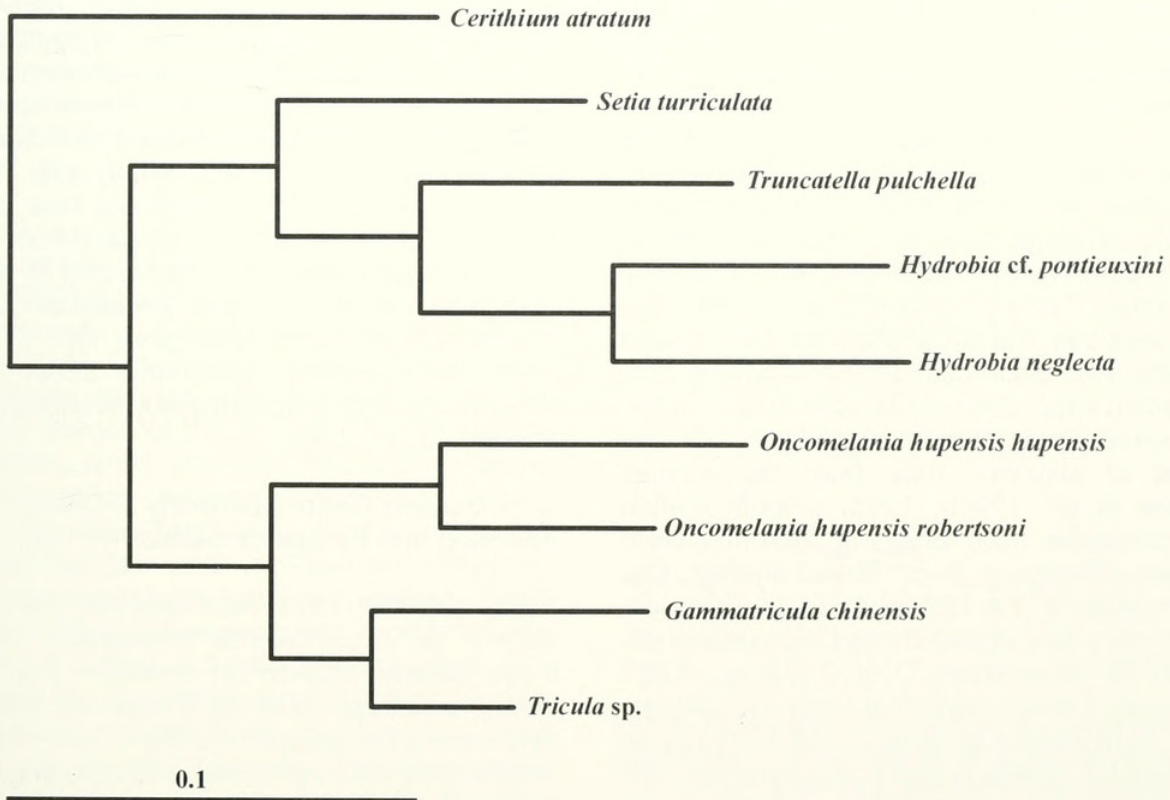There has been the question of the monophyly of the Pomatiopsidae with the Triculinae

FIG. 4. Maximum likelihood tree based on partial sequences of the COI gene. *Cerithium atratum,* the outgroup, is a ceritheacean snail; all others belong to the superfamily Rissoacea. *Setia* belongs to the Rissoidae; *Truncatella,* the Truncatellidae; *Hydrobia,* the Hydrobiidae. The lower cluster, greatly divergent from the Hydrobiidae, belongs to the Pomatiopsidae, with *Oncomelania* in the Pomatiopsinae and *Tricula, Gammatricula* in the Triculinae (adapted from Davis et al., 1998).

and Pomatiopsinae sister taxa. The question was raised because there are significant differences in the female reproductive anatomy between the subfamilies, and the inclusion of both in the Pomatiopsidae required considering the differences in how sperm enter the female reproductive system to have evolved from a common ancestor. In the Pomatiopsidae, sperm entry is via the spermathecal duct that extends from the bursa copulatrix to the anterior end of the mantle cavity. In the Triculinae, sperm enter into a spermathecal duct that ends at the posterior end of the mantle cavity, either beside the pericardium, or from passage through the pericardium.

Allozyme data (Davis et al., 1994b, 1995) and the COI data are concordant in demonstrating the monophyly of the Pomatiopsidae with the two sister subfamilies. Both data sets perhaps give insight into the evolution of the Chinese Pomatiopsidae. The COI data show *Tricula* sp. from Sichuan weakly supported in the triculine clade with some alternative trees placing it basal to the *Oncomelania* clade (Davis et al., 1998; fig. 3), other trees have *Tricula* sp. basal in the triculine clade. This is because the genetic distance between *Tricula* and *Oncomelania hupensis robertsoni* from Yunnan is less than it is between *Tricula* and *O. hupensis hupensis* (0.144 vs. 0.188). The anatomically derived *Gammatricula* is more genetically distant (0.171 vs. 0.195). The allozyme data set showed that smooth-shelled *Oncomelania hupensis* from Zhejiang Province was much more similar to triculine (*Gammatricula songi*) than two triculines were to each other *Neotricula lilii* vs. *Gammatricula chinensis* (Nei's D of 0.100 vs. 1.236). Because of this, the UPGMA phenogram based on the Nei distances did not show a pronounced separation of *Oncomelania* from the triculines (Davis et al., 1994b; fig. 4). On the basis of allozyme data from two studies (Davis et al., 1994b, 1995) smooth-shelled *Oncomelania* from Zhejiang Province were greatly divergent from ribbed-shelled *Oncomelania*. In the 1994b study, two smooth-shelled vs. one ribbed shelled populations differed by an average D of 0.173 vs. 0.051 between the two smooth-shelled populations. (We here correct an error in the 1994 paper. In figure 2, shells D and E are reversed; the smooth shell shown as D comes from population 1, not 2, etc.) In the 1995 study, the least divergent among 14 populations was between the smooth-shelled population from Zhejiang Province and smooth-shelled *O. hupensis*

robertsoni from Dali, Yunnan Province (D = 0.007), some 2,000 km away.

The following is a synthesis of the foregoing: (1) The Triculinae and Pomatiopsinae are highly divergent on the basis of robust morphological data; there are considerable differences in the ground-plans of the female reproductive systems. They are ecologically distinct. Molecular data support the monophyly of the Pomatiopsidae with the two divergent subfamilies. The subfamilies are soundly based. (2) *Tricula* from Sichuan is morphologically very similar to species of *Tricula* from Yunnan, China, to northern India (the type species is *Tricula montana* Benson, 1843, from northern India). *Neotricula* and *Gammatricula* are derived triculine genera distributed, in China, below the Three Gorges of the Yangtze River. (3) The primitive shell condition is small, smooth and without varix. Species of *Tricula* and *O. h. robertsoni* are small, smooth and without varix (Davis, 1979). (4) The direction of evolution is from the northern Indian Plate into northern Burma and western Yunnan, China, and subsequently down evolving river systems (Davis, 1979, 1980, 1992). (5) Considering molecular genetics, *Tricula* is more closely related to *O. h. robertsoni* than to downstream, derived, ribbed-shell *O. h. hupensis*. (6) The comparatively low molecular divergence between *Tricula* and *O. h. robertsoni* and between some triculine taxa and smooth-shelled *Oncomelania* that have close genetic affinity with Yunnan and Sichuan *O. h. robertsoni,* may indicate the relative recent divergence (Miocene) of the ancestral taxon into *Tricula* and all subsequent triculine taxa, and a small smooth-shelled *Oncomelania* that gave rise to all other *Oncomelania* dispersing down the Yangtze River and subsequently evolving ribs and the varix.

Oncomelania Genetic Diversity in China: Allozyme and Sequencing Data

Davis (1992, 1994) has reviewed the taxonomy of *Oncomelania* throughout Asia; these papers provide an overview that includes a discussion of the anatomical uniformity among the subspecies, the *Oncomelania hupensis* polytypic complex, and breeding genetics. The 1994 paper provides a species definition uniformly used for all of our studies of the Pomatiopsidae. The question then is: how genetically diverse is *Oncomelania* throughout China? On the basis of allozymes

O. hupensis hupensis

GuiPing

Yue
Yang PengZe

HanYang

JiangLing

NingGuo

GuiChi

TongLing

O. h. tangi

FuQing

XiaPu

JianLi

Dali
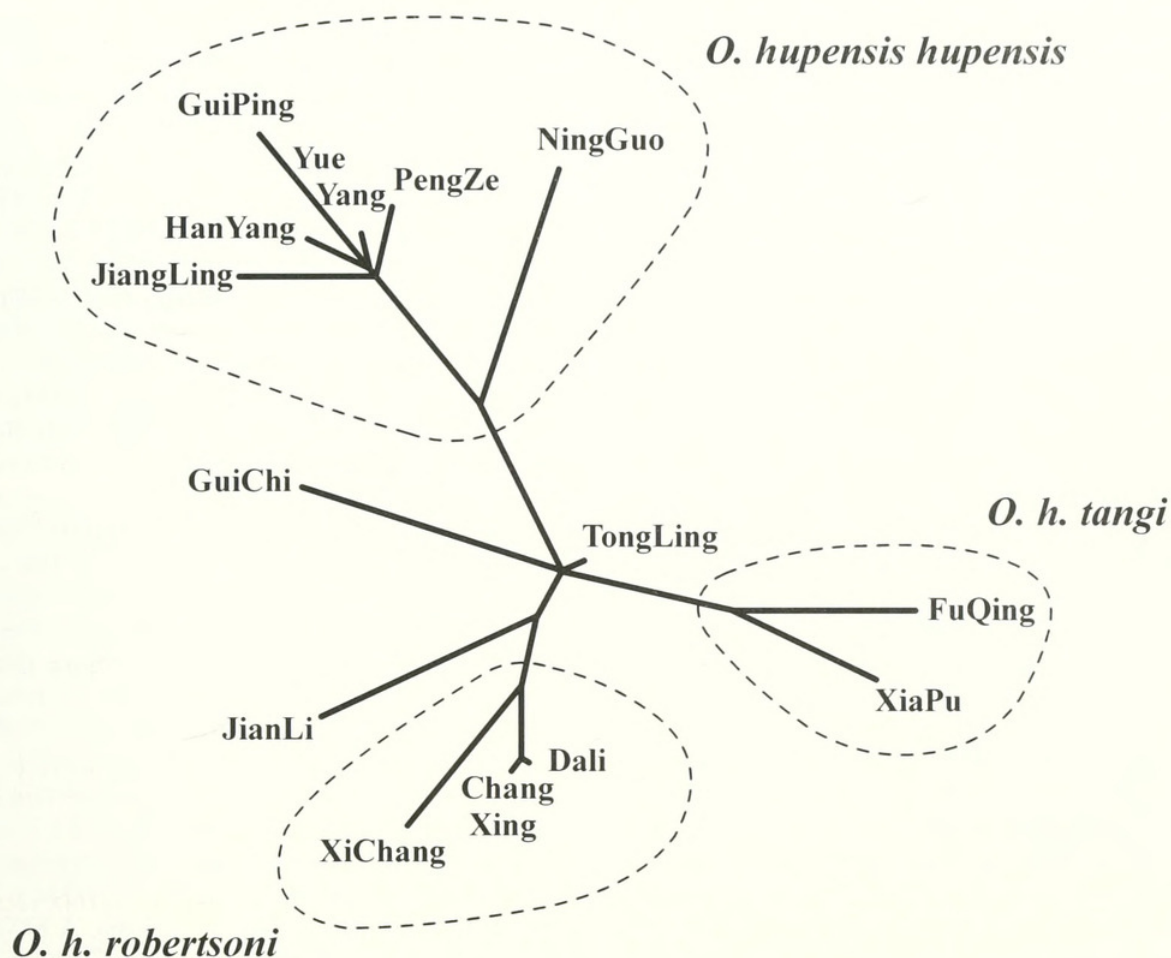Chang
Xing

XiChang

O. h. robertsoni

FIG. 5. FITCH tree based on allozyme data showing three clusters that are classified as subspecies. Three populations (Gui Chi, Jian Li, Tong Ling) are intermediate among the clusters and were considered "hybrids" by Davis et al. (1995). We now recognize these to be "unstable populations," aggregates of snails deposited from diverse locations by the annual floods of the Yangtze River.

(8 provinces, 14 populations, 30 loci), Davis et al. (1995) found that populations throughout China could be grouped into three subspecies (Figs. 5, 6). *Oncomelania hupensis robertsoni* occurs in Yunnan and Sichuan above the Three Gorges of the Yangtze River. It has a relatively small smooth shell without varix. *Oncomelania hupensis hupensis* occurs throughout the Yangtze River basin below the Three Gorges and in Guangxi Province. These populations have a relatively large shell of the same allometry as *robertsoni,* are primarily ribbed and with a strong varix. *Oncomelania hupensis tangi* is from Fujian Province, isolated from the Yangtze River drainage by tall mountains. This subspecies changes shell allometry; the shells are proportionally much wider than those of the other subspecies, are smooth, and have an exceptionally thick varix. Within *O. hupensis hupensis,* there is considerable genetic diversity;

O. h. robertsoni
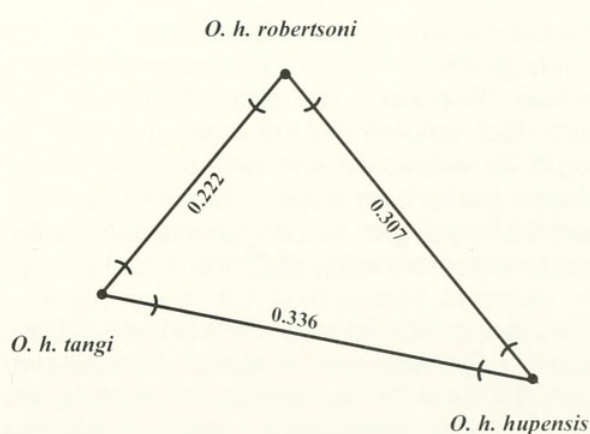
0.222

0.307

0.336

O. h. tangi

O. h. hupensis

FIG. 6. Nei's minimum distances (in scale) among subspecies of *Oncomelania hupensis* based on allozyme data (standard deviations are marked as curved lines).

Nei's minimum D = 0.204 ± 0.085 (N = 21). Sequence data from mitochondrial COI (Fig. 4) and Cyb genes (Spolsky et al., 1996) confirm the divergence between *O. h. hupensis* and *O. hupensis robertsoni*.

Smooth vs. Ribbed Snails: Systematic Implications: The question of the taxonomic status of smooth-shelled *Oncomelania* in China has been an ongoing debate for many years (Liu et al., 1981; Lou et al., 1982; Kang, 1998a). Above, we discussed the genetic relationships of two smooth-shelled populations from Zhejiang Province. Zhou et al. (1995) studied the allozymes of 34 populations from nine provinces. They used 16 loci of which five were esterase loci. In UPGMA clustering of Nei's (1978) "D", they also found that Sichuan and Yunnan snails clustered apart from *O. hupensis hupensis* (*sensu* Davis et al., 1995), as did the one population they had from Fujian (*O. hupensis tangi*). One population from Anhui with a smooth shell and varix, clustered with the ribbed-shelled populations. Using Fitch-Margoliash least-squared cluster analysis, all smooth-shelled *Oncomelania* clustered together, but the Anhui smooth-shelled population was basal and distinctly apart, rather intermediate between the smooth and ribbed-shelled populations. Zhou et al. (1995) concluded that there were two taxonomically distinct groups: ribbed and smooth-shelled types. Aside from the Sichuan, Yunnan and Fujian populations, they examined only three other smooth-shelled populations, one from Anhui and two from Jiangsu Province. They did not consider the shell morphological and biogeographical differences that separate *hupensis, robertsoni and tangi*. They did consider that smooth-shelled population groups might be separated into subspecies, for example, those from Fujian, Yunnan, Sichuan, and the hilly region of Jiangsu Province at the extreme eastern edge of China.

The Taxonomic Status of Smooth and Ribbed Shelled Oncomelania in China: We initiated work in 1994 in an attempt to resolve the smooth vs. ribbed-shell problem in the Yangtze River below the Three Gorges, having determined that *robertsoni* and *tangi* were distinct taxa. It has long been known that the ribbed-shelled *Oncomelania* occur only in China, in the marshes and flood plains of the Yangtze River and flood plains of the lower tributaries of the Yangtze River. Smooth-shelled *Oncomelania* were to be found in "hilly" regions (Liu et al., 1981; Lou, et al., 1982). We used the Miao River of Hubei Province as a natural experiment (Davis et al., 1999). This small river, 25 km long, has ribbed-snail populations along the lower half and smooth-shelled populations in the upper half. The ribbed-shelled populations live on the flood plains of the Miao River, and are subjected to annual flooding. The smooth-shelled populations live above the effects of flooding. There is a bridge across the river, 13 km from the mouth of the river, just above the point reached by the annual floods. We studied the allozymes (starch gel electrophoresis, 35 loci) from four populations above the bridge and three populations below the bridge (example of shell types shown in Fig. 7). There were three control populations outside the Miao River drainage. The mean number of individuals studied from each population ranged from 44 to 122. The results were conclusive: ribbed-shelled and smooth-shelled populations did not assort into discrete clusters; one species is involved, *O. hupensis hupensis.* The mean overall Nei's (1978) D was (0.038 ± 0.035). The mean D for populations above the bridge was (0.024 ± 0.016); for below the bridge, (0.045 ± 0.036). A FITCH tree, based on Wright's modified Rogers' D, shows intermixing of ribbed and smooth-shelled populations (Fig. 8).

Based on this natural experiment, populations of *Oncomelania* below the Three Gorges of the Yangtze River, within the Yangtze River drainage, that are smooth (but with varix), and with the same allometry as snails of ribbed populations in the lower Yangtze drainage, are one subspecies, *O. hupensis hupensis.* This excludes the smooth-shelled populations in the hills of Jiangsu and Zhejiang Provinces. Ribbing is associated with annual flooding of the Yangtze River and its tributaries. Snails from any elevation, or a man-made situation that removes a population from the annual floods, attain a smooth shell but still retain the varix. Molecular genetic data do not support the concept of different taxonomic status for these two shell types. Accordingly, *Katayama fausti* Bartsch, 1925, is a synonym of *O. hupensis hupensis* Gredler, 1881. (*Katayama* used to be used as a genus to include all smooth forms of *Oncomelania hupensis*.) This same conclusion was reached by Lou et al. (1982), who noted that *fausti* and *hupensis* lived over the same geographic region and that the ribbing vs. smooth condition was strictly related to elevation above flooding.
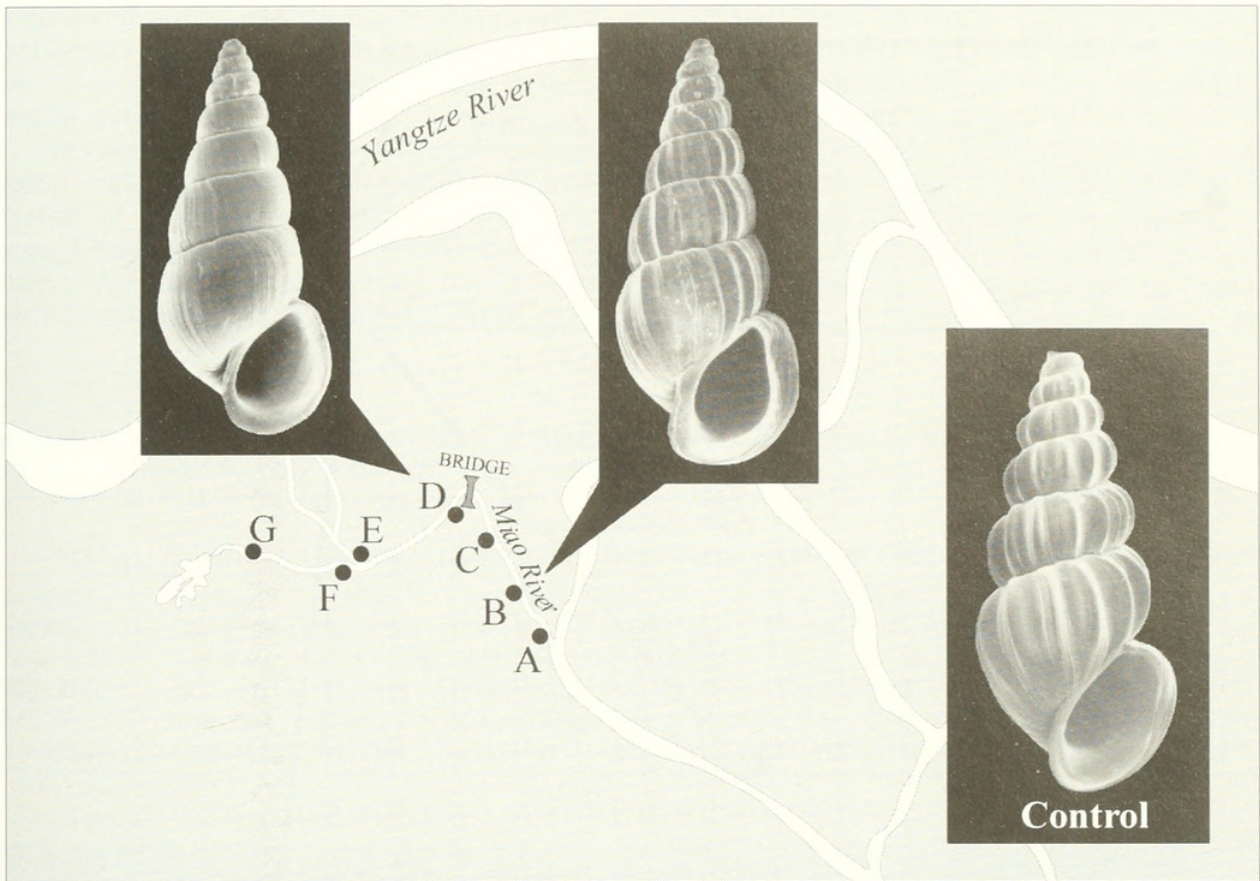
FIG. 7. Miao River localities (Hubei Province) of smooth-shelled and ribbed-shelled populations of *Oncomelania hupensis.* Populations with ribbed shells are below the bridge (A, B, C) and are inundated each year by the annual flooding of the Yangtze River. Populations above the bridge (D–G) have smooth shells; they are not flooded each year. The control snail is from the flood planes of the Yangtze River in Gui Chi County, Anhui Province.

Based on allozymes, smooth-shelled *O. hupensis guangxiensis* Liu et al., 1981, from Guangxi Province is also a synonym of *O. h. hupensis* (Davis et al., 1995).

It has yet to be determined how the eastern Chinese hill-dwelling populations of Jiangsu and Zhejiang relate to smooth-shelled snails studied here from the Miao River and Guangxi Province. These few special populations require intense study. Are they truly genetically divergent from *O. h. hupensis?* Are they part of the *robertsoni* complex, but have independently evolved a varix? Are they a distinct subspecies?

Ecology and Genetic Instability: Three populations studied by Davis et al. (1995) did not group with populations forming the *hupensis, tangi* or *robertsoni* clusters: GuiChi and Tong Ling from Anhui Province and Jian Li from Hubei Province (Fig. 5). They were called hybrids between the *robertsoni* and *hupensis* genomes. After revisiting and collecting snails

on Lao Zhou Island (an island in the Yangtze River in Tong Ling County, Anhui Province), we now understand why these populations did not cluster with one of the three subspecies clusters. Using COI sequence data, it is clear that these populations are indeed *O. h. hupensis.* What accounts for these results? These three localities are flooded, covered by water and swept during the annual floods. Snails found in these locations are not populations, really, but aggregates of snails imported form diverse areas and deposited with the receding floodwaters. Such populations have been called "genetically unstable" aggregates (Davis et al., 1999) in which true population structure does not attain and HWe is not attained in polymorphic loci.

Floatation during Yangtze River flooding is a major source of dispersion for *Oncomelania hupensis hupensis* and the schistosomes they transmit. This phenomenon is apparently not known outside China. During the floods, snails are lifted of the islands in the Yangtze
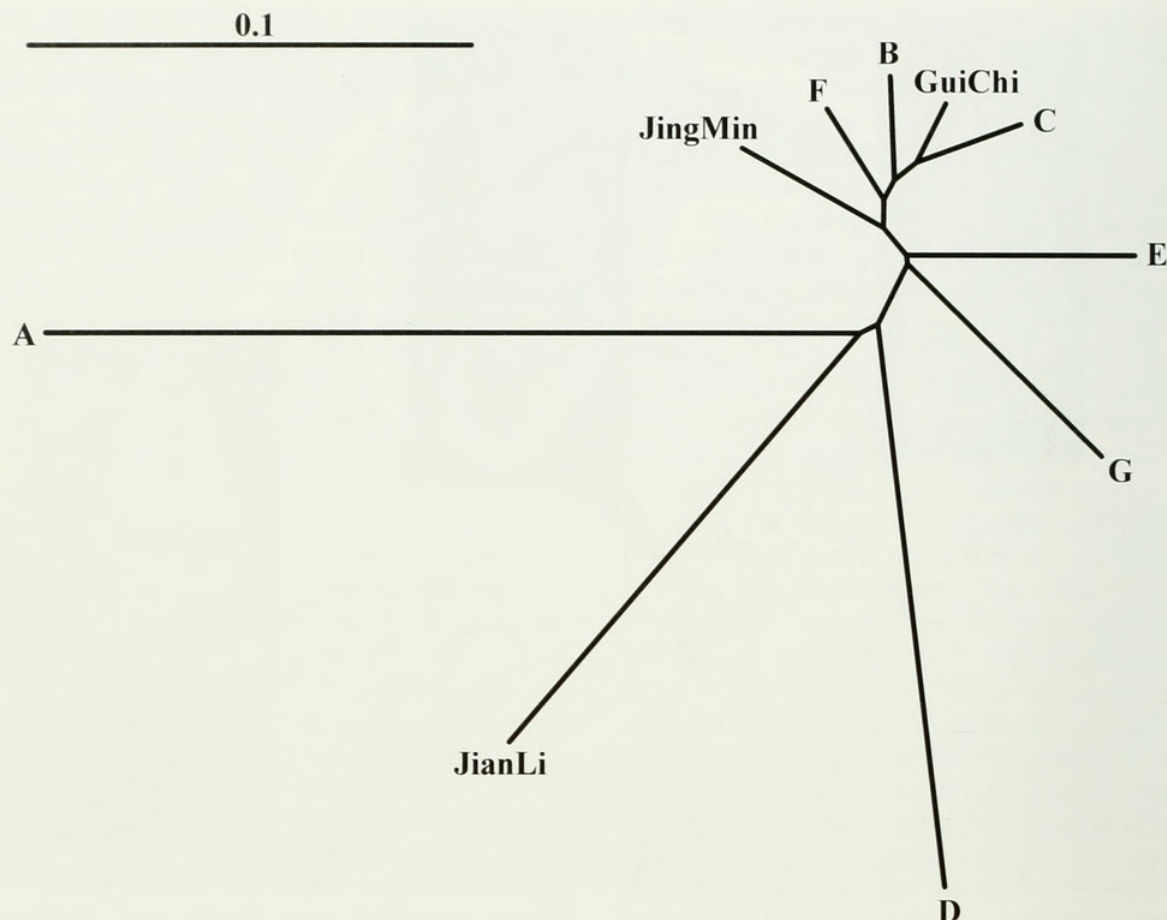
FIG. 8. FITCH tree based on Wright's modified Rogers' genetic distance (allozyme data) for ribbed (A–D) and smooth-shell populations (D–G) from the Miao River. Control populations are from Jian Li, Jing Min and Gui Chi.

and flood plains and floated by the millions down the river to be deposited on downstream flood plains or swept into canals when the flood gates are opened. The impact on importation into the canals of Hubei Province have been documented by Xu & Fang (1988), Xu et al. (1989, 1993), and Yang et al. (1992). While snails on the islands either float off or drown, snails on the flood plains often escape flooding by climbing tree trunks, often to heights of more than three meters.

As the vast flood plains of the Yangtze River below the Three Gorges are swept by floods each year, causing snails to be dislocated in vast numbers and carried over considerable distances, the TMRC is researching the effects of such dislocation and mixing on the genetics of both snails and the schistosomes these snails transmit. These aggregates of flood-deposited snails are some of the most highly infected snails in China. We have selected benchmark sites of stable populations and unstable aggregates of snails in different provinces. A stable population is one beyond

the effects of annual flooding and sufficiently removed in tertiary stream systems that there is the possibility that there has been no immigration or emigration for many years, and the population of snails has been out-breeding within the confines of the isolated population. Schistosomes in this stable populations would, theoretically, be genetically uniform, in contrast to the schistosomes isolated from unstable snail aggregates. What one discovers in analyzing schistosome genetics very much depends on schistosome breeding structure, how much one can truly sample of the schistosome population overall when one has to screen 6,000 snails to find four to six infected snails and one is limited to assessing the genetics of worms derived from the cerceriae of only these few snails. Such low rates are the rule in endemic areas and in sites with stable populations. While coevolution of snails and schistosomes has been clearly demonstrated through breeding and infectivity studies (reviewed: Davis, 1980, 1992), it is not at all sure that studies of structural genes or mitochon-

drial genes will demonstrate the pathways of schistosome genetic diversification. Studies are in progress to attempt to answer some of these questions.

We have found a way of demonstrating genetic instability within and between aggregates of *Oncomelania hupensis hupensis* using haplotypes of the mitochondrial COI gene. We used six populations from Hunan Province as an example. Table 1 gives local-ity data. Figure 9 provides a topographic map of China showing Dong Ting Lake in relation-ship to the Yangtze River. Figure 10 is a GIS map of the locality data with reference to Dong Ting Lake, the Yangtze River, and ele-vation contours. Table 2 gives the number of nucleotide differences among ten individuals from each population, the accumulative num-ber of differences and the number of haplo-types (mitochondrial genes are maternally in-

TABLE 1. Locality data for six populations of *Oncomelania hupensis hupensis* from Hunan Province used for COI sequence analyses. Coordinates are in decimal degrees. These localities are mapped in Fig. 10.

Locality No.	Prefecture	County	Township	Administra-tive Village	Natural Village	Latitude	Longitude
MG96.16	Changde	Li Xian	Fang Shi	He Jia	He Jia Grp	29.79°N	111.44°E
MG96.17	Changde	Hanshou	Po Tou	San Xing	Huang Long Shan	28.92°N	112.13°E
MG96.19	Yue Yang	Yue Yang	Jun Shan	Mu Hu Pu	He Hua Zhou	29.37°N	113.00°E
MG96.20	Zi Yang	Yi Yang	Zi Hu Kou	Shi Ma	Huang Jia Hu	28.74°N	112.55°E
MG96.21	Yue Yang	Xiang Yin	Qing Tang	Shang Shan	Chen Jia Wan	28.86°N	112.89°E
MG96.22	Changde	Li Xian	Jiu Wan	Gan Jia	Meng Jiang Wai	29.56°N	111.92°E

FIG. 9. Topographic map of China showing the lowland basins of China, and the relationships of Dong Ting Lake and Poyang Lake to the Yangtze River. Dong Tink Lake of Hunan, China, is residual from a vast lake that filled the great basin of central China, i.e. Hubei Province north of the Yangtze Rriver and Hunan Province south of the river. The great basin involves a vast area on either side of a line from Yichang to the east of Wuhan. This basin, full of marshes, lakes and canals is the most impacted by *Schistosoma japonicum* today. Poyang Lake, the largest lake in China, is likewise a vast area of marshlands heavily impacted by schistosomiasis.
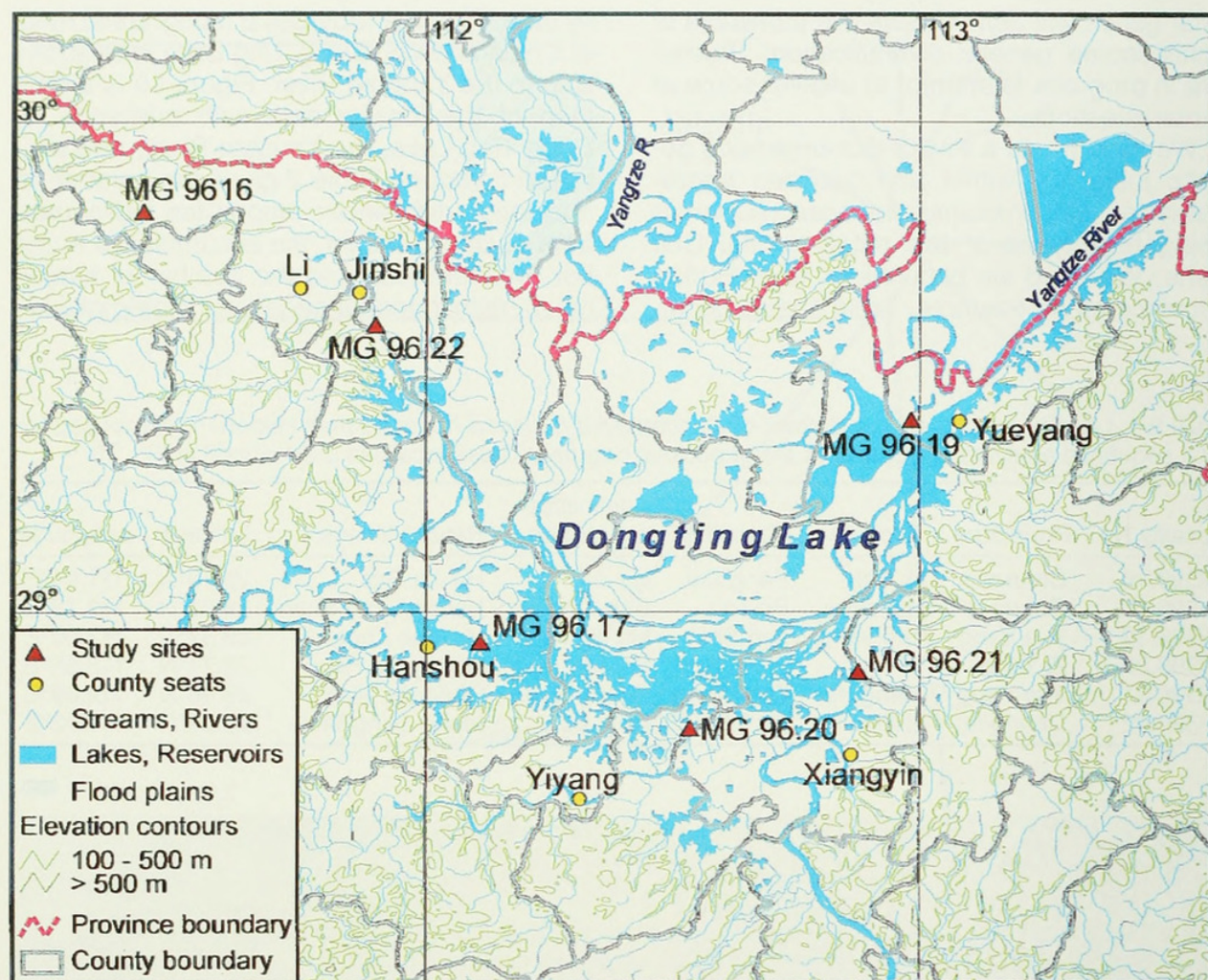
FIG. 10. GIS-facilitated map of the Dong Ting Lake district.

TABLE 2. Genetic diversity, based on COI sequencing data, within and among populations of *Oncomelania hupensis hupensis* from Hunan Province. A–F represent these populations in Table 3 and Fig. 11.

	A MG96.16	B MG96.17	C MG96.19	D MG96.20	E MG96.21	F MG96.22	A–F TOTALS
No. individuals	10	10	10	10	10	10	60
No. and % polymorphic sites	8 (1.30%)	24 (3.80%)	25 (3.90%)	25 (3.90%)	23 (3.60%)	22 (3.50%)	75 (11.80%)
No. of nucleotide diff. (mean + stand. dev.)	2.84 ± 2.2	7.24 ± 11.0	6.82 ± 9.9	7.11 ± 10.7	7.18 ± 10.8	9.18 ± 17.0	9.75 ± 19.8
No. haplotypes	2	9	10	10	8	6	40

herited and thus only the female complement of DNA is involved). Table 3 provides the pairwise comparisons among these populations (total number and percentage of nucleotide differences). In Figure 11, a maximum likelihood tree shows the relationships among the haplotypes from these "populations."

Most of these populations are from islands or flood plains of Dong Ting Lake, areas that, unlike Hubei Province across the "punch bowl" of the Yangtze River, are unprotected by a continuous series of dikes that keep flood waters out. Note that at Yueyang, the Yangtze River connects to Dong Ting Lake, and here is the outlet for the four rivers of Hunan that flow into the lake. During flood season there is consid-

TABLE 3. Pairwise comparisons of six Hunan populations of *Oncomelania hupensis hupensis* for nucleotide differences (total number and percentage) in a 638 basepair fragment of the COI gene.

Populations	A	B	C	D	E	F
A	—					
B	11.5 (1.8%)	—				
C	13.2 (2.1%)	7.8 (1.2%)	—			
D	12.3 (1.9%)	7.1 (1.1%)	7.4 (1.2%)	—		
E	12.4 (1.9%)	7.8 (1.2%)	8.1 (1.3%)	7.8 (1.2%)	—	
F	11.5 (1.8%)	11.9 (1.9%)	12.8 (2.0%)	12.0 (1.9%)	11.0 (1.7%)	—
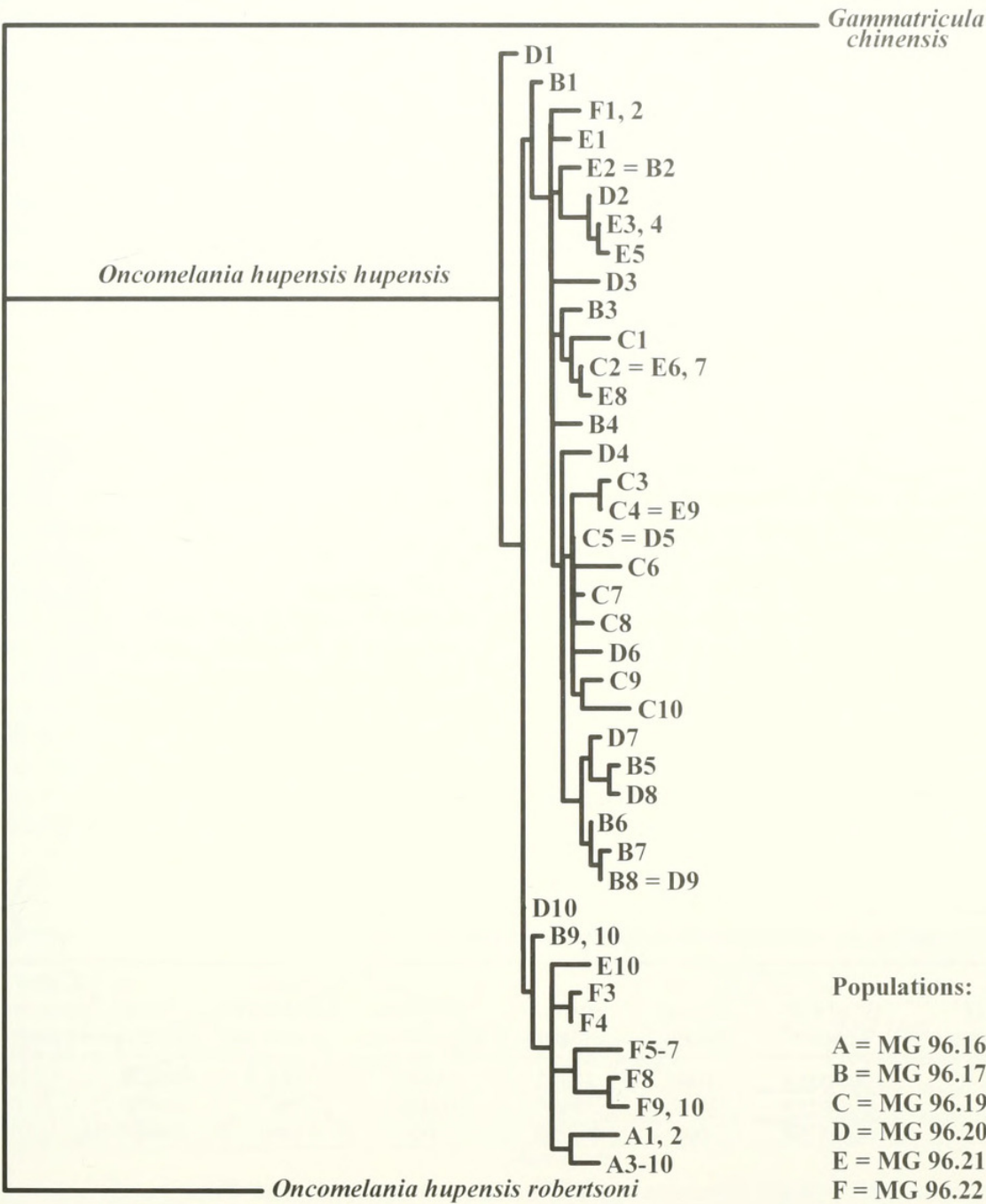
FIG. 11. Maximum likelihood tree based on partial sequences of the COI gene showing the interspersion of haplotypes. Population A is stable. Populations B, C, D, E, and F are "unstable" populations, affected by flooding.

erable increase in the volume of the lake and inundation of grazing lands and marshes.

In a stable population, one would expect an average of 0 to 0.5% nucleotide differences within populations (0 to 3 nucleotide differences), or one to two haplotypes per ten individuals. As seen, only population A (MG 96.16) is stable with two haplotypes involving eight polymorphic sites; all individuals of this population are in a unique cluster apart from clusters with individuals from other localities. Its closest genetic relationship is with six individuals of population F (MG 96.22) and one individual from population B (MG 96.17). These two localities are the closet ones to A. Population A is far west of Dong Ting Lake and removed from the effects of the annual flood. It is in slightly elevated terrain, in the 100 to 500 meter contour lines on the map.

All the other so-called populations are "unstable," aggregates of individuals with a significant number of different haplotypes, indicating import from different areas of Hunan along Dong Ting Lake. For example, individuals from locality B (MG 96.17), at the west end of the lake, share haplotypes with individuals in localities C, D, E and F. Population C (MG 96.19), the closest locality to the Yangtze River, forms its own subcluster, except that the cluster includes an individual from D, and one C individual is found clustering with individuals from B and E. In two populations (C and D), there were ten different haplotypes for ten individuals; we had not reached the limit of numbers of haplotypes to be found. What we consider unstable aggregates of individuals had 60% to 100% different haplotypes per ten individuals.

These data clearly show that the flood-impacted areas around Dong Ting Lake host aggregates of snails swept together and de-posited from diverse areas along the Yangtze and Dong Ting Lake, and that there is a surprising number of haplotypes to be found among these aggregates. These are not natural populations in the usual sense of the term. These findings have considerable implications for the genetics of schistosomes transported by these snails. Our data also indicate that with sequence data from four individuals of a population, when only one haplotype is found, the probabilities are very good that the populations are "stable", that is, a Yunnan population from Dali (Yunnan and Sichuan populations are in high plateaus or hilly regions unaffected by annual floods).

We will use haplotype diversity to map pathways of dispersals and introductions throughout China based on gene flow analyses (Hudson et al., 1992). Wilke & Davis (1999) have shown how this can be done with gastropod populations using European *Hydrobia ventrosa* and *Hydrobia ulvae* as a paradigm.

Ecology, Modes of Schistosome Transmission, and the Three Gorges Dam

Modes of Schistosme Transmission: Epidemiological models of schistosome transmission and control in China might be improved taking into consideration the fact that there are four distinctly different modes of transmission (Table 4). The fundamental differences are major. (1) There are genetically distinct subspecies of *Oncomelania hupensis* involved in transmission. (2) There are distinctly different snail ecologies associated with the genetic differences. (3) There are different snail life tables associated with the ecological differences. (4) Transmission to man is primarily caused by cattle infections (water buffalo and other cattle) in some areas, not in

TABLE 4. Modes of transmission of *Schistosoma japonicum* in China.

Mode of transmission	Snail sub-species	Annual floods	Environ-ment	Short life expectancy	Stable population structure	Three Gorges	Cattle > 85% responsible for transmission
I	*hupensis*	yes	lake*	yes	yes	below	yes
II	*hupensis*	yes	river*	mixed	no	below	no
III	*hupensis*	no	canals* canal net- works	no	yes	below	no
IV	*robertsoni*	no	high eleva- tion*	no	yes	above	no

*High elevation = 500–2000m; below the Three Gorges along mid-to lower Yangtze River, elevations mostly 0–200 m; see text for details.

others. (5) Transmission is by snails of either genetically unstable aggregates, or by snails in stable populations.

The different modes may be better understood by discussing the biogeographical regions that set up different modes of transmission in China.

I: Poyang Lake. The subspecies of snail is *O. h. hupensis.* The lake, located in Jiangxi Province, is the largest lake in China (Figs. 12, 13). It is a major focus of transmission in China. We consider that over 85% of transmission is attributable to cattle (the hypothesis of the current TMRC epidemiological study (EPI) on Poyang Lake to assess the importance of cattle in maintaining the life cycle of *Schistosoma japonicum* relative to infecting man). The life expectancy of snails is considered to be about one year (Zhang et al., 1996).

All transmission to man occurs in the lake! All living accommodations for man and domestic animals are outside the dikes that contain the lake. All snails are found on the flood plains and on the numerous islands inside the dikes. The annual dynamics of the lake dictate the life cycle of the snails. One may consider a "bathtub" model. With the annual flood of the Yangtze River, the lake fills like a bathtub, covering most of the islands with water contained by the dikes that have been built over centuries. Snails have no place to retreat to and adult snails presumably, for the most part, drown. Subspecies of *Oncomelania* are capable of living four to five years. In nature, their life expectancy exceeds two years. In Poyang Lake, life expectancy is about one year.

With reference to drowning, *Oncomelania hupensis* is an amphibious species. The young stay submerged during their early stages of development, often floating upside down, feeding on the surface of quiet water. As adults, the snails are found out of but near water, on the banks of irrigation ditches

FIG. 12. Topographic map of China showing the position of the dam across the Yangtze River and the reservoir that will eventually drown the Three Gorges. The dark tent-like bars focus ones attention to the mountain chains that serve as a funnel for the Yangtze River with the Three Gorges at the narrow end of the funnel. These mountains and the constraint of the Three Gorges have kept in isolation snail faunas and parasites in Yunnan and Sichuan provinces above the Three Gorges from faunas below the Three Gorges.

FIG. 13. GIS-facilitated map of Poyang Lake using ArcView 3.0a in connection with the Digital Map Database of China (ESRI Inc.) showing localities of the TMRC epidemiological study in progress. The lake is shown in full flood during the rainy season (late May to August or September).

and swamps, on shaded moist soil. During drought, the adults move down into the soil and aestivate. Adults cannot withstand continual submersion; they will drown. Because of this, drowning is a method used to control schistosomiasis in some areas of China.

The lake is connected to the Yangtze River by a very narrow outflow channel. It is probable that there is little immigration or emigration of snails in the lake during the annual floods. The flooding Yangtze backs up the flow of water from the lake to the river so that it is probable that there is little transport of snails in the lake. Accordingly, the snails on lake islands and flood plains are considered to have a "stable" population structure. However, after centuries of truncating the life history of the snails due to the annual floods, the question is: has selection altered the genetics of life history strategy? If it has, what is the effect on the dynamics of schistosome transmission?

In Poyang Lake, schistosomiasis is primarily a disease of cattle. People becoming infected are those who contact water during the low-water season within the lake basin, such as cattle herders, fisherman, and other boatmen.

II: Yangtze River Islands and Flood Plains. The subspecies of snail is *O. h. hupensis.* There is no stable population structure. Snails are aggregates of snails swept away during the annual floods and deposited with the lowering of water levels post flooding. Many aggregates of snails are old snails of varying age, from one to four or five years. Efforts to control snails in places such as the flood plains of Nanjing are thus far ineffective. Clearing out snails from a large section of flood plains here, close to the end of the Yangtze River in Jiangsu Province, is thwarted each year with the deposition of millions of heavily infected snails swept downstream with the floods.

III: Canals of Hubei Province; Canal Networks of Southern Yangtze River. The subspecies is *Oncomelania hupensis hupensis.* In the terminal ends of the greatly branched canal systems of this province, snail populations are presumably stable and with the normal life span. The influence of cattle on maintaining infections varies from place to place but does not approach the situation in Poyang Lake. At the Yangtze River, great flood gates keep the flooding Yangtze from the interior, contained by great dikes. When the flood gates are opened, snails from the Yangtze are swept into the lower canal systems (Xu & Fang, 1988; Xu et al. 1989, 1993; Yang et al., 1996). Migration along the lower canal systems would presumably cause unstable population genetics.

The Chinese literature discusses "hilly habitats" for some populations of *Oncomelania hupensis hupensis* and *O. h. robertsoni* (see Liu et al., 1981). The latter subspecies, discussed below, occurs at high altitude on the plateaus and hills, mountains of Yunnan and Sichuan Provinces, mostly at elevations ranging from 500 to 2000 m. A few populations live on lower plateaus or basins at 200–500 m. The snails live on more or less horizontal areas associated with agricultural practices such as rice farming (for example on terraces) although in Yunnan, *Oncomelania* has been found in small, trickling perennial flows of water flowing down hills with a slope of some 25 to 30 degrees at 1,000 m.

The former subspecies, as discussed above in the section on the taxonomic status of smooth and ribbed-shelled *Oncomelania,* primarily lives at low altitudes (0–200 m) and is affected by the annual floods of the Yangtze River and associated rivers; they have ribbed shells. When populations occur above the effects of the annual floods, the shells are smooth. Chinese workers have called the habitat hilly where the smooth-shelled populations are found, even if the altitude is slight (e.g., 90 m). We here limit the term "hilly" to populations dwelling at 500 m or greater.

There are a few populations of *O. h. hupensis* that live in mountain valleys such as found along the border of Zhejiang and Anhui provinces at an altitude of about 1,000 m. Populations are found in the northwestern parts of Guangxi Province, along the Yu Jiang and Hongshui He rivers at altitudes of 200 to 400 m, a few at about 1,000 m (Liu et al., 1981). These are hill-dwelling populations, few in number, and genetically all *O. h. hupensis.*

IV: Yunnan and Sichuan Provinces. The subspecies is *Oncomelania hupensis robertsoni.* The environment is in the hills and low mountains above the Three Gorges of the Yangtze. Populations are not affected by flooding and are presumed stable with a normal life expectancy. The contribution of horses, goats and cattle on maintaining infections varies from place to place but presumably does not reach the magnitude found in Poyang Lake. As the snails are highly divergent genetically from *O. hupensis hupensis,* presumably *S. japonicum* is likewise genetically divergent above and below the Three Gorges.

We do not place *O. hupensis tangi* in the discussion of modes of transmission because

this subspecies is now irrelevant to this issue. The subspecies, restricted to the coastal areas of Fujian Province, has been brought to the edge of extinction, with only one population maintained and guarded to save an example of this taxon.

Because *S. japonicum* has coevolved with its snail hosts (Davis, 1980, 1992), a number of questions arise. Has severe natural selection in Poyang Lake coupled with schistosome zoonosis in cattle modified *S. japonicum*'s genetics, pathogenicity and reactivity to drugs? Do flood-deposited aggregates of snails, demonstrably diverse genetically, host similarly genetically diverse aggregates of schistosomes? As *robertsoni* and *hupensis* are so diverse genetically, the schistosomes they transmit must also be diverse genetically. Is this true? A number of factors impact on this question. Much depends on the breeding structure of *S. japonicum* in wild populations. Are the worms normally outbreeding, or are they parthenogenetic? Are genetic factors involved with coevolution reflected in changes in structural genes or mitochondrial genes? Can one differentiate schistosomes from stable and unstable populations using standard genetic markers currently available?

These questions are under investigation within the Shanghai TMRC.

Implication of the Three Gorges Dam: The construction of the Three Gorges Dam across the mainstream of the Yangtze River constitutes man's largest alteration of the environment to date. The river is the third largest in the world, some 3,900 miles long. More than 400 million people live in the Yangtze River drainage system. The superdam, some 185 m high and 2.15 km wide, will back up a 600 km-long reservoir (Fig. 12). The huge reservoir will have an impact on the global environment as well as all of China. The Three Gorges is 250 km long, a churning caldron hemmed in by tall cliffs. Today, the cliffs and a barrier of mountains isolates Yunnan and Sichuan provinces from the rest of southern China to the east. These barriers have effectively kept the biodiversity of two vast regions separated; for example, *O. hupensis robertsoni* from *O. h. hupensis.*

With the advent of the dam, the immense reservoir, and climate changes, we make a number of predictions. Because of the locks and lateral canal systems enabling boats to traverse the dam and travel the reservoir, there will be movement of snails and parasites in both directions across this previously unassailable barrier. Snails and parasites will colonize suitable sections of the immense reservoir area. The considerable increase in the height of the water level behind the dam will cause significant backup of rivers that now flow into the future reservoir basin, creating new marshlands and bridges to *O. h. robertsoni* and *Tricula* habitats.

The vast changes in ecology will promote emerging diseases of a variety of types, snail-borne diseases and schistosomiasis in particular, among them. The stabilization of the Yangtze River, with the elimination of the great annual flooding, at a slightly higher level than today will mean stabilization of vast habitat areas for snails and considerably increased marshland habitats for snails. Poyang Lake should undergo a transformation with considerable land, formerly flooded annually, left above water all year. This means that prime snail habitat will be free from flooding all year. Should this materialize, there will be profound changes in selective pressure on snails in Poyang Lake and along the Yangtze River including its islands. There should be a massive shift from unstable aggregates of snails to stable population structure with concomitant shifts in parasite genetics. Snail population density should increase considerably.

The best way to monitor and track such sweeping changes is to combine remote sensing (RS) via satellite images and geographic information systems (GIS). The Shanghai TMRC has undertaken such studies with a special focus on the dynamics of Poyang Lake. Figure 13 shows a GIS-facilitated map of Poyang Lake during full flood and with TMRC epidemiological study sites marked. We are currently benchmarking selected sites for water levels: the lowest and highest water levels and the dates in relationship to rainfall patterns, temperature, humidity and incident radiation, as well as ecological factors supporting snail populations and schistosome infections.

We have placed special attention on four administrative villages where the TMRC is conducting the EPI study to assess the significance of water buffalo in the transmission of *S. japonicum.* Figure 14 is an enlargement of that section of Figure 13 showing the locations of the epidemiological study sites. Figure 15 is an overlay of a Landsat image on the study sites, showing the lake at lowest water in March. About 90% of the water during peak flood empties out leaving exposed countless

FIG. 14. GIS-facilitated map of an enlargement of that section of Poyang Lake of concern to the TMRC epidemiological study.

islands, floodplains suitable for cattle grazing, and vast mud and sand flats. Flooding covers all but the highest islands in the lake, and thus man and cattle are excluded from the lake basin. During low water, there is maximum use of the emergent grasslands for cattle grazing and availability to man. The RS image shows the extensive expanses of uncovered flood planes, marshes and mud-sand flats.

The focus of the TMRC epidemiological study is on the interaction between infected water buffalo and snail host populations throughout the buffalo's grazing range. GIS studies require a long-term view, because these grazing range environments are not static, and annual fluctuations may be considerable. On top of annual cycles, the Three Gorges Dam will cause permanent dramatic shifts in numbers of environmental variables. An example of annual dramatic environmental changes is the early spring flood in 1998 that devastated southern China, the worst flood of
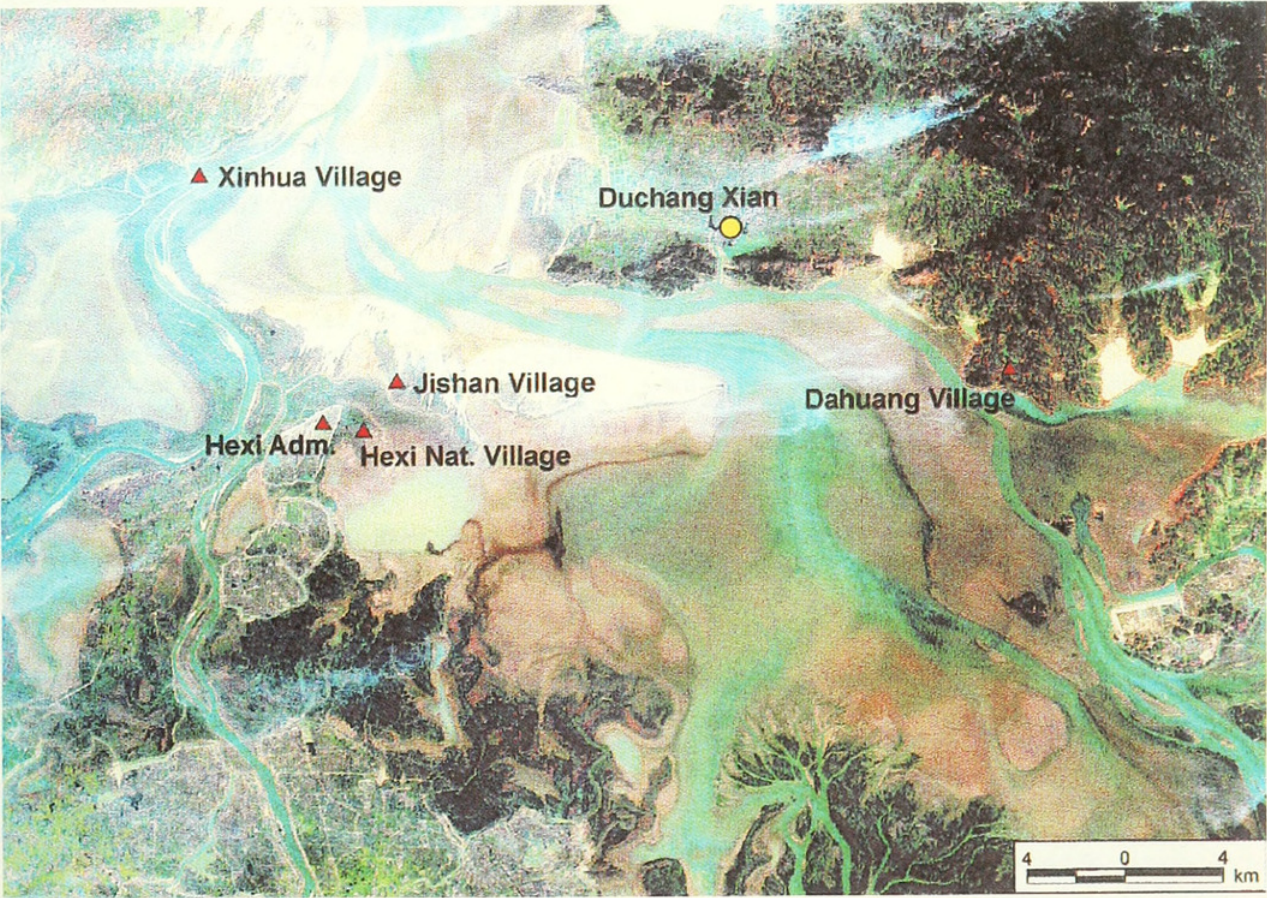
FIG. 15. Landsat image of the epidemiological sites shown in Fig. 14. The image was taken during lowest water in March (courtesy of NASA). During low water only 10% of the water remains from full flood leaving exposed islands and vast flood planes as well as mud and sand flats.

some 40 to 50 years. In our EPI study, one administrative village's grazing land was dramatically changed. One section was swept clean of snails, and no living snails were found there in the fall of 1998 or the spring of 1999; in another section, an area that had no snails recorded in previous years was found to have snail populations; none were infected. This grazing land was the lowest of our study areas in altitude above mean low water, and was deeply impacted not only by the flooding but by the severe cold temperatures affecting reproduction. The factors of temperature, time and duration of rainfall and flooding had a negative impact on snail survival and reproduction. Increased depth of water brought on by the Three Gorges Dam will have a dramatic impact on this particular site; we predict that snails will not survive there.

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