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REVIEW

Human and Animal Fascioliasis: Origins and Worldwide Evolving Scenario

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SUMMARY Fascioliasis is a plant- and waterborne zoonotic parasitic disease caused by two trematode species: (i) *Fasciola hepatica* in Europe, Asia, Africa, the Americas, and Oceania and (ii) *F. gigantica*, which is restricted to Africa and Asia. Fasciolid liver flukes infect mainly

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herbivores as ruminants, equids, and camelids but also omnivore mammals as humans and swine and are transmitted by freshwater Lymnaeidae snail vectors. Two phases may be distinguished in fasciolid evolution. The long predomestication period includes the *F. gigantica* origin in east-southern Africa around the mid-Miocene, the *F. hepatica* origin in the Near-Middle East of Asia around the latest Miocene to Early Pliocene, and their subsequent local spread. The short postdomestication period includes the worldwide spread by human-guided movements of animals in the last 12,000 years and the more recent transoceanic anthropogenic introductions of *F. hepatica* into the Americas and Oceania and of *F. gigantica* into several large islands of the Pacific with ships transporting livestock in the last 500 years. The routes and chronology of the spreading waves followed by both fasciolids into the five continents are redefined on the basis of recently generated knowledge of human-guided movements of domesticated hosts. No local, zonal, or regional situation showing disagreement with historical records was found, although in a few world zones the available knowledge is still insufficient. The anthropogenically accelerated evolution of fasciolids allows us to call them "peridomestic endoparasites." The multidisciplinary implications for crucial aspects of the disease should therefore lead the present baseline update to be taken into account in future research studies.

KEYWORDS human and animal fascioliasis, initial steps and historical landmarks, *Fasciola hepatica* and *F. gigantica*, hybrid intermediate fasciolids, lymnaeid snail vectors, mammal reservoirs, livestock, domestic ruminants, equids, camelids, swine, paleobiogeographical origins of fasciolids, evolution in the predomestication period, *Fasciola* spread in the postdomestication period, mankind history, fasciolid evolution, the Near East cradle, human-guided movements of domesticated hosts, *F. gigantica* spread in Africa and Asia, *F. hepatica* spread in Europe, Asia, Africa, the Americas and Oceania, geographical distribution, livestock export/import, multidisciplinary repercussions, new research baseline, fasciolid origin and spread, human and animal movements, pre- and postdomestication periods, worldwide distribution

INTRODUCTION

Fascioliasis is a freshwater snail-borne, zoonotic, parasitic disease caused by the trematode species *Fasciola hepatica* and *F. gigantica*, as well as by genetically hybrid forms showing phenotypically intermediate characteristics (1). The adult stage of these fasciolid flukes infects the biliary canals and gallbladder of the liver of many species of herbivore mammals, as well as omnivores such as humans and pigs (Fig. 1) (2). Sylvatic ruminants are the original hosts and domestic ruminants are the main hosts, ensuring the presence of these helminths almost everywhere. Livestock, mainly sheep and cattle but also goats, equines, and camelids, are the species having contributed to the worldwide spread of this disease due to human-guided movements of these domestic animals from the beginning of the postdomestication period, i.e., in the past, recent times, and in the present throughout the period from 10,000 to 8,000 years BC (1).

Fasciolid flukes follow a two-host life cycle (Fig. 2). They are transmitted by snails of the family Lymnaeidae (Fig. 3), a very wide group of very old gastropods, including species on all continents excepting the two poles (3). Although there are fasciolid species/lymnaeid species specificity restrictions and not all lymnaeid species are able to transmit a fasciolid, the original existence of susceptible lymnaeid species in Europe, Africa, Asia, the Americas, and Oceania allowed for the worldwide spread of the disease. Passive transport of several lymnaeid species in mud attached to the hooves of animals, but also with plant exchange commerce, without excluding additional potential passive transport by birds, underlay the spread of lymnaeid intermediate hosts, transmitters, or vectors from one continent to another, which additionally facilitated the spread of this disease (1, 4). This is why fascioliasis has become the only trematodiasis of cosmopolitan distribution, whereas all other trematodiases show smaller defined geographical distributions linked to the restricted distributions of their specific snail vector species.

This worldwide distribution adds to an emerging picture in the last 3 decades. Several areas of human fascioliasis endemicity have been described in many countries, and the

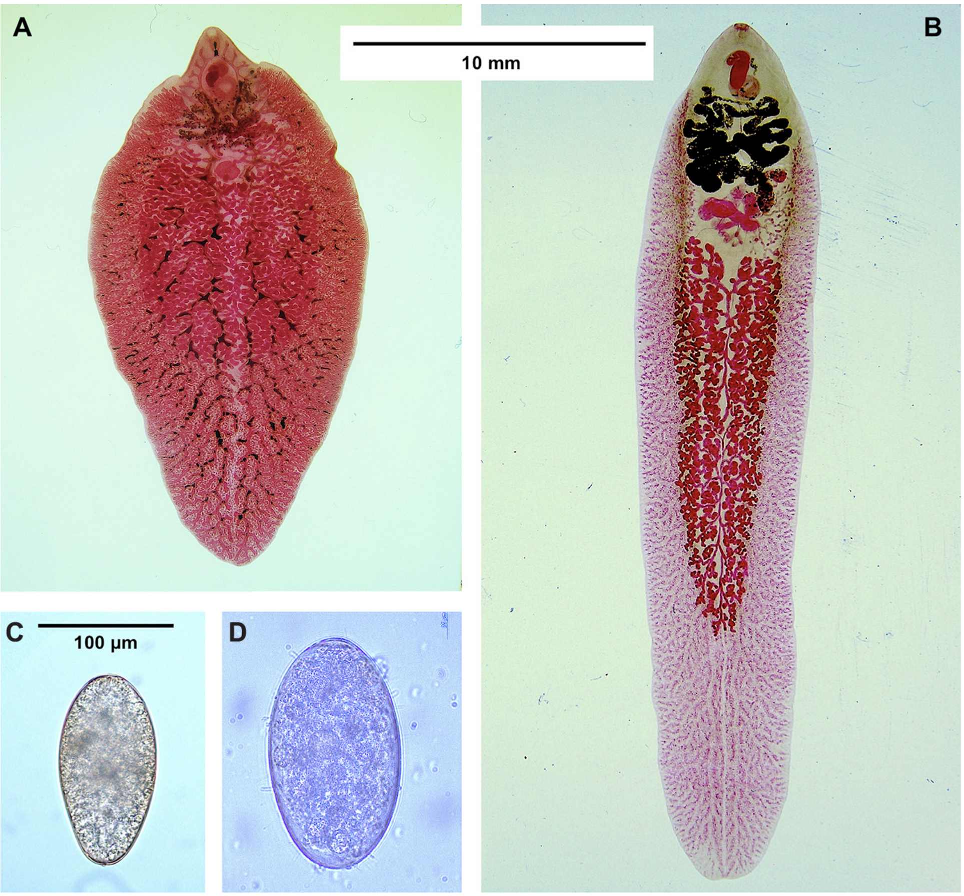

FIG 1 Adults and eggs of fasciolid species. (A) Adult stage of *Fasciola hepatica* in ventral view. (B) Adult stage of *F. gigantica* in ventral view. (C) Egg of *F. hepatica*. (D) Egg of *F. gigantica*. Note the pronounced shoulders and the nonparallel lateral body borders in panel A but the absence of shoulders and almost parallel lateral body borders in panel B. (A and B) At the same scale; (C and D) At the same scale.

number of case reports has been progressively increasing in the five continents (5). The high pathogenicity of fascioliasis in humans (6–8), the virulence of fasciolid excretory products (9), and the immunosuppression in both disease periods—the acute phase (10) and the chronic phase (11, 12)—present a worrying scenario. Indeed, long-term post-treatment sequelae have been described in developed countries (13), and even mortality has been reported in areas of hyperendemicity in mainly low-income countries (14), as well as in developed countries (15). The immunosuppression liver flukes induce (i) underlies usual coinfections with other pathogenic protozoans and helminths, leading to high morbidity in depauperated rural areas of developing countries (16, 17), (ii) causes public health problems affecting children, and (iii) underlies community underdevelopment. In addition, resistance to triclabendazole, the drug of choice for the treatment of fascioliasis, has recently begun to be reported in areas of human endemicity (i.e., in areas where the disease is endemic for humans), which poses a great problem due to the lack of efficient alternative drugs (18).

As a result, the World Health Organization (WHO) has included fascioliasis among the group of Foodborne Trematodiases in the priority list of Neglected Tropical Diseases (NTDs) in its WHO NTD Roadmaps for 2020 and 2030 (19, 20). The WHO has also very recently emphasized the convenience of achieving the roadmap targets noted for these trematodiases through a cross-cutting One Health approach (21).

The pronounced complexity of fascioliasis, the great number of biotic and abiotic factors involved in the circulation of the liver flukes, the diversity of transmission patterns, and the heterogeneity of epidemiological situations, with one or another *Fasciola* species

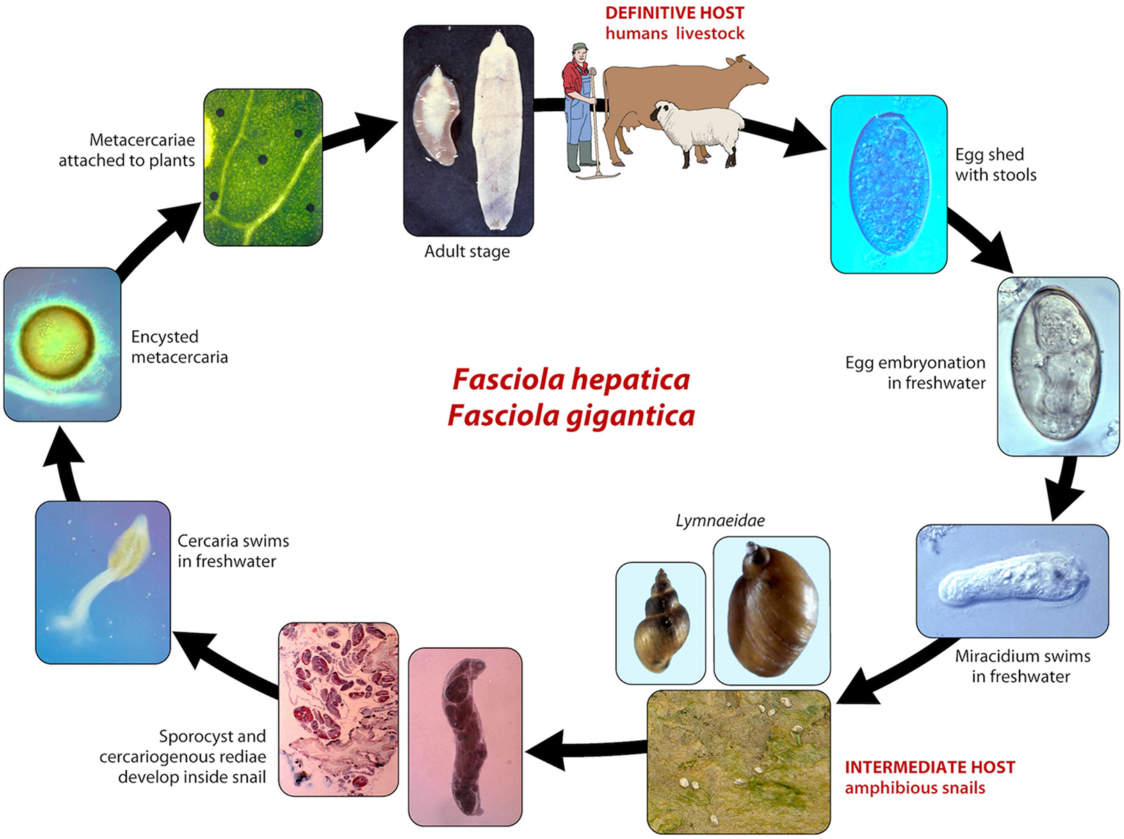

FIG 2 Life cycle of the fasciolid species *Fasciola hepatica* and *F. gigantica* following a similar transmission pattern.

and potential hybrid intermediate forms in local and/or zonal overlap situations, together with one or more lymnaeid vector species with different behavior, seasonality, and ecological requirements, and different domestic and wild mammal species playing the role of reservoirs, hinder the correct interpretation of results obtained in local studies of areas of endemicity. To sidestep such difficulties, the usual recourse to explain the findings is comparison with situations in other countries, which in turn may lead to the risk of increasing the misunderstandings when a comparison is made to another area where fasciolids, lymnaeids, reservoirs, factors, and characteristics of human communities vary.

To facilitate the interpretation of the results of local studies and countrywide assessments, a first worldwide baseline, including multidisciplinary analyses of fasciolid flukes and lymnaeid vectors, together with description of the appropriate analytical tools, was provided in 2009 (1). This baseline has proved to be very helpful and has been used in very numerous research studies in all continents. During the time elapsed since then, new knowledge has been obtained in crucial multidisciplinary aspects. Among them, genetic, molecular, archeological, paleontological, paleoclimatic, linguistic, and historical studies have furnished impressive contributions about the historical movements of humanity throughout the world. The appropriate consideration of all this new knowledge allows us now to make a significant step forward from the point of view of fasciolid flukes, lymnaeid vectors, mammal reservoirs, and fascioliasis.

This review highlights *Fasciola* and fascioliasis in the history of parasitology and neglected diseases, reanalyzes the paleobiogeographical origins of both *Fasciola hepatica* and *F. gigantica* and respective repercussions in the predomestication period, and provides a detailed description of the spread and evolving scenario of fasciolids and lymnaeid vectors throughout all continents along the postdomestication period since today. This new evolving scenario should be the baseline on which to carry out multidisciplinary research on this disease in the future.

FIG 3 Lymnaeid snails are characterized by their screwed shell with whorls decreasing in width toward a pointed or obtuse apex, dextral (right-handed) aperture, absence of operculum, and small eyes located at the base of distinctive triangular tentacles.

FASCIOLA SPP. IN THE HISTORY OF PARASITOLOGY AND NEGLECTED DISEASES

Present multidisciplinary knowledge indicates that *F. hepatica* and *F. gigantica* started the large widening of their geographical distributions as a consequence of the early domestication of livestock species occurred around 12,000 to 10,000 years ago at the dawn of the Neolithic period, mainly in the Fertile Crescent, in the Near and Middle East. By adapting to early domesticated animals, *F. hepatica* was able to initially colonize almost the whole Old World by spreading westward into Europe, eastward into Asia and southward into Africa. It subsequently also spread into the New World with livestock transocean transport in old ships during the last five colonization centuries (1). Regarding Oceania, the introduction of *F. hepatica* into Australia occurred in the mid-XIX century (22) and into Tasmania only a few years later (23). In that way, *F. hepatica* was able to reach the present worldwide distribution of the disease (1).

Findings of *F. hepatica* eggs in coprolites in archeological remains of different old periods from 3,900 years BC in Europe are numerous (24). *Fasciola* infection should have been frequent in Europeans during the Roman period and the Middle Ages (25). Interestingly, some plants in the herbal folk-medicine culture of the ancient Etruscans, a distinctive group who dominated central Italy from the Iron Age (ca. 1000 BC) to the later first millennium BC and whose divination priests were experts in "reading" the livers of sheep to decide where settlements should be founded, would have been efficient against *F. hepatica* infection (26). Eggs of this fasciolid have recently also been found in paleofaeces of a donkey, probably the present-day endangered Persian onager *Equus hemionus onager*, in northwestern Iran, in biological remains dating back to the Sassanid period, 224 to 651 AD (27), which represents the first archeological finding of *Fasciola* in Asia and the Near East (28).

In its turn, *F. gigantica* spread thanks to the domestication of herbivore ruminants throughout Africa and Asia from its origin in the East African region up to give rise to its present restricted distribution on these two continents (1). In Egypt, where only *F. gigantica* was present in old times (29, 30), the effects of the infection by this fasciolid

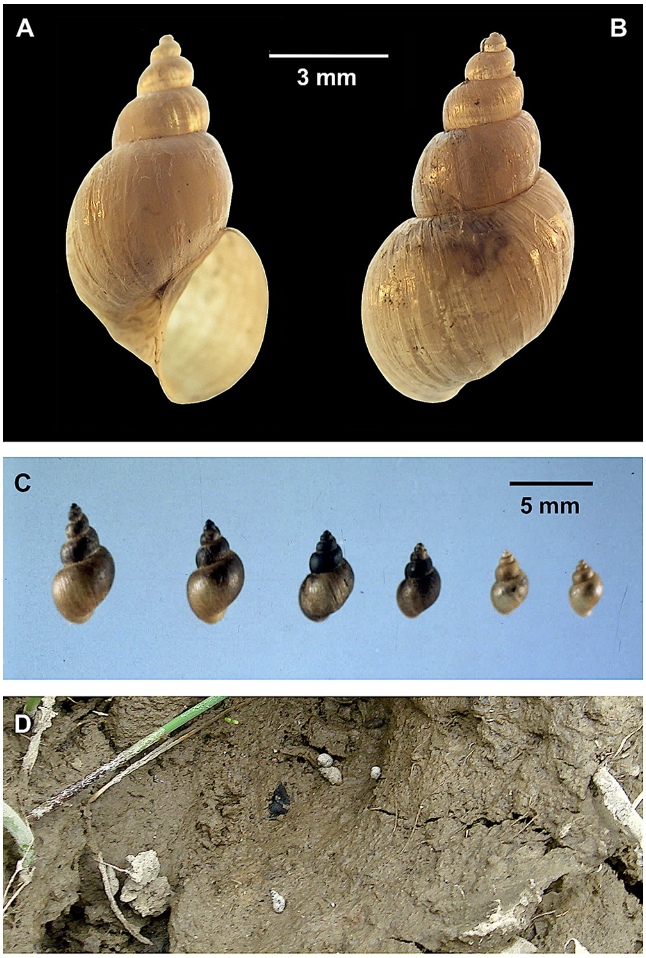

FIG 4 *Galba truncatula*, main vector of *Fasciola hepatica*, is a small lymnaeid species belonging to the *Galba/Fossaria* group. (A) Ventral view. (B) Dorsal view. (C) Shell growth. (D) Specimens on mud outside water.

in livestock appears illustrated in the graves of Old Egypt, and remains of the parasite itself were found in histological sections of the liver of a mummy (31). Whereas the existence of amphibious susceptible lymnaeid species of the *Galba/Fossaria* group played a crucial role in facilitating the cosmopolitan spread of *F. hepatica* (Fig. 4), (i) the distribution of the *F. gigantica*-specific lymnaeid vectors of the *Radix* group limited to the Old World and (ii) the less amphibious characteristics of radicine lymnaeids not appropriate for long distance passive transport on mud attached to animal hooves (Fig. 5) appear to be the two factors explaining why *F. gigantica* never succeeded in colonizing continents outside the Old World via a maritime route such as the Americas and Oceania (32).

The wide distribution of the disease, worldwide with *F. hepatica*, additionally with *F. gigantica* in Africa and Asia, together with the relatively big size of the fasciolid adult stage easily visible by naked eye when in the livers of sacrificed animals, as well as its impact on livestock husbandry (33) known in the veterinary field since very long ago, underlies the important role played by *Fasciola* and fascioliasis in the old history of parasitology and helminthology (34, 35).

FIRST CITATIONS

The first reference to the detection of *F. hepatica* in an animal corresponds to Jehan de Brie, whose original manuscript about sheep husbandry by shepherds in Ile-de-France, France, in 1379 (36), written after request of the King of France Charles V le Sage because of the importance of this practice at that time, has been lost but was reprinted later in Paris in

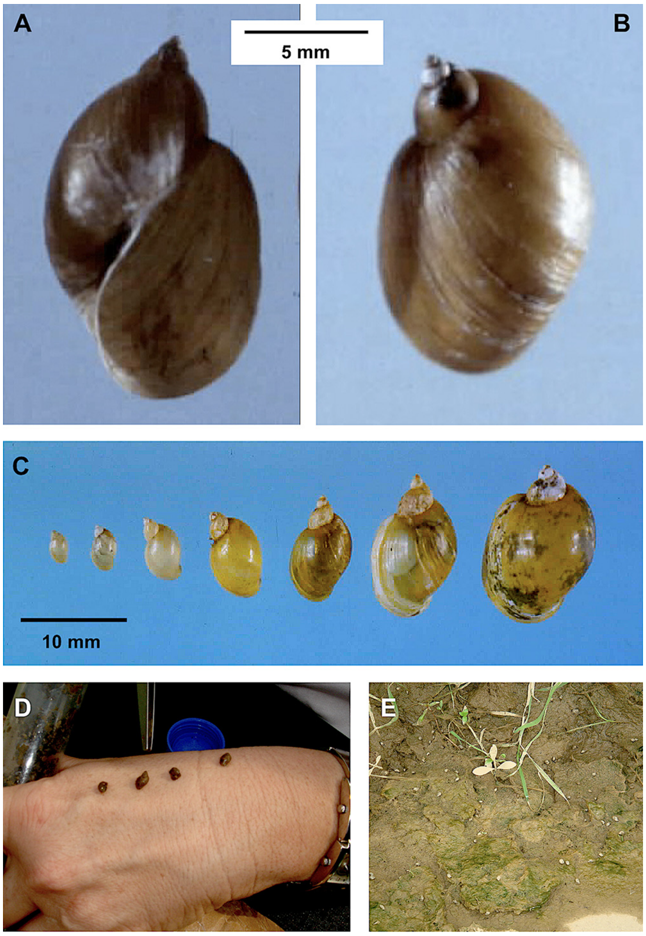

FIG 5 Lymnaeid species of the *Radix* group transmit *Fasciola gigantica*. (A and B) Large *R. natalensis* from Africa on ventral and dorsal views. (C) Shell growth in the large-sized species *R. auricularia* from eastern Europe. (D) small-sized *R. viridis* from Vietnam. (E) Vietnamese *R. viridis* specimens on mud outside of water (radicine vectors are less amphibious than fossarines, but *R. viridis* is a remarkable exception).

1514 (36). Not only De Brie was the first to describe fascioliasis in sheep, including infection of the liver which led to sheep death, but he also suggested that the disease was acquired by consumption of a bad grass, he called "dauve," growing on swampy ground. The present-day French term "douve du foie" indeed derives of the term "dauve" used by De Brie.

Two centuries were needed to find the subsequent reference to *F. hepatica* infection in the liver of sheep by Hieronymus Gabucinus in 1547 (37), who described the worm as having the form of a "squash seed." Shortly thereafter, the first epizootic by this fasciolid in sheep was reported to have occurred in 1552 in The Netherlands and published by Cornelius Gemma in 1575 (38), who also suggested that in such a kind of plague the animals were the front-runners or harbingers of such a disease in humans. Another century later, cattle were also observed to be infected in another epizootic event that happened from 1663 to 1665 in the duchy of Cobourg, as reported by Joh Frommann in 1676 (39). Two first simple drawings in dorsal and central views of the liver fluke found in sheep, clearly showing the typical anterior shoulders, were provided in a "Letter by Mr. Pecquet" in 1668 (40), which were subsequently reproduced by Nicolas Andry in 1774 (41) in the first volume of his book on the generation of worms in the human body.

The first observation of *F. hepatica* in the biliary canals of a human was made by Pierre Borel and cited is on page 282 of a book by Clericus Daniel Le Clerc in 1715 (42), although it was up to Pallas in 1760 (43) to provide the first report on infection of the common bile duct by *F. hepatica* in a woman in Berlin.

Whereas *F. hepatica* was named by Linnaeus in his *Systema Naturae* in 1758 (44), *F. gigantica* was described one century later from a 9-day-dead giraffe, *Giraffa camelopardalis*, in the Anatomical Museum at the University of Edinburgh, Edinburgh, United Kingdom, by Cobbold in 1855 (45). The giraffe specimen dissected by Cobbold came from the traveling animal collection known as George Wombwell's Travelling Menagerie, where the giraffe was kept for less than 8 months (46). The elongated and slender varieties *F. hepatica* var. *angusta* described for fasciolids infecting bile ducts of oxen in Senegal (47) and *F. hepatica* var. *aegyptica* reported from the livers of buffaloes, cattle, sheep, and goats in Egypt (48) were both later synonymized with *F. gigantica* (49).

The first report of *F. gigantica* in a human concerned a 25-mm-long fluke expectorated during an attack of coughing associated with hemoptysis by a French naval officer in Rio de Janeiro, Brazil, and was published by Hilario de Gouvea in 1895. This patient had made a stay of several weeks in Dakar, Senegal, during July of the same year, and the ectopic parasite was highlighted to resemble the liver flukes infecting buffaloes in Egypt (50). Many years later, the infection of a Uzbekistani child in Tashkent was reported to be caused by *F. gigantica* only based on the detection of fasciolid eggs in the stools (51). Simultaneously, in April 1927, the infection of a 44-year-old male patient in Hanoi, Vietnam, and who was living in Indochina since 1907 was also reported to be caused by *F. gigantica* by considering the length of 170 to 180 μm of the fasciolid eggs found both in the stools and in the gallbladder (52).

INITIAL STEPS AND HISTORICAL LANDMARKS

Fasciola hepatica was the first trematode whose life cycle was elucidated. These old experiments constitute a landmark in parasitology, because these studies served as progenitors of all modern life cycle studies (53). The life cycle of the liver fluke was simultaneously discovered by Leuckart in 1881 (54, 55) and Thomas in the same and two subsequent years (56–58) in independent studies. Although both authors were able to show the complete development of the successive larval stages, none of these two authors demonstrated the development of the adult stage from the metacercarial stage. This last step of the life cycle was described a few years later by Lutz in 1892 and 1893 (59, 60), although not in *F. hepatica* but in *F. gigantica* of Hawaii. The invasive stages of *F. hepatica* in mammals were the subject of two famous articles published in the early beginnings of the *Advances in Parasitology* by Dawes and Hughes (61, 62). The life cycle of *F. hepatica* has been more recently reviewed in detail (63), although there are still question marks on given phases of the development of the liver fluke to be further clarified (64).

The veterinary importance of liver fluke affection in livestock and its worldwide distribution led to an increasing number of studies and a very wide literature on *Fasciola* infection in domestic herbivore animals, mainly sheep and cattle, during the whole 1900s century. Concerning fascioliasis in humans, reports of individual cases, case series, and even the description of outbreaks, mainly familiar but sometimes also on epidemics caused by rainfall increases, were progressively published during the first half of this century and up to the 1980 decade. This long period was therefore dominated by very numerous clinical studies, above all in western Europe, including France as the country with a higher number of reports, usually related to the tradition of eating watercress, and secondarily Russia, northern African countries, and Latin America. Argentina, Chile, Venezuela, and Peru in South America, Cuba in the Caribbean, and Mexico in Central-North America reported most of the cases in the New World. Interestingly, countries that acquired public health notoriety for fascioliasis during the whole first middle of the past century due to the several human cases published, such as Russia and Algeria (65, 66), almost disappeared from such a priority list due to a decrease in reporting presumably linked to national governmental changes (67). In other countries, such as Argentina, the lack of awareness of physicians about this disease during several decades of the 1900s frequently led to pronouncedly delayed diagnostics when unexpectedly finding the liver fluke in surgery interventions after lithiasis suspicion (68).

Among the studies which represented important steps ahead in crucial aspects of fascioliasis during this 1900s period, the following may be counted: studies by Pedro Kouri about diagnostics, but above all on the treatment with emetine in Cuba (69); studies by Joseph

E. Alicata on the transmission of *F. gigantica* linked to human infection in Hawaii (70); Bengt Hubendick's immense malacological review of all lymnaeid species of the world furnishing the baseline for subsequent studies on fascioliasis snail vectors (3); the contributions of the British C. B. Ollerenshaw on the relationships of fascioliasis transmission with the meteorological factors and the way to apply them for forecasting fascioliasis (71–73), which became the baseline for the present research field of environmental studies on climate-based risk by this disease by means of mathematical modeling, remote sensing assessments, and geographical information systems (GIS) analyses; the contributions on veterinary control, including a wide work in treatment and drugs, as well as the epidemiological studies on livestock fascioliasis, including the role of lymnaeid snails, by Joseph C. Boray in Australia (74), an author unanimously recognized by other specialists on the liver fluke and who really merits a chapter for himself in the history of fascioliasis research (see the "In Memoriam" dedication in reference 18); and a usually overlooked article describing the histopathology of this disease in 16 patients by the Uruguayans Acosta-Ferreira, Vercelli-Retta, and Falconi (75) that furnished the base knowledge on the pathology caused by the liver fluke infection in humans.

RECENT AND PRESENT IN HUMAN FASCIOLIASIS

The 1990s represents a decisive decade for human fascioliasis. Kenneth E. Mott, Chief of the "Schistosomiasis and Other Trematode Infections Unit" of the at-that-time running "Parasitic Disease Program" at the headquarters of the World Health Organization (WHO) in Geneva (76), realized the increasing importance of human fascioliasis due to the reports of human cases arriving at WHO from different countries, at the end of the 1980s and in the early 1990s. Under his auspices, a first initiative was launched on the French Mediterranean island of Corsica, where field and experimental results demonstrated the use of the black rat *Rattus rattus* as a sylvatic reservoir host underlying an ecological niche widening that allowed researchers to observe and explain human infection in nontypical habitats (77, 78). These results suggested a higher capacity of adaptation of the liver fluke which did not fit well with the knowledge available at that point, but the findings motivated Mott to request action on the Northern Bolivian Altiplano. Internal reports from Bolivia to the WHO were referring to human infection in that part of the Andean Altiplano, between Lake Titicaca and the city of La Paz. These reports concerned an area at the very high altitude of 3,800 to 4,000 m above sea level (m a.s.l.) that was posing a question, as indeed it was mentioned that lymnaeid populations living at European altitudes of 1,500 to 2,000 m a.s.l. were not susceptible to liver fluke infection. Several years of work, started on 1992, demonstrated this to be an area of human hyperendemicity with the highest prevalences and intensities ever reported (79, 80).

In between the aforementioned two initiatives, K. E. Mott, together with M. G. Chen, reviewed the different aspects of human fascioliasis, including around 2,500 reports published worldwide between 1970 and 1990 (6). This review furnished a multidisciplinary update of the disease in humans; the review sections on clinics, on pathology, and on symptomatology should be highlighted. The review furnished an extremely useful baseline at the beginning of the 1990s and showed how delayed the knowledge on human fascioliasis was compared to that on schistosomiasis, a fact which was evidently due to the secondary focus it always received compared to livestock fascioliasis of well-known veterinary impact. At the end of this decade, two articles further helped from the epidemiological and treatment-control points of view, namely, (i) the first classification of epidemiological scenarios of human fascioliasis, which allowed the countries to define their disease situations (81), and (ii) the agreement of WHO with Novartis Pharma AG (Basel, Switzerland) for the donation of Egaten (triclabendazole for human use), which was subsequently approved not only for treatments but also mainly for control through national initiatives of preventive chemotherapy in countries of endemicity (82).

During the period from 1990 to the present, several teams followed long-term research lines that have significantly increased our knowledge of crucial aspects of this disease and whose results have proved to be highly useful for human fascioliasis. The French researcher Daniel Rondelaud focused on different lymnaeid vector species and their role in the transmission of mainly *F. hepatica* but also *F. gigantica*, including deep studies on *Galba truncatula*, the main vector species of *F. hepatica* (83). The Irish school maintained its tradition of focusing on fascioliasis, with Ian Fairweather as the main author in the field of fasciolicide drugs (18, 84). Substantial knowledge was also gained throughout the research studies by John P. Dalton and his team on serological diagnosis, on the role of cathepsines in liver fluke infection, and on the immune response induced by infection of the liver fluke in animals (10), as well as the on physiological systems underlying pathogenicity and virulence (85).

Decisive steps were undertaken by WHO Headquarters Geneva from 2000 to 2010 following receipt of sufficient evidence clearly showing that overcoming neglected tropical diseases makes economic and development sense. The WHO Department of Control of Neglected Tropical Diseases (NTDs), led by Lorenzo Savioli, with the advice of a few selected worldwide experts composing the Strategic and Technical Advisory Group on NTDs (WHO-STAG), initiated a paradigm shift to control selected priority neglected tropical diseases through a new approach using integrated interventions, mainly by large-scale campaigns using a few, safe, highly efficient drugs. Many generous donations enabled the WHO to mobilize further resources and scale up implementation activities. Bold steps were taken to develop a framework to tackle these diseases, among which human fascioliasis was the only worldwide-distributed disease included in the group of Foodborne Trematodiases, together with clonorchiasis, opisthorchiasis, and paragonimiasis. Triclabendazole for fascioliasis and praziquantel for the other three foodborne trematodiases were the drugs on which to rely for this disease group regarding treatments and preventive chemotherapy.

Within this framework, human fascioliasis was the objective of an expert meeting in 2006 in which the decision was taken to implement a worldwide initiative, including two axes (86).

The first axis concerned interventions for the use of triclabendazole in areas of human endemicity. The initial steps included different strategies according to four selected countries of human endemicity. In Vietnam, infected subjects were treated in hospitals by passive detection after appropriate radiophonic diffusion. In Egypt, subjects were treated after active detection by qualitative coprological diagnosis. In Bolivia and Peru, a long-term preventive chemotherapy strategy by means of annual mass drug administration with a single 10-mg/kg triclabendazole treatment to decrease morbidity, mainly in schoolchildren as the most affected age group, was launched after the successful results on the absence of secondary effects obtained in a pilot study (87). Many other countries presenting areas of endemicity subsequently also benefitted from this Egaten donation.

The second axis concerned the worldwide assessment of the scenarios of human fascioliasis throughout, although preferentially in low-income countries, including field surveys on the transmission and epidemiology in different areas, but also experimental studies on applied multidisciplinary aspects.

The designation of an official WHO Collaboration Centre on Fascioliasis and its Snail Vectors (WHO CC SPA-37 and later SPA-53) to work monographically on human fascioliasis in direct link with WHO Headquarters Geneva proved to be key for the implementation of the aforementioned worldwide initiative. The subsequent designation of the same expert team of Valencia, Spain, as international Reference Centre of FAO/United Nations on zoonotic parasitic diseases, mainly on livestock helminthiases, directly linked to FAO Headquarters Rome, further facilitated the tasks on the zoonotic aspects of the disease in areas of human endemicity. It should be noted that keeping a long-term research line on human fascioliasis is very difficult, because of the need for (i) multidisciplinary approaches and consequent very different methods, techniques, and respective infrastructures; (ii) field work to cover all continents, because of the high heterogeneity of this disease in the different areas of endemicity lead to the need of personal work *in situ* (i.e., a cow behaves similarly everywhere, but humans do not); (iii) laboratory studies needing for the experimental, highly complex maintaining of many lymnaeid vector species, plus ruminants in given cases; and (iv) availability of continuous high-level funding to cover the corresponding costs of all these aspects plus the expert personal team for their management. This explains why publications on this human disease by other author teams do not appear repeatedly and throughout a long period when looking at the literature.

An additional step forward was launched by WHO at another meeting held in Lao PDR in 2009 (88), in which appropriate rapid-impact intervention packages, community diagnosis, and monitoring were evaluated for public health prevention, with emphasis on control/elimination tools to be improved. In that sense, the usefulness of a new coproantigen test for human fascioliasis monitoring was evaluated in areas of endemicity in Bolivia and Peru with successful results (89). International focus on human fascioliasis was further maintained thanks to its inclusion in the priority list of NTDs tackled by the WHO Roadmap 2015 to 2020 (19) and subsequent Roadmap 2030 (20).

Among the major milestones on human fascioliasis reached in the aforementioned WHO initiative during the last 2 decades, the following may be highlighted because of their wide repercussions, in chronological order.

In 2001, DNA marker sequencing demonstrated its usefulness for lymnaeid species classification (90). Malacological methods traditionally used for the classification of lymnaeid snail species proved to be insufficient, mainly in the groups of species transmitting fasciolids in which shell and anatomy similarities may lead to confusion and misclassifications (4). This opened a broad research field to reassess previous species classifications and fasciolid species/lymnaeid species specificity aspects.

In 2005, the overall situation of human fascioliasis and the factors underlying its emergence/re-emergence were analyzed, with emphasis on environmental changes, the capacity of the liver flukes to colonize and adapt to new hosts and environments, the geographical expansion of given efficient vector species, and its adaptation to other lymnaeid species autochthonous in the newly colonized areas (91). This analysis of the emergence/re-emergence of human fascioliasis proved to have a wide impact, according to the citation number, very high for a neglected among the neglected diseases as human fascioliasis (92).

Also in 2005, in another article of wide diffusion, fascioliasis was also highlighted to be the vector-borne disease presenting the widest latitudinal, longitudinal, and altitudinal distribution known, and the lack of correlation between human and animal fascioliasis was emphasized for the first time (93). A newly proposed classification of transmission patterns offered a new baseline for the assessment of the characteristics of areas of human endemicity, by referring to the fact that thus far well-known patterns of fascioliasis may not always explain the transmission characteristics in a given area and control measures should consider the results of eco-epidemiological studies undertaken in the zones concerned (93).

In 2009, a new baseline for the human disease with many new concepts, plenty of new data, results and proposals, and even new terminology, was published, highlighting that human fascioliasis is very different from a simple extrapolation from fascioliasis in livestock as traditionally considered before (1). The marked heterogeneity of human fascioliasis was illustrated by the different epidemiological situations and transmission patterns in the different areas of endemicity. The paleobiogeographical origins of *F. hepatica* and *F. gigantica* were defined, and their geographical spreads in both livestock predomestication times and the postdomestication period were analyzed. Paleontological, archeological, and historical records, together with genetic data on recent dispersal of livestock species, were taken into account to establish an evolutionary frame for the two fasciolids in all continents. Genotyping tools as the complete sequences of the nuclear ribosomal DNA (rDNA) markers ITS-2 and ITS-1 and mitochondrial DNA (mtDNA) gene markers *cox1* and *nad1*, as well as phenotyping tools by morphometric analyses of adult-stage parameters, were defined in detail, and their variability characteristics in *F. hepatica* and *F. gigantica* were established. A standardized methodology for fasciolids and lymnaeids was proposed for future contributions to have significant value and comparable characteristics (1). Baseline concepts and tools provided within this new worldwide framework proved to be very successful in stimulating research in the subsequent years, as demonstrated by the numerous studies using or referring to it. Indeed, this article was highlighted to be the one receiving more citations per year among the many published in the journal *Advances in Parasitology* in a bibliometric analysis at the occasion of the centenary of this journal (94).

In 2014, a significant step forward was made in the field of pathology and symptomatology, with emphasis on two aspects (7): (i) the focus on the chronic phase of the disease was reoriented from being previously considered of secondary importance, because in developed countries diagnosis was mostly made at the symptom onset during the acute phase, to crucial because almost all infected subjects in low-income countries were diagnosed during the chronic phase; and (ii) the terms of neurofascioliasis and ophthalmofascioliasis were restrictedly proposed for the rare cases in which the direct affection of the central nervous system or the eye is caused by a migrant ectopic fasciolid fluke. Such cases should be differentiated from the more frequent cases in which whether migrating fasciolid juveniles during the acute phase or liver flukes infecting the liver during the chronic phase are the indirect, at-distance cause of genuine neurological, meningeal, psychiatric, and ocular manifestations. The great impact of such situations had never been considered when estimating the global burden of fascioliasis (7).

Also in 2014, human diagnosis availabilities were analyzed in front of the new scenario of human fascioliasis after mainly, including the different epidemiological situations in areas of human endemicity in low-income countries (5). The study focused on advantages and weaknesses, sample management, egg differentiation, qualitative and quantitative diagnosis, antibody and antigen detection, posttreatment monitoring, and post-control surveillance. Difficulties caused by the different infection phases and parasite migration capacities, clinical heterogeneity, immunological complexity, different epidemiological situations, and transmission patterns were highlighted. The lack of a diagnostic technique covering all needs and situations indicated the convenience of a combined use of different techniques, at least including a stool technique and a blood technique (5).

In 2016, owing to the increasing number of *F. gigantica* infection reports in humans, a long-term comparative physiopathogenicity experimental study was made for the first time in the same animal model host. The higher *F. gigantica* pathogenicity proved to be linked to its bigger size and biomass and contrasted with previous studies that only reflected the faster development of *F. hepatica* observed in short-term experiments (95).

In 2018, human fascioliasis infection sources were analyzed for the first time in front of the new worldwide scenario of this disease (96). These infection sources proved to include wild and cultivated freshwater plants, wild and cultivated terrestrial plants, traditional local dishes made from sylvatic plants, raw liver ingestion, drinking of contaminated water, beverages and juices, ingestion of dishes and soups, and washing of vegetables, fruits, tubercles, and kitchen utensils with contaminated water. The high diversity of infection sources and their heterogeneity in different countries were highlighted to underlie the large epidemiological heterogeneity of human fascioliasis throughout (96). In the same research focus, a very recent long-term study has additionally emphasized other influencing aspects such as housing, behavioral traditions, social aspects, knowledge of the inhabitants on *Fasciola hepatica* and the disease, and livestock management, with special attention on problems in infancy and gender (14).

A key point in the last years of human fascioliasis has been the availability of triclabendazole for the treatment, both for infected subjects (97) and for preventive chemotherapy (82). Triclabendazole has rapidly become the drug of choice for human fascioliasis, the only trematodiasis known to not respond to praziquantel. The advantages of triclabendazole include its high efficiency with one treatment course of two doses, its lack of secondary effects, the fact that it the only drug with action on adult flukes in the liver as well as on migratory juveniles, and its usefulness for both adult subjects and children. A recent study has shown that triclabendazole may even be used to treat very-early-infected children less than 1 year in age (98).

Unfortunately, the wide use of triclabendazole for livestock treatments has derived in the appearance of resistance to this drug in many countries. Although initially reported only for areas of animal endemicity, in which the resistance was also faced when treating sporadic human cases, the problem has already reached several areas of human endemicity, mainly in South America. Many studies on this worrying scenario, including from individual patient treatments and field surveys up to experimental research, have shed light on this important question in recent decades (18).

The results of a recent bibliometric analysis on fascioliasis research highlight the gradually increasing scientific focus that fascioliasis is generating (92). The increasing knowledge on human affection worldwide is undoubtedly underlying this ascendent progression of fascioliasis attraction.

MULTIDISCIPLINARY REPERCUSSIONS OF FASCIOLID GEOGRAPHY

The two-host life cycle of both *F. hepatica* and *F. gigantica* and consequently the transmission of fascioliasis include many phases that are strongly dependent on abiotic and biotic factors of the external milieu (Fig. 2): presence in freshwater collections in the area, egg embryonation in fresh water, hatching and swimming of the miracidium, presence of appropriate amphibious/aquatic snail species of the family Lymnaeidae, finding and penetration into a lymnaeid vector or intermediate host, intra-molluscan larval-stage development inside the poikilothermic snail, shedding of cercariae into fresh water, swimming of cercariae looking for an appropriate aquatic plant support (or in water surface), attachment and encystation of the metacercariae, and definitive host behavior enabling oral infection.

The number of abiotic and biotic factors influencing each one of these phases is very high. Therefore, *a priori* one would expect these factors to more or less restrict the viability of such a life cycle to given regions/zones/areas and consequently the disease to follow some kind of biogeographic orientation. This would considerably help the understanding of fascioliasis and facilitate the follow-up of transmission patterns, the assessment of epidemiological situations, the defining of prevention measures, and the designation of control initiatives. With such an aim, a very wide study of areas of human fascioliasis endemicity in Europe, Asia, Africa, and Latin America was undertaken. The results of this comparative analysis showed an impressive variation in areas of endemicity at such a level that no common useful factor could be discerned (99). Moreover, not only the very different diet characteristics of the human communities throughout the world (96) but also the pronounced differences in water accessibility, housing types, behavioral traditions, social and religious aspects, and livestock management procedures linked to the disease transmission and infection sources (14) define a highly complicated scenario for fascioliasis. The liver flukes have developed an outstanding opportunistic capacity to benefit from all this instead of to become constricted. Thus, the two fasciolid flukes show such a wide geographical distribution that the conclusion is that fascioliasis has a worldwide distribution, covering Europe, Asia, Africa, the Americas, and Oceania, with the only exception of northernmost regions of the Holarctic due to extreme cold temperatures and given islands such as Greenland, Iceland, and Sri Lanka, where fasciolids and/or lymnaeid vectors probably never arrived. When taking into account that the two *Fasciola* species are of a relatively recent evolutionary origin, the question is posed about how were parasites with such an environmental dependence capable to colonize almost the whole world? It should be considered, however, that lymnaeids are very old gastropods, as can be deduced by the very high number of lymnaeid species and their existence in all the continents (3).

We have been in need for about 30 years of field work and surveys of liver fluke areas of endemicity in different continents and parallel experimental studies in the laboratory until catching the guiding thread allowing for the understanding of the spreading strategies used by these two trematodes to successfully colonize the whole world. In that sense, fascioliasis in unique among trematodiases. Indeed, human fascioliasis is a very heterogeneous and complicated disease, and it should be considered that there is human infection risk in each area of animal endemicity (81). The many different transmission patterns and epidemiological situations of fascioliasis require direct research in the field to correctly assess all factors underlying the transmission and epidemiology characteristics in a given area. In fascioliasis research, the risk of misinterpretations is very high when working with biological samples collected in slaughterhouses (in many low-income countries, the original geographic origin of the slaughtered infected animals is either unknown or linked to doubtful information) or by other people without having personally visited and surveyed the corresponding area of endemicity. Field observational analyses are crucial to understand how the circulation of the fasciolid fluke evolves in an area of endemicity. The scientific method to analyze these field aspects needs repeated visits to the same area of endemicity by the same person and numerous annotations and registers of field observations, triangulation of observations by different participants, individual and group interviews, photographical records and measurements of biotic and abiotic factors, in order to allow for statistically significant results. The results of more than 35 years of such a work in the same areas of human endemicity have recently illustrated the high complexity of human and animal fascioliasis (14).

History, a Key to Understanding Fascioliasis

We progressively realized the importance of human behavior in fascioliasis. First, within an area of endemicity, we observed the link between the type of management of the livestock by local inhabitants and the freshwater collections inhabited by lymnaeid snail vectors (14). Second, we saw the historical relationships between the human communities of close areas of endemicity, such as in the case of the Bolivian and Peruvian Altiplanos (80, 100). Third, we became aware of the historical exchanges or goods transported by domestic pack animals between areas of different countries, such as between Andean highlands and eastern lowlands of South America for the silver of the Potosi mine (101), or transhumance pastoralism between countries south and north of the Sahara Desert (67). Fourth, DNA sequencing allowed us to detect genetic identities or similarities between liver flukes and also lymnaeid vectors between Bolivia and Spain, subsequently verify the trans-Atlantic livestock transport with old vessels by Spanish colonizers, and make the first constatation that not only fasciolids but also lymnaeid vectors were able to be passively transported by livestock (102).

In summing up, there was a factor that we had overlooked when trying to understand the distribution of human fascioliasis areas of endemicity, namely, history concerning human-guided movements of livestock. This led to the differentiation between the pre- and postdomestication periods in the evolution of fasciolid flukes, whose baseline was for the first time exposed in 2009 (1). This distinction has not only evident multidisciplinary implications for the causal agents and snail vectors of fascioliasis but also many repercussions on aspects of the disease. Indeed, a consequent founder effect is observed when analyzing genetic results, both at the local level in the clonic constitution of lymnaeid vectors reproducing by selfing as for instance in the Northern Bolivian Altiplano (103) and at the level of a whole continent in fasciolids of Latin America because of their first original source from Spain (32). Such a founder effect is also detectable at the world level, because in fact the original animal domestication events mainly occurred in the western Asian region of the Near East, the so-called Fertile Crescent comprising modern-day Irak, Turkey, Syria, Lebanon, Jordan, Iran, and the Caucasus. *Fasciola hepatica* originated in this region, and *F. gigantica* found an ideal evolutionary crossroad in this Near East region; consequently, most of the fasciolids everywhere concern lineages descending from this region (1).

Field studies performed in the areas of endemicity of many countries of different continents along repeated visits to the same area of endemicity throughout different seasons and different years has allowed us to verify the surprisingly timely persistence of lymnaeids when the habitat has not been destroyed by a natural disaster or markedly modified by human activities. We have been able to find the lymnaeids in exactly the same corner or stone of a water collection where they were described to be present in detailed articles published centuries ago. This indicates that lymnaeids are able to ensure fascioliasis transmission in an area for very long time. Repeated livestock movements over time allow lymnaeids to increase their adaptation to a given area and in that way “strongly attach” to the conditions of that area, thus maintaining and, with an increased number of populations, ensure fasciolid transmission for the future.

In the predomestication period, fasciolids should have infected the wild fauna in Africa and Near East Asia, although there is thus far no molecular data suggesting that they were able to spread throughout wide regions. The capacity of fasciolids to infect different appropriate herbivore and also several omnivore mammal host species of the wild fauna from introduced livestock sources has been repeatedly observed in many places where such wild and domestic hosts overlap.

For the postdomestication period, the past human movements and historical events accompanied by domesticated fasciolid hosts that should be considered to have been involved in the spread of *F. hepatica* and *F. gigantica* and have marked the present distribution of fasciolids include the following: (i) those followed by ancient peoples who were pastoralists or herders, i.e., humans who moved with their livestock; (ii) movement processes occurring throughout long periods of thousands or at least many hundreds of years (lengthy time span) so as to offer a sufficiently wide number of occasions for fasciolids to expand with their domestic animals and ensure the colonization of new geographic zones; (iii) these human-guided livestock movements should have occurred throughout an appropriate chronological period; (iv) these movements should have evolved according to a spreading convenient direction from a region presenting fasciolids at the beginning to another region where they were absent; and (v) the highly successful fasciolid spread which took place in the Americas during 2 to 3 centuries with the European introduced livestock by the Spanish conquerors gives an idea about the speed a fasciolid colonization process may follow throughout a wide region where these parasites were previously absent.

Sources for the needed information are found in the fields of history, archeology, paleontology, paleoclimatology, and paleobiogeography concerning the last 12,000 years. In the present article, we performed the widest analysis of all this type of knowledge to offer a baseline to understanding the situation of fasciolids, lymnaeids, and fascioliasis in all regions, zones, and areas of the whole world.

Fasciolid Spread Linked to the History of Human Movement

It should be considered that, before the appearance of motorized vehicles, all kinds of transport of goods, materials, and even humans occurred using pack or draft animals along terrestrial routes (Fig. 6). The smaller sheep and goats, although used as pack animals in only given areas due to their limited weight transport capacity, were fundamental for life sustainability of human populations following long movements. Sailing ships were the first manufactured vehicles used for the passive transport of livestock, initially along short trips as inside the Mediterranean and later along long journeys through oceans between different continents. Fasciolids benefited therefore from human-guided movements of domesticated animals during thousands of years. Behavioral aspects (mainly defecation habits ensuring the arrival of fasciolid eggs to freshwater collections inhabited by lymnaeid vectors) do not suggest infected humans to have played a significant role as fasciolid spreaders compared to livestock, despite archeological findings indicating that liver fluke infection in humans was relatively frequent at least in Europe during the Neolithic.

In infections by *Fasciola* species, it is important to differentiate between a definitive host and a reservoir host. A mammal species may be successfully infected and the fasciolid adult stage be able to follow the complete intraorganic migration until reaching the biliary canals and gallbladder, but this does not ensure this host will further transmit the liver fluke. A host may not be suitable for the adult stage to reach sexual maturity and produce eggs, or these eggs be not viable, lose their transmission capacity at any of the subsequent larval phases, or even so do it in a number insufficient to ensure the transmission in the long term. Illustrative examples are (i) humans in many present-day European countries in whom the liver fluke does not produce eggs (5), (ii) European pigs that are resistant to liver fluke infection (2), (iii) many horse breeds that do not become infected by the liver fluke (104), or (iv) South American camelids that shed eggs of low viability and always in dung piles far away from water collections (105). This means that pigs and horses are not convenient spreaders,

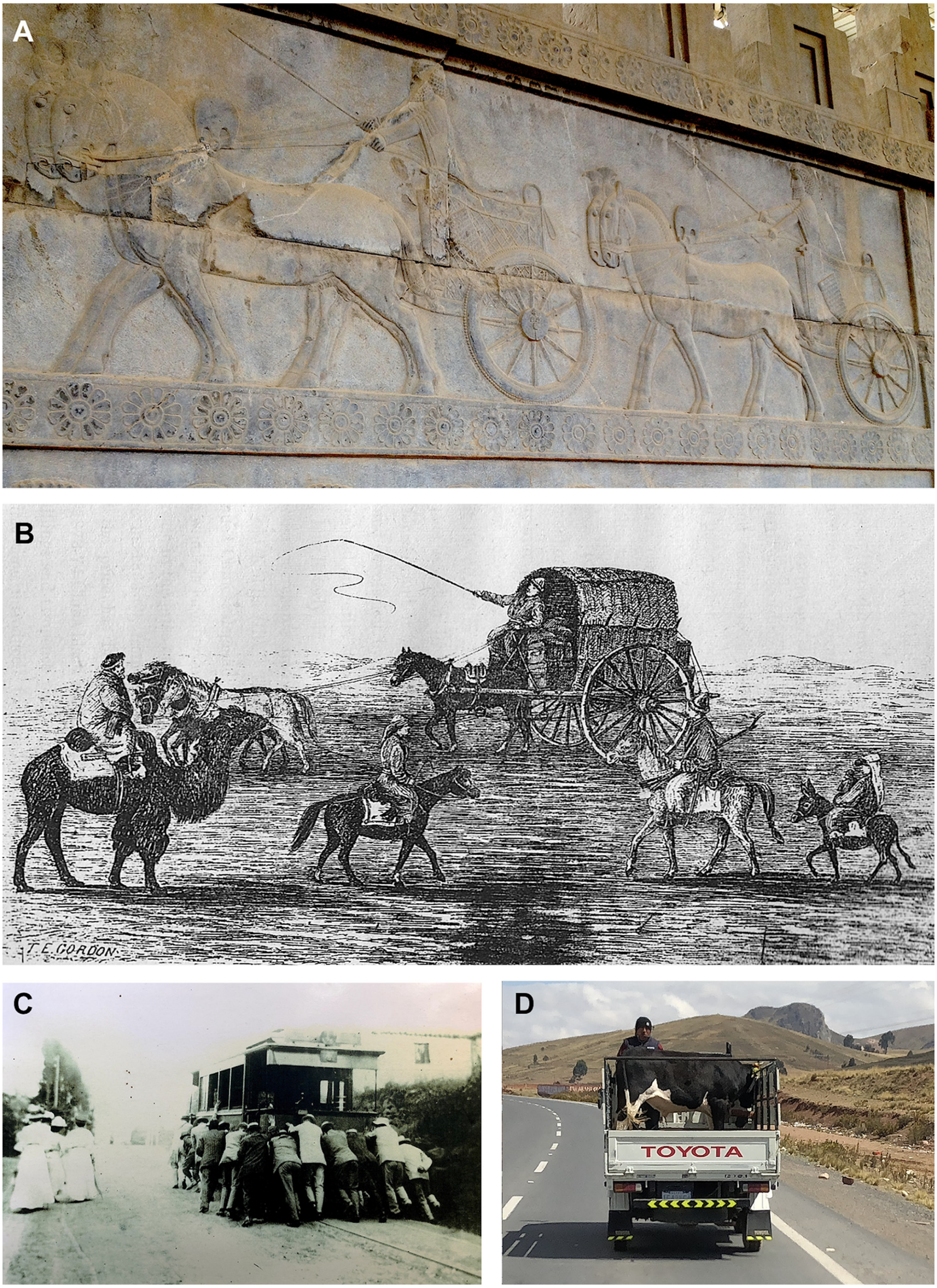

FIG 6 The wheel invention pronouncedly facilitated good transport, and mainly equids but also cattle additionally became draft animals, although initial carts with wooden wheels were not appropriate for mountain routes or steppe crossing. (A) Horse-drawn small carriages sculpted on the stone walls of the ruins of the ancient city of Persepolis dating back to 515 BC, at a 1630-m altitude in present-day Iran. (B) Transport cart drawn by horses in a picture representing a road scene additionally showing human transport by Bactrian camel, mule, horse, and donkey in the Silk Road trading center of Kashgar city, Xinjiang, China, in the 1870s (drawing by Thomas Edward Gordon, 1876, from the book *The Roof of the World*, uploaded to Wikipedia by John Hill in 2005 and available in the public domain). (C) Initial difficulties posed by motorized vehicles prolonged the use of pack and draft animals, as in La Laguna, Tenerife, Spain (reproduced from an uncredited picture in an unidentified old newspaper, available at Mesón La Hijuela, La Laguna, Tenerife, Canary Islands, Spain). (D) Trucks are now also used for the transport of animals in rural areas of low-income countries, such as in the area of human fascioliasis human hyperendemicity of the Bolivian Altiplano.

although some local susceptible breeds may develop a spreader role in given regions, and that South American camelids should be ruled out for such a role.

Main domestic mammal species sharing the characteristics of being a definitive host and a reservoir host requested to play the role of a potential fasciolid spreader include ruminants such as sheep, goats, taurine cattle, zebu cattle, buffalo, and yak but also equines such as donkeys and mules (and rarely horses) and Old World camelids such as the two-humped Bactrian camel and the one-humped dromedary. Pigs might have also contributed to fasciolid expansion, as in the case of South America, where they prove to be appropriate hosts (2).

When analyzing whether a large human movement occurred in the past could have contributed to the spread of fasciolid flukes, key information to look for concerns (i) which domesticated mammal species were accompanying the moving human populations, the quantity of domestic animals involved (high number of animals or their repeated use if in lower numbers define greater fasciolid spread probabilities), (ii) in what period of history did this movement take place, and (iii) how were these animals used and how were they managed.

Many concepts and notions should be extrapolated to past epochs to assess their importance in the expansion of fasciolid flukes, including nomadism, pastoralism, herders, and vertical and horizontal transhumance. These are in their turn linked to crucial aspects in fascioliasis. One such aspect is seasonality, which concerns the population dynamics of fasciolid and lymnaeid species along the monthly changing climatological factors and surface freshwater availability, and consequently varying disease transmission risk. Another factor is altitude, e.g., warm lowlands versus cool highlands, when considering the maximum and minimum development temperature thresholds of the two *Fasciola* species.

Interactions between seasonality and altitude should carefully be considered. For instance, the presence of the warm-climate-preferring *F. gigantica* in a country such as Kazakhstan, where temperatures reach 30°C under zero but with a short summer window of high temperatures, allows for a short fascioliasis transmission season every year. Areas of the so-called “zonal overlap” comprising neighboring mountains and lowlands (1) is another situation unexpectedly showing *F. hepatica*-infected livestock in the lowlands and *F. gigantica*-infected animals in the highlands because of vertical transhumance, such as in the Iranian province of Guilan (106). This differs from the situation of the so-called “local overlap,” where both *F. hepatica* and *F. gigantica* appear to infect humans and animals in the same locality (1) due to historical events having led to the geographical confluence of both fasciolid species and their respective specific lymnaeid snail vector species, as in the Nile Delta in Egypt (107).

Lymnaeid Vector Transport and Fascioliasis Spread

Fasciolid flukes could not have been successful in their worldwide expansion unless the existence of appropriate lymnaeid vector species would have not allowed for it. Lymnaeidae is a very old family of gastropods, including autochthonous species with the capacity to play as vicariant vectors in all continents. Freshwater snails are known to be passively transported (108), whether by birds, with the exchange of exotic or ornamental plants for botanical and/or private gardens, with the trade of aquatic edible vegetables, or with the commerce of ornamental pets as in aquaria. Given species of lymnaeid vectors have been able to go for a pronounced intercontinental spread by one or other of these ways, such as *Pseudosuccinea columella* (109) and *Radix rubiginosa* (110). The trend to resort to a potential passive transport of lymnaeids by birds is repeatedly used in the literature in front of situations for which the authors lack explanatory arguments. Although the involvement of bird carriers cannot be ruled out (Fig. 7), it is evident that such a type of jumping spread do not show the characteristics needed for a gradually chronologically progressive expansion allowing for the spread and colonization of new areas by fasciolid flukes. Moreover, the postdomestication period is evolutionarily too short. Fasciolid spread required fast lymnaeid transport and colonization capacity, a characteristic offered by livestock movements and local rural animal management but not by the too sporadic transport by migratory birds.

There is no doubt that human-guided movements of livestock have altered the original geographical distribution that many lymnaeid vector species reached in the predomestication period. Such a distributional modification appears to have been more pronounced in

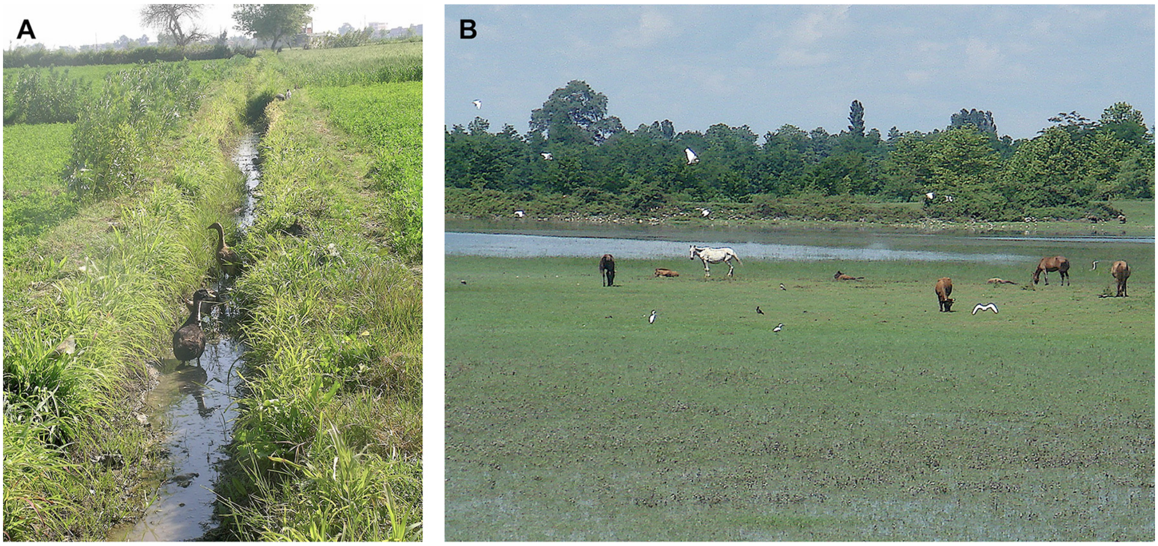

FIG 7 Birds may play a discrete role in the spread of lymnaeid snail vectors. (A) Domestic anatids can expand lymnaeid snails at a local level, such as in irrigation canals inhabited by *Galba truncatula* between agricultural fields in the human fascioliasis hyperendemic area of the Nile Delta, Egypt. (Photograph courtesy of Patricio Artigas, Valencia; used with permission.) (B) Freshwater-frequenting birds such as ardeids may spread lymnaeid snails throughout longer distances, such as in the flatland lagoons of areas of fascioliasis endemicity of Georgia, but do not follow human movements as livestock does.

the cases of lymnaeid species showing a marked amphibious trend, as in most of the small species of the *Galba/Fossaria* group (Fig. 4). Such species as *G. truncatula*, *L. cubensis*, *L. neotropica*, and *L. schirazensis* are evident examples. Indeed, the arrival and colonization of Andean countries by the European *G. truncatula* is an irrefutable proof of long journey passive transport in mud attached to the hooves of livestock transported in the ancient vessels of the Spanish conquerors crossing the Atlantic Ocean during the early years of the New World colonization (1, 102). After crossing the Panama isthmus, livestock was mainly introduced by the Europeans into South America through the Pacific coast. The markedly amphibious *L. schirazensis* has demonstrated its usefulness as marker of livestock movements, including even transocean crossing when passively transported with livestock in ships, although its small size and extremely terrestrial behavior underlie its incapacity to act as a fascioliasis transmitter (4).

Three characteristics of lymnaeid snails facilitate their capacity for passive transport in mud attached to the hooves of livestock. First, their amphibiousness-linked capacity to enter in hibernation/estivation by burying inside mud and return to activity when recontacting water allows them to jump from a freshwater collection to another. Second, their multiplication capacity by selfing allows them to successfully colonize a new area with only one founder specimen, as demonstrated by the clonic *G. truncatula* populations throughout the whole area of human fascioliasis hyperendemicity of the Northern Bolivian Altiplano, where all DNA markers show identical sequences (103). Third, lymnaeids are typical r strategists (organisms of rapid multiplication capacity), with a fast precocious multiplication speed able to give rise to a numerous population from only one or a very few specimens in a very short time of a few weeks. In lymnaeid vector species belonging to the *Galba/Fossaria* group, the three aforementioned characteristics are well developed, which increases the probabilities of their passive transport in long-term repeated livestock movements involving a high number of domestic animals.

Species of the *Radix* group are usually bigger lymnaeids and are less amphibious (Fig. 5). In fact, this might have been one or the main reason why they never colonized the New World. The present geographical restriction of *F. gigantica* to Asia and Africa is always argued on the base of the limited distribution of *Radix* vector species to only these two continents. There is, however, an important exception in this group, namely, the small vector species called *R. viridis*-*R. ollula*-*G. pervia* whose evident amphibious trend underlies its link to rice cultures and explains how it has been able to widely expand throughout southeastern and eastern Asia (Fig. 5). Nevertheless, the lack of a logical biogeographical distribution of *Radix* species throughout the Tibetan Plateau (111) suggests that livestock movements occurred during very long time periods might also have modified the distribution of *Radix* lymnaeid species in this wide region (see more detailed analysis of the Tibetan Plateau later in this article).

Stagnicolines is another group of lymnaeids of Holarctic distribution in which a few species have been found infected by *F. hepatica* in nature. These findings concern rare local situations (90, 112), which in no way indicate this lymnaeid group to have been involved in fasciolid spread.

The question of specificity regarding the *Fasciola* species/lymnaeid species relationships is posed when analyzing the geographical spread of fasciolid flukes. A worldwide analysis indicates that *F. hepatica* is transmitted by *Galba/Fossaria* species, whereas *F. gigantica* is transmitted by *Radix* species. There are, however, several contradictory results that do not fit such a simple restricted specificity. For instance, the Asian species *R. viridis-R. ollula* has been experimentally demonstrated to successfully transmit *F. hepatica* from Australia (113). Other experimental assays have shown *Galba/Fossaria* species transmitting *F. gigantica* and, vice versa, *Radix* species transmitting *F. hepatica*, although in all these cases there was a lack of DNA sequencing to verify that no hybrid fasciolids resembling one or the other species were involved in the experiments. A recent detailed phenotyping and genotyping study about *F. gigantica*-like sized flukes in Ecuador (only *F. hepatica* exists in the Americas) has reached the conclusion about a vector specificity filter acting as a driving force in accelerating the evolution of fasciolid hybrid lineages, originated after *F. gigantica* introduction with livestock importation from another continent, toward *F. hepatica* due to the *Radix* absence. In other words, the lymnaeid vector species specific for a *Fasciola* species present in an area, when lymnaeids belonging to the other lymnaeid group specific for the other fasciolid species are absent, would in the midterm of a few decades ensure the return of hybrid intermediate lineages, originated by the introduction of the other fasciolid together with imported livestock, toward the fasciolid species originally existing in the area in question (32).

SPECIES OF FASCIOLINAE STILES ET HASSALL, 1898

The last review of Fasciolidae Railliet, 1895 furnished a detailed systematic-taxonomic analysis of all taxa of this trematode family (1). New knowledge obtained afterwards leads to a few but important modifications in the subfamily Fasciolinae.

Fasciolinae includes species presenting branched ceca, dendritic testes and ovary, adult stages developing in the liver and rarely duodenum and lungs, and flukes transmitted by freshwater snails of the family Lymnaeidae. This subfamily includes three accepted genera, namely, *Fascioloides* Ward, 1917, *Tenuifasciola* Yamaguti, 1971, and *Fasciola* Linnaeus, 1758 (1). *Fascioloides* comprises only the large American liver fluke species *F. magna* (Bassi, 1875) Ward, 1917, which infects North American and European bovids and cervids. *Tenuifasciola* also includes only one species, *T. tragelaphi* (Pike et Condy, 1966) Yamaguti, 1971, known to parasitize the sitatunga *Tragelaphus spekei* in Zimbabwe and also found in cattle in Africa (1).

Fasciola is the genus in which more species and varieties have been described. The following should be counted in chronological order of description: *F. hepatica* Linnaeus, 1758, known to infect mainly livestock but also a large variety of other domestic and wild animals in the five continents; *F. gigantica* Cobbold, 1855, parasitizing domestic and wild herbivorous animals, especially ruminants, and restricted to parts of Africa and Asia; *F. jacksoni* (Cobbold, 1869) in the Asian elephants *Elephas indicus* and *E. maximus* in India, Burma, Sri Lanka, and Indochina; *F. hepatica* var. angusta Railliet, 1895, in cattle of Senegal; *F. hepatica* var. aegyptiaca Looss, 1896, from cattle in Egypt; *F. nyanzae* Leiper, 1910, in *Hippopotamus amphibius* and transmitted by the lymnaeid *Radix natalensis* in Africa; *F. californica* Sinitsin, 1933, from the lymnaeid *Lymnaea bulimoides* in California and adult experimentally obtained in the rabbit; *F. halli* Sinitsin, 1933, from cattle and sheep in Texas and Louisiana and transmitted by the lymnaeid *L. bulimoides*; and *F. indica* Varma, 1953, from *Bos indicus*, *B. bubalus*, *Capra hircus*, and *Sus cristatus* and transmitted by the lymnaeid *Lymnaea acuminata* in India (see review in reference 1).

Another species, named *F. hepatogigantica* Khalifa, El-Hady, Omran et Ahmed, 2013, has been recently described from sheep and cattle of Egypt (114).

Regarding *F. jacksoni*, a molecular comparison of the sequences of markers of the rDNA (28S, ITS-1, and ITS-2) and the mtDNA (*nad1*) with those of *Fascioloides magna* (115) suggested the convenience of reclassifying this species as *Fascioloides jacksoni* (Cobbold, 1869) Heneberg, 2013 (116). However, genetic distances appeared to be almost equidistant and this reclassification remained doubtful, even after additionally analyzing the mtDNA *cox1* gene (117). The taxonomic change received, however, definitive support later when analyzing the complete mitochondrial genome (118).

The North American species *F. californica* and *F. halli* were synonymized with *F. hepatica* a long time ago. Indeed, three different morphotypes of *Fasciola* were described in animals in the United States (119). Flukes proved to fit the characteristics of *F. hepatica* in several parts of the country, whereas those from Texas and Florida resembled *F. gigantica*, and the flukes from the area of the Gulf coast area showed intermediate characteristics. These forms could be considered the consequence of importations of zebu cattle and buffalo from India occurred in 1875 and 1906, and perhaps also from Africa, after introgressions of imported *F. gigantica* into US-native *F. hepatica*. The absence of lymnaeid snail species of the *Radix* group was argued to understand the impossibility for *F. gigantica* to adapt, but crossbreeding could easily take place inside the imported animals because there were directly released into the field without prior quarantine as always made at that time. Hybrids unable to develop in *Radix* absence could not survive, but others may have kept through viable introgressed lineages able to be transmitted by US-native lymnaeids (1).

Flukes resembling *F. gigantica*, considering mainly size characteristics, were also reported from Mexico (120, 121) and Ecuador (122). However, liver flukes from Mexico only showed small genetic distances when molecularly compared by ISSR-PCR to Spain (123). Moreover, the size range reported for the Mexican *F. gigantica* was verified to enter in the morphometric range of *F. hepatica* (124). In addition, an introduction of *F. hepatica* into Mexico with livestock transported from Spain was concluded by genetic rDNA intergenic region haplotyping (124). Therefore, an evolutionary phenomenon similar to the aforementioned in the United States has been discarded in Mexico for the moment, unless *F. gigantica*-sized specimens are found in as-yet-unexplored areas of this country in the future.

On the contrary, Ecuador is the only South American country in which flukes surpassing the maximum size known for *F. hepatica* have been verified to be present after an exhaustive morphometric phenotyping compared study with computer image analysis system (CIAS) (32). Fasciolid flukes infecting sheep in this country showed an abnormally big size for *F. hepatica*, the only liver fluke species known to inhabit South America. The morphometric study demonstrated their size to be intermediate between standard *F. hepatica* and *F. gigantica* populations. Such a characteristic has thus far only been found in several fasciolids in areas of Africa and Asia where the two *Fasciola* species and their specific lymnaeid vector species overlap, except the aforementioned cases in the United States (119). This unexpected feature suggests a past introduction of *F. gigantica* with imported livestock from Pakistan in 1974 or *F. gigantica*-like intermediate hybrids from the United States in 1986 (32). Sequencing of rDNA and mtDNA markers in their complete length evidenced a lack of heterozygotic positions differentiating the two species at the rDNA level and the absence of introgressed fragments and heteroplasmic positions at the mtDNA level. The period elapsed since the introduction leading to hybridization was considered to be sufficiently long as to enable the complete homogenization of the rDNA by concerted evolution, as well as for the mtDNA to return to homoplasmy. The existence of several lymnaeid species with the capacity to transmit *F. hepatica*, together with the absence of *F. gigantica*-specific lymnaeids of the *Radix* group, undoubtedly underlay the gradual selection of *F. hepatica* genomic characteristics (32). These results further explain the variability of forms described in the United States (119) and the appropriateness to synonymize *F. californica* and *F. halli*. In addition, this study demonstrated the importance of the evolutionary filter developed by the specificity regarding the lymnaeid vector species and highlighted the difficulties, if not to say, incapacity, of *F. gigantica* to colonize the New World unless susceptible *Radix* lymnaeids are introduced. This is crucial from the health point of view, when the higher pathogenicity of *F. gigantica* is considered (95).

The species *F. indica* was synonymized with *F. gigantica* by several authors (125–127). Indeed, *F. indica* was described in Lucknow, Uttar Pradesh, in the northern part of India, where *F. gigantica* is overall found transmitted by *Radix acuminata* at very high rates (128). Along the same Central Asian plain, intermediate forms have been described in Pakistan in the West (129) and also in Bangladesh in the East (130). A recent DNA sequencing study showed Indian *F. gigantica* to have a rDNA 28S 618-bp fragment differing at a nucleotide position regarding *F. hepatica* and at another position from African *F. gigantica*, although in aberrant flukes. Moreover, 26 substitutions were found between Indian *F. gigantica* and African *F. gigantica* in a 1,400-bp mtDNA fragment enclosing cytochrome *c* oxidase subunit III (cox III), tRNA histidine (tRNA-His) and cytochrome *b* (cob) (131). This means a genetic distance of only a 1.85%, which is lower than the 1.95% (30 variable positions in the 1,533-bp *cox1* gene) found in *F. hepatica* inside Ecuador, a country with frequently imported livestock from neighboring countries (32). Again, all information indicates phenotypes and genotypes resulting from the mixing of fasciolid populations and justify the *F. indica* synonymy with *F. gigantica*, which is easily understandable when considering the history of human migrations throughout many centuries along this Asian region (1).

The same arguments apply to the most recently described species *F. hepatogigantica* in Egypt (114), proposed after the description of a new strain in this country (132). The description of *F. hepatogigantica* was genetically supported only after banding patterns obtained by means of restriction fragment length polymorphism (RFLP) of a 618-bp fragment of the rDNA 28S (114). Indeed, it is well known since the end of the 1800s (48) and beginning of the 1900s (29) that only *F. gigantica* was present in Egypt, and that *F. hepatica* began to be detected in livestock imported from Europe later (133–135). The existence of lymnaeid species able to transmit *F. hepatica* throughout the whole Mediterranean northern Africa, including Egypt, such as *Galba truncatula* already in ancient times (136), allowed European *F. hepatica* to be introduced and progressively spread throughout many parts of the country, thus giving rise to situations of local overlap (1). The description of intermediate forms in Egypt (137) speaks about the consequences of fasciolid hybridization. The coexistence of lymnaeid species specific for both fasciolids in the same place allow for frequent coinfections in livestock and therefore for the production of recent hybrids in which concerted evolution does not have yet time for rDNA homogenization. The illustrations of immature and mature worms of the so-called new strain (132) (Fig. 4 and 5) also fit with intermediate forms usually found in areas of fasciolid species overlap. It is therefore appropriate to synonymize *F. hepatogigantica* with *F. gigantica pro parte* and *F. hepatica pro parte*.

In this context, only three species remain valid, namely, *F. hepatica*, *F. gigantica*, and *F. nyanzae*; of these, only the two first can infect humans. Although the capacity of *F. hepatica* and *F. gigantica* to crossbreed and give rise to viable hybrids has been used to pose their validity as separate species is in doubt, several arguments were already raised to justify keeping them as valid species (1). Among these arguments, a different paleobiogeographical origin and different worldwide spreading routes were concluded and multidisciplinary analyzed within a new framework which was established and proposed as baseline for subsequent research (1). Analysis of the many studies published afterward on aspects related to this new baseline framework demonstrates how useful it has been and may continue to be in the future.

Two periods were chronologically distinguished in this evolutionary framework: (i) the predomestication period and (ii) the postdomestication period (1). New knowledge obtained in recent years allows for a tuning up of given steps within this framework.

FASCIOLID ORIGIN AND EVOLUTION IN THE PREDOMESTICATION PERIOD

A wide multidisciplinary analysis based on paleontological data on host species, chronological paleoclimatic variations along the geological periods, inter-region faunal migrations in the past, present wild and domestic host species spectrums of fasciolid species, and genetic/molecular knowledge on host species, together with DNA marker sequence data, geographical distribution, adaptation, development, life span, pathogenicity, and immunity/resistance of the fasciolid species, were considered to build up an evolutionary framework about the origins and geographical spread of *F. hepatica* and *F. gigantica* in both the ruminant predomestication times and the livestock postdomestication period (1). This evolutionary framework furnished a new baseline from which to interpret the results of modern genetic techniques applied to *Fasciola* and lymnaeids from different regions of the world. Researchers were encouraged to take this framework into account when interpreting the results of their genetic studies and to suggest an appropriate modification to the framework when nonfitting results were obtained (1).

It was concluded that the ancestor may be found in an ancient fasciolid form infecting old Artiodactyla in Africa during the early Oligocene when the first Pecoran radiation occurred. The origin of *F. gigantica* was estimated to probably be the result of an adaptation of this ancient fasciolid to bovids, such as bovid ancestors of Alcelaphinae, Reduncinae, and Bovinae, during the second Pecoran episode, resulting in an explosive radiation during the early Miocene. This origin was probably in the warm, eastern Africa, where the lymnaeid snail *Radix natalensis* ensured the transmission (1). On the other hand, the origin of *F. hepatica* was concluded to have probably occurred in the Eurasian Near East, as a derivation from the same ancient fasciolid or a *F. gigantica*-close old form introduced with ruminants from Africa during a major sea level lowering in the early Miocene. The origin of *F. hepatica* was suggested to have likely been the result of colonization of and subsequent adaptation to a new, more northern and temperate-colder region, as well as the result of two host capture phenomena to smaller lymnaeid species of another lineage such as *Galba* and to midsized ovicaprine ruminants (1).

Results of wide analyses of the whole genomes of *F. hepatica* and *F. gigantica*, together with that of the intestinal species *Fasciolopsis buski* (Lankester, 1857) Stiles, 1901, a member of the subfamily Fasciolopsinae Odhner, 1910, performed recently (138) fit also well within the aforementioned scenario. Therefore, these results provided more support and did imply no need for any modification.

However, the more recent DNA marker sequencing of the complete rDNA genes and spacers and mtDNA coding genes, typically used for the molecular characterization of fasciolids, of the species *F. nyanzae* from hippopotamus has furnished crucial new data which led to a few but important reconsiderations on the paleobiogeographical origins of both *F. hepatica* and *F. gigantica* (139). Although the few single nucleotide polymorphisms (SNPs) at the spacers ITS-1 and ITS-2 showed an equidistance between *F. nyanzae* and *F. hepatica* on one side and between *F. nyanzae* and *F. gigantica* on the other side, the highly conserved, 1,980-bp small ribosomal unit or 18S gene showed three nucleotide differences between *F. nyanzae* and *F. gigantica*, whereas there were surprisingly none between *F. nyanzae* and *F. hepatica*. A molecular clock translation of this genetic distance indicates that the chronological divergence between the origins of *F. hepatica* and *F. gigantica* may be estimated around 8.41 million years. This dating estimate proved to perfectly fit the present paleontological knowledge about past host group radiations and migrations. In addition, the intraspecific variability with a number of different haplotypes in the two mtDNA coding genes *cox1* and *nad1*, including but a few mutations found in the hippopotamus populations studied, suggests a high conservationism degree of the host habitat of such a wild animal in the southern latitudes of Africa (139).

The following main conclusions may be obtained from these results (Fig. 8).

Hippopotamids and *Fasciola* Origins

The role played by hippopotamids in the paleobiogeographical origins of both *F. hepatica* and *F. gigantica* deserves more focus. These herbivorous mammals have an amphibious life style, which is reflected in morphological adaptations such as their eyes or orbits, which are elevated above the skull, and their nostrils and ears that can be closed while diving (140). Common hippopotamids inhabit areas of peaceful waters, which they leave during night to graze terrestrial vegetation (141). This amphibious behavior make them ideal hosts to participate in fasciolid transmission. Indeed, the aforementioned observations fit well with paleontological data which illustrate that the oldest known Hippopotamidae are from

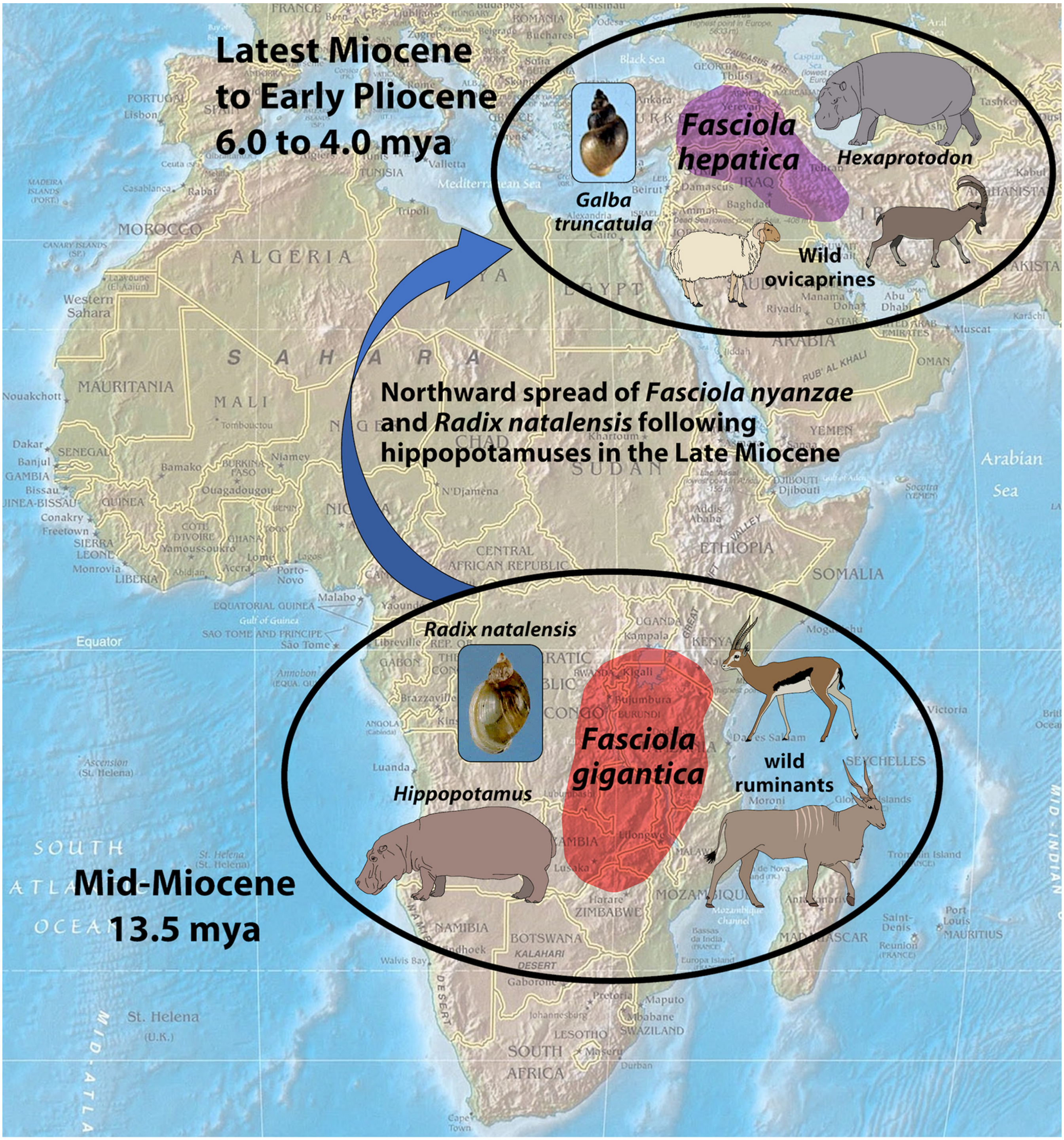

FIG 8 Paleobiogeographical origin of *Fasciola gigantica* by host capture derivation from *F. nyanzae* of *Hippopotamus* in wild ruminants in southeastern African warm lowlands, by keeping the same lymnaeid vector species *Radix natalensis*, around 13.5 mya in the Mid-Miocene. The northward spread of *F. nyanzae* and *R. natalensis* following African hippopotamuses reaching westernmost Asia in the Late Miocene is depicted. The subsequent paleobiogeographical origin of *F. hepatica* occurred by host capture from *Hexaprotodon* hippopotamuses and *R. natalensis* in wild ovicaprines and *Galba truncatula* in the cooler mountainous area in Near East Asia around 6.0 to 4.0 mya in the Latest Miocene to Early Pliocene periods.

the Early Miocene of sub-Saharan Africa, where they later reached their major diversity. From there they dispersed northward to North Africa and Europe, and eastward to the Indian subcontinent and South East Asia (140). The Asian fossil lineage is included in the genus *Hexaprotodon*, in which the non- or less-elevated eyes indicate a less amphibious, i.e., more terrestrial life style than in their African *Hippopotamus* relatives (142).

Paleobiogeographical Origin of *Fasciola gigantica*

Fasciola gigantica should have derived from *F. nyanzae* ancestors in southeastern Africa, most probably in or around the territories of present-day Uganda, Congo, Kenya, Tanzania, Zambia, and Zimbabwe by a host capture phenomenon from early hippopotamids to ancient bovids (139). This means that this capture event should have occurred later than previously considered, i.e., during the third phase in the split of the bovids corresponding to the radiation of mainly Reduncinae in Africa at about 13.5 million years ago (mya) (143), instead of during the second Pecoran episode during the early Miocene (20.2 to 16.9 mya) (1). This period of the mid-Miocene was marked by an important global cooling (144) and fits well with present data about the frequent *F. gigantica* infection reported in African species of Reduncinae (*Kobus defassa* in Congo, *Kobus kob* in Uganda and Congo and *Kobus varondi* in Zambia) and Bovinae (as the African water buffalo *Syncerus caffer* in Uganda and Sudan) (46, 145, 146). Important aspects to be considered here are the high prevalences by *F. gigantica* found in wild populations in Uganda, such as a 47% of the kob *K. kob*, 58% of African buffalo, and 47% in Jackson's hartebeest *A. buselaphus* (145). In Uganda, parasitological surveys of the wild fauna evidenced that *F. gigantica* can be maintained in wildlife in the absence of domestic ruminants (147). Moreover, it should be taken into account that *F. gigantica* has also been reported from *Hippopotamus amphibius* in Zambia (46). On the contrary, *F. nyanzae* has never been found in a host species different from the hippopotamus. Moreover, attempts to experimentally infect cattle, goats and rabbits failed, which supports the specificity of *F. nyanzae* for hippopotamus (148).

In a review of infection by *F. gigantica* and *F. hepatica* in wild animals in East and Southern Africa in more recent years, the results highlighted a similar scenario (149), including wildlife reports of *F. gigantica* in three countries: (i) in Zimbabwe infecting the Blue wildebeest (*Connochaetes taurinus*), Bushbuck (*Tragelaphus scriptus*), Common duiker (*Sylvicapra grimmia*), Eland (*Taurotragus oryx*), Giraffe (*Giraffa camelopardus*), Impala (*Aepyceros melampus*), Kudu (*Tragelaphus strepsiceros*), Sable antelope (*Hippotragus niger*), and Tsessebe (*Damaliscus lunatus*); (ii) in Swaziland infecting the Impala; and (iii) in Zambia infecting the Kafue lechwe (*Kobus leche*). The prevalence of infection by *F. gigantica* was highest in the Kafue lechwe (52.5%) in Zambia, followed by Kudu (12.5%) in South Africa. Wildlife reports on *F. hepatica* in Kudu in South Africa, as well as Sable antelope and Common or Gray duiker in Zimbabwe, were linked whether to closeness to domestic livestock or to accidental infection (139).

Such a host capture event from *F. nyanzae* in hippopotamus to bovids giving rise to a speciation phenomenon leading to the origin of *F. gigantica* is additionally supported by the fact that both species use the same lymnaeid species as vector, namely, *Radix natalensis* (139), as verified in Uganda (148). Recent molecular studies have again confirmed this lymnaeid species as transmitter of *F. nyanzae* in Zimbabwe. The other two lymnaeid species in which this fasciolid was molecularly detected, *Pseudosuccinea columella* of large worldwide distribution and *Radix plicatula* of Asian Far East distribution, are snails introduced into the surveyed, hippopotamus-inhabited, artificial lake of Zimbabwe from abroad (150, 151).

Summing up, for *F. gigantica* we may already designate (i) southeastern Africa as its paleobiogeographical region of origin; (ii) the mid-Miocene, around 13.5 mya, as the chronological period for its appearance; (iii) ancient wild bovid species of Reduncinae, Bovinae, and Alcelaphinae as the most probable original definitive hosts; (iv) *Radix natalensis* as the original lymnaeid snail vector species; and (v) warm lowlands surrounding peaceful freshwater collections inhabited by hippopotamuses as the original ecological habitat (Fig. 8).

Paleobiogeographical Origin of *Fasciola hepatica*

The rDNA 18S molecular dating estimates and mutations at the level of the internal transcribed spacers indicate that *F. hepatica* should have derived directly from *F. nyanzae* ancestors and again support its younger origin compared to the origin of *F. gigantica* (139). The paleobiogeographical origin of *F. hepatica* in the Near East region, including fasciolid lineage adaptation to Palearctic ovicaprines, the *G. truncatula* snail vector, and a cooler environment (1), keeps its validity. No contradicting argument with contrastable support has appeared in recent published related research.

Such an evolutionary event and its dating estimate perfectly fits the spread of past *Hexaprotodon* hippoptamids from northern Africa into Asia. The oldest hippopotamids in Asia were found in the Siwaliks (*Hexaprotodon sivalensis*) and their radiometric and paleomagnetic dating indicated this presence to have occurred in the latest Miocene to Late Pliocene, between 6.1 and 5.9 mya (142, 152, 153). Fossil remains show that they used the so-called Levantine corridor to expand from northeastern Africa through the eastern Mediterranean Near East and up to the Caucasus, according to hippopotamid fossil findings in present Georgia around 1.40 mya, in the Israel-Libano area around 1.40 to 0.70 mya, and in Syria around 0.30 to 0.25 mya (140). Moreover, it should be noted that the present *Hippopotamus amphibius* was still present in Palestine in the Neolithic and even until very recently in the northern Nile River basin (154).

The species diversification within the two genera of midsized ruminants *Ovis* and *Capra* occurred 6.8 to 5.1 mya (143), overlaps chronologically and geographically, and may have facilitated the definitive host capture phenomenon from an hippopotamid fasciolid lineage following a trend toward less amphibious/more terrestrial behavioral characteristics. Wild goats or bezoar *Capra aegagrus* (155) and wild sheep or mouflon *Ovis gmelini* (=*O. orientalis*) (156, 157), ancestors of the domesticated *Capra hircus* and *Ovis aries*, respectively, are known to have inhabited the region from the southern Levant through southeastern Turkey and northern Syria to the high Zagros Mountain pastures and lowland plains of Irak and Iran (158). Indeed, the normal habitats of wild sheep are steep mountainous woods near tree lines and are known to migrate to lower altitudes in winter. Today, the range of the wild argali sheep *Ovis ammon* and the Siberian ibex *Capra ibex siberica* extends into the Hindu Kush, whereas the smaller wild urial sheep *Ovis orientalis* and wild bezoar goat *Capra hircus aegagrus* are only found in Afghanistan and northern Pakistan further south (139).

The possibility for wild boars *Sus scrofa* to have played an intermediate evolutionary role in this capture phenomenon may not be ruled out (139). Indeed, Suidae are evolutionarily related to hippopotamids (154), and the wild boar is an animal living both in warmer lowlands and cooler highlands (159). *Sus scrofa* was present in the Asian Near East (160–162). Moreover, the wild boar in western Europe (163) and feral black pigs in Sicily (164) are known to be infected by *F. hepatica*. In addition, recent wide experimental and field studies have demonstrated not only that *F. hepatica* can develop in the pig but also that this host is able to play an important role of reservoir for this parasite, at least in South America (2).

The freshwater characteristics of the hippopotamid milieu, the many reports on *F. nyanzae* infection in different African places where this animal has been parasitologically surveyed (46), and the wide geographical distribution of its vector, the aquatic, warm-habitat preferring *R. natalensis*, including a northeastern spreading arm along the Nile River course up to the Nile Delta (165), indicate that *F. nyanzae* should have followed the northward geographical expansion of its hosts (139). *Radix natalensis* appears to be still present in Jordan nowadays, a country where the amphibious, cool-habitat-preferring lymnaeid snail species *G. truncatula* is also present (166). Moreover, *G. truncatula* is known to transmit *F. hepatica* throughout all this area of the Levant up to the mountains of Iran (106, 167) and northern Pakistan (168, 169).

The confluence of all needed factors fits an original appearance of *F. hepatica* in the Near East occurred from the period around 6.0 to 4.0 mya, which does not mean this speciation event by definitive and vector host capture phenomena to have occurred in this period but perhaps somewhat later, as the aforementioned factor coexistence was maintained for subsequent long time (139).

Summing up, for *F. hepatica* we may already designate (i) the Near East of Asia as its paleobiogeographical region of origin; (ii) the period of the latest Miocene to Early Pliocene, around 6.0 to 4.0 mya and perhaps a little bit more recent, as the chronological period for its appearance; (iii) wild ovicaprine species of goat, the bezoar *Capra aegagrus*, and sheep, *Ovis orientalis* (Bovidae: Caprinae), as the most probable original definitive hosts, without ruling out a possible evolutionary intermediate passage through the wild boar *Sus scrofa*; (iv) *Galba truncatula* as the original lymnaeid snail vector species; and (v) cooler areas and/or mountainous zones, where large hippopotamuses and the aforementioned midsized mammals overlapped, as the original ecological habitat (Fig. 8).

WORLDWIDE SPREAD DURING THE POSTDOMESTICATION PERIOD

Archeological data on human and animal coprolites, and historical records of human migrations, as well as genetic data on recent dispersal of domestic ruminant species and key influencing aspects of livestock management such as transhumance, intranational movements, and international import/export, were added to all the aforementioned multidisciplinary knowledge sources in the analysis to establish the evolutionary framework for *F. hepatica* and *F. gigantica* across all continents (1).

The very few nucleotide differences that both *F. gigantica* and *F. hepatica* show at the level of the sequences of the complete rDNA ITS-2 and ITS-1 spacers, and the mtDNA coding genes *cox1* and *nad1*, the four markers traditionally analyzed in the genetic studies of fasciolids throughout, indicate that the broad dispersal took place quite recently and occurred from the same founder zone or region. In other words, the data therefore suggest that neither *F. gigantica* in southeastern Africa nor *F. hepatica* in the Near East were following any broad geographical expansions during the Neogene or the Pleistocene.

The only additional transcendent event that occurred in the predomestication period concerns the emigration of *F. gigantica* into southwestern Asia together with many large African bovids and *R. natalensis* through the Levantine corridor. This happened by taking advantage of the northward extension of the East African Rift, with ecological and climatic characteristics similar to those of eastern Africa along the Plio-Pleistocene period. This allowed *F. gigantica* to be present in the Asian Near East before the Neolithic (139).

Consequently, all multidisciplinary data lead to conclude that the crucial phenomenon allowing for the wide spread of fasciolids was the evolutionary very recent animal domestication event occurred only around 12,000 to 10,000 years ago and their subsequent human-guided movements, mainly of ruminants but also equids and camelids (1). More recent results of the comparative analysis of the genomes of *F. hepatica* and *F. gigantica* further support the same scenario and dating (138). The subsequent human-guided movements of ruminants, equids, and camelids, together with human migrations, explain the fasciolid spreads throughout the Old World, the New World, and Oceania (Fig. 9). In the following sections, new information appeared in recent years is analyzed to tune up the steps of the evolutionary scenarios followed by the two fasciolid species in the postdomestication period according to continents, originally furnished in 2009 (1).

The original detailed baseline proposed in 2009 should be here considered to avoid repetitions, since only needed improvements and modifications are analyzed in the following sections. The postdomestication evolutionary scenario for *F. gigantica* is restricted to Africa and Asia because the absence of lymnaeids of the *Radix* group does not seem to allow this fasciolid to colonize the Americas (32), whereas for *F. hepatica* all continents are analyzed except the very high latitudes and extreme polar regions because of the minimum development temperature threshold of this fasciolid.

FASCIOLA GIGANTICA SPREAD THROUGHOUT AFRICA AND MADAGASCAR

The key element for the analysis of the spread of *F. gigantica* throughout the African continent is its lymnaeid vector *R. natalensis*. This lymnaeid should be considered its only transmitter host because other lymnaeids found in Africa, such as *P. columella* or the radicines *Radix rubiginosa* and *R. plicatula*, only concern very recent introductions (151, 170, 171). The snail *R. natalensis* has a wide distribution covering the lowlands from the southernmost South Africa up to sub-Saharan latitudes from the Nile River in the East up to Senegal in the West (165, 172). The ethology of this snail is not as amphibious as in species of the *Galba/Fossaria* group, so that it does not appear to be able to be passively transported by terrestrial animals as livestock. Consequently, it should have been for hippopotamuses and crocodiles to play the role of the spreaders of this lymnaeid vector during the Late Pleistocene and Early Holocene, even including its trans-Saharan distribution, according to wide paleohydrological studies (173).

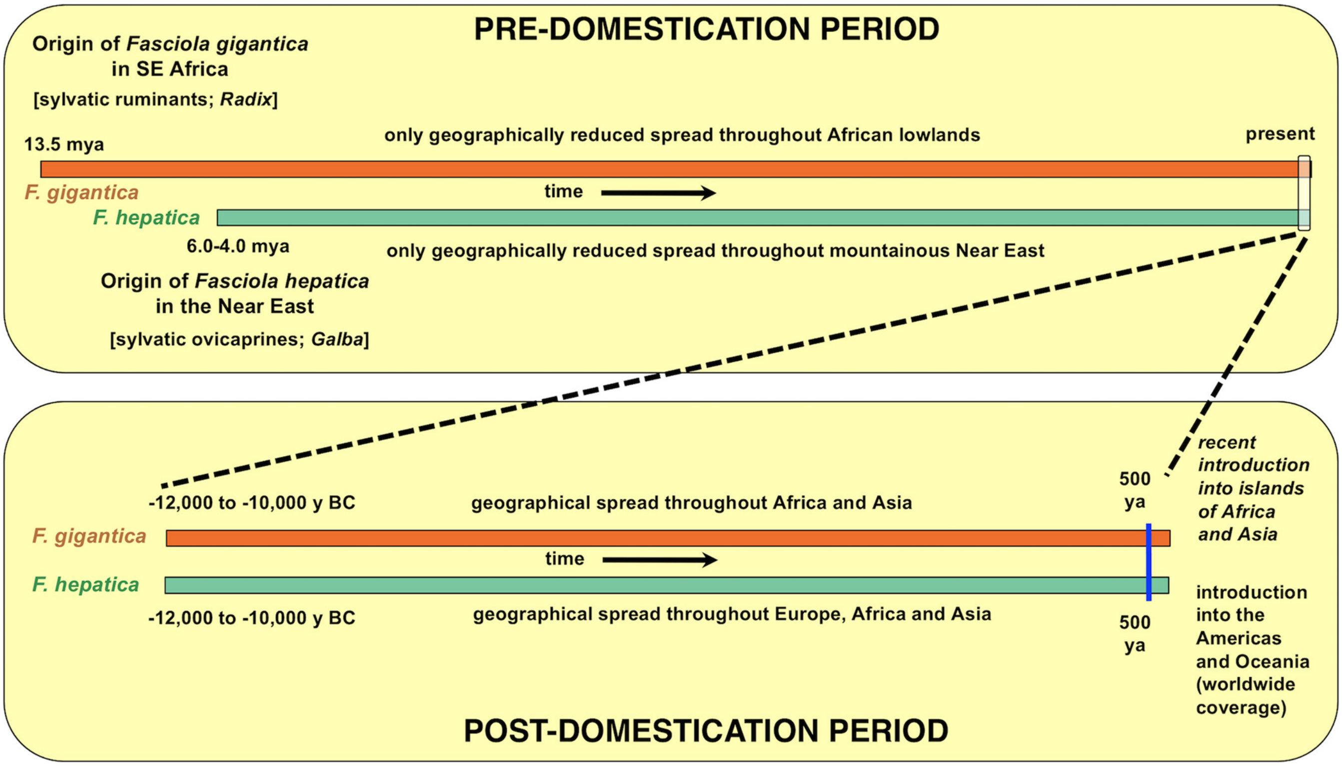

FIG 9 Chronological schema of the paleobiogeographical origins and evolution of *Fasciola gigantica* and *F. hepatica* along the predomestication period and the postdomestication period, including transoceanic spread in the last 500 years.

The distribution of *R. natalensis* throughout Africa fits well with the distribution of *F. gigantica*, so that only in countries lacking *R. natalensis* there is also *F. gigantica* absent, such as Morocco, Algeria, and Tunisia (1). The northernmost latitudes for *F. gigantica* include Mauritania in the West (174), southern Algeria in the center (67), and the Nile Delta in the East thanks to a northward expanding arm represented by the passive drag with animals and/or the Nile waters from the South (139).

Reports about *F. gigantica* infection in the wild fauna suggest that this fasciolid might have been able to spread considerably since its origin around 13.5 mya and before the arrival of humans, up to cover the large southeastern region, including lowlands of at least Uganda, Congo, Rwanda, Burundi, Kenya, Tanzania, Zambia, and Zimbabwe. Species such as the wildebeest, with well-known long north-south yearly migrations, may have contributed to this spread.

Domesticated animal species that, once infected by *F. gigantica*, could have participated in the spread of this fasciolid throughout Africa as a consequence of human-guided movements of livestock included goats, sheep, pigs, zebus, dromedaries, and donkeys (175). The human introduction of goats, sheep, pigs, and zebus into Africa is evident, because of the absence of their wild ancestors in this continent. Goats, sheep, and pigs began to appear in the Sinai and Egypt from 6000 BC and along the Nile, the Sahara, and northern African coasts from 6000 BC.

There was an additional introduction route from the Arabian Peninsula by which the dromedary entered. This animal first appears around 3000 BC in the Arabian Peninsula, a crucial animal for human development of numerous African populations. Remains of this camelid were found in Lower-Nubia beginning around 1000 BC, and its progressive spread up to the Sahara occurred subsequently. However, the way for its introduction either through Sinai-Egypt or the horn of Africa is still pending clarification. Another Asian species, the zebu, domesticated around 4000 BC in Pakistan, shows sufficient genetic probes indicating that it was introduced through the horn of Africa. The first documentation of its presence is in a painting from an Egyptian grave dated from ca. 1500 BC (175).

Another domesticated animal having played a crucial role in the development of African peoples is the donkey (176). The appearance/introduction of the domesticated donkey in Africa is not yet elucidated because the distribution of the wild donkey *Equus africanus* covered the North and East of Africa, the Near East, and the Arabian Peninsula. The first remains of domestic donkeys are from northern Egypt between 4500 and 3500 BC, and somewhat later more south in Sudan, whereas domesticated donkeys appear in the center and north of Africa only from the first millennium AC. Findings in the Near East and Arabian Peninsula from around 4000 to 3000 BC suggest possible additional domestication sites, although genetic diversity analyses furnish more support to northeastern Africa as the original area for its domestication (175).

The dromedary is known to be infected by *F. gigantica* in Egypt (177, 178) and, similarly, the donkey in Egypt (179), Ethiopia (180), and Chad (181). Donkey isolates of *F. hepatica* have recently experimentally been proved to be viable and infective throughout all life cycle stages until infection of a new definitive host. The donkey was therefore demonstrated to be able to play the role of reservoir (182). It may *a priori* be considered that it will be similar with *F. gigantica* in the donkey.

The relatively few nucleotide differences between the complete sequences of the haplotypes of the mtDNA *cox1* and *nad1* genes, with maxima of only 1.17 and 1.21% in pairwise comparisons, respectively, found in *F. gigantica* from Senegal, Burkina Faso, Algeria, Niger, and Nigeria (1, 67) indicate that the spread occurred in recent times. Moreover, the two haplotype groups which appear in the network analysis suggest a spread from East to West. This fits with the oldest civilizations such as Old Egypt and Nubia along the Nile and their connections with western Saharan human settlements, with certain nomadic tribes becoming specialized in large-scale breeding of cattle, sheep, and dromedaries during the 7th to the 15th centuries. Niger was the center of large commercial trans-Saharan caravan routes between North and South, East, and West (Fig. 10), and good transportation was ensured by donkeys and cattle as well as slaves (1). The recent findings of *F. gigantica* sequence identities and similarities indicating a south-north trans-Saharan geographical origin, with introduction from Ghana, through the Sahel countries of Burkina Faso and Mali into Algeria (67) further supports this evolutionary scenario (Fig. 11). In Chad, infection by *F. gigantica* in transhumant herds of nomadic people was highlighted already time ago (183) and has been put in focus again recently (184). In the neighboring Nigeria, there is ample literature on *F. gigantica* currently infecting sheep and goats (185), as well as cattle (186).

The report of a trematode in histological sections of a mummified liver in one of two brothers found in a tomb of the 12th dynasty of Old Egypt dating from 1938 to 1756 BC, classified as *F. hepatica* based on a flattened group of cells with thickened walls of the parasite (31), should have probably belonged to *F. gigantica* (but see the explanation below in section on the spread of *F. hepatica* in Africa).

The finding of new DNA haplotypes in a short 381-bp fragment of mtDNA *cox1* found mainly in Zimbabwe and one in South Africa (187) also fit the African scenario of *F. gigantica*. Indeed, a higher diversity may be expected in zones close to the area of origin of the species. The richness of 1,400-bp mtDNA haplotypes enclosing the regions coding for cytochrome *c* oxidase subunit III (coxIII), tRNA histidine (tRNA-His), and cytochrome *b* (cob) found in *F. gigantica* from Tanzania (131) speak in the same sense. It may be concluded that the wild fauna might have conserved old *F. gigantica* haplotypes and that several of them may have been transferred to livestock sharing the same areas inhabited by *R. natalensis*.

Bantu peoples, farmers and shepherds, probably also contributed to the spread of *F. gigantica* by means of their migrations 5,000 to 4,000 years ago (188) and when they gradually adapted to an agricultural and pastoralism life style. Starting from areas in the river Niger basin, the present Nigeria and Cameroun, they spread throughout central Africa up to the East and South along the so-called iron routes during the first centuries AC (1, 189). The southward spread of Proto-Khoisan peoples from Tanzania, Zambia, Malawi, and Mozambique around 1,000 years AC (190), originally hunters but later adopting agricultural life style due to their mixing with Bantu people (191), may have subsequently contributed to the further spread of *F.*

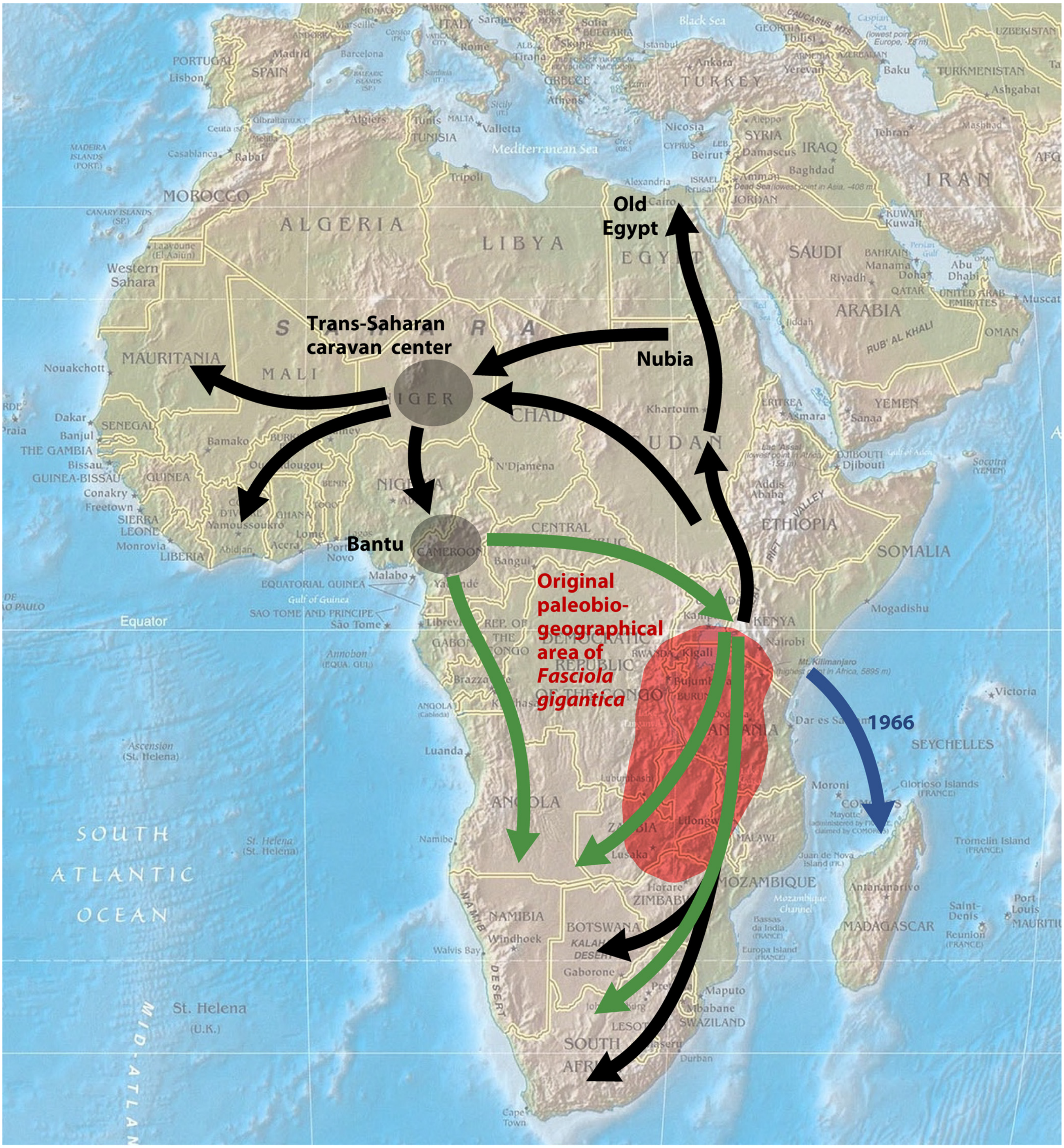

FIG 10 Main ancient spreading routes of *Fasciola gigantica* throughout Africa from its original paleobiogeographical area in southeastern Africa. Black arrows indicate the progressive westward spread of *F. gigantica* from the eastern oldest civilizations of Old Egypt and Nubia, with Niger as the center of trans-Saharan caravan routes; green arrows indicate the southward spread of *F. gigantica* with migrations of Bantu pastoralists from their origin in the river Niger basin. The approximate date of introduction into Madagascar is 1966 AC. For the domestic animal species involved and the dating of the movements, see the additional discussion in the text.

gigantica to more southern latitudes (Fig. 10). Indeed, *F. gigantica* poses a veterinary problem in lowland livestock along the whole East and South of Africa today (171).

In Madagascar, fascioliasis was imported only quite recently. The first reports of liver flukes in animals are from 1896, 1904, 1905, 1917, 1929, and up to mid-1900s, and even 1966 (192), although they only concerned *F. hepatica* imported with sheep from France and northern

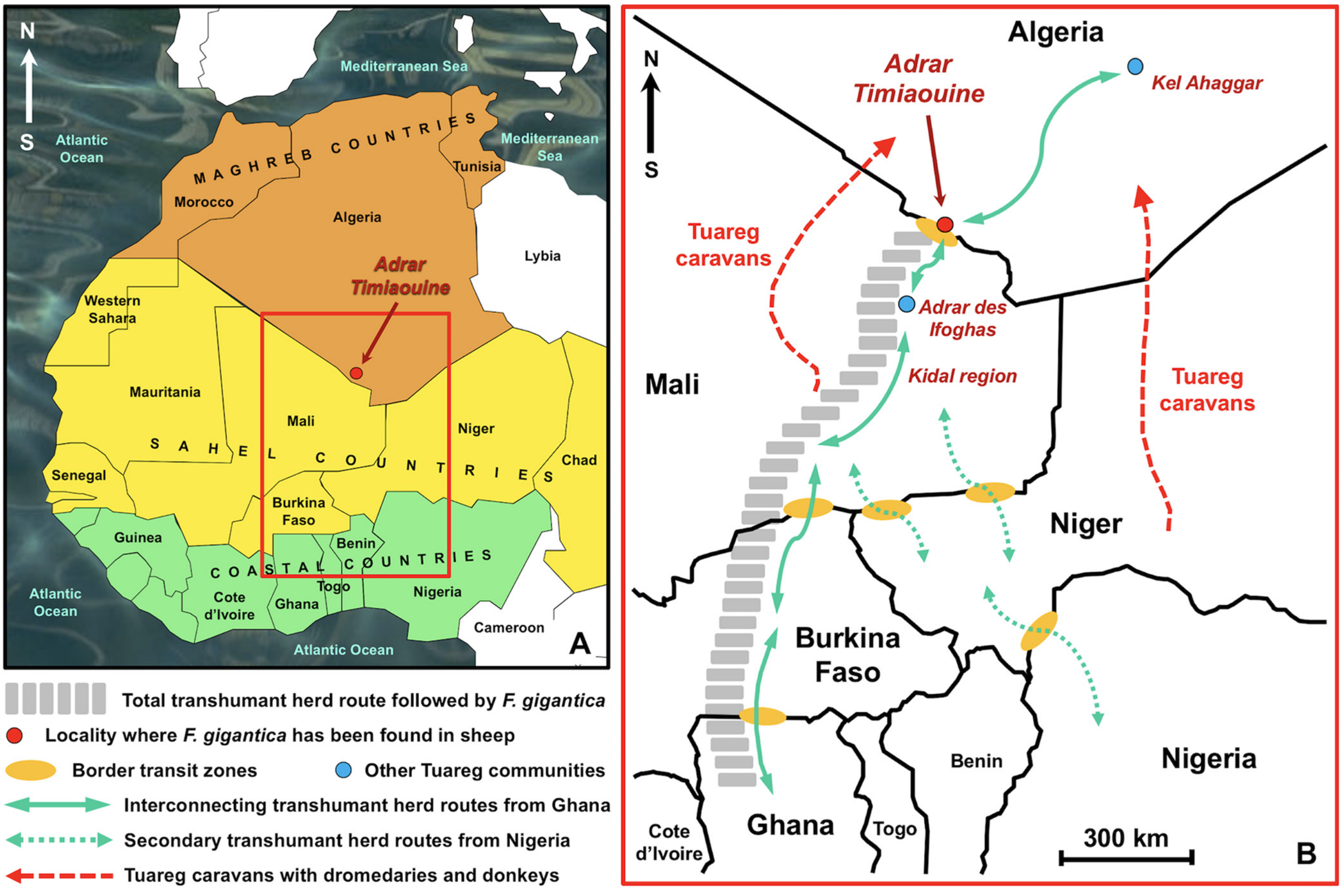

FIG 11 *Fasciola gigantica* infecting sheep in Algeria. (A) Map of western Africa showing the location of Adrar wilaya, Timiaouine community, where the fasciolid specimens were found in southern Algeria, among the frames of Maghreb countries, Sahel countries and coastal countries. (B) Map showing the introduction route of *F. gigantica* into Algeria, following several interconnecting herd transhumance routes by nomadic pastoralists from Ghana and through Burkina Faso and Mali. Other transhumance routes potentially involved cross Mali and Niger up to Nigeria. Dromedaries and donkeys in Tuareg caravans may have also contributed to its northern spread from Mali and Niger up to southern Algeria. Information on transhumance routes and caravans comes from various sources.

Africa, and later also cattle. Importations of sheep, goats, cattle, and pigs were also performed in 1945 and 1949 from South Africa (192, 193), where *F. hepatica* is also present. However, in 1966 *F. gigantica* was reported in zebu cattle for the first time in Madagascar, namely, in an area where temperature-tolerant Sahiwal zebu cattle imported from Kenya had been released (Fig. 10) (194). This fasciolid succeeded in its introduction thanks to its adaptation to native *R. natalensis*, the only lymnaeid species known to exist in Madagascar since long time (192, 195) and whose insular forms have been molecularly assessed (196). Subsequently, this fasciolid begun quickly to spread in 1967 up to cover almost the whole island in 1971 (193, 197). This progressive record history suggest that *F. hepatica* cannot be introduced in an area where *Galba*/*Fossaria* lymnaeid species are absent, despite the presence of *Radix* vector species able to transmit *F. gigantica*. This is a very important fact which is in agreement with the same conclusion recently reached in Ecuador about the crucial evolutionary role of the lymnaeid vector filter but concerning the opposite situation of the impossibility for *F. gigantica* to colonize *Radix*-absent areas inhabited by *Galba*/*Fossaria* lymnaeid species (32).

THE NEAR EAST, CRADLE REGION FOR THE POSTDOMESTICATION SPREAD

The wide multidisciplinary analysis at worldwide level indicated a founder effect for the postdomestication spread of *Fasciola* species, which converged on the Fertile Crescent as the area of origin (1). Recent results of multidisciplinary studies of *F. hepatica* and *F. gigantica* reinforce this conclusion. However, recent archeological investigations have shown that the region of western Asia where domestication of goats, sheep, cattle, and pigs occurred was wider and that all this phenomenon began to happen earlier than initially considered

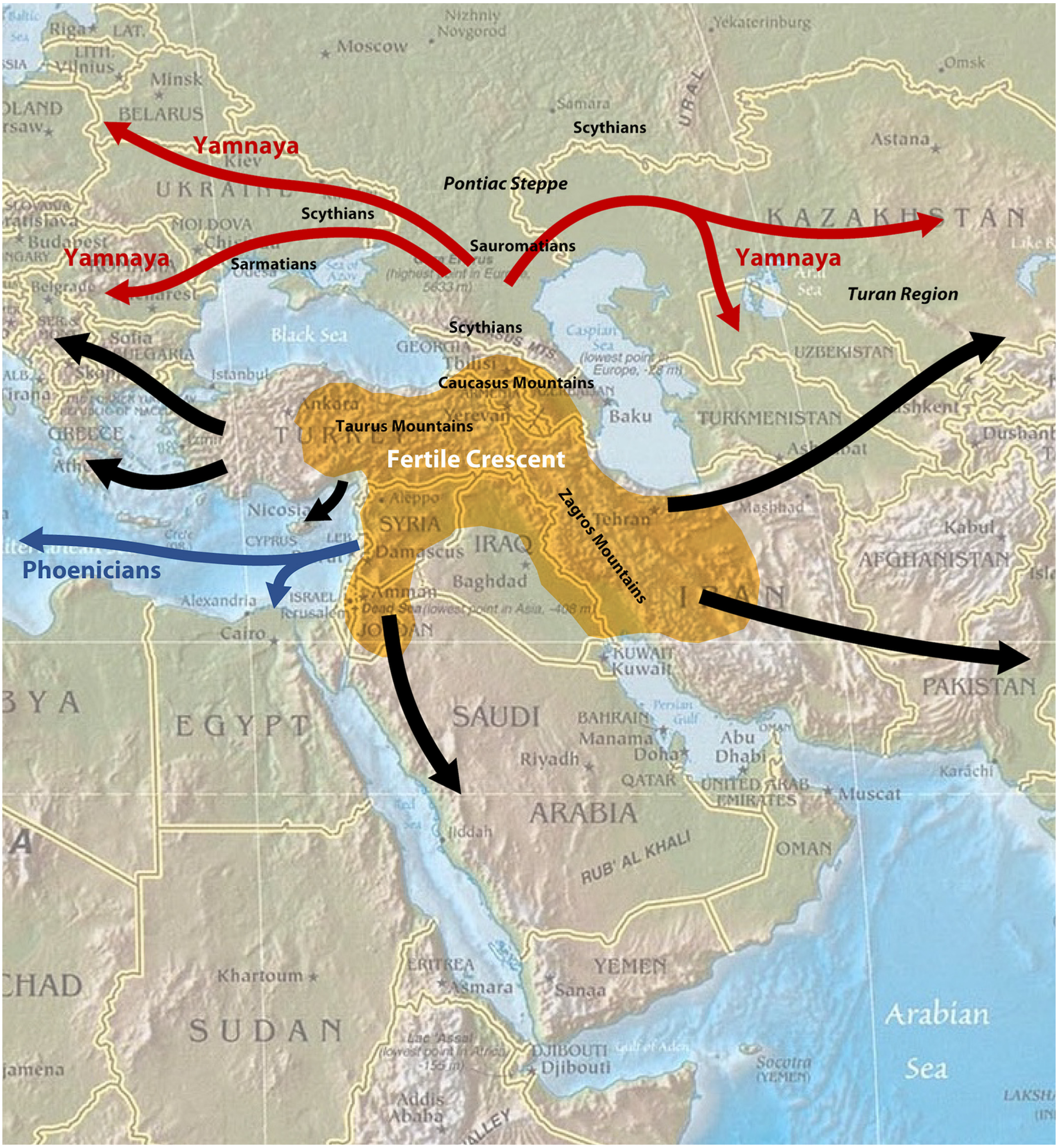

FIG 12 Main Neolithic spreading routes from the Near East cradle used by fasciolid flukes to expand into Europe, Asia, and the Arabian Peninsula. Black arrows indicate the oldest ways from the Fertile Crescent. Red arrows indicate the westward and eastward spreading routes by the Yamnaya seminomadic pastoralists from the steppes northward of the Caucasus. Blue arrows indicate the westward spread by the Phoenician navigators with their old vessels along the Mediterranean Sea from their Levantine origin. For route dating, see the text. Note the absence of an arrow between the Fertile Crescent and Old Egypt, illustrating that *F. gigantica* colonized Egypt from the South and *F. hepatica* is supposed to have entered through the Nile Delta only by recent livestock importation from Europe in the first half of last century.

(158). Thus, the region in question includes from the Zagros mountains and eastern Mesopotamia flatlands in Iran, in the East, up to the Taurus mountains and Anatolia in present Turkey, and current Syria, Lebanon, Jordan, and Israel in the Levant, in the West (Fig. 12) (158, 198).

The past existence of potential hosts of fasciolids such as bovids, cervids, suids, and equines has been detected in a cave in the western-central Zagros, whose remains were dated around 75,000 years BP (199). All suggests that midsized bovids as the ovicaprines played a crucial role in the host capture phenomenon leading to the speciation of *F. hepatica* (1). The goat's wild ancestor, known as the bezoar *Capra aegagrus*, was widely distributed from the Taurus mountains and easter Anatolia in Turkey in the West, along all the Zagros mountainous chain throughout present Irak and Iran, down to the Iranian plateau (155, 200). The sheep's wild ancestor, the mouflon *Ovis gmelini* (=*O. orientalis*) characterized by presenting 54 chromosomes, was distributed from southeastern Anatolia to the Zagros mountains (201). Initial domestication steps with goats and sheep occurred around 11,000 years BP, and pigs and cattle domestication in that area started around 10,500 and 10,000 years BP, respectively. These dating estimations were 9,000 to 8,500 years BP in Mesopotamia, 10,000 to 8,500 years BP in Anatolia, and 9,600 to 8,500 years BP in the Levant (158).

In Irak, the two fasciolid species are present. *Fasciola hepatica*, together with *F. gigantica*, has been molecularly identified in livestock in the Duhok governorate, Kurdistan region, northern part of the country, at the foothills of the Zagros mountains (202, 203). The diagnosis of fascioliasis in several patients in the governorate of Sulaimaniyah, as well as in the Zagros foothills but in northwestern Irak, is geographically also worth mentioning (204). *Fasciola hepatica* has also infected children and cattle, sheep, and goats in Babylon City (205) and animals even southward, in Mosul, coinfected with *F. gigantica* (206). *Fasciola gigantica* has also been reported in central Irak (207), with a high prevalence in water buffalo (208), and in the southeastern Basrah close to the Iran border (209).

In Iran, *F. hepatica* and *F. gigantica* were reported in buffaloes, cows, sheep, and goats in the Khuzestan, in the southwest of the country neighboring Irak (210) and have more recently been molecularly confirmed (211). Interestingly, the morphological studies showed the existence of specimens with slight differences, which were noted to resemble *F. indica* from India (210), today recognized as intermediate forms originated by hybridization of the two fasciolid species.

In Turkey, fascioliasis causes great economic losses (212), and the existence of the two fasciolid species has molecularly been confirmed (213–215). The species *F. gigantica* is distributed throughout the country (216), usually infecting livestock at low rates, except in coastal regions and the eastern provinces (217). This fasciolid has also been diagnosed in humans in southeastern Anatolia (218–220). *Fasciola hepatica* is also widely distributed in livestock in Turkey, from localities as Bursa in the West and Samsun in the North up to the whole eastern and southeastern parts of the country (221–225). The pasture-grazing seminomadic sheep, goat, and cattle populations, maintained since ancient times, have been progressively decreasing over recent years because of the decrease of total coverage of grasslands, people preferring modem life in the cities, and industrial animal husbandry replacing the old style, especially for cattle breeding (224). There are many human cases of reported infection by *F. hepatica* in Turkey, but unfortunately in most of the reports the specific diagnosis remains unverifiable because they rely on serology, symptomatology, image techniques, lack of egg measurements, and an absence of scale in the photograph of an egg shed by the patient. In cases in which at least a photograph of the fasciolid adult recovered is furnished allowing for the ascription to *F. hepatica*, the following areas were concerned: Van (226), Elazig (227), Giresun (228), Cukurova (229), Adana (230), and Antalya (231). The geographical origin of the patient in these reports concerns the eastern and southeastern parts of Turkey, namely, the old zone where the earliest animal domestication took place or close to it (158).

In Syria, both *F. hepatica* and *F. gigantica* infect sheep (232) and goats (233). *Fasciola hepatica* appears to be restricted to the wet regions of Syria, especially the former marsh areas, where up to 60% of the sheep are infected. This fasciolid is also found in 97% of the cattle, in which only 3% are infected by *F. gigantica* (234).

In Lebanon, *F. hepatica* was found by ERCP in the common bile duct of a Lebanese native (235). In Israel, the epidemiology of *F. hepatica* was assessed (236). In Jordan, there were also regular patterns of transhumance, following traditional routes and associated with particular tribal and subtribal groupings (237). *Fasciola hepatica* also appears to infect domestic animals in this country, but the numerous importations from other countries such as Romania, Australia, Turkey, Saudi Arabia, and Somalia undoubtedly mask the picture because many imported animals are left grazing in open fields (238). Interestingly, *F. hepatica* has also been detected in the dromedary (239). In this country, *F. gigantica* has also been reported in Azraq (240).

The northernmost extent of the geographical distribution of *R. natalensis* in Jordan in the past and today (166, 241) and also Palestine (139), an area which probably was continuous with the presence of this lymnaeid species in the Arabian Peninsula (242), indicate a coexistence of this *F. nyanzae*/*F. gigantica* original vector with the past and current coexistence of *R. auricularia* and *G. truncatula* throughout this region. This coexistence of the three lymnaeid species is unique.

The species *R. auricularia* appears to be widely present throughout the aforementioned region, including highly polymorphic shells in the Quaternary in the plain of Lower Mesopotamia (243), and currently appearing as pronouncedly aquatic in permanent, still, or slow-flowing water and temporary collections of water of this plain (244), as well as in central Irak (207). The marked polymorphism of this species in the lowlands of southeastern Irak has been recently malacologically and molecularly analyzed (245, 246). This lymnaeid has also been reported from the lowlands of Khuzestan Province (247) in Iran, in the Mediterranean Region, in the Central Anatolia Region, in the Black Sea Region, and in the Southeastern Anatolia Region in Turkey (248), in Lebanon (249), in Israel (250, 251), and in Jordan (166).

The lymnaeid *G. truncatula* is also widely distributed throughout this Near East region, including Irak (205, 244, 252), in the Khuzestan Province (247) and in the Zagros mountains (M. D. Bargues et al., unpublished data), both in Iran. In Turkey, it has been reported in the regions of Marmara, the Aegean, the Mediterranean, Central Anatolia, the Black Sea, Eastern Anatolia, and Southeastern Anatolia (248). It has been also found in Lebanon (249), Israel (253), Palestine (254), and Jordan, both in an archeological site between 14,000 and 15,000 years BC, close to Lebanon and Syria (255), and also today (166). In Israel, the invasive lymnaeid vector *P. columella* has additionally been reported (251).

The wide geographical distribution shown by both the two fasciolid species and the lymnaeid species throughout this Near East region should have undoubtedly been a consequence of pastoralism and transhumance, including movements of sizable herds over large areas, as it was along great distances in eastern Turkey (224), and in the Central Zagros mountains with neighboring plains (256–258). Sheep and goat were mainly involved in these movements, although cattle, swine, domestic horse, and Bactrian camel have been also found to participate (259).

The aforementioned summarized frame of fasciolids and lymnaeids in the Near East is a worldwide unique scenario of overlap of *F. hepatica*, *F. gigantica*, *G. truncatula*, and *R. auricularia*, to which *R. natalensis* is added in its southern part. Moreover, there are other aspects worth mentioning. On one side, both newly hatched and old specimens of *G. truncatula* from this region proved to be susceptible to infection with *F. gigantica* (244). The opposite was also observed, i.e., *R. auricularia* (=*R. gedrosiana*) transmitted *F. hepatica* at 25 to 28°C (260). Although one might think that hybrid fascioIds could be involved in these old experiments, this local cross-susceptibility may be also interpreted as evolutionary remains of the original area where speciation began. Another feature to be emphasized is the high temperature under which *F. gigantica* and *R. auricularia* interact in this region: *F. gigantica* egg hatching occurs at 25°C (215), with a minimum time required for hatching found even at 30°C (210), and maximum production of cercariae at 25°C, significantly decreasing at 19 and 30°C (261). This characteristic is crucial to understanding how *F. gigantica* was able to spread eastward throughout the warm flatlands of southern Asia up to Southeast Asia. It is evident that this Near East region reunites all the characteristics supposed for the geographical origin and the cause of the founder effect nowadays observed in the worldwide distribution of *F. hepatica* and the Asian spread of *F. gigantica* (Fig. 12) (1).

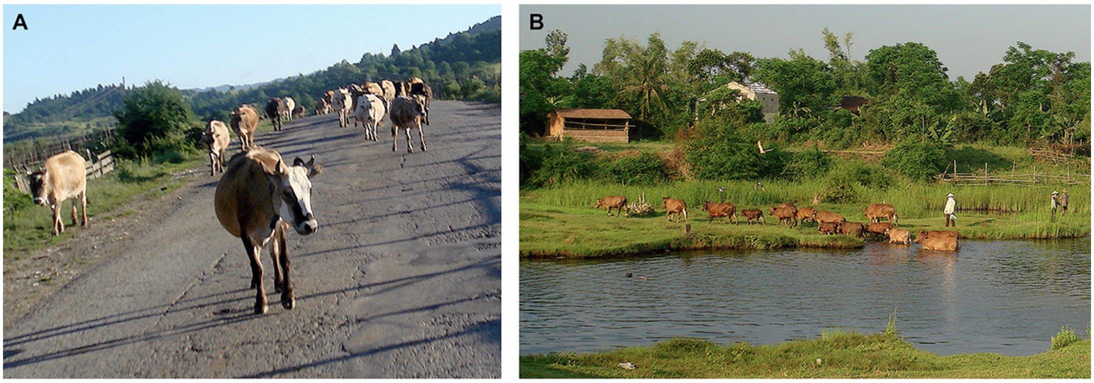

FIG 13 Livestock management plays an important role in the spread and seasonality of fascioliasis. (A) Vertical transhumance in the mountains of the Caucasus. (B) Management of cattle herds to and from lymnaeid-inhabited freshwater collection in Quang Nam, Vietnam.

FASCIOLA HEPATICA SPREAD INTO EUROPE

Fasciola hepatica is the only *Fasciola* species in mainland Europe, covering the whole continent except the northernmost higher latitudes of Norway, Sweden, and Finland because of liver fluke temperature thresholds, as well as Iceland because of the absence of appropriate lymnaeid vector species. *Fasciola gigantica* never colonized Europe, except its southeasternmost Caucasian part. In Georgia, *F. gigantica* was reported in humans twice (262, 263), although recent wide prospections throughout the country were not able to rediscover it (S. Mas-Coma et al., unpublished data). This fasciolid is, however, still present, although less frequently than *F. hepatica*, in livestock in the southern neighboring Armenia, where the mtDNA *nad1* gene showed a maximum genetic distance between haplotypes in global networks of 49 nucleotide steps for *F. gigantica* compared to only 15 for *F. hepatica* (264), and one interesting transversion was detected in the rDNA ITS-2 of *F. hepatica* specimens (265). An old colonization by *F. gigantica* from Anatolia (present-day Turkey) in the East and present-day Iran in the South may be interpreted (Fig. 12), whereas additional recent introduction(s) by *F. hepatica* from mainland Europe may not be ruled out. This fits the knowledge on pastoralism in the Anatolian-Transcaucasian interaction in the fourth to the third millennium BC (266). The Kura-Araxes ethnic groups inhabited the South Caucasus, including the current nation-states of Georgia, Armenia, Azerbaijan, and northeastern Turkey, from 3,500 to 2,450 years BC. In the Caucasus Mountains, vertical transhumance is still practiced today (Fig. 13). These ethnic groups migrated southwest across a wide area from the Taurus Mountains down into the southern Levant, southeast along the Zagros Mountains, and northward across the Caucasus Mountains (267). Mobile sheep and goats guided by these ethnic groups are known to have increased in the third millennium BC (268).

The extreme rarity of *F. hepatica* infection in the wild fauna of eastern Europe, just found only in a few mammal species (269, 270), of which only *Alces alces*, *Capreolus capreolus*, and *Cervus elaphus* among the ungulates (269), indicates secondary infections from local livestock sources. Infection by *F. hepatica* has also been reported from different populations of the European bison *Bison bonasus*. This animal was totally exterminated from Europe in the 18th century, and only a few specimens survived in zoos. The Bialowieza National Park, in the Poland-Belarus border, was the first to launch a reintroduction of this species in 1929, with specimens from the Western Caucasus where the bison was to become extinct just a few years later, and from various zoos. Other east-European parks have followed this initiative recently (271). The molecular characterization of *F. hepatica* specimens infecting the bison in Bialowieza clearly indicated an infection source from domestic livestock (272, 273). Indeed, *F. hepatica* is known to infect bovines in the area around Bialowieza (274), and *G. truncatula* is widely distributed throughout Europe (90).

Regarding western and northern Europe, it is well known that the Neolithic expansion into Europe was by migration from an origin in settlements in the Near East around 10,500 years BP, and through subsequent settlements in the Aegean and Balkans established around 2,000

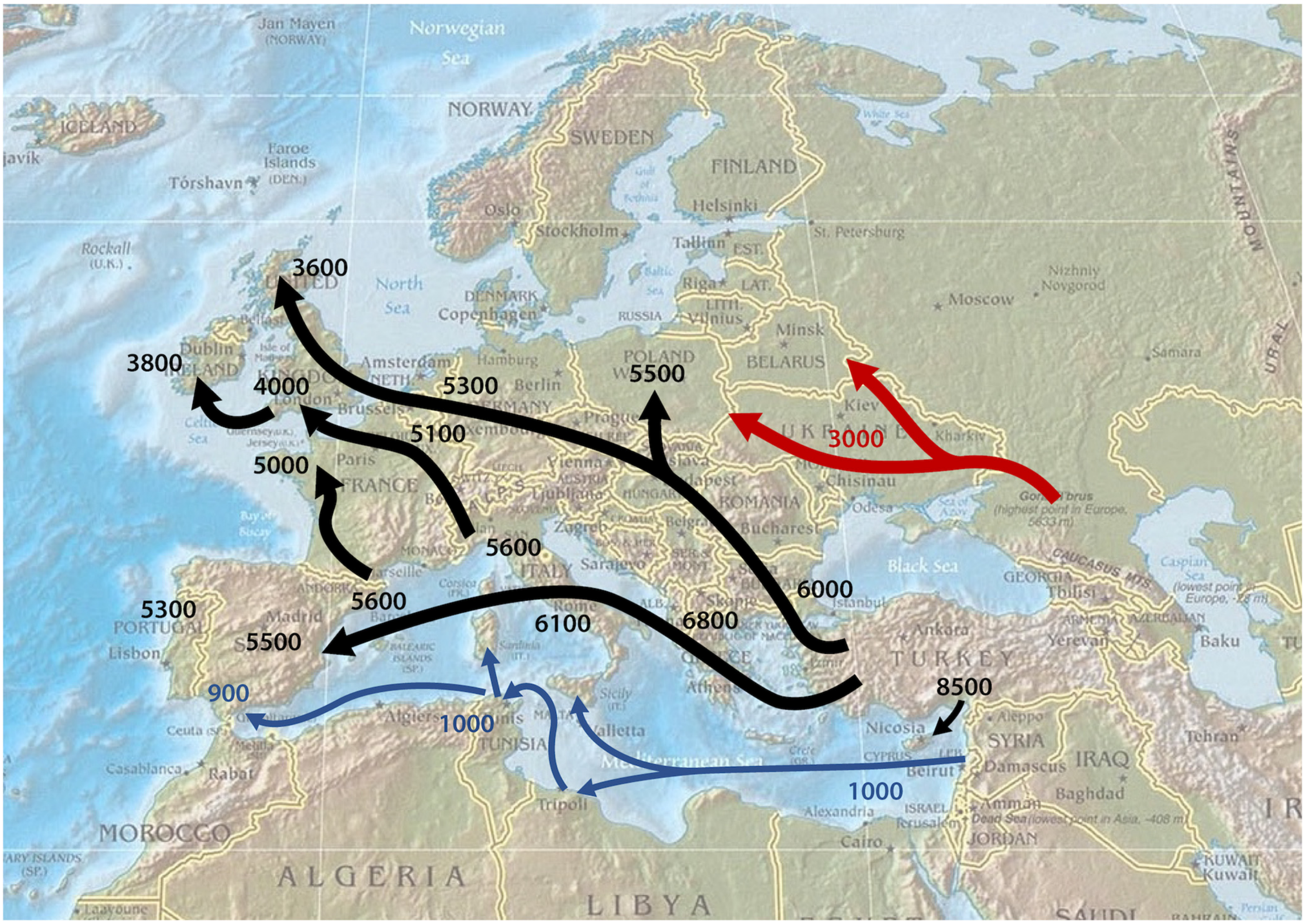

FIG 14 Main Neolithic spreading routes from the Near East cradle used by *Fasciola hepatica* to expand into Europe. Black arrows and respective dating indicate sheep spread according to archeological remains. Red arrows indicate the Yamnaya pastoralists. Blue arrows indicate the routes of Phoenician navigators. Dates are expressed in years BC. For more information, see the text.

later (Fig. 12). All archeological data indicated a crucial role of the eastern Mediterranean coastal areas in this initial spreading process, among which the geographical strip of the Levant, including present-day western Syria, Lebanon, and Israel (Fig. 14). Stéphanie Harter, in her Ph.D. thesis, prepared while on the Paleoparasitology expert team of the Université de Reims, Champagne, France, found four *F. hepatica* eggs with length/width measurements of 126.2 ± 7.96/78.38 ± 9.36 μm in a latrine of the archeological site of Beit Shean in Israel dated 200 to 300 years AC (275).

Of higher interest is, however, the discovery of five *F. hepatica* eggs measuring 123.18 ± 6.27/78.52 ± 5.08 μm in sepultures found in the archeological site of Shillourokambos, on the island of Cyprus, dated in the 8th and 7th millennia BC (275, 276). These findings are by far the oldest archeological record of *F. hepatica* (277), and they indicate that this fasciolid was infecting domesticated animals probably from the earliest beginning of the domestication process in the initial core area (Fig. 12) and that it spread throughout the Near East quite rapidly as a consequence of the aforementioned human-guided animal movements.

Indeed, more recent data have indicated that human migration from the mainland into Cyprus took place between 11,100 and 10,600 years BP, that is, shortly after the beginning of agriculture, although they introduced goats, sheep, cattle, and pigs to the island only a short while later, around 10,400 years BP (278), The absence of ungulates on the island at the early beginning of the Cyprus colonization agrees with the absence of domestic ungulates in the immediately neighboring region of the mainland, even during the Late Pre-Pottery Neolithic A (PPNA = 11,500 to 10,500 years ago) or the beginning of the Early Pre-Pottery Neolithic B (PPNB = 10,500 to 9,000 years ago), although an insufficient capability of earlier seafaring vessels cannot be ruled out. Traveling the 60 km to Cyprus by boat, these colonists from somewhere in the Northern Levant transported the complement of mainland fauna, including all four major livestock species of sheep, goats, cattle, and pigs (279). The parasite *F. hepatica* was able to maintain its life cycle thanks to the presence of *G. truncatula* in the past, as it was found in an area of the island neighboring wells dated from 7,200 to 6,800 years BC (280), and continues to be present on Cyprus still nowadays (281). This lymnaeid could have been passively introduced in mud attached to the hooves of the livestock species transported from the Levant, although an earlier arrival to Cyprus cannot be discarded if the previous insular fauna is considered, as it is the case of the pygmy hippopotamus *Phanourios minutus* (also known as *Hippopotamus minor*) whose extinction on the island is dated only 11,000 to 9,000 years ago, probably accelerated by the presence of humans (282).

After the early colonization of Cyprus, the subsequent westward spread of Near East farmers into Europe seems to have followed two main ways, both showing an initial step in the Aegean (Fig. 14).

One would have used the maritime way from Cyprus to Crete and further westward through the Mediterranean by pioneer seafaring colonization (283, 284), additionally supported by similarities with Sardinian inhabitants (285), and subsequent migration northward into the mainland as southern France and in the Apennine Peninsula (286). Recent data suggesting that the old Etruscans, a distinctive group who dominated central Italy through the first half of the first millennium BC, did know about the infection of their sheep by *F. hepatica*, provides additional support (26), although in that case an arrival through northern Italy as part of the second mainland trajectory cannot be ruled out (see below). The feasibility of the introduction of domesticated animals within sea vessels has been already highlighted in the aforementioned analysis of Cyprus. Midsized goats and sheep could have been transported from island to island and/or along the Mediterranean coast without great problems. It should be taken into account that neither goats nor sheep were present in Europe before this point, which means that these midsized ruminants were introduced from the Near East (201). The genetic relationships between the sheep of the Iberian Peninsula and sheep lineages only found in the Middle East and Asia (287, 288) suggest an old flow from the Near East to the Iberian Peninsula (1), dated around 5500 BC (289). Taurine cattle were also involved in this Mediterranean trajectory by boat, although a low genetic diversity points to a low effective cattle population arriving in the Western Mediterranean (290), easily understandable when considering the less manageable big size of this animal inside the sea vessels of that time. Iberia subsequently played a role in the additional eastward spread into Europe from 4,500 years BC until connecting with the terrestrial spreading wave coming through mainland Europe from Anatolia and present-day Greece (Fig. 14). The findings of the markedly amphibious lymnaeid snail *L. schirazensis*, a well-contrasted biological marker of livestock movements, in the Nile Delta and the Mediterranean coast of the Iberian Peninsula (4) further support this maritime trans-Mediterranean spread.

The other way is concluded from recent genomic analyses which have furnished evidence suggesting that it was for the farmers of northwestern Anatolia, i.e., present-day Turkey, to have been also involved in the initial spread into Europe, according to the studies performed in the Kumtepe archeological site, at only 2.5 km away from the Strait of the Dardanelles and 2 km away from the Aegean Sea (Fig. 12 and 14) (285). It is evident that the introduction of livestock through this narrow Strait would have been markedly easier than jumping from Cyprus throughout many Aegean islands until entering the European mainland. Another approach by mathematical modeling concluded that this way was the origin of a wider European mainland colonization (289). Concerning this nonmaritime spreading way, it should be emphasized that the recent results demonstrate that the spread into and across Europe occurred together with the migration of people and not by the dissemination of ideas (284). This means that people migrated with their livestock, which allows us to understand the spread of both *F. hepatica* and *G. truncatula* until covering whole Europe. Estimations date the arrival of domesticated sheep into Greece around 6,800 years BC, northern Italy and Switzerland around 5,600 years BC, Poland around 5,500 years BC, Germany and northern France around 5,300 to 5,100 years BC, Great Britain around 4,000 years BC, and Ireland around 3,800 years BC (Fig. 14) (201). Taurine cattle introduced by this second mainland trajectory appear to have followed a parallel spread finally reaching Central and Western Europe around 5,500 years BC and Northern Europe around 4,100 years BC. Interestingly, the taurine cattle gene flow between the Near East-Anatolia region and Europe shows a total reduction to 0 around 5,000 years BC (290).

The Phoenicians around 3,000 to 1,200 years BC were engaged with livestock breeding, including sheep, goats, and cattle (291), and also the Greeks and later the Romans should have further contributed to the spread of the liver fluke throughout the Mediterranean, and in the case of Romans also deep northward into mainland Europe. Indeed, there are signs of animal farming in Greece dated around 6,500 years BC, and in the ancient Hellenic societies, animals were essential for the practicalities of farm work and food production, manufacture of garments, transport, war, hunting and sacrifice (292). The Romans had a great interest in improving mainly cattle breeds and secondarily sheep and goat breeds, while during the Middle Ages most effort was put in improving sheep breeds (293, 294). The Arabs should have probably also influenced liver fluke distribution by livestock improvements throughout northern Africa and into the Iberian Peninsula through the Gibraltar Strait (1), as observed in Portugal (295).

Moreover, recent genetic studies have shown the role developed by the Yamnayas, a tribe of seminomadic pastoralists originated from the steppes northward of the Caucasus, in the period from around 4,500 years BP during the transition from the Copper Age to the Bronze Age. During the Early Bronze Age, the Caucasus was in communication with this Eurasian steppe of the grasslands north of the Black Sea and the Caspian Sea of present-day Russia. The Yamnayas steppe herders had an important influx by migrating westward into Europe (Fig. 14) and also eastward into Asia (Fig. 12). This influence proved to be higher in northern Europe, and explain some Indo-European languages in eastern Europe in the third millenium BC. They might represent an additional entry for *F. hepatica* and perhaps also *G. truncatula* into Europe (296, 297).

Findings of *F. hepatica* eggs in archeological sites in Europe are numerous, considering the difficulties posed by this type of archeoparasitological studies and suggest that liver fluke infection in both animals and humans was usual, which in its turn indicates (i) proximity and interdependence of humans and livestock, (ii) the inclusion of freshwater vegetables in the human diet in these old times, as for instance watercress as highlighted by the existence of watercress sellers in the streets noted in a book published in 1545 (298), and (iii) drinking of natural water from freshwater collections inhabited by *G. truncatula* and frequented by livestock as drinking source, a scenario still found nowadays in many rural areas of human fascioliasis in low-income countries (14, 96).

The oldest discoveries of liver fluke eggs in mainland Europe are from Switzerland around 5,900 to 4,900 years and 5,384 to 5,370 years BP, France around 5,600 years BP, the Netherlands around 5,400 to 5,230 years BP, and Germany around 4,500 years BP (277). In the Middle-Age period, *F. hepatica* egg findings throughout mainland Europe concern the centuries XI to XIII, XIII to XV, XIV and XV, XVI, XVII and XVIII, XVIII, and 800 years AC (298, 299). All these archeoparasitological reports fit well with the dating estimations of the aforementioned gradual spread of the domesticated ruminants throughout Europe.

The DNA marker sequencing of *F. hepatica* flukes infecting sheep and cattle from Greece, Bulgaria, and Poland furnished basic lineages but also additional mtDNA haplotypes differing between the populations of the three countries (300). These results were in need for speculation referring to different hypotheses tracing back to predomestication times. The aforementioned scenario of *F. hepatica* spreading ways with livestock introduced into Europe by pastoralists from different geographical sources now allows for an explanation that appropriately fits the postdomestication period. Considering the geographical location of these three eastern European countries, peculiar haplotypes in Greece should be linked to the first Aegean step of the Mediterranean spread originated in the Levant (201), the specific haplotypes in Bulgaria should be related to the second mainland spread entering through the Strait of the Dardanelles (285), and the singular haplotypes in Poland may be understood by considering the third liver fluke colonization way by the Yamnayas (296).

FASCIOLA HEPATICA AND *F. GIGANTICA* SPREAD INTO ASIA AND THE PACIFIC ISLANDS

In the Asian continent, the historical movements of mankind offer the highest complexity of spreading and mixing routes for fasciolid expansion during the postdomestication period. A great amount of new knowledge about human-guided movements of livestock and pack animals throughout Asia has been obtained in the last 2 decades, and there are still many regions for which our historical knowledge is still incomplete. The newly provided historical background, together with very recent international exchanges of livestock by country importation and exportation, is, however, sufficient to understand and explain the highly remarkable spectrum of phenotypic forms, hybrids, spermic and aspermic, diploid, triploid, and even mixoploid fasciolids reported from this continent.

Zagros and Northern Caucasus as Geographic Origins for the Eastward Expansions

There are two regions that might have played a role in the evolutionary origin for the eastward spread of *Fasciola* species into Asia during the Neolithic period. One was the southern original region including the Zagros long mountainous chain, which is considered the main cradle of eastward spread (301) and from which the first herders began to expand around 8,000 to 7,000 years BC, mainly by vertical transhumance from the mountains down to the eastern plains, routes still followed by pastoralist tribes in recent times (Fig. 12) (302).

Archeological remains have illustrated the faunal exchange linked to pastoralism that existed between the Zagros mountains and the eastern neighboring plains during the Neolithic period (256–258). The two main species involved in these movements proved to be goats and sheep, although cattle (Fig. 15), swine, domestic horses, and Bactrian camels (Fig. 16) were also participating (259). Domestic goats were the only ungulates archeologically found 1,000 km eastward from Zagros around 7,000 years BC, namely, in the Alburz in the North and in the Fars in the South of present-day Iran. Domesticated sheep and cattle appeared in the same places a little bit later. These findings proved that the eastern expansion took place simultaneously in the South along the Persian Gulf and in the North of the Iranian plateau (Fig. 12) (201).

The first archeological report of *F. hepatica* eggs in whole Asia concerns the finding of fasciolid eggs measuring 111 to 134 μm and 71 to 94 μm in coprolites from a donkey. The biological remains were found in the Chehrabad salt mine archaeological site, at an altitude of 1,663 m a.s.l. in Zanjan province, northwestern Iran, and dated back to the Sassanid period, from 224 to 651 AD. This donkey was probably the present-day endangered Persian onager *Equus hemionus onager*, the Asiatic Wild-ass native to the deserts of Iran. This finding proves the past existence of *F. hepatica* in that region and indicates that, at least at the time, this fasciolid had already spread through the Zagros mountains eastward from the Fertile Crescent. Nevertheless, the findings suggest that it should have been there since long before. Eurasiatic wild asses were present in the region and neighborhood of the Fertile Crescent when the domestication of the livestock reservoirs of *Fasciola* began (27). That study concluded that during the Sassanid period, sheep, goats, cattle, and also horses, mostly used for the army, as well as humans, are likely to have been infected by *F. hepatica*, with the infection risk mainly focused in the highlands. Note that, around 620 years CE, the Sassanid Empire expanded up to present-day Pakistan in the East and even, although only during a short period of 26 years, up to cover the whole Fertile Crescent zone (27). Consequently, both *Fasciola* species could benefit from this period for their spread. It should also be emphasized that the Chehrabad salt mine archeological site lies very close to the Talesh Mountains, where (i) recent archeological evidence has suggested that vertical transhumance pastoralism with the eastern lowland was practiced around 6,500 years ago (303), (ii) a zonal overlap of *F. hepatica*/*G. truncatula* in the highlands and *F. gigantica*/*R. auricularia* in the lowlands has been observed and has been both phenotypically (106) and genetically (167, 304) verified, (iii) Guilan lowlands, where *L. schirazensis* showing DNA marker sequences identical to those in the Nile Delta of Egypt and the Iberian Peninsula has been found in several places (4) and from which molecularly identical snail species specimens have recently been found in three additional localities farther

FIG 15 Large and midsized ruminants are the most efficient spreaders of fasciolid flukes and lymnaeid snail vectors. (A and B) Zebu cattle and sheep illustrated on the stone walls of the ruins of the ancient city of Persepolis dating back to 515 BC, at a 1,630-m altitude in present-day Iran. (C) Sheep and goats in night camp of Bakhtiari nomads practicing vertical transhumance in the Zagros Mountains in present-day Iranian province of Yasuj. (D) Herd of sheep and goats besides irrigation canal inhabited by *Radix natalensis* in an area of human fascioliasis hyperendemicity in the Nile Delta, Egypt. (E) Water buffaloes refreshing in freshwater collection in the Caucasus flatland endemic area of fascioliasis. (F) Yak for selling at the market of the Silk Road trading center of Kashgar city, in Xinjiang, China. (Panel F is slightly modified from https://commons.wikimedia.org/wiki/File:Kashgar_yak.jpg, by Bernard Gagnon, licensed under the Creative Commons Attribution-Share Alike 3.0 Unported, 2.5 Generic, 2.0 Generic, and 1.0 Generic licenses.)

southward in the Iranian province of Kerman (305), and (iv) Guilan lowlands, where the greatest public health problem caused by human fascioliasis is known to occur in Iran (106).

In Iran at present, *Fasciola* infection has been reported in sheep, goats, cattle, buffalos, camels, donkeys, horses, and also humans, geographically covering most of the country but with higher infection rates in its northern part because of the more appropriate ecological and climatic characteristics for the liver fluke transmission (306). The distribution of *F. gigantica* and *F. hepatica* inside Iran appears to be marked by the distribution of the respective specific lymnaeid vectors *R. auricularia* and *G. truncatula*. Whereas *R. auricularia* appears widely present throughout the country except in altitude zones and appears as the only lymnaeid in the southern warm and arid zones, *G. truncatula* shows a preference for cooler areas in the North and also in altitude areas, including a distribution overlap of both fasciolids in several zones.

FIG 16 Domestic camelids played a crucial role in the spread of fasciolid flukes in Asia and Africa during the postdomestication period. (A and B) Two-humped Bactrian camel and one-humped dromedary appear already domesticated on the stone walls of the ruins of the ancient city of Persepolis dating back to 515 BC, at a 1,630-m altitude in present-day Iran. (C) Dromedary caravan in Asia illustrated in the Catalan worldmap created by the Majorcan cartographic school in the year 1375 and preserved in the Bibliothèque Nationale de France, Paris (reproduced from facsimile in private collection). (D) Dromedaries grazing on lymnaeid-inhabited water drainage canal of neighboring crop field in an area of fascioliasis hyperendemicity of the Nile Delta, Egypt. (E) Caravan of pack Bactrian camels on the Silk Road. (Panel E is slightly modified from https://commons.wikimedia.org/wiki/File:Silk_Road_1992.jpg, posted by fdecomite, licensed under Creative Commons Attribution 2.0 Generic license.)

This frame resembles that known in Irak, and there is no information suggesting that it was different during the postdomestication period BC, except, of course, the climate change from the initial fertile orchard to the subsequent more aridity (307), mainly in the southern parts, which still prevails today.

Nomadic pastoralist tribes may have been crucial in keeping the distributional scenario of fasciolids and lymnaeids unchanged or only slightly changed along thousands of years. The Bakhtiari tribe is still practicing the annual movement back and forth with their herds all along the Zagros Mountain range from Azerbaijan in the extreme North and the Arabian Sea in the extreme South between valleys and foothills according to seasons (Fig. 17). In southern Iran, the Qashqai (Kashkai) is a tribe practicing transhumance since very ancient times in the Fars province, near the Persian Gulf, following

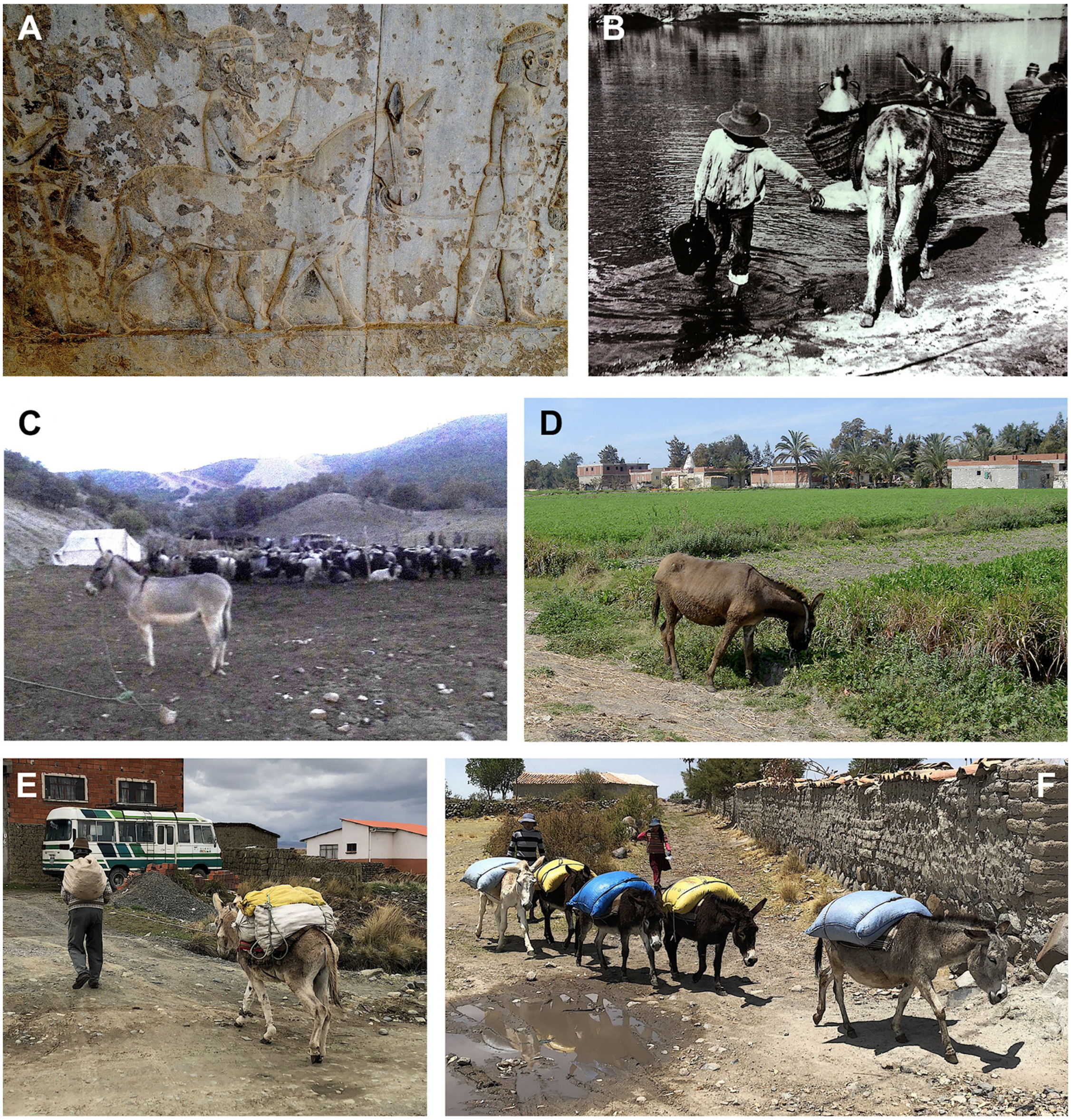

FIG 17 Pack donkeys participated in the spread of fasciolid flukes in the five continents. (A) Domesticated donkey illustrated on the stone walls of the ruins of the ancient city of Persepolis dating back to 515 BC, at a 1,630-m altitude in present-day Iran. (B) Use of the donkey for the transport of water collected from the Tajo River at Toledo, Spain, in the 1920s. (Modified from a picture by Pedro Román Mariana, available at the Centro de Estudios Juan de Mariana, Diputación de Toledo, Spain.) (C) Donkey in night camp of Bakhtiari nomads practicing vertical transhumance in the Zagros Mountains, in present-day Iranian province of Yasuj. (D) Donkey grazing on *Galba truncatula*-inhabited water drainage canal of crop field in an area of fascioliasis hyperendemicity of the Nile Delta, Egypt. (E and F) Pack donkeys are used throughout an area of human fascioliasis hyperendemicity of the Bolivian Altiplano, whether individually for rural route combined with bus for ulterior transport to La Paz city or by donkey groups for the transport inside the Altiplano.

yearly migrations driving their livestock from South to North for fresh pastures. The Qashqai migrate with large flocks, numbering seven million head (308).

The other northern region concerns more recent times and included the steppes northward of the Caucasus, which were inhabited by the seminomadic Yamnayas pastoralists around 4,500 years BP (296, 297) and where fasciolids should have arrived by pastoralism during the Anatolian-Transcaucasian interaction around 4,000 to 3,000 years BC (266, 267). By considering the respective development temperature thresholds of the two liver fluke species, it may be concluded that, although not exclusively, the eastward spread from the Zagros region, through present-day Iran, could be used preferentially by *F. gigantica*, whereas the Asian spread occurred northward of the Caspian Sea, through present-day Russia, and should have been *a priori* more convenient for *F. hepatica*.

The northern way for the initial eastern expansion into north-central Asia. The steppe zone of Eurasia is a very wide, relatively monotonous, woodless landscape extending from modern Hungary in the west and the plains of Mongolia far to the east, with the Eastern European forests and the Siberian taiga in the north, the Black Sea coast and the Caucasus in the southwest, and the great Central Asian deserts and the Pamir and Sayan mountains in the southeast.

The Yamnaya expansions from the western steppe into Europe and central and south Asia during the Early Bronze Age (~3,300 to 3,000 years BC) are believed to have brought with them Indo-European languages and also horse husbandry (296). The vast grasslands making up the Eurasian steppe zones from present-day Ukraine through Russia and Kazakhstan to Mongolia have served as a crossroad for human population movements during the last 5,000 years (309). From their original northern Caucasus region, the Yamnaya steppe pastoralists followed an early eastward split along the Central Steppe of the Turan region (including present-day countries of Turkmenistan, Uzbekistan, Tajikistan, northern Afghanistan, and Kyrgyzstan) through present-day Kazakhstan up to reaching the Altai Mountain region around 3,000 years BC (Fig. 12). Interestingly, recent genetic studies indicate that pastoralists furthermore diversified southward, whether through the western shore of the Aral Sea and/or preferentially through the Tian Shan way down to the Pamir mountains in Tajikistan at the Middle Late Bronze Age circa 1,700 years BC and the Swat Valley of Pakistan around 1,000 years BC, to finally reach the Indus Valley, where they mixed with people arrived from a western origin of Iran and southern Afghanistan, on one side, and from an eastern origin of Southeast Asia and India, on the other side (310).

Chronologically later, the Scythians emerged in the Pontiac steppe around 2,000 to 1,000 years BC (Fig. 12). This ancient nomadic people were a mixture related to the Yamnaya culture (311) and inhabited the Eurasian steppes from eastern Ukraine up to today Kazakhstan, along the Russian steppes of the Siberian, Urals, Volga, and Southern regions (312, 313). They created the so-called first Central Asian nomadic empire, dominating from the Carpathian Mountains in the west to the Ordos Plateau in the east during the Iron Age (314), including landscapes appropriate for fasciolid colonization such as temperate grasslands, savannas, and shrublands. The combination of rich water resources, mountains, and steppes with versatile grass meadows attracted ancient peoples to these regions.

The early first millennium BC was characterized by the formative development of nomad pastoralism throughout the Eurasian steppes. This lifestyle was conducive to population mobility and to the formation of military units necessary to protect herds and to conquer new territories. The dominant position occupied by these peoples in the ecological niche they frequently shared with neighboring agrarian tribes is a result of their nomadic military superiority and is thought to have originated in the North Caucasian steppes as early as the 8th century BC. This occurred concurrently with the advance of Scythian nomad tribes from the steppes east of the Volga River and Caspian Sea to the North Caucasus (Fig. 12), including the Kuban region (315, 316).

The Sauromatian and Sarmatian tribes were groups of nomadic tribes living in the steppes between the Volga and the Ural rivers and in the South Uralian steppes (Fig. 12). Their lifestyle was similar to that of the Scythians, and their history began in the late seventh to the early 6th century BC and continued to the 4th century AD (317). Saka was another name used to denote the steppe nomads. They subsisted mainly on cattle breeding and mastered horseback riding, which enabled them to move quickly through vast territories. This allowed for interactions between populations of quite distant areas. In the Kazakh steppe, people could migrate 800 to 1,000 km with their cattle (318). In Central Asia, these nomadic tribes were actively involved in the political and military expansion of the Achaemenid Empire. In the fertile territory in southern Kazakhstan, they developed agriculture and semisedentary cattle breeding, thus contributing to the development of the earliest Central Asian civilizations. These tribes practiced horizontal transhumance throughout great distances throughout the year. Their herds comprised sheep, camels, and horses. However, a seminomadic cattle breeding developed in eastern Kazakhstan, as well as in the foothills and alpine valleys

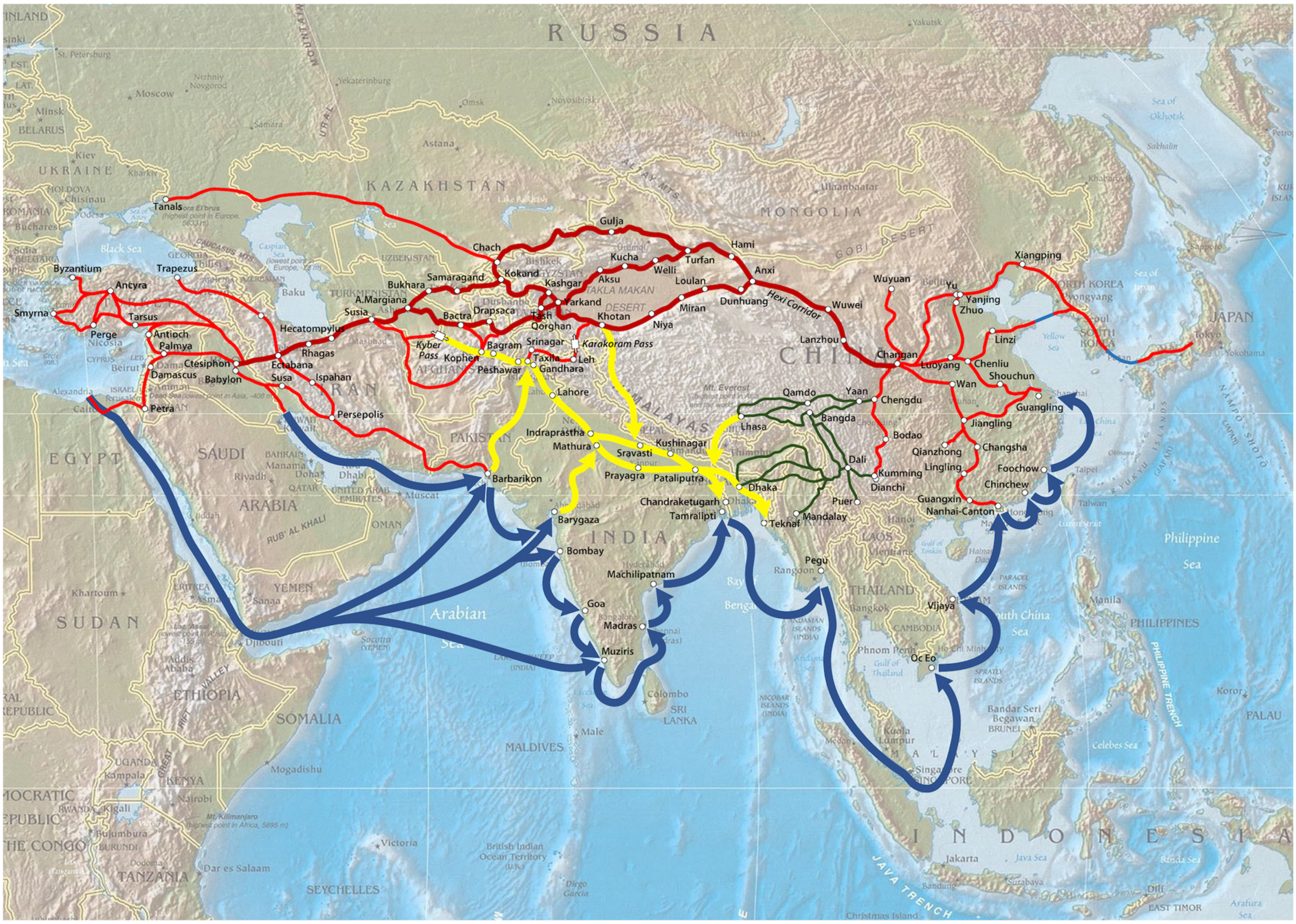

FIG 18 Spread of fasciolid flukes by means of the Silk Road (dark red), Grand Trunk Road and its connections (yellow), Tea-Horse Road (green), Maritime Silk Road (blue), and secondary expansion and interconnecting routes (red). Only main routes used from 300 years BC are included. Details on which routes were used by *Fasciola hepatica* and/or *F. gigantica* are noted in the text. For present-day names of localities, see text. The data on this map were derived from many historical sources.

of the Tian Shan and in the Sayan and the Pamir-Altai mountainous zones, characterized by vertical transhumance. Winter grazing took place in the valley and the herds migrated to highland pastures in the summer, whereas populations were predominantly sedentary and cattle grazed within a limited territory in the deltas of the larger rivers and on the borders of agricultural oases (318, 319). This cattle breeding economy continued to function from ancient times throughout many centuries (318).

In addition, all of these tribes played a crucial part in the Silk Road, the vast trade network connecting eastern Europe and the Near East with eastern China (Fig. 18), thus contributing to the prosperity of those civilizations (320).

The long period from the 3rd millennium BC to the 4th century AD during which the aforementioned pastoralist nomadic tribes dominated the vast Eurasian steppes should have provided many opportunities for fasciolid flukes to expand eastward into north-central Asia. Today, *F. hepatica* and *G. truncatula* are known to be present in modern Russia according to a very wide distribution, including from the southern part of the country (321), the Moscow area (322), the northern Novgorod region (323), the Tyumen province eastward of the Urals (324), and up to the eastern extreme Altai region (325).

In present-day Kazakhstan, *F. hepatica* is found in livestock in areas with harsh climates throughout (326, 327), although with higher prevalences along the southern parts and the western part of the country, mainly in sheep but also infecting goats, cattle, camels, and horses. On the contrary, *F. gigantica* appears to be restricted to floodplains of the southern desert zone (328), in the southwestern Kazakh flat landscape with dry, hot climate, as in the lowlands near the Syrdarya River (326, 327). A 10 to 11% infection rate by *F. hepatica* has been reported in sheep from the easternmost Tian Shan and up to a 21% infection rate from the Karatau mountains and the foothills in the southernmost central Shymkent Province, including sporadic infection by *F. gigantica* (329). Interestingly, in a parasitological study of several species of wild ungulates throughout the whole territory of Kazakhstan, infection by *F. hepatica* was only found in two species, namely, the Bukhara or Bactrian deer of the lowland subspecies *Cervus hanglu bactrianus* in the Karachingil State hunting farm of the southeastern Almaty Province and the Asiatic mouflon or argali *Ovis ammon* in the Karatau mountains of the southcentral Shymkent Province (330). This distributional frame of fasciolids is the consequence of seasonal movements of sheep and other livestock as a crucial animal husbandry in Kazakhstan from the earliest times, including traditional herd migration routes following a pattern with a northward movement of several hundred km in spring, grazing of the northern steppe zone in summer, and a return to the milder south for winter grazing. Such livestock management adapts to the extreme continental climate of Kazakhstan, with long and very cold winters in the north (minimum temperatures of −48 to −52°C and summer temperatures averaging 18 to 20°C), and temperatures varying between +41 and −49°C in Central Kazakhstan, whereas in the south the mean temperature in June is 25°C and snow may lie on the ground between December and January. Rainfall is low, except in the mountains, and decreases from north to south. With the temperature gradient, this generates a transition from wooded steppe in the north through grassland to semidesert and desert biotopes. The global warming phenomenon may be related to the recently detected range expansion of *R. auricularia* eastward up to the Lake Baikal (331), which in its turn may allow for a future eastward spread of *F. gigantica* in these latitudes.

The central way for the initial eastern expansion into Central Asia. The remains of domesticated goats, sheep, and cattle recently found in archeological excavations on northeastern Iran have been dated back to the early domestication period in the Zagros mountains, suggesting that the initial domestication zone could be wider than previously considered and may have included the Iranian plateau and eastern bordering mountainous areas (Fig. 12) (302). The importance of these findings relies on the way they may represent for the spread of fasciolids into the southern part of present-day Turkmenistan. Indeed, *F. hepatica* and *F. gigantica* and their respective specific lymnaeid vectors *G. truncatula* and *R. auricularia* (=*R. gedrosiana*) are now present and even overlapping in several places of the province of Mazandaran, southward of the Caspian Sea (332). Several additional findings also indicate that domesticated sheep and goats could have followed a more septentrional route from Alburz around 6,800 years BC, through present-day Turkmenistan, Uzbekistan, Tajikistan, and Kyrgyzstan around 6,000 years BC (333), up to even China, where the first sheep findings date back to around 3,000 years BC (201). In the eastern part of Iran, fascioliasis in livestock has been reported from Northern Khorasan (334, 335) and the presence of both fasciolid species molecularly verified in Razavi Khorasan (336), two provinces located neighboring Turkmenistan.

The current distribution of both *F. hepatica* and *F. gigantica* fits perfectly with this early postdomestication geographic expansion. The two *Fasciola* species have been molecularly diagnosed by rDNA ITS-2 sequencing infecting goats, sheep, and cattle in Turkmenistan (265, 337). A similar infection picture with the two fasciolids is found in the domestic ruminants in southern Uzbekistan (338) and up to the Fergana zone in the easternmost part of the country, where intermediate forms (at that time designed as *F. indica*) were reported (339). In Uzbekistan, the presence of *F. gigantica* has been genetically confirmed (265, 337) and even been detected to infect children in the western country zone of Tashkent since long ago (51). In Tajikistan, human fascioliasis has been reported (340), including *F. hepatica* infection in children in western Tajikistan (341) and *F. gigantica* infection molecularly confirmed in livestock (265, 337). In Kyrgyzstan, liver fluke infection is known in livestock (342), including reports of *F. hepatica* in children in the western part of the country (343).

The southern way for the initial eastern expansion into South Asia. Another way for the early eastward spread of fasciolid flukes, together with early domesticated ruminants, was into southeastern Asia along the southernmost zone of the Persian Gulf. Archeological remains of domesticated sheep suggest that this expansion was probably from zones as the Fars, up to even the Indus valley in present-day Pakistan (Fig. 12), where the first domestic lineages are dated back to around 6,500 years BC, at the same period in which the first local evidences of zebu domestication (201). The initial data revealed a highly complex genetic and demographic history of the region covering from the Iranian plateau throughout the Indus Valley in present-day Pakistan and Central Asia, mainly present-day India (344, 345). Because of the arid conditions of this southern region, but also due to the impact of the Indian summer Monsoon (346), this Neolithic dispersal from the Near East through the Middle East and to the Indian subcontinent has been estimated to have occurred at a lower rate of 0.65 km/year than that followed by the westward spread across Europe from 9,000 to 6,000 years ago, which was at an average rate of about 1 km/year and was probably facilitated by the large river beds as in the cases of the Danube and the Rhine (347). Relationships running between southeastern Iran, southern Afghanistan, southwestern Pakistani Baluchistan, and the wide Indus Valley have been concluded from results of archeological prospections (348). It has more recently been concluded that the Indus Valley civilizations mixed with people in the southeast to form one of the two main ancestral populations of South Asia. Moreover, there was a mixing with descendants of steppe pastoralists who spread via Central Asia after 4,000 years ago to form the other main ancestral population (310).

In the South, goats are present from the Himalaya high altitudes to the Thar desert or Great Indian desert lowlands and the humid coastal areas of India (1). There are circa 123 million goats in India, and all Indian domestic goat lineages analyzed thus far fall into a monophyletic group that probably accompanied the Indo-Aryan speakers entering India about 3,500 years ago. The only exceptions are goats adapted to the cold high-altitude environment in the Himalayas, which appear to have had a different demographic history from the other breeds (349).

The expansion of taurine cattle into South Asia and the opposite spread of zebu cattle into Mesopotamia and the Levant are considered part of the prehistoric exchange between these two primary centers of agricultural innovation (350). Data indicate a Neolithic transition and independent domestication of zebus in India (351). Taurine cattle of northeast Asia diverged recently from cattle of Europe and Africa but are well separated from Indian zebus. mtDNA studies of the Bali-zebu in Indonesia and the yak-zebu in Nepal suggests that genetic introgression occurred with other domestic cattle during the process of domestication (352).

The current fasciolid picture in this region also fits the aforementioned eastward spread from southeastern Iran into Afghanistan and Pakistan.

In southeastern Iran, the prevalences of fascioliasis in livestock are currently low (306), which is related to the aridity conditions because of the pattern of low rainfall. Cattle appear to be more infected than sheep and goats in the southern Jahrom region, with infections peaking in winter (353). However, it is the goats that show liver fluke infection in the southeastern part of the country neighboring Pakistan (354), and infection by *F. hepatica* has been reported in a patient living in Zahedan, very close to the border of both western Pakistan and southern Afghanistan (355).

In Afghanistan, *F. gigantica* has been reported from sheep, cattle, and buffalo in infections showing a frequency higher than those by *F. hepatica* in goats, sheep, and cattle (356). However, *F. hepatica* was the only fasciolid detected in sheep from northern Kabul, and its molecular characterization showed a mixture of haplotypes similar to those found in western countries and China (357).

In Pakistan, both fasciolid species appear to be widely distributed, with *F. hepatica* preferentially in higher altitudes such as in Quetta in western Balochistan (129) and other parts of this wide province neighboring both southeastern Iran and southern Afghanistan (358), also in the Mardan district, Khyber Pakhtunkhawa province in northern Pakistan (169), close to high Himalayan foothill altitudes where *G. truncatula* is known to be present (168). This fasciolid has even been reported from localities of southern Punjab (129), where intermediate forms have been described (359), given that *F. gigantica* is distributed throughout all the warmer lowlands (129).

In India, *F. gigantica* is widely distributed in ruminants, with prevalences varying between 30 and 80% according to the different geoclimatic regions, mainly in cattle and buffaloes during winter months, whereas infection rates are lower in sheep and goats and peak during the monsoon rainy season (360, 361). In this country, *F. hepatica* has been reported only from sheep in the highland mountains of Gulmarg, Kashmir valley (362). The radicine lymnaeid species *R. acuminata* and *R. luteola* appear to be the main snail vectors throughout, whereas *Galba/Fossaria* lymnaeid species only occur in the high mountains of the Northern Himalayan region (363, 364). This lymnaeid vector distribution therefore agrees with that of *F. gigantica* and *F. hepatica* in the country. The livestock population of the northern Indian state of Uttar Pradesh comprises about 15% of the total livestock of India, including small ruminants such as goats and sheep, but mainly large ruminants such as cattle and, predominantly, buffaloes (128). It should be highlighted that in the Gorakhpur district, in the northeastern part of Uttar Pradesh, an impressively high infection rate of up to 94% has been reported in slaughtered buffaloes, and that the 72.0% rate of infection by *F. gigantica* in *R. acuminata* snails found in one transmission focus appears to be the highest worldwide record of fasciolid infection in a lymnaeid population (128). Indeed, infection rates by *F. hepatica* in *Galba/Fossaria* snails are usually lower than 2%, rarely up to 5 to 10%, and only exceptionally reaching around 30% in very particular concentrated habitats.

Further Eastward Dispersal of Fasciolids and Lymnaeids throughout Asia

The chronologically subsequent wide movement event to be considered in the eastward spread of fasciolids in Asia is the so-called Silk Road (Fig. 18). The appropriateness of the chronology and long-time span, geographical coverage, and pack animal use of the Silk Road for the expansion of *Fasciola* species was first highlighted in 2009 (1).

Although commonly referred to in the singular as the Silk Road, the very wide phenomenon of trans-Eurasian exchanges between China and the Mediterranean should better be labeled in the plural as Silk Roads, given the many different interconnection routes followed according to a complex system of interactions, people movements, goods transport and trade, and material exchanges, where silk was indeed only one of the products involved. There were many paths, tracks, and roads, fluctuating seasonally for timely appropriate crossing of rivers, mountain passes, wide valleys, and steppes and also changing over time to overcome regional conflicts or adapt to changing markets.

Northern Silk Road, China, and Tibetan Plateau. There is a very wide literature on the Silk Roads since long time, but it has been in the last 2 decades that significant advances have been published on the secondary routes and interconnections of the Silk Roads with other important coexisting trade network systems, such as the Grand Trunk Road in Pakistan and northern India, the Maritime Silk Road along the sea coasts of southern Asia, and the Tea Horse Road, reaching South East Asia (Fig. 18). All of them may have been involved in the spread of *Fasciola* species because of the use of pack animals with fasciolid reservoir capacity and the very long periods for these networks. The numerous transhumance grazing systems throughout Asia, including both horizontal and vertical movements, should have additionally participated in the expansion and mixing of *Fasciola* at a more local scale.

After the unification of many of the northern nomads and China from the 2nd century BC, a Chinese mission to Central Asia in 138 BC opened further contacts, and this is usually considered the beginning of the Silk Roads. There is, however, evidence about sporadic, time-limited long-distance trade between Mesopotamia lowlands and mountainous present-day Pakistan from the 4th millennium BC (365), between Mediterranean cities and western India around 2500 to 2000 BC (366), and between the Mediterranean and China by the 6th century BC onward (367). Such exchanges were not only between the long distance extremes in China and the Mediterranean through Central Asia but also in transports between intermediate nodes and stations, several including the so-called Caravanserais for the recovery of travelers and animals (Fig. 19) at shorter distances and moved not only east-west but also north-south across the Tibetan plateau and high mountain passes linking Central Asia and South Asia, through Afghanistan and Pakistan, and allowing for connections with India, Nepal, Bhutan,

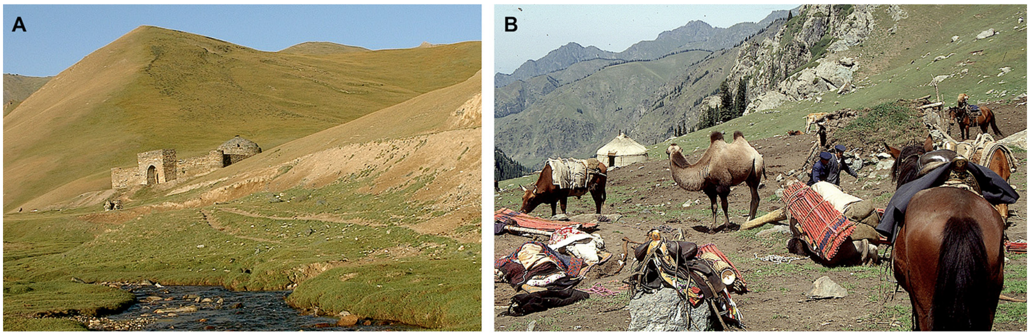

FIG 19 Resting places were available along the trade routes of the Silk Road for the recovering and overnighting of travelling merchants and their animal caravans, in several cases also for good exchanges, distributed from China to the Near East and the Indian subcontinent although mainly present across Central Asia. (A) The 15th century caravanserai (large guest house) Tash Rabat located at a 3,105-m altitude in the Torugart mountain pass, besides small water course typically adequate for the existence of freshwater lymnaeid snail vectors, in the At-Bashi district, Naryn province, Kyrgyzstan, on the route between the Fergana valley and Kashgar city in China. (Modified [magnified to highlight details of interest] from https://commons.wikimedia.org/wiki/File:Caravanserai_Tash_Rabat_on_Silk_Road_in_Kyrgyzstan.jpg, by Peter Schepens, licensed under the Creative Commons Attribution, Attribution 2.0 Generic license.) (B) Caravan including Bactrian camel, taurine cattle, and horses after overnighting in a permanent tent in the high mountains. (Reproduced from https://commons.wikimedia.org/wiki/File:Silk_Road_1992_(4368174878).jpg, by Magnus Manske and posted by fdecomite, licensed under the Creative Commons Attribution 2.0 Generic license.)

and Bangladesh (Fig. 18) (368–371). The activity along the Silk Roads increased during Islamic empires of the Middle East and Central Asia, the Tang Dynasty in China, and the Byzantine Empire in the Mediterranean, and particularly the Mongol empire by enhancing interactions between nomadic and settled agricultural communities in the 13th and 14th centuries CE. The land routes declined in the early 16th century CE, when the seas routes became dominant, which means a total duration of at least 15 centuries for the traditional Silk Roads. Overland exchange between Central Asian markets still continues today, although motorized transport is progressively replacing the typical animal caravans (370).

The main routes of the Silk Roads transited through a wide variety of topographic, hydrographic, climatic, and ecological regions, with considerable elevation variation and including zones of mountains, mountain passes, and surrounding high mountain ranges (altitudes of up to 2,900 m in Kopet-Dag, 7,000 m in Pamir, and 7,000 m in Tian Shan), steppes, grasslands, dry deserts, desert margins and desert floodplains, river valleys and river crossings, and deltas (Fig. 18). Water access was evidently crucial for life sustainability, pasture irrigation, and population development and was needed to deal with changing climate, hydrology, and ecology over time. In Middle Asia, perennial and seasonal streams peak in spring and summer and lead to a very high irrigation potential, sufficiently allowing for vast agriculture zones. The high mountainous massifs act as collectors of precipitation, mainly in the form of snow and ice, which is discharged by a few rivers across piedmonts and desertic flood plains down to interior reservoirs (372).

These main land routes connected many trading centers, including from Babylon in Mesopotamia up to Changan (present-day Xian), throughout latitudes northern from the Chinese Kunlun Mountains (Fig. 18). Worth mentioning are the secondary routes linking (i) Babylon westward with the Mediterranean shore of the Levant and Anatolia (present-day Turkey), (ii) Chach (present-day Tashkent) westward through the north of the Aral Sea and the Caspian up to the Black Sea, (iii) Bactra (present-day Balkh) southward along the Hindu Kush passes to Kophen (present-day Kabul), and (iv) from Kashgar (present-day Kashi) also southward to Purushapura (present-day Peshawar) in that way connecting with the southern Grand Trunk Road along northern India and with the Maritime Silk Road through the Pakistani port of Barbarikon at the Indus Delta (Fig. 18). (v) Finally, emphasis should be given to the secondary routes connecting Changan (Xian), first southward along the eastern Tibetan plateau and through mountain passes up to Nepal and Bhutan and subsequently with the Indian Grand Trunk Road, then eastward with the Pacific shore at Nanhai (present-day Guangzhou) representing another link with the Maritime Silk Road, and finally also the eastward connection with Korea and through short sea route further to Japan (Fig. 18). In total, there were >75,000 km of connecting terrestrial routes using pack animals able to spread fasciolid flukes in repeated back and forth travels along many directions and orientations for more than 1,500 years.

Animals are really an essential part of the history of the Silk Road. They played an irreplaceable role in Silk Road transportation. While midsized ruminants such as sheep and goats supplied the necessities in everyday life for numerous folks (Fig. 15), bigger and stronger species were mainly used as pack animals. Among them, the two-humped Bactrian camel was crucial for cold and arid northern regions, whereas it was substituted for the one-humped dromedary in the warmer southern routes of the Grand Trunk Road (Fig. 16) (373). Similarly, taurine cattle were used in the northern routes of the Silk Road, whereas they were substituted by yaks in the high and cold altitudes of the Silk Road and by buffaloes and zebu cattle (Fig. 15) in the southern warmer routes of the Grand Trunk Road (1). Equines were used throughout in these Asian Roads, including horses, mules, and donkeys (176). These equines are infected by both fasciolid species in Iran (374). In the western Chinese locality of Kashgar (Fig. 6), traders exchanged their yaks and exhausted pack-horses for Bactrian camels to take their merchandise into the desert (Fig. 16).

Bactrian camels show the infection by *F. gigantica* in the Gurevo region of Kazakhstan today (375); both Bactrian camels and dromedaries are infected by the two fasciolid species in Iran (376), and dromedaries have also been infected by *F. gigantica* in Egypt (178). Taurine cattle have recently demonstrated to be very efficient fasciolid reservoirs even at very high altitudes (377), yaks show liver fluke infection in the northwestern Chinese province of Gansu (378, 379) and also in the Qinghai-Tibet plateau (380), and buffaloes show very high prevalences of infection by *F. gigantica* along the warmer Upper Pradesh in northern India (128). Mules and donkeys (Fig. 17) have recently proved to be efficient fasciolid speaders (104, 182).

Humans should have also been affected by the two fasciolid species throughout the routes of the Silk Road. In the city of Samarkand, a crucial node of the Silk Road in present-day Uzbekistan, *F. hepatica* was found in 45, *F. gigantica* in 25, and both species in 11 inhabitants of a total of 81 examined postmortem and in whom fascioliasis infection were found incidentally in all subjects (381). In China, it should be highlighted that the higher risk of human infection in the present-day province of Gansu (382) coincides well with the so-called Hexi corridor or Gansu corridor of the Silk Road transiting south of the Gobi Desert and north of the Qilian Mountains. This corridor originated after the joining of the routes bordering the Taklimakan desert in southwestern Xinjiang in Northwest China, i.e., the northern route coming from Turfan (present-day Turpan) and the southern one from Dunhuang, and ran eastward to Wuwei and in the Lanzhou direction to Changan (Xian).

In additio to the main routes of the Silk Road, the role of pastoralists following transhumance grazing systems (308), including both vertical and horizontal transhumance types, should be taken into account concerning the expansion of fasciolids at a more local level because such herding movements were current throughout the zones where the Silk Road transited. In China, for instance, pastoral areas are found along the provinces of Xinjiang, Gansu, Qinghai, Tibet, Sichuan, and Inner Mongolia, where different types of stock raising systems are still used today (383). Similarly, as in mountainous zones of the neighboring west Asian countries, in Burjin in the extreme northwest of China, herders of cattle, sheep, goats, horses, and some pack camels still follow a traditional seasonal vertical transhumance pattern up to 400 km long, with winter grazing on the desert fringe at a 300- to 1,000-m altitude and summer high pastures at just below 3,500 m today (384). In the vast grasslands of Tibet, the populations of livestock and humans were low in the past, probably in equilibrium with grazing resources (385).

Lymnaeids of the *Radix* group collected throughout the Tibetan Plateau were phylogeographically analyzed by sequencing of fragments of the nuclear rDNA and mtDNA (111). Diversity results suggested multiple colonization events combined with long intraplateau evolution but did not offer a logical biogeographical picture as the one observed in freshwater fishes, i.e., intraplateau distribution could not be explained by drainage-related dispersal. Therefore, a possible passive dispersal by water birds was the mechanism proposed to explain the scenario detected (111). Unfortunately, a potential passive transport by human guided movements of livestock was overlooked. Indeed, when analyzing the geographical distribution of the snail samples studied, an overlap with the great movements (Roads) and local secondary smaller routes (transhumance) of livestock is easily observable. Thus, (i) the phylogenetic clade 1 found in that study shows a distribution that agrees with the routes of the Silk Road and its southward connection with the Tea Horse Road, (ii) clade 2 shows overlap with the western routes of the Silk Road and also with the easternmost Tea Horse Road, (iii) clade 3 fully agrees with the Tea Horse Road, (iv) clade 5 agrees westward with the Grand Trunk Road and eastward with its connection with the Tea Horse Road, (v) clade 9 with the Tea Horse Road and its northward connection with the Silk Road, (vi) clade 10 seems linked to only a part of the Silk Road, and (vii) clades 11 and 12 appear related to an Indian part of the Grand Trunk Road. All this indicates that radicine lymnaeids, despite being markedly less amphibious than *Galba/Fossaria* lymnaeid vector species, are also susceptible to be passively transported by livestock if the time period is long enough, even when livestock populations were not numerous. This evidently facilitates the spread of *F. gigantica* with livestock movements.

Influences of the wide Mongol Empire. The evolution of the Mongol Empire overlaps with almost the whole 15 centuries of the northern trans-Asian Silk Roads. The Mongols were followers of the earlier steppe empires originated in Mongolia between the 3rd century BC to circa 840 CE. Another nomadic state emerged in regions with coexisting nomad and sedentary populations in Manchuria or Central Asia instead of in Mongolia from the 10th century and began to create an empire in which a nomadic (or seminomadic) minority, backed by a strong military machine, ruled a multiethnic nomad and sedentary population (386). This empire reached from Korea to Hungary and Moscow in Russia and from Yunnan in southern China to Irak, with their most enduring legacy in Central Asia, and evolved until its collapse in 1368. The Mongols conquered the sedentary civilizations, thus breaking down the boundaries between the eastern steppe, a Chinese sphere of influence, and the western steppes, influenced by Islam. Despite their wide westward expansion by an army of horse-riding soldiers, the Mongols concentrated more on the control of people and herds than on their territorial gains. The wide-ranging mobilization and expanding trade led to continuous moves of people, goods, ideas, plants, and even virus diseases, such as the Bubonic plague, throughout Eurasia. There were plenty of traders who benefited from the safe roads and access to the postal stations ensured by the Mongols (386). Consequently, the Mongols should not have contributed to the spread of fasciolids into new geographic zones by themselves, as indeed horses are not appropriate fasciolid reservoirs (104) and their expansion was from the East to the West. However, they may have developed a key role as facilitators of long-term exchanges between steppe nomads and neighboring sedentary civilizations and, in that way, contributed to the secondary local spread of fasciolids northward and southward from the main routes of the Silk Road. In addition, they may have pronouncedly increased the mixing of fasciolid populations, which can still be observed in the results of molecular studies of fasciolids in regions of present-day central Asian countries such as Turkmenistan, Uzbekistan, Tajikistan, and Kyrgyzstan they dominated (337).

Silk Road eastward mainland extension and northern Pacific islands. Although Changan (Xian) was initially the final trading center because of its silk production, the development of the so-called Maritime Silk Road with mainly the two Pacific ports of Nanhai-Canton (present-day Guangzhou) and Chinchew (present-day Quanzhou) and additional smaller ports along the Chinese coast further north led to new secondary land routes that represented a southeastern and eastern terrestrial expansion for the transport of goods (Fig. 18). The results of a recent study demonstrating the presence of both *F. hepatica* and *F. gigantica* infecting goats, cattle, and water buffaloes in the provinces of Hubei and Anhui, located in this eastern part of China (387), fit well with the aforementioned expansion of the Silk Road. Moreover, trade activities also extended further north up to the Korean Peninsula and subsequent sea connection with Japan (Fig. 18). The spread of fasciolids and lymnaeid vectors also occurred from the Chinese Pacific ports to the Hawaiian Islands in more modern times.

In Korea, the earliest evidence of domesticated cattle was found in archeological sites dating as early as 2,300 years BP (388), and cattle footprints on the rice paddy fields at another site indicate that cattle were well integrated into rice farming as draft animals by the period of 1,700 to 1,400 years BP (389). These dating estimations chronologically coincide well with the Silk Road. Indeed, the Peninsula of Korea was connected to the easternmost extreme of the Silk Road at Changan (Xian) through an eastward terrestrial route which reached two further main trading centers at Luoyang and Yanjing (present-day Beijing), although a more direct link from Luoyang along the course of the Huang He River (present-day Yellow River) via the Shandong Peninsula and subsequent oversea connection through the Yellow Sea also functioned (Fig. 18). The Korean Peninsula should therefore have been the easternmost land extreme where accumulating fasciolids arrived from all around Asia, but also the bridge of the Silk Road to the islands of Japan (Fig. 18). It should also be considered that the Japanese islands, thanks to their geographically strategic situation in the Pacific, also benefited from direct maritime trade with several ports located along the Chinese coast of the Pacific Ocean, and also ports from other more southern Pacific large islands such as those of the present-day Philippines and Indonesia during long time past periods. All this, and in the case of Japan together with its insularity characteristics, undoubtedly underlie the complexity of fasciolid forms reported from these two countries.

In Korea, the presence of *F. hepatica*, *F. gigantica*, and intermediate forms in livestock has genetically been verified by nuclear rDNA marker sequencing (390). Early on, the fasciolids of Korea were already observed to show three karyotypes, namely, diploid specimens presenting 20 chromosomes, triploid specimens with 30 chromosomes, and mixoploid 2n/3n specimens presenting a 20/30 mosaic constitution (391). The differentiation of aspermic specimens from normal spermic specimens was also reported, and parthenogenesis was suggested to have occurred (392–394). It should, however, be considered that recent knowledge indicates that in fasciolid flukes, despite being hermaphroditic flukes, cross-fertilization seems to be more usual than previously thought, above all in high-burden infections in livestock facilitating the coexistence of and consequent contact between several flukes inside the same biliary canal (a common situation in many areas of endemicity). Hence, the production of eggs in aspermic flukes may in such situations be the consequence of cross-fecundation between a normal spermic specimen and an abnormal aspermic fluke with nonfunctional testes but with normal ovary. Nevertheless, parthenogenesis has been evoked in other hermaphroditic trematode species, and testicular abnormalities have been not rarely observed in nonfertile specimens in other trematode groups. Unfortunately, fasciolids lack a seminal receptacle as an independent organ in the female genital system which could facilitate the observation of sperm inseminated by a normal spermic specimen into an aspermic one after crossbreeding. In fasciolids, this function is in charge for the proximal coils of the uterus, which have been therefore also called "receptaculum seminis uterinum" (395).

It should be highlighted that the results of the molecular studies indicated a founder effect with the transfer of the fasciolids from Korea into Japan by means of the introduction of infected domestic ruminant hosts from the 1st to the 2nd country (394, 396). The snail *R. viridis* seems to be involved in the transmission of both *F. hepatica* and *F. gigantica*, even in the same area (397, 398).

In Japan, historical data suggest that cattle were first introduced into Japan as domestic animals from the Asian continent by early immigrants between the years 500 BC and 300 AD coinciding with the introduction of rice cultivation (399, 400). Cattle strain mtDNA sequencing allowed for the detection of a second immigration of cattle from North China via the Korean peninsula around 200 AD and then expanded from the western region to all of Japan (401). Moreover, two old documents written in the 16th and 17th centuries describe how hundreds to thousands of cattle and horses were imported from Mongolia and Siberia from 1454 to 1456 to the northern part of Japan. In addition, Shorthorn and Devon breeds were imported for strain improvements in 1868, including about 2,600 animals from British and Continental breeds over a 10-year period and were crossed with native cattle in several prefectures (402). Summing up, the ancient arrival of fasciolids into Japan may have included several liver fluke lineages from different geographical origins. Together with the past introduction of lymnaeid species of the *Galba/Fossaria* and *Radix* groups and the consideration of the evolutionary characteristics of insularity, these findings indicate a high complexity of fasciolids in Japan. The coexistence of different fasciolid lineages and lymnaeid species arrived from abroad concentrated in Japan, and the crossing reproduction capacity allowing for hybridization of these hermaphroditic flukes increased due to the consequent crowding originated by the insularity phenomenon, thus leading to a pronounced lineage mixing.

When comparing *F. hepatica*, *F. gigantica*, and intermediate forms by means of the morphological study of adult specimens and eggs, as well as the characteristics of the life cycle, it was concluded that *F. hepatica* was not present in Japan and that Japanese fasciolids in fact belonged to *F. gigantica* and to intermediate forms which were designed as the "Japanese species" (403). Elucidation of the complexity of fasciolids in Japan required decades of effort by many scientists; among these, the excellent work by Tadashi Itagaki of Morioka and his team in the last decades has been crucial in clarifying this situation, especially with regard to spermic, aspermic, diploid, triploid, mixoploid, parthenogenetic, and hybrid flukes.

Sequencing of nuclear rDNA and mtDNA markers showed the existence of two major fasciolid forms in Japan resembling those of *F. hepatica* and *F. gigantica* in all the four molecular markers and were mainly distributed in northern and eastern-western parts of Japan, respectively (396). A recent genetic study of Japanese parthenogenetic fasciolids infecting domestic cattle and wild sika deer by means of DNA microsatellites showed a diversity whose geographical distribution was hypothetically linked to cross infections between the two host species and past human-guided livestock movements (404). Another molecular study using two other nuclear rDNA single-copy, coding genes to assess the fasciolid situation of Japan by comparing with liver flukes collected in other countries of Asia demonstrated (i) the hybrid origin of aspermic *Fasciola* lineages, (ii) the absence of *F. hepatica*, and (iii) the presence of *F. gigantica* and fasciolid hybrids in eastern India, Nepal, Bangladesh, Myanmar, Thailand, and Vietnam, and (iv) that only hybrids, however, were found in Korea and Japan (405). Unfortunately, the original picture of fasciolids in several of the southern and southeastern Asian countries was probably masked by the numerous livestock importation events from other countries, where only *F. hepatica* is present, which occurred in recent years because of the increasing population demands.

The fauna of lymnaeid vector species underlying the aforementioned fasciolid scenario on the island of Hokkaido, Japan, includes the *Galba/Fossaria* species *G. truncatula* and the two *Radix* species *R. viridis*/*R. ollula* and *R. auricularia*. Molecular analyses also demonstrated that three different haplogroups within *R. auricularia* merited further studies because of genetic distances at the species level (406). Local fasciolids have been verified to use *G. truncatula* both experimentally (407) and under natural conditions (408). Interestingly, however, *F. hepatica* from Australia was demonstrated to infect *R. viridis*/*R. ollula* but not *G. truncatula* nor *R. auricularia* from Japan (113). In addition, *F. gigantica* has been observed to be transmitted by *R. viridis*/*R. ollula* (409). It is evident that more studies are needed to completely elucidate which lymnaeid species transmit which fasciolid in Japan.

Fasciola gigantica was also able to colonize Hawaii, where it caused public health problems reported in the past (70, 410, 411). In the Hawaiian Islands there are two lymnaeid species evidently introduced via a maritime route from different Pacific sources and which have been classified as *R. viridis* or *R. ollula* and *Pseudosuccinea columella*. Both are known to be able to transmit *F. gigantica*, although it is the smaller species and not *P. columella* that plays the main role in disease transmission on these islands. The detection of abnormal spermatogenetic fasciolids in Hawaii (412) may be interpreted as the past existence of hybrid lineages resulting from a reduced, insufficient *F. hepatica* introduction and slowly evolved to the disappearance.

In an exhaustively detailed study on livestock in Hawaii, all importations of livestock, including that from the first introduction of each domestic species up to the date of the publication in 1929, highly useful information shows the impressive complexity of livestock import/export during the period from the last decade of the 1800s (413). The first cattle of longhorn Spanish type from California arrived to Hawaii in 1793 and rapidly increased and spread. Different cattle breeds were later imported from Scotland (in 1811 and 1889), from the state of Washington (in 1894), from the British Islands brought around Cape Horn (around 1890), from Boston (in 1855), from New Zealand (sometime after 1880), again from California (in 1898), from New Hampshire (about 1920), from Ireland around Cape Horn (in 1868), from Australia (in 1880), from New Zealand (about 1895), and from Oregon (in 1908). After 1854, numerous Shorthorns were imported during the next 50 years, and in the 1890s the importation of Holstein cattle from California began until it became—by far—the most populous dairy breed in Hawaii.

The first sheep were imported into Hawaii from California in 1793 and 1794. Sheep merino breed from Sydney, Australia, arrived later (1845 and 1852), from Germany (1853 to 1862), from France (1862), from Tunisia (1900 and 1910), and from Indiana (1904). It should be noted that sheep were—unusually—transported inter-island from Molokai to Niihau in 1864. The first goats were apparently brought by Captain Cook in 1778. Swine were seemingly found in the Hawaiian Islands when Captain Cook made his 1773 voyage, but there is, unfortunately, no record available showing how they got to Hawaii. Cook left a pair of English breed pigs in 1778. The first horses were imported from California in 1803. Also in the 1880s, other horses arrived from England, and in 1884 horses were imported from Arabia and India (413). Thus, all of the exporting countries were geographic sources for *F. hepatica*, but only the latter nations, Arabia and India, could have furnished *F. gigantica*. Even so, where did *R. viridis/R. ollula* widely distributed in the Hawaiian streams and swampy lowlands come from?

Knowledge of the presence of liver flukes in Hawaii dates back at least to 1892 when Lutz reported the presence of fasciolids infecting cattle, horses, and probably also pigs on the islands of Oahu, Maui, and Kanai, and probably Hawaii as well (59). A short while later, flukes were again collected from a horse on the island of Hawaii in 1894 (70). Although at that time the parasites were reported as *F. hepatica*, subsequent studies showed them to be *F. gigantica* (414). The introduction of *F. gigantica* into Hawaii is believed to have come from China, together with water buffaloes. In fact, Asian water buffaloes (*Bubalus bubalis*) were introduced into Hawaii around 1890 by Chinese rice farmers and were used for plowing, puddling, and leveling rice and old wetland taro (*Colocasia esculenta*) pond fields. Hundreds of these water buffaloes existed in Hawaii from this period until the 1940s (415). These data not only fit chronologically well but also shed light on the introduction source of the lymnaeid vector *R. viridis/R. ollula*, which is known to be widely distributed along the Pacific coast of China up to Korea. The numerous livestock imported before 1890 should have introduced *F. hepatica*, which could not colonize due to the absence of lymnaeid vectors, whereas *F. hepatica* arrived with cattle and sheep imported later than this date could have at least hybridized with previously existing *F. gigantica*, thus explaining the abnormal spermatogenetic fasciolids found in Hawaii in recent times (412).

Land Connections of the Maritime Silk Road

Maritime routes along all south Asian sea coasts developed later, extending westward through Turkey to Istanbul and involving the Mediterranean on to Italy and eastward to Korea and Japan (Fig. 18). The Maritime Silk Road shaped the historiography of trade in the Indian Ocean and the historical development of Southeast Asia and the South China Sea (416). Land and maritime routes were interlinked, especially through the interaction with terrestrial routes bringing material to and from the ports. Although slowly beginning by 200 BC, the Maritime Silk Road flourished later on until the 15th century CE, taking advantage of already existing older trade networks. Accounts of large trading vessels from Southeast Asia date back to at least the 3rd century CE (417). Arab dhow traders ventured into the routes by the 7th century CE, thus leading to the earliest spread of the Islam throughout southern Asia (416). Before the 10th century, maritime activities primarily concerned Southeast Asian traders, although Tamil and Persian traders also participated. China gained prominence from the 10th to the 13th centuries by sailing trading expeditions southward to the Suku Sea and the Java Sea. The Chinese trading colonies established in Southeast Asia underlay the emergence of the port of Nanhai-Canton (present-day Guangzhou), located on the Pearl River about 120 km north-northwest of Hong Kong and 145 km north of Macao, and also that of the more northern Chinchew (present-day Quanzhou), in front of Taiwan, as key regional exchange centers in China (416).

Important connections between the maritime routes along Indian Ocean coasts of central south Asia and eastern Pacific coasts and the land routes of the Silk Roads and Grand Trunk Road included, from West to East, the ports of Barbarikon, Barygaza, Tamralipti, and Nanhai (Fig. 18). The Barbarikon port was located in the Indus Delta, and pack animals followed a northward way along the Indus Valley through Multan until Taxila, near present-day Pakistan's Rawalpindi-Islamabad, which was an important staging point on the Silk Road, along with Peshawar and ancient Bactria, north of the Hindu Kush mountain range in northern Pakistan. Interestingly, there was a terrestrial route between Babylon in Mesopotamia up to Barbarikon along the northern coast of the Persian Gulf and the Arabian Sea during the first centuries of the Silk Roads, around 300 BC and 100 AD, and which included Persepolis as an intermediate trading center. It should be noted that Persepolis, located at a 1,630-m high altitude, was a city of great importance from 515 BC to the 10th century AD and that sheep, goats, zebu cattle, donkeys, horses, camels, and dromedaries appear repeatedly in the scenes shown on the stone walls of the ancient Persepolis ruins (Fig. 15 to 17), illustrating a close relationship between humans and these fasciolid host species (418). The port of Barygaza, present-day Bharuch, was found at the Gulf of Khambhat, western India, and the materials followed a northward way until Mathura, relatively close to present-day Delhi and westward up to Punjab and eastward to Upper Pradesh. These two ports of Barbarikon and Barygaza at the Arabian Sea developed an important role in the direct sea connections with Mesopotamia through the Persian Gulf and also with Egypt through the Red Sea (419–421), like the ancient haven of Muziris at the southernmost cone of the India subcontinent (Fig. 18). Tamralipti, present-day Tamluk in West Bengal, a port city and capital of Suhma Kingdom in ancient Bengal, located close to Calcutta (present-day Kolkata) on the coast of the Bay of Bengal, in eastern India, developed an important role at the easternmost extreme of the Grand Trunk Road because of its westward connections through ancient Pataliputra and throughout northern India until the Pakistani Peshawar along trade exchanges that existed for a very long period of ~2,500 years. Therefore, the Bay of Bengal may even have been the center of the Maritime Silk Road (416). Nanhai, present-day Guangzhou, Guangdong, Canton province, on the Pearl River Delta in southeastern China, between present-day Macao and Hong Kong, was the eastern end of the Maritime Silk Road and was connected with the terrestrial Silk Roads following a northward way until Changan (Xian) (Fig. 18).

Pack animals used to transport the materials from and to the ports of Barbarikon, Barygaza, and Tamralipti were needed for long land passages to reach the northern trade centers along the Grand Trunk Road of northern India and Pakistan. Similarly, the Nanhai port was connected to Changan (Xian) along routes between several inland trade centers throughout easternmost China (Fig. 18). There is no mention in the literature suggesting that vessels used throughout the Maritime Silk Road (Arab vessels, sailing trade dhows, Chinese "Junk Ships," etc.) (417, 422) were taking livestock. Some large dhows, however, were used for livestock transport, at least in Yemen (423), and this indicates that additional studies on this subject are needed.

The wide distribution of fascioliasis throughout the Indus Valley up to the Indus Delta (359) and, similarly, in old Bengal (130) illustrate how the land connections of the ports of Barbarikon and Tamralipti could have influenced the spread of *F. gigantica* throughout the respective interconnected zones. In China, the correlation of the higher risk of human infection in the present-day neighboring provinces of Hubei, Jiangxi, and Fujian (382) with the land routes connecting Changan (Xian) in present-day Shaanxi province with the Maritime Silk Road ports of Chinchew (present-day Quanzhou) and Nanhai-Canton (present-day Guangzhou) should be highlighted because of its geographical evidence (Fig. 18).

Worth mentioning also is the geographical distribution of the lymnaeid species known as *R. viridis* or *R. ollula*. This is an efficient fascioliasis vector whose wide distribution in Southeastern Asia and along the Pacific coast up to Korea and Japan suggests having taken advantage of transport livestock movements in the inland routes of the Tea Horse Road and also by sea via the Maritime Silk Road and other trade shipping farther north. Indeed, this small lymnaeid species displays two peculiar characteristics within the *Radix* group that are similar to those in vector species of the *Galba/Fossaria* group (in part explaining why it was included in *Galba* as *G. pervia*): it is markedly amphibious and therefore facilitates its passive transport in mud attached to the hooves of domestic animals, and it has been experimentally demonstrated to transmit both *F. hepatica* and *F. gigantica* (397, 424).

Grand Trunk Road and South Asia

The Grand Trunk Road is the name applied to the trade route connecting Kophen (today known as Kabul) in modern-day Afghanistan, in the West, with the coastal locality of Teknaf in the southernmost extreme of eastern Bengal (present-day Bangladesh) besides the Burma (present-day Myanmar) border, in the East, running roughly 2,600 km in total (Fig. 18) (425). Thus, this long trade route linked Central Asia to the Indian subcontinent for at least 2,500 years from the 3rd century BC (426, 427).

Indeed, in the western extreme, through the Khyber Pass, Gandhara (Islamabad in present-day Pakistan) became a regional center of trade connecting Bagram in Afghanistan to Taxila in Pakistan. The Khyber Pass is a mountain pass in the Khyber Pakhtunkhwa province of Pakistan, on the border with Afghanistan. It connects the town of Landi Kotal to the Valley of Purushapura (present-day Peshawar) at Jamrud by traversing part of the Spin Ghar mountains. This was a route connecting the Indian subcontinent with the Silk Road in Central Asia via the Grand Trunk Road by two courses: (i) one from Purushapura and Kophen, the route followed westward up to old Bactria through the Hindu Kush mountainous region, and subsequently to the Near East, and (ii) another from Taxila, an old site located only 25 km northwest from Islamabad-Rawalpindi, from which the caravan route connected northward with Kashgar through the Karakoram Pass via Srinagar and Leh covering Western Himalaya (Fig. 18) (425).

From Taxila, the Grand Trunk Road followed a very long eastward way throughout northern Pakistan and India along a course southward from the Himalaya chain, including many ancient trading sites. In northern Punjab, the route connected Taxila and Islamabad-Rawalpindi with Lahore (428) and subsequently to Amritsar and the old site of Indraprastha, close to modern Delhi, and then to Mathura and Prayagra (present-day Allahabad), near the confluence of the Ganges and Yamuna rivers in Utter Padesh, and to Pataliputra (present-day Patna) in the confluence of the Ganges with the Son River in Bihar. From Pataliputra, the route divided whether southward up to the port of Tamralipti (Tamluk), close to present-day Kolkata in the eastern part of the Ganges Delta, or continued eastward into Bengal up to Dhaka Chittagong and later southward to the most extreme port of Teknaf on the coast of the Bay of Bengal (426, 427). This most extreme end of the Grand Trunk Road represented the link with the network of the Tea Horse Road and therefore the connection with South East Asia (Fig. 18).

Along the journey in India, the Grand Trunk Road also received routes coming from the Silk Road at least along two additional passes through the Himalayan mountains: (i) one was coming from the Silk Road at Khotan after crossing the Kunlun Shan westward of the Tibetan plateau and through the western part of modern-day Nepal down to the Indian Shravasti in Uttar Pradesh and (ii) another from the Tibet capital Lhasa through the Himalayan pass between present-day Bhutan and Nepal and subsequently reaching Chandraketugarh in West Bengal, close to modern Kolkata, and representing further links to both the Silk Road in the north and the Tea Horse Road toward the East (Fig. 18) (425).

In addition, the Grand Trunk Road connected with the Maritime Silk Road by trade transporting caravans in charge for the materials arriving or being shipped at the ports of Barbarikon in Pakistan, Barygaza in western India, and Tamralipti in eastern India (Fig. 18).

The pack animals used throughout the Grand Trunk Road and its aforementioned northward and southward connections belonged to the same species as those used in the Silk Road, although one-humped dromedaries were incorporated to replace the two-humped Bactrian camels in the routes through Afghanistan, Pakistan, and India because of their better adaptation to warmer climates (Fig. 16) (373).

In addition to the long distance pack animal movements throughout the Grand Trunk Road, very old traditions of transhumance may have also participated in the spread of fasciolids and lymnaeids at a more local scale (429) in the zones such as the following: (i) the mountain ranges running down to the Turkestan plain; (ii) the Hindu Kush in Afghanistan; (iii) the foothills of the Karakoram and also of the Himalaya (430, 431), as well as the uplands of Balochistan in Pakistan; and (iv) the northwestern Indian Himalayas, such as Himachal Pradesh (432), to the west of the Nepalese border. In all of these zones, transhumance systems similarly follow the vertical type, with overwintering in the warmer plains, foothills, or the desert fringe, and stocks moving upward to reach mountain or alpine pastures in summer. Sheep and goats represent the basis of most of these systems, although cattle and buffalo also migrate with the Gujar tribe in Pakistan and India, and camels are important in Balochistan and Afghanistan (308).

Interestingly, not only goats but also sheep were also regularly used as pack animals during the Neolithic period. Pack goats can carry 30% of their weight over 24 km of mountainous terrain daily and are more agile and adaptable to a greater variety of environments. Pack goats and sheep were crucial for the survival of nomadic pastoralists, such as, for instance, those practicing transhumance in the Chang Tang plateau in western Tibet, extending into the southern edges of Xinjiang, as well as southeastern Ladakh, in India. Sheep have recently also been demonstrated to be very efficient fasciolid reservoirs at very high altitudes (377). The use of pack ovicaprines, especially pack goats, may explain how obsidian and other goods that circulated in exchange networks were transported across long distances and mountainous terrain (433).

Transhumant herding is also important in Nepal (434), Bhutan (435, 436), and also in the Indian mountainous Sikkim state located in between these two countries. The eastern Himalayan region is wetter than western Himalaya. This underlies the differences in livestock species. In the eastern Himalaya, large ruminants including yak and gayal (*Bos frontalis*) for high-altitude areas and cattle and buffaloes for lower-altitude zones, are much more important than sheep and goats. The systems are more stratified altitudinally, since yaks prefer low temperatures and can survive in colder places than other stock. The same pasture may be used by different species and different herding groups in different seasons of the year (308).

Summing up, the Grant Trunk Road should have been undoubtedly involved in the eastward spread of fasciolids throughout southern Asia as the bridge between the Near East and southern China and South East Asia. The many northward and southward secondary interconnecting branches and transhumance systems should also have developed an intense influence toward an important mixing of fasciolid populations from different Asian geographic origins.

The areas of animal fascioliasis endemicity in Kabul, Afghanistan (357), the two areas of human endemicity in Pakistan caused by *F. hepatica* in the Mardan area (169) and by *F. gigantica* in the eastern zone of Lahore (359, 437), and the area of animal hyperendemicity throughout Upper Pradesh in India presenting surprisingly high prevalences by *F. gigantica* in buffaloes and also lymnaeids (128), follow the Grand Trunk Road with evident exactitude. This route harbors *F. gigantica* transmitted by Asian races or species of the *R. auricularia* superspecies complex, with *R. luteola* being also involved in its eastern Indian part. The existence of *F. gigantica* in southern India shows a parallelism with the southward spread of

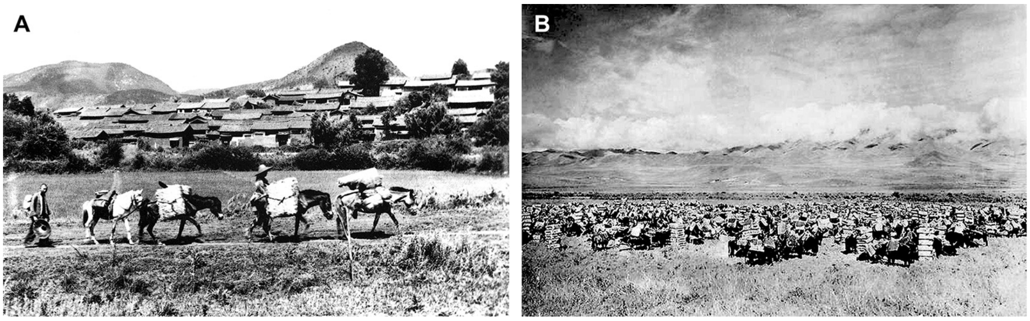

FIG 20 The main routes of the Tea Horse Road traversed across the Hengduan mountain range spanning the west side of the Chinese provinces of Sichuan and Yunnan and the southeast of Tibet. Mules are powerful long distance pack animals ideal for difficult rugged mountainous routes and were therefore the main pack animals used in the caravans along the narrow and steep mountain ways. (A) Mule caravan in southern Yunnan, around the year 1900. (B) Massed caravan of pack animals carrying tea at Songpan, northwestern Sichuan, China, in 1922, illustrating the magnitude of animal transport involved in the Tea Horse Road. (Both panels are uncredited historical pictures contributed by CPA Media Pte, Ltd., Singapore, through Alamy Limited, Abingdon, UK.)

these two radicine lymnaeid vector species, as observed in the Tamil Nadu area besides Chennai city (old Madras) (438). In the Mardan area close to Peshawar, of the Khyber Pakhtunkhawa province in northern Pakistan, closeness to the northern mountainous zone of the westernmost part of the Himalaya chain explains the presence of *G. truncatula* allowing for the transmission of *F. hepatica* (168). The Khyber Pakhtunkhawa province is a zone where transhumance pastoralism has been well studied (439) and is a southern continuation of livestock transhumance practiced in the mountainous areas further north (440).

The Tea-Horse Road, Southeast Mainland, and Insular Asia

The Tea-Horse Road is one of the least studied overland routes. The name is given to a network of corridors linking southwestern and northwestern China, Tibet, Nepal, Bhutan, Bengal (present day Bangladesh), northeastern India, and Burma (present day Myanmar) (Fig. 18). The main core of this network included routes between the two Chinese provinces of Yunnan and Sichaun and Tibet (441), whose aim was the exchange of Chinese tea produced in the Yunnan area of Puer and the temperate rainforests of Xishuangbanna/Sibsongbanna, besides the modern-day northern borders of Thailand, Laos and Vietnam, and also in the Sichaun area of Yaan via Changdu, for Tibetan horses needed by China for military purposes (368). This was a network of routes extending more than 3,000 km in total through sparsely populated mountain ranges and passes, river gorges and crossings, high plateaus, and grasslands. The passage is only open for 4 or 5 months per year. These exchanges began in the Tang Dynasty (618 to 907 CE), became more prominent during the Song (960 to 1279 CE), and were afterward followed through the Ming dynasty (1368 to 1644 CE) and Qing dynasty (1644 to 1911 CE) (442).

The caravans from China to Tibet used a variety of pack animals, including horses, mules, donkeys, yaks, oxen, and also hardy human porters for the transportation of goods (443), sometimes including an impressive number of pack animals (Fig. 20). Mules were key elements in the caravans along the Tea Horse Road because they are powerful pack animals, mainly in long distance movements along difficult rugged mountainous routes but also in semiarid flatlands because of their capacity to replace oxen in ploughing (Fig. 20) (176). Mules were also included in the tribute paid to the Chinese overlords (444), along the long routes of the Silk Road. It should be noted that mules have recently proved to be effective reservoirs for fasciolid spreading across mountain chains (104). Yaks were used at higher, cooler altitudes and conditions. Return trade caravans included herds of sturdy horses from Tibet for military and official use in China, among which the so-called "heavenly horses" from Uzbekistan.

This core network was also northward connected from the Tibetan capital Lhasa to the Shaanxi-Gansu part of the Silk Road at Dunhuang heading toward Xinjiang and Central Asia (442). In its southwestern extension, the Tea-Horse Road connected through the mountain pass between Nepal and Bhutan, with the easternmost Indian extreme of the Grand Trunk

Road leading up to the port of Tamralipti, near present-day Kolkata, and Bengal. Finally, the northward Chinese route from Puer had a westward derivation from the locality of Dali, including a more northern route through Kamrup in Indian Assam and another southern one up to easter Indian Manipur, both through upper Burma ending in Bengal (445, 446). Assam was considered the Eastern Gateway to the "eastern world," i.e., for the passage of people, goods and ideas between India, Burma and China since the 2nd century BC (447). This eastern India-Burma-Yunnan Road was very difficult to traverse in ancient times and still is so in the present. By the 7th century AD, various other branches emerged to create a web of trading routes (448).

This means there were a total of at least four zones through which fasciolids could initially enter and spread through the Tea Horse Road and colonize southwestern Sichuan and Yunnan, and subsequently more-eastern China up to even Beijing, but also Thailand, Cambodia, Laos, Vietnam, and all remaining South East Asia (446, 447).

In China, the geographical overlap of the higher risk of human infection in the present-day neighboring provinces of Sichuan and Yunnan (382) with the core routes of the Tea Horse Road concerning the tea production zone of Yaan along Chengdu and Kangding in Sichuan and mainly that of Puer, Kumming, and Dali in Yunnan should be emphasized because of the geographical distributional evidence. Indeed, in this zone an outbreak of human fascioliasis caused by *F. gigantica* has been recently reported (449, 450).

Knowledge of fascioliasis indicates that only *F. gigantica* is present in the lowlands of northeastern India and Bangladesh, where it is transmitted by two lymnaeid species of the *Radix* group, namely, *R. acuminata* and *R. luteola* (128). In the eastern parts of Burma and South East Asia, on the other side of the mountains separating Assam and Manipur from the zone of Dali, Kumming, and Puer, *F. gigantica* appears similarly to have been the only fasciolid present throughout the southern lowlands down to the southernmost extreme of South East Asia, where it is transmitted by other radicine species such as *R. viridis* and *R. rubiginosa*, although *R. swinhoei* has also been incriminated (451). These radicine snail species have recently been proposed to be included in other lymnaeid genera (452, 453). Throughout this southeastern Asian region, the recent repeated importation of livestock from other regions where only *F. hepatica* is present has led to widely extended hybridization; this has been observed when molecularly analyzing flukes from many provinces in Vietnam (Fig. 13), where both *F. gigantica* and admixed and/or introgressive hybrids can currently be found (454).

All this is further supported by the exclusive presence of *F. gigantica* in peninsular Malaysia (455), and neighboring insular Indonesia, including Sumatra (456), Borneo (457), Java (458), and Sulawesi (459). The main lymnaeid vector species in these islands appears to be *R. rubiginosa* (460, 461), a snail species even found in fossil form in Java (462), suggesting it to be a species probably original of these islands. The invasive capacity of *R. rubiginosa* by means of plants (110) has allowed it to spread throughout all Indonesian islands and even farther away.

It should be considered that, in mainland South East Asia, the first domestic cattle concerned zebus (*Bos indicus*), and these animals have been archeologically recorded from Thailand dating back to circa 3,600 years BP (463), *a priori* too early for the possibility of fasciolids to have reached such eastern longitudes, and that any evidence of domestic bovines is completely missing from Neolithic sites in northern and southern Vietnam (464, 465). Domestic ruminants were also absent in the Indonesian islands, where only the archeological remains of pigs, dogs, and chickens can be found from the Neolithic period (466).

The absence of lymnaeid species of the *Galba/Fossaria* group throughout this eastern and southeastern Asian region should be emphasized in order to understand the lack of *F. hepatica*. Three aspects concerning this assumption should be considered: (i) the species named *Galba pervia* (467) in this southern Chinese region is in fact a radicine and had indeed already synonymized with *R. viridis* some time ago (3), (ii) the reports of intermediate forms and hybrids of *Fasciola*, even sometimes ascribed to *F. hepatica* itself, are the results of very recent introductions by ship importation from countries of Oceania and Europe, and (iii) the reports of *F. hepatica* infecting yaks in eastern Tibet (468) and also humans and animals, although still requiring confirmation in Nepal (469), as well as the presence of *G. truncatula* in western Bhutan (470), suggest that this fasciolid and related specific *Galba/Fossaria* species might have been introduced eastward into the mountainous altitude areas crossed by the Tea Horse Road caravans, although, unfortunately, local field studies in these high altitudes are still lacking.

The very recent finding of the markedly amphibious lymnaeid snail *L. schirazensis* in Thimphu district, west of Geneykha, around 5 km east of Chuzom, at an altitude of 2,750 m a.s.l. in Bhutan (471), poses an interesting question about how this snail reached such an extreme eastern location, because it has been demonstrated that this snail species is a very useful marker of livestock movements (4). This finding could also be *a priori* considered as a further argument supporting the spread of *F. hepatica* with livestock following the Grand Trunk Road southward of the Himalayas (472) through India (473), a hypothesis for which significant evidence is still lacking. Indeed, this finding of *L. schirazensis* in Bhutan has been confirmed by sequencing of a *cox1* fragment (471), which shows no mutation regarding the same sequence of this lymnaeid in Iran, but also in Egypt, Spain, the Caribbean, and Central and South America (4). This molecular result indicates a recent spread in Asia and rejects an old existence in altitude refugia. Fascioliasis is recognized to be one of the major problems affecting health and productivity of cattle in Bhutan (474). Moreover, *G. truncatula* and *R. acuminata* have been reported from this country (470), which means that lymnaeid vectors for both *F. hepatica* and *F. gigantica* are present in Bhutan, which resembles the situation in the neighboring and physiographically similar Nepal (469, 475). There are four long land routes which could *a priori* allow for the arrival of *L. schirazensis* to the neighboring zone of western Bhutan: (i) from the East by the Tea Horse Road through the eastern Indian Assam; (ii) from the North by the Tea Horse Road through the Tibet capital Lhasa; (iii) from the North by the northern Silk Road southward connection also with Lhasa; and (iv) from the West by the Grand Trunk Road along the lowland belt of the Terai region northward of the Indo-Gangetic Plain and southward from the outer Sivalik foothills of the Himalayas, throughout the south from Nepal, where fascioliasis is mainly transmitted by *R. acuminata* and is highly endemic in livestock (126) and where there was a secondary south-north road which started at the Jaldhaka river in India, just northward from present-day Bangladesh. This secondary route followed a northward way through the southeastern corner of present-day Bhutan and continued through valleys up to Pagri and subsequently to the Tibetan plateau. These four route possibilities would share their last way through the Indian mountainous pass at the Sikkim state located in between Nepal and Bhutan. Transhumant agropastoralism has traditionally been practiced in western Bhutan since ancient times (436), and there was certainly a pastoralist migratory route from southern Samtse to Haa in southwestern Bhutan (476). This transect formed a bridge between the aforementioned secondary route of the Grand Trunk Road in northern India and all the way up to just besides the valley connecting with Geneykha in the Thimphu district (477) and further northward up to the Tibetan Lhasa.

The first three routes should be ruled out because of the long distances, low temperatures, and lack of areas where this snail is known to be present throughout North, Central, East, and South-East Asia. The fourth route would involve passive transport by livestock over too long a distance from southern Iran through Afghanistan, Pakistan, and India along warm and—in some places—arid lowlands, and it is therefore difficult to envisage occurring for such an extremely amphibious snail.

However, there was a gift importation of 69 Friesian cows/heifers and 3 bulls/bull-calves in 1989 from Spain (S. A. Ahasan and S. Mas-Coma, unpublished data), a country where *L. schirazensis* is well known to be present and expanded (4). These animals should have arrived by a maritime route via the Bay of Bengal, and the wet conditions of Bangladesh allowed *L. schirazensis* to expand northward up to the Indian Sikkim state and subsequently by transhumance to reach Bhutan. The possibility of a past arrival by another sea route in an old Arab dhow transporting livestock following the Maritime Silk Road from Egypt via the Red Sea or from Iran via the Persian Gulf and Oman Gulf and subsequently sailing in between southern India and present-day Sri Lanka and finally up to the Tamralipti port in the Bay of Bengal does not seem realistic.

The aforementioned hypothesis linked to recent livestock import fits all of the requirements posed by our present knowledge of this lymnaeid species. Indeed, *L. schirazensis* has proved to be a snail able to be passively transported along intercontinental routes by ships when in mud attached to the hooves of the transported livestock and, once arrived in the new mainland zone, to be also able to spread with livestock movements, although not along very long distances (4). All of this explains how *L. schirazensis* could be easily introduced up to where it has been found and, moreover, similarly suggests ways by which fasciolids and lymnaeids colonized Bhutan. Indeed, fascioliasis is known to infect cattle in the Haa district (478).

A similar way for fascioliasis introduction into Nepal, located westward just besides the aforementioned Indian mountainous pass at the Sikkim state, may logically be envisaged. It should be considered, however, that Nepal was using additional south-north transects through other mountain passes in the western part of the country for its exchanges with India and China (479). Both *F. hepatica* and *F. gigantica*, as well as intermediate fasciolid forms, have been described in Nepal. The four lymnaeid species initially reported in this country all belong to the *Radix* group: *R. auricularia* race *rufescens*, *R. auricularia sensu stricto*, *R. viridis*, and *R. luteola* (475). However, the presence of *G. truncatula* has recently been reported after DNA sequencing (149), which indeed means that *F. hepatica* may be transmitted in this country by both *G. truncatula* and *R. viridis*.

FASCIOLA HEPATICA SPREAD INTO AFRICA

The distribution of *F. hepatica* in Africa is restricted to two regions. On one side, it is found throughout the western Maghreb countries of northern Africa, including Morocco, Algeria, and Tunisia. On the other side, it is known throughout the long eastern region from the Nile Delta in Egypt down to South Africa, although along a discontinuous distribution.

The report of *F. hepatica* verified by ITS sequencing in Central Africa, i.e., Niger (480), should be considered a result of recent livestock introduction, as already discussed (1).

Fasciola hepatica Spread into Northwestern Africa

Regarding Morocco, Algeria, and Tunisia, the results of recent studies fit well with the scenario previously described (1). In this region, *G. truncatula* is present, *R. natalensis* is absent, and *F. gigantica* apparently never reached such northern latitudes of the African continent (67). The recent finding of *F. gigantica* in southernmost Algeria only represents an introduction with transhuman herds guided by nomads (Fig. 11), and its unique report in donkeys of Morocco has been proved to be a misdiagnosis with *F. hepatica* (67). An initial introduction of *F. hepatica* with midsized ruminants as mainly goats, but also sheep, may be envisaged to have occurred along the northwestern African coast of the Mediterranean around 6,800 to 6,400 years BP from animals of the eastern Mediterranean Levant (158, 481). It should be emphasized that *G. truncatula* from Morocco shows a peculiar mutation in the position 132 of the rDNA ITS-1 (haplotype ITS1-B), which allows its differentiation from *G. truncatula* of Europe (haplotype ITS1-A) (102). This supports a westward spread along northern Africa that is different from the westward spread throughout Europe.

Subsequently, first the Phoenicians with their vessels coming from the Levant around the 1st millennium BC (Fig. 21) and then the Empire of the Romans who gave a great importance to livestock, followed by the Arabian expansion carried out from the Near East along the southern Mediterranean shore up to the Iberian Peninsula, which opened a long-time exchange through the Gibraltar strait, should undoubtedly have influenced livestock movements and mixing throughout many centuries (1). It should also be considered that livestock importations into the Maghreb countries from mainland Europe (e.g., France and Spain) took place in recent times, and thus an overlap of chronologically different introductory

FIG 21 Main ancient spreading routes of *Fasciola hepatica* throughout eastern and northern Africa from its original paleobiogeographical area in Near East Asia. Red arrows indicate the spread through the Arabian Peninsula in the predomestication period. Black arrows indicate the spread through the Arabian Peninsula in the postdomestication period and through the Mediterranean Sea from the Levant with the Phoenicians.

F. hepatica waves may have been at the origin of highly mixed populations of this fasciolid in this region. Such mixing has been observed in studies on the genomes of goats (482). All this needs to be considered to correctly interpret results of the recent molecular characterization of *F. hepatica* in Algeria and Tunisia, which fit well the aforementioned scenario (483–485). However, the suggested introduction from Spain into Algeria of given mtDNA *nad1* haplotypes and the suggested origin of *F. hepatica* in Algeria from

Europe because of the lack of genetic relationships of *F. hepatica* of Algeria with Egypt and Afghanistan (486) does not fit adequately. Indeed, mainly midsized ruminants but also taurine cattle were originally introduced into the Italian Peninsula and the Iberian Peninsula from the Levant around 5500 BC (Fig. 14) (289, 290), and a subsequent introduction following a maritime route might have occurred along the north-African coast with the Phoenicians around 1,000 to 900 years BC (Fig. 21) (291). The shared mtDNA haplotypes should have been probably introduced in the Maghreb and in the Iberian Peninsula during these Neolithic spreading waves. Moreover, neither in the aforementioned first old arrival nor in the second Phoenician wave was there a previous transit through Egypt and, of course, a relationship with Afghanistan can geographically be ruled out. Nevertheless, the hypothesized dispersal direction from the northern high Bordj-Bou-Arreridj plateau, close to the Mediterranean coast, to the southern Djelfa steppe, closer to the desert (486), appears to agree with these historical records.

Fasciola hepatica Spread into Eastern Africa

Concerning the presence of *F. hepatica* along eastern Africa, new data have appeared in the last decade that indicate the need to reformulate the puzzle of this evolutionary scenario in great part.

The evolutionary enigma of *Fasciola hepatica* in Egypt. For the analysis of the arrival and spread of *F. hepatica* in eastern Africa, the first unavoidable step should refer to Egypt.

The remains of a trematode found in histological sections of a mummified liver in one of two brothers found in a tomb of the 12th dynasty of Old Egypt, around between 1938 and ca. 1756 BC was classified as *F. hepatica* based on a flattened group of cells with thickened walls of the parasite (31), although with such material the differentiation from *F. gigantica* should not have been evident.

Stéphanie Harter, in her Ph.D. thesis (275), reported the finding of four *Fasciola* eggs in a sarcophagus, dated 400 to 300 years BC at the archeological site of Saqqara, south of Cairo, in Upper Egypt. The sizes of these eggs, ranging from 118.32 ± 13 by 74.22 ± 6.17 μm, undoubtedly indicate that they belonged to *F. hepatica*. In the same thesis, she further reported the findings of *Fasciola* eggs in human and animal mummies in three archeological sites of Upper Nubia, northern North Sudan: (i) for Sai island, 20 eggs, measuring 131.37 ± 2.03/70.26 ± 0.11 μm, in two places dated from 700 to 300 years BC and 275 years BC to 350 years AC; (ii) for the site of Kerma, 5 eggs, measuring 133.24 ± 3.13 by 66.78 ± 7.7 μm, dated from 2,400 to 1,750 years BC; and (iii) for the site of Sedeigna, 2 eggs, measuring 121.5 ± 16.3 by 81.7 ± 13.4 μm, dated from 300 to 1,500 years AC. According to these measurements, there is no doubt that they all belonged to *F. hepatica*.

These archeological data appear to fit old reports on the presence of *G. truncatula* in Lower and Upper Egypt (165, 172, 487). One subfossil finding of *G. truncatula* in Lower Egypt and another in Upper Egypt were noted (172). Nevertheless, *G. truncatula* cannot be malacologically differentiated from *L. schirazensis*, another lymnaeid also known to be present in the broad Nile Delta area and which has been demonstrated both experimentally and in the field to not transmit *F. hepatica* (4). In Sudan, appropriate studies on lymnaeids are unfortunately lacking, although the presence of *G. truncatula* should *a priori* not be ruled out (488), and both *F. hepatica* and *F. gigantica* have been reported to infect sheep imported from Sudan into Saudi Arabia (489).

Surprisingly, however, these data do not agree with the literature on fascioliasis in Egypt. Although the old literature coincides in highlighting that *Fasciola* was posing big health problems in sheep in Egypt since as early as 1834 (490), there are many reports noting that the only fasciolid species present in sheep, goats, and cattle, as well as in pigs (491), was *F. gigantica* (29, 30, 48). The lymnaeid *R. natalensis* ensured its transmission (165, 172). Subsequent studies emphasized that livestock was later imported from European countries where *F. hepatica* was present (492, 493). Although *F. hepatica* was initially noted to be absent in native animals of Egypt (494). It began to be detected in imported animals (133–135), which indicated that it succeeded in adapting to local lymnaeids. A little later, both fasciolid species began to be reported together in coinfections of domestic animals (495) and, with time, *F. hepatica* became a common parasite in Lower Egypt (493, 496). The construction of the extensive irrigation canals throughout the wide Nile Delta provided the ideal biotopes for the spread of *G. truncatula* passively transported by the freely moving numerous livestock species (Fig. 15) (107). The previous absence of this large irrigation system might not have facilitated such a quick spread of *F. hepatica*.

It may be concluded that *F. hepatica* was present in Egypt for a period BC. There are two possible ways for such an introduction into Egypt to be considered: (i) whether it was introduced from the Near East thanks to the exchanges the Old Egypt civilization performed with Mesopotamia (1), since indeed goats, sheep, and cattle are known to also have entered Africa by this northeastern way (497), which would explain the introduction of the markedly amphibious snail *L. schirazensis* from the Near East to the Nile Delta (4), or (ii) whether it arrived from the south thanks to the interaction the Egyptians maintained with the Nubia civilization until they finally conquer the Nubian Kerma around 1,550 years BC (275). This southern route implies the acceptance of another previous *F. hepatica* entry way through the Arabian Peninsula and the horn of Africa (see below) (Fig. 21).

Following one way or another, this means that *F. hepatica* should have disappeared (or remained in undetectable small local foci) from Egypt until a recent livestock importation from Europe allowed it to be reintroduced around the middle of the last century. The only way to understand such a disappearance is considering the desertification process suffered by Egypt (498). Progressively increasing aridity, including an onset of hyperaridity around 5500 BP, led societies to become increasingly fragmented, with groups retreating to refuges such as the Nile Valley, returning to specialized pastoralism or migrating southward back to the Sudan (498).

The recent report of *F. hepatica* infecting both cattle and *G. truncatula* but not coexisting *R. natalensis* in the Dakhla Oasis, at a latitude similar to that of Luxor in Upper Egypt, but deep in the desert, 350 km away westward from the Nile, verified by *cox1* sequencing (499), should be considered. This finding illustrates the pronounced colonization capacity of this liver fluke species and its lymnaeid vector by taking advantage of livestock movements. This isolated focus in the middle of the desert recalls the similar extreme situation described in a desert-arid focus of human fascioliasis in Argentina, in which all disease transmission factors are concentrated in a small area where humans and animals go for water supply (500).

***Fasciola hepatica* spread in eastern and southern Africa.** The changing fascioliasis scenarios in Egypt suggest to the need to look for an alternative introduction route of *F. hepatica* that allows for its observed eastern spread in Africa. Indeed, the presence of *F. hepatica* is currently known in a first focus linked to the plateaus of Ethiopia, a second large focus related to the highlands of Kenya and Tanzania, and a third large area of endemicity in South Africa (1). Although an introduction of at least zebu cattle at the end of the 7th century was already evoked to potentially represent a major entry point through the Horn and the East Coast of Africa (1), the real role of the Arabian Peninsula in the introduction of *F. hepatica* could not be envisaged until the new knowledge obtained during the last decade on the importance of this Peninsula in the faunal exchange between Asia and Africa occurred in the Late Miocene and earliest Pliocene, including bovids (501). Additional research has shown the presence, at least periodically, of substantial water bodies and open grassland habitats, as well as animals with African and Asian affinity in Saudi Arabia in the Middle Pleistocene (502). This includes a period following the appearance of *F. hepatica* in the Near East Asia and consequently the possibility for this fasciolid to have entered with wild animals through the horn of Africa largely before the appearance of humans and animal domestication. Moreover, paleoclimatic data and the fossil record support an environmental instability facilitating several spatial and temporal refugia in East Africa which manifested in the ungulate assemblages along the Pleistocene (503).

In the Arabian Peninsula, lymnaeid vector species reported include *R. auricularia*, *R. natalensis*, and *G. truncatula* (242)—a faunistic assemblage representing a continuation of that known in Jordan (166). In Saudi Arabia, fascioliasis has been present for a long time (504). More recently, a new species of *Galba*, *G. robusta*, has been proposed for specimens collected in Yemen and which were larger than *G. truncatula* (505). In this zone, the presence of *G. truncatula* had been previously reported from the highlands of North Yemen and South Yemen (506, 507). Although it is still not known whether *G. robusta* can transmit *F. hepatica*, its size suggests such a possibility, because small *Galba/Fossaria* species such as *L. schirazensis* do not (4). The additional interest of *G. robusta* is due to the fact that it has been linked to the small *G. mweruensis* of northern Kenya, also considered to be present in Ethiopia, and to *G. umlaasiana* originally described in South Africa (505).

Moreover, *Galba/Fossaria* specimens were collected in the highlands of Ethiopia, the Eastern Arc Mountains of Tanzania, Mt. Elgon in Uganda, and the highlands of Lesotho in southern Africa (Fig. 21), molecularly characterized, and ascribed to the species *G. mweruensis*, although no specimens from the type locality of Kenya could be collected and analyzed (149). Populations of this lymnaeid from Kenya Highlands were shown to transmit both *Fasciola hepatica* and *Fasciola gigantica* (508, 509), which is surprising not only because of its small size of only up to 8.5 mm in height (149), when considering that the similarly small *L. schirazensis* does not transmit because it does not survive the fasciolid infection (4), but also because it undoubtedly belongs to the *Galba/Fossaria* group of vectors of *F. hepatica* that appear to be unable to maintain the life cycle of *F. gigantica* in the long term (32).

An explanation may be found in its old origin. Indeed, estimations based on a 658-bp mtDNA *cox1* fragment led to conclude that the split between *G. mweruensis* and *G. truncatula* was dated around 3.9 and 7.8 mya and that diversification of *G. mweruensis* populations occurred between 1.7 and 3.4 mya (149). Unfortunately, the accelerated evolution of mtDNA repeatedly reported in pulmonate gastropods and for which many explanatory hypotheses have been proposed, among which one refers to the influence of isolation in refugia (510), was not considered. All this suggests that these dating estimations concerning the early Pleistocene, the Pliocene, and even the Miocene may perhaps be overestimations. Nevertheless, there is no doubt that *G. mweruensis* has an old origin, which brings it evolutionarily close to the dating of the origin of *F. hepatica*. Parasite specificity is an evolutionary process that requires a long period of parasite-host coevolution (except in fast speciation events linked to capture phenomena from a host group to another phylogenetically distant group, which is here evidently not the case). This may underlie the capacity of *G. mweruensis* to transmit both fasciolid species. However, it cannot of course be ruled out that the sympatry of the two fasciolid species in the Kenya Highlands gave rise to hybrid fasciolids which were unknown to exist at that time (508, 509) and which we know today to be able to lead to morphological classification confusion and consequently to fasciolid/lymnaeid specificity misdiagnoses when working with organisms that are not molecularly assessed.

In Ethiopia, *F. hepatica* is known to infect sheep in the Ethiopian Central Highlands (511) and appears to be more prevalent than *F. gigantica* in cattle, including also mixed infections, in given areas (512). The infection rates by *F. hepatica* greater than those by *F. gigantica* in donkeys, horses, and mules in the highlands of southern Ethiopia should be emphasized (513). Recently, the donkey has been experimentally proven to play a role as a reservoir of fascioliasis (182) and, similarly, although at an intermediate level between donkey and horses, this capacity has been verified for the mule (104). The infection risk by the two fasciolids throughout Ethiopia was already analyzed according to geographical zones and seasons by means of a geographical information system (GIS) forecast model, which showed that there is an *F. hepatica* infection risk in most parts of the country (514). The same GIS approach additionally extended to Sudan and Kenya showed that areas with an average annual mean temperature higher than 23°C were generally unsuitable for *F. hepatica*, whereas highland zones of Ethiopia and Kenya proved unsuitable for *F. gigantica* due to an inadequate thermal regime (515).

In Kenya, the presence of *F. hepatica* infecting cattle has been known for a long period (516), and its recent molecular characterization by rDNA ITS-2 and ITS-1 sequencing has shown no differences regarding *F. hepatica* from Switzerland, Spain, Tunisia, and China (517). In Uganda, studies on the prevalence of fascioliasis in indigenous cattle slaughtered in Kampala City abattoir showed that infection by *F. hepatica* was very low compared to that by *F. gigantica* in this lowland plain (518). On the contrary, the prevalences of *F. gigantica* proved to decline with increasing altitude in the Mount Elgon National Park, where *R. natalensis* populations disappeared from 1,800-m altitudes upwards and *G. truncatula* populations begin to appear from 3,000 m upward, although no *F. hepatica* could be found in the cattle analyzed (519).

In Tanzania, *F. gigantica* is widely present in the lowlands (520), but *F. hepatica* appears in high-altitude areas such as the Kitulo Plateau of the Southern Highlands inhabited by *G. truncatula* (521). In a subsequent study of a 1,400-bp mtDNA sequence enclosing the regions coding for cytochrome *c* oxidase subunit III (coxIII), tRNA histidine (tRNA-His), and cytochrome *b* (cob), four haplotypes formed a clade separated from European *F. hepatica* by at least 71 nucleotide substitutions, which is estimated to have differentiated ~1 mya. Another fifth haplotype was within the range of *F. hepatica* known in Europe, indicating that these specimens were the result of a recent introduction of cattle and sheep into the area (131). This result suggests an old introduction of *F. hepatica* and subsequent survival in altitude refugia, which agrees with the results of the studies on the local species of *Galba/Fossaria* obtained later and analyzed above (149), as well as with the several spatial and temporal refugia in East Africa observed in the ungulate assemblages along the Pleistocene (503).

In Zimbabwe, both *F. gigantica* and *F. hepatica* have been reported (522), although *F. gigantica* is the prevalent species, and *F. hepatica* has even been found to infect wild herbivores. However, DNA sequencing did not show haplotypes markedly differing from European *F. hepatica* haplotypes (187). In Lesotho, *G. truncatula* was malacologically identified (523–526), given the wide distribution of *G. truncatula* in the neighboring South Africa (527), so the question remains as to whether both *G. truncatula* and *G. mweruensis* (149) coexist in the country or all *G. truncatula* populations in Lesotho should be ascribed to *G. mweruensis*.

In South Africa, the existence of *F. hepatica* and *F. gigantica* has been known since long ago (528). It is now generally accepted that the earliest sheep and cattle appeared in southern Africa around 2,000 years ago (529, 530). However, recent new information suggests small-scale infiltrations, including Khoe-language-speaking pastoralists along the East, along the westers Atlantic coast from Namibia, and through the middle reaches of the Limpopo River Basin (529, 531, 532). The presence of *G. mweruensis* in Lesotho supports an entry for fasciolids and lymnaeids along the eastern part of the country, as does the distribution of both *F. hepatica* and *F. gigantica* in the eastern and southern parts of South Africa (Fig. 21) (171). However, the presence of *P. columella* and the recently detected *Radix rubiginosa* of Asian origin (170) indicate more recent introductions. Indeed, these two snails are considered invasive species taking advantage of the ornamental pet trade (110).

The question is now posed regarding *G. truncatula* in South Africa. If its molecular analysis confirms that it is *G. truncatula*, we will need to consider its introduction by a way different from eastern Africa, where all populations previously ascribed to this species seem to indeed belong to *G. mweruensis*. If all populations in South Africa belong to *G. mweruensis* instead of to *G. truncatula*, this would support an eastern entry of *F. hepatica*, and peculiar haplotypes of this fasciolid should be detected as ancestral remains, as found in Tanzania (131). Nevertheless, studies of DNA markers of *F. hepatica* from South Africa have not yet detected any peculiar sequence (187), and only moderate sequence variation was found in *F. hepatica cox1* (533), which proved to be far from the peculiar sequences found in Tanzania.

If both lymnaeid species coexist in South Africa, their geographical distribution inside the country should be assessed to see which one predominates in the eastern part and which one predominates in the west southern part of the country. The malacologically assessed distribution of *G. truncatula* in South Africa appears clearly concentrated in the eastern part of the country (527), which also supports an eastern entry of *F. hepatica*. An introduction of *G. truncatula* with imported livestock by ship should *a priori* not be ruled out, since this lymnaeid colonized South America in that way (1).

When considering all of the aforementioned data, there is an agreement indicating three potential introduction waves of *F. hepatica* into eastern and southern Africa.

A first old entry from around the Middle Pleistocene, perhaps in the Early Pleistocene, coincides with a faunal interchange of large mammals between the Near East and eastern

Africa (534), through the Arabian Peninsula and the Horn of Africa. This old wave would explain the peculiar haplotypes of *F. hepatica* in Tanzania (131), as well as the *G. mweruensis* populations isolated in refugia in eastern Africa (149). This wave did therefore occur in the predomestication period, without any intervention by humans (Fig. 21).

A second wave occurred in the postdomestication period, through the Arabian Peninsula and the Horn of Africa, and is based on the introduction of domesticated zebu and perhaps also the dromedary along the period between 4,000 and 1,000 years BC (175). Indeed, the Arabian Peninsula was populated by many dispersing human populations during the Quaternary (535). A large-scale movement of people has been proposed to occur through the Arabian Peninsula into Ethiopia around 3,000 years ago, probably associated with the D'mt kingdom and the arrival of Ethiosemitic languages. This resulted in the dispersal throughout eastern Africa, followed by a migration of admixed populations, probably pastoralists related to speakers of the Khoe–Kwadi languages, from eastern Africa to southern Africa, around 1,500 years ago (536). Other domesticated animals, mainly sheep and secondarily also goats and donkeys, introduced into Africa long before, probably played a role in the subsequent spread of *F. hepatica* along eastern Africa, as for instance in a northward spread up to Upper Nubia and Egypt. Indeed, the genetically distinct fat-tailed sheep would have been introduced from the Arabian Peninsula through the Horn of Africa to subsequently spread southward throughout the eastern part of Africa (497). Rock art representations of this sheep in the Horn of Africa and South Africa indicate that it was already there by the second half of the Holocene. The archeological *F. hepatica* egg findings in Kerma, dated around 2,400 to 1,750 years BC (275), indicate an early introduction in this postdomestication period, namely, around 4,000 and 2,500 years BC. A role for sheep, goats, and zebu in a spread throughout southeastern Africa may also be envisaged (Fig. 21). Interestingly, different goat subclusters could be found corresponding to the breeds from (i) Kenya, Tanzania, and Ethiopia, (ii) Uganda and Burundi, and (iii) Malawi, Zimbabwe, and Mozambique (482).

A third wave of introductions refers to importation events into different eastern African countries that occurred in recent decades, such as, for instance, several recent exports of highly productive or specialized breeds of European goats into Tanzania and Kenya (482), or the haplotype present in the highland Tanzanian *F. hepatica* population, which was within the range of nucleotide diversity seen in European flukes (131), most probably introduced together with cattle and sheep imported into this area in recent times (521).

The aforementioned first and second waves further support the origin of *F. hepatica* in the Near East, its original wide spread from this region together with domesticated animals, and an early extent of this crucial region southward into the Arabian Peninsula (Fig. 21). The scenario of *F. hepatica* in northwestern, northeastern, eastern, and southern Africa should be henceforth considered to facilitate the correct diagnosis of patients, as well as to assess the related epidemiology and to define the appropriate control measures, which differ pronouncedly between *F. hepatica* and *F. gigantica*.

FASCIOLA HEPATICA SPREAD INTO THE AMERICAS

Fasciola hepatica was introduced into the Americas from western Europe around 500 years ago with the transport of livestock by the Spanish "conquistadores" in their old vessels. There is very wide documentation on which livestock species were transported, how were they transported inside the vessels, where in Spain they originated, and where in the Americas they were released. This anthropogenic process allowed for repeated introductions mainly along the first decades of the colonization period, from the end of the 15th century throughout the 16th century. DNA marker sequencing of *F. hepatica* has further supported such an introduction into South America (1, 32, 102) and North America (124), as well as the subsequent spread throughout South America (Fig. 22) (101). The simultaneous introduction of markedly amphibious lymnaeid species from the Old World, such as *G. truncatula* and *L. schirazensis*, passively transported

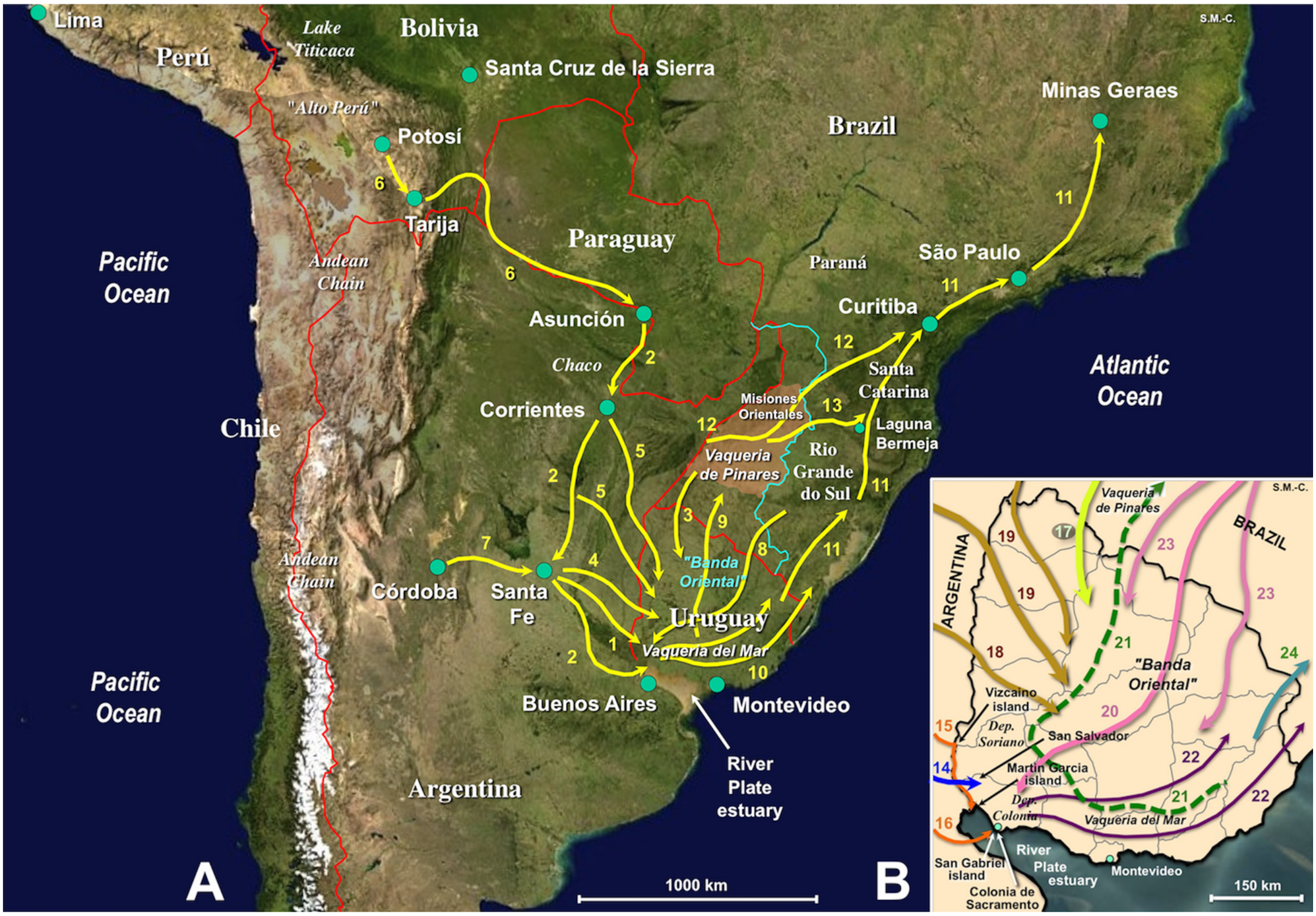

FIG 22 *Fasciola hepatica* was mainly introduced and colonized South America from the coast of the Pacific. Livestock movement timeline in the early introduction and spread of *Fasciola hepatica* and lymnaeid vectors from the Bolivian Andes down to the eastern lowlands of South America. (A and B) Maps of South America (A) and Uruguay (B) showing main livestock passageways during the early Spanish and Portuguese colonizations. The analysis concerns the time of the old Viceroyalty of Rio de la Plata, from Buenos Aires in the South and the "Banda Oriental" in the Southeast up to "Alto Peru" in the North. Numbers: 1 and 14, first introductions of pigs in 1541, horses in 1574, and goats in 1577; 2, 15, and 16, first and second introductions of cattle derived from Corrientes population in 1611 and 1617; 3 and 17, introduction of cattle from Misiones by Jesuits at the beginning of 17th century; 4, 5, 18, and 19, first introduction of sheep from Santa Fe in 1727 (4, 18) and subsequent large-scale cattle introductions with "faeneros" from Asuncion, Corrientes, and Santa Fe (4, 5, 18, 19); 6, livestock route for silver transport from Potosi mines from mid 16th century; 7, original route for introduced goats in 1611 to 1618; 8 and 20, introduction of sheep by the Portuguese in 1734 to 1735; 9 and 21, largest rustle of more than 400,000 cattle in 1705, from Vaqueria del Mar to Vaqueria de los Pinares, at the southern part of the Jesuit Misiones Orientales area (brownish area); 10 and 22, Livestock spread at mid and end of 17th century; 11, livestock route (Camino Real, Ruta del Viamont or Caminho do Viamão) for gold transport from Minas Geraes mines from 1690; 12, interconnection livestock route (Ruta de las Misiones or Caminho das Missões); 13, interconnection livestock route (Ruta de la Vaquería or Caminho da Vacaria); 23, groups of Portuguese "bandeirantes" also using livestock; and 24, northward spread of livestock. (Reproduced from reference 101.)

in mud attached to animal hooves, furnishes additional molecular support to confirm all this scenario. *Galba truncatula* played a decisive role in this introduction phenomenon because of its adaptation to wide territories of South America (103, 537–539) and its great liver fluke transmission capacity of isolates from different domestic animal species, even under the extreme conditions of a very high altitude (2, 182, 377). *Lymnaea schirazensis* is not a vector species, but it has proven to be a very valuable marker of livestock movements, even between continents (4). American autochthonous lymnaeid snail species of the *Galba/Fossaria* group and the genus *Pseudosuccinea* subsequently facilitated this liver fluke colonization of, and spread throughout, the New World thanks to their liver fluke transmission capacity, such as *L. bulimoides* and *L. humilis* in North America, *L. cubensis* and *P. columella* in the circum-Caribbean region, *L. cousini*, *L. neotropica*, and also *P. columella* in South America, and *L. viator* in the Southern Cone.

A recent report on the detection of eggs of *F. hepatica* in deer coprolites in Patagonia dated back to 2,300 years BP (540, 541) has again focused on the possibility that this liver fluke was already present in the Americas before the first arrival of the Europeans with their livestock. More-recent analyses have tried to find explanations for such an earlier existence in the New World (542–544). A multidisciplinary analysis is consequently appropriate to see whether there are verifiable arguments which could justify such an assumption, in the way to provide a well-defined scenario on which to base and fit future research on fascioliasis in the Americas.

North America and the Bering Land Bridge

The first report of a potentially ancient presence of a fasciolid in the New World was in a sample of 168 coprolites (ca. 250 to 800 years old) from Lovelock Cave and in the reconstituted intestinal contents of a mummified body (age unspecified) from a cave at Pyramid Lake, Nevada, western USA (545). Unidentified eggs were seen in small numbers in three samples, among which one egg was of an apparent fasciolid trematode. Unfortunately, no subsequent study was later published on this egg so as to get an adequate conclusion.

There is logically the temptation to link the aforementioned archeological fasciolid report to the reports of the presence of *G. truncatula* in the North Gulf Coast of southwestern Alaska, south central and/or extreme northwestern British Columbia, and Yukon River drainage in the western Yukon (3, 546–548). Unfortunately, this specific lymnaeid classification has never been molecularly confirmed, and it should be considered that traditional malacological methods relying on shell morphology and anatomy are largely insufficient for snail specimen classification in lymnaeids of the *Galba/Fossaria* group (4, 90, 549). Indeed, an expert as Burch already emphasized that the aforementioned North American *G. truncatula* populations may in fact belong to a different species (John B. Burch, Curator of Mollusks. Museum of Zoology, University of Michigan, Ann Arbor, MI, personal communication to J. Cordeiro, 2000, in Nature Serve Explorer, 18 October 2018, https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.107211/Galba_truncatula) (accessed 2 January 2022).

Paleoclimatic and paleobiogeographical knowledge does not support an introduction of *F. hepatica* and/or *G. truncatula* from the Palearctic Region into the Nearctic region through a past Beringia land bridge, either with animals or with humans. Beringia played an important role in the exchange of faunas between eastern Asia and North America during several geological epochs until 10,000 to 8,000 years ago when the land bridge disappeared (550). However, this northern connection was not geographically permanent nor climatically stable, a varying evolution that should carefully be considered to reach timely correct interpretations of faunal turnover events. Environmental temperature is a key factor at such an extreme latitude in this evolving Beringia scenario. For instance, Quaternary glacial and interglacial cycles, driven by changes in the orbital pattern of the earth, followed periods of about 20, 40, and 100 Ka and led to pronounced changes of sea level and to the corresponding opening/closing of this land bridge.

Within the evolution of fasciolid trematodes, Beringia could have played a role since the beginning of the Neogene, around 23 million years ago. From the early to the middle Miocene, the warm climate of the so-called Miocene Climatic Optimum underlay a great radiation of ruminants. Around 15 mya, the sea levels fell due to cooling climate in the high latitudes, forming ice sheets (551). In the late Miocene, the temperature gradient from equator to pole was weak, and higher latitudes were warmer than today (552).

The rDNA 18S gene molecular clock indicates a divergence in the old lineage of *Fascioloides* in the Holarctic, estimated to be at 19.8 mya between *F. jacksoni* of elephants of southern Central Asia and southern East Asia, on one side, and *F. magna* of cervids and caribou in North America, on the other side (139). This fits well with a faunal exchange between the Palearctic and Nearctic regions allowed by the aforementioned temperature evolution in Beringia during the Miocene. There is broad consensus that ancestral odocoileine cervids entered America from Siberia via the Bering Strait in the late Miocene/early Pliocene (551). This dating estimation appears however to be chronologically too early for *F. hepatica*, according to its pronouncedly distant Near East geographical origin around the Miocene-Pliocene boundary, 6.0 to 4.0 mya or even somewhat more recently (139). Moreover, there is no paleontological information suggesting a fast ruminant migration from the extreme West up to the extreme

East of Asia in this epoch as to think that *F. hepatica* could reach the Far East and the Beringia region so early.

Paleontological evidences from bovid fossils indicate subsequent turnover events between the two continents (553). The first arrival of a bovid from Asia into North America is ascribed to the immigration event occurred around 7.5 to 6.8 mya (554), which also took place too early, similarly as the subsequent short one at 5.9 to 5.8 mya. However, the following intercontinental connections around 4 mya and the subsequent at the interval of 3.0 to 2.0 mya (555) could fit for the Nearctic entry of *F. hepatica* provided this fasciolid to have reached the Far East at that time, perhaps with the earliest sheep appeared in China (approximately 2.42 mya) (553). This, of course, depends on whether a migration and host capture from late Miocene Near East caprine ancestors had happened previously. Unfortunately, there is no fossil argument to support such an eastward movement of ovicaprines potentially infected by *F. hepatica* and passively transporting *G. truncatula* snails.

The evolution of camelids, the only remaining mammal group of potential interest regarding a possible ancient introduction of *F. hepatica* from Asia into the Americas does not shed any light on that question. In fact, the Camelidae family originated in North America during the Eocene period around 40 to 45 mya (556) and the Old World domestic dromedary *Camelus dromedarius*, the domestic Bactrian camel *C. bactrianus*, and the surviving, undomesticated, wild *C. ferus* are only evolutionary consequences of an introduction, although following the opposite trans-Beringian directionality from the Nearctic into the Palearctic that occurred after the late Miocene (557).

Crucial information has been progressively obtained about the evolution of Beringia during the late Miocene, Pliocene, Pleistocene, and Holocene by studying many parasite-host assemblages focusing mainly on helminths of artiodactyls and rodents, but also lagomorphs, insectivores, and carnivores. The conclusions of this study of interest for our *F. hepatica* analysis refer to the fact that the Bering Land Bridge was not a feasible colonization route for many temperate organisms and that helminths included cestodes and nematodes, but no trematodes (558). In the regions with a more Arctic climate, there is a clear tendency toward a lower species diversity of trematodes, up to their total disappearance in the high Arctic. This may be conditioned by the severe environmental conditions in the Arctic coastal zone which impede the transmission of the trematode free-living larval stages of miracidia and cercariae. The only trematodes in Beringia are those infecting fish and intertidal digeneans in birds circulating through molluscs such as marine snails, bivalves, and cephalopods (559).

This argues against a freshwater lymnaeid snail faunal exchange between northeastern Asia and North America through the cold Beringia in the last geological periods. A very recent review of the present knowledge of the malacofauna of the circumpolar Arctic emphasized the depauperate species richness of snails compared to temperate regions, referring to only 12 lymnaeid species in Beringia, 16 in Siberia, and 8 in North America, with only one species, *Lymnaea stagnalis*, present on both Palearctic and Nearctic sides, although there are authors arguing that the two populations of this species are not conspecific (560). Studies indicate that the more northerly an island or archipelago lies, the poorer its molluscan fauna is. This impoverishment of the aquatic malacofauna of all Arctic islands does not favor the hypothesis of intensive migration of molluscs via land bridges. Moreover, potential refugia represented by hydrothermal sites in different regions within the Arctic and Subarctic zones do not seem to have played a role in migrations beyond being just a specialized niche for freshwater organisms, because snails in such "hot waters" have it apparently very hard, i.e., impossible, to return to normal local cold-water collections.

According to literature reports of malacological classifications, lymnaeid species that have been potentially involved in *trans*-Beringian migrations through different past geological epochs include (i) two lymnaeid stagnicoline species to have migrated from North America to Asia, namely, *Ladislavella atkaensis* and *L. catascopium*, and (ii) five lymnaeid species from Asia to North America, comprising the stagnicoline *Lymnaea stagnalis*, two radicine as *Kamtschaticana kamtschatica* and *Radix auricularia*, and the two *Galba/Fossaria* species *Galba sibirica* and *G. truncatula* (560). The presence of *Radix auricularia* (and also reports of *R. peregra*) is known to be just a consequence of a recent anthropogenic introduction from

Europe (3, 90, 546), and *Kamtschaticana kamtschatica* is a species of hot springs, warm puddles, and geyser fields (560). Hence, the wide presence of lymnaeid species of the *Radix* group in Asia northward from the Himalayas (452) and the total absence of these radicine lymnaeids in North America further supports the great difficulties or impossibility of lymnaeid snails to cross through such a cool northern Bering land bridge.

Galba sibirica is a species reported from Mongolia, Siberia, Russian Far East, and northeastern China, a northern geographical distribution indicating an adaptation to cold habitats, and whose shell height typically does not exceed 7.5 mm (561, 562). This small size suggests this species to be probably not susceptible to *F. hepatica* transmission, as already confirmed in another small species of the same group (*L. schirazensis*) (4).

Like *L. schirazensis*, *G. truncatula* is a markedly amphibious species, a characteristic that underlies a great dependence on climatic factors but also facilitates its passive transport by staying in mud attached to the hooves of the animals. This allowed these two lymnaeid species to colonize the New World, including very-high-altitude areas in equatorial latitudes. However, *G. truncatula* does not show an unlimited altitudinal spreading capacity (103), which is defined by its minimum development temperature threshold, Moreover, it should be considered that in altitudinal habitats of such extreme conditions, the high daily temperatures counteract the low nightly temperatures (563), allowing for the development of lymnaeid populations in mainly permanent freshwater collections due to the high evapotranspiration rate at such extreme altitude (564). Although the minimum development temperature threshold of *F. hepatica* is at 9 to 10°C, the counteraction of day/night temperatures at such altitudes has been experimentally verified to enable *F. hepatica* transmission in spite of a mean environmental monthly temperature maintained below the aforementioned minimum (377). At Arctic and Subarctic latitudes, the absence of high temperatures during the short sunshine hours and even the total absence of sunshine during long periods of the year do not provide such a counteraction. Sunshine is additionally needed for the growth of freshwater algae on which this lymnaeid in great part feeds. All in all, this explains the absence of *F. hepatica* transmission throughout all extreme latitudes.

In the same sense, it is hard to understand why the *G. truncatula* populations reported from Alaska and northwestern Canada did not spread southward, unless these populations indeed belong to another *Galba/Fossaria* species adapted to cold environments, in agreement with Burch's opinion.

Although the richness of species of the *Galba/Fossaria* group, also called fossarine species, may be interpreted as an evidence of the paleobiogeographical origin of this group in the Nearctic region, the very great genetic distances between Palearctic *G. truncatula* and North American *Galba/Fossaria* species indicate a trans-Beringian migration from North America to Asia occurring a very long time ago, which should have taken advantage at least of the Miocene Climatic Optimum, if it did not occur even earlier. This supports the widely accepted paleobiogeographical origin of *G. truncatula* in the Paleartic region as an evolutionary derivation from the migrant fossarine ancestors. The genetic distances also indicate a similar origin and evolution for *L. schirazensis*. Such an evolution also agrees with the general East to West migration trend within the Palearctic region observed in studies of the malacofaunas (560).

The complexity of the evolutionary geographical and climatic changes occurred along the many different geological epochs, the very different characteristics and requirements of the organisms involved, and the many multidisciplinary factors to be considered in these types of analyses underlie the inappropriateness of simplistic interpretations of phylogenetic trees based on a few DNA sequences, usually based on incomplete gene sequences. The risk of misinterpretations of DNA sequence phylogenies when not supported by fossil data are well known (565). The conclusion about the origin of *G. truncatula* in North America and its subsequent worldwide spread from there, reached in such a phylogenetic study (566), is such an example which may lead to subsequent misinterpretations about the spread of human and animal fascioliasis.

Even humans have been suggested to have played the role of hosts involved in the introduction of *F. hepatica* from Asia into the Americas through Beringia (543). Such a hypothesis suffers from the lack of considering many crucial aspects.

A part of the confusion may derive from summaries referring to humans as having introduced the liver fluke into a new area. It should be clarified that this does not mean that humans infected by *Fasciola* were the hosts originating the spread of this trematode. Humans only collaborated or facilitated such a spread when accompanied by or moving their livestock that was infected by *Fasciola*. This happened (i) when in the new area there were susceptible lymnaeids enabling the liver fluke to adapt, be transmitted, and thus become established, and (ii) also when in the new area there were no lymnaeids, but the imported livestock passively transported susceptible lymnaeids in mud attached to their hooves, which adapted to freshwater collections of the new area and subsequently allowed for liver fluke transmission in that area (1).

Although we know today that humans are viable hosts in areas of human endemicity, mainly when they are hyperendemic (567), this concerns areas where the human lifestyle is sedentary. Liver fluke infection has also been reported in nomads (568), but such cases refer to tribes following livestock transhumance, i.e., yearly ups and downs along the same altitudinal transect. The humans crossing Beringia in old times were not accompanied by livestock, because the dates of the trans-Bering entry of humans into North America, at least 13,000 years ago, are anterior to the earliest herbivore animal domestication events (12,000 to 10,000 years ago). In contrast to animals, it is difficult to think of human migrants passively transporting lymnaeid snails. The cold northern temperatures of Beringia neither allow to think at the possibility for fasciolid eggs shed by humans to be able to embryonate. Indeed, one of the traditional methods for experimentally keeping liver fluke eggs from starting embryogenesis is by applying a low temperature, such as 4°C.

Interestingly, two other alternative routes of human arrival to the Americas have been proposed to have occurred before the European colonization of 500 years ago as a way to explain the detection of human specific nematodes in human coprolites found in archeological prospections in the New World (569). To understand the presence of *Trichuris trichiura*, *Strongyloides stercoralis*, and ancylostomid hookworms in archeological findings, it has been argued that humans could have reached the Americas more than 7,000 years ago using either (i) a coastal migration route by means of natural watercraft (570), without excluding a jumping migration from the Kamchatka Peninsula to southern Alaska along the Aleutian Islands, or (ii) a northern trans-Pacific migration (569). In this way, they would have avoided the extreme cold of the Arctic. However, these assumptions are based on human-specific nematode parasites following a one-host life cycle, i.e., without the need for a vector or intermediate host for their transmission. There are therefore no arguments to support the feasibility of an introduction of *F. hepatica* by infected humans by one of these two alternative routes. As already highlighted in studies on Arctic and Subarctic latitudes, the environmental conditions impede the existence of trematodes other than those infecting fish and birds (558, 559). It should be considered here, moreover, that the circulation of winds throughout the Pacific does not allow a more southern west-east navigation, as established by the early Spanish precursors of the 250-year-old commercial route known as the Manila Galeon in 1565 (571). The easterly course across the Pacific, known as "Tornaviaje" or "Urdaneta's Route," was by no means an easy voyage due to the difficulties in mastering the Pacific trade winds underlying very long trips lasting to 6 to 8 months.

South America and the Panama Isthmus

Three reports have referred to the potential presence of *F. hepatica* in South America before the Spanish colonization. The first concerned a communication presented to a scientific meeting that referred to the remains of a necrotic parasite with spines and a cuticle (note, however, that trematodes have no cuticle, but they do have a tegument) with an immature ovary seen in some sections of the liver of a child between 3 and 10 years of age from 1,200 years ago, found during a radiographical review of 60 livers from pre-Columbian, Peruvian mummies. The helminth in question was classified as an immature *F. hepatica* (572). No eggs were found that could verify this diagnosis. Unfortunately, no subsequent, more detailed study was published, so this communication may be included within the many paleoparasitological reports from South America classified as never verified and anecdotal (573).

The second report concerns the finding of a single egg in one of 13 sacrificed domestic camelids recovered from the pre-Hispanic Chimú culture site of Huanchaquito-Las

Llamas, close to Trujillo, Peru (574). The archeological excavations furnished remains of 140 children and 206 camelids deposited together during one major event dated around 1400 to 1450 AD. This egg is ovoid, with a length of 130 μm and a width of 85.7 μm, was noted to present an operculum and a knob at the abopercular side and appears deformed in the published photograph (see Fig. 3E in reference 574 [p. 4]). Although these characteristics agree with those of a trematode egg of digeneans of the order Plagiorchiida and the size coincides with that of *F. hepatica*, the authors judiciously declined to conclude a specific ascription, since only one egg was found. Despite this, a misleading publication subsequently appeared referring to the finding of eggs (plural) belonging to *F. hepatica* (as if the diagnosis had been definitive) in the Huanchaquito-Las Llamas archeological site noted in the aforementioned article (543). Several observations may be added regarding this finding in Peru. On the one hand, South American camelids are known to be infected by *F. hepatica*, but certain aspects indicate that these animals may not be considered *F. hepatica* reservoirs able to maintain the life cycle of the liver fluke by their own: (i) their defecating behavior in dung piles, which is always far away from freshwater collections, (ii) the low viability of *F. hepatica* eggs shed in their feces, (iii) the high pathogenicity of the liver fluke in these camelids, suggesting a low parasite-host adaptation, and (iv) their infection always in areas where they cohabit with liver fluke-infected domestic livestock (105). On the other hand, the Huanchaquito-Las Llamas archeological site is located near the Pacific coast in northern Peru, very close to the Tumbes area where the first Spanish colonizers arrived with their ships repeatedly importing livestock at the end of the 15th century and the beginning of the 16th century (575), which is only roughly a few years later than the dating estimation of the aforementioned large sacrifice event.

The third report concerns the recent finding of eggs ascribed to *F. hepatica* in coprolites belonging to native deer identified as the southern pudu *Pudu puda* or the huemul *Hippocamelus bisulcus* (Cervidae) found in the Cueva Parque Diana archeological site, at an altitude of 964 m a.s.l. in the Lanín National Park, North Patagonia, Argentina, dating back to 2,300 years BP (540–542) and which has further been the focus of explanatory analyses (543, 544). This archeological report also merits some consideration.

The measurements reported for these eggs from Patagonian cervids were noted to be 120.0 to 147.5 by 62.5 to 87.5 μm (mean, 131.7 $\pm$ 7.82 by 72.8 $\pm$ 5.96 μm) length/width, which corresponds well with the measurements of "pure" *F. hepatica* in areas without overlap with *F. gigantica* and hybrid absence (576), as is the case for all of South America. These eggs are also described as well preserved, as shown in photographs, with the form, width, and color of the shell and the operculum undoubtedly corresponding to *F. hepatica*. The egg illustrated in the Fig. 1 of the first article (see reference 540, p. 84), which is the same egg but rotated appearing in Fig. 1 of the subsequent article (542), shows a shell and a still-closed operculum, both in perfect status as that of standard *F. hepatica* eggs recently shed in feces of typical reservoir host species. This is a little surprising in such an ancient egg. Indeed, the egg shell in *F. hepatica* is thin and not very resistant, which underlies an easily understandable deformation with time and the total disappearance or opening of the operculum in ancient eggs found in archeological sites (27, 574). Moreover, in the two photographs of the aforementioned ancient egg (540, 542), the egg is empty. Considering that the operculum is still present and closed, this means that this egg was unfertile and not viable. In fasciolids, the eggs are shed nonembryonated with the feces of the definitive mammal host. Recently shed fertile eggs contain a highly visible single germinal cell, located in the mid-axis and anterior half of the egg and surrounded by vitelline material (see, for instance, Fig. 7A in reference 182 [p. 14]). During embryogenesis, which requires adequate environmental conditions, eggs evolve through the following four clearly visible development phases: (i) eggs that include an early developing morula, (ii) eggs with an advanced morula, showing vitelline granules and/or spheroidal cells, (iii) eggs that include an outlined miracidium, in which a miracidial form begins to be observed, and (iv) eggs with a developed miracidium, in which a fully developed miracidium is observed inside. Degenerated, empty, and broken eggs may also be found, mainly when eggs have been shed by an animal belonging to a non-well-adapted reservoir host species (see the photographs in reference 182). Finally, a large morphological and morphometric intraspecific variability was also observed in *F. hepatica* fertile eggs, even in feces shed by individuals of the same host species (576).

This last observation on the absence of inner content is crucial when considering that two relatively long mtDNA marker fragments were noted to be sequenced from these ancient eggs (542). From where was the DNA extracted if the eggs where empty? In the article, it is noted that ". . . eggs . . .. were manually isolated by the use of a micropipette . . ." and that ". . . isolated eggs were used for ancient DNA (aDNA) extraction, amplification, and sequencing . . ..," and later it is added that "before DNA extraction, 30 eggs were washed three times in ultrapure water . . .. and a first disruption step . . .." The finding of 30 eggs is surprising because the numbers of eggs in archeoparasitological remains is usually pronouncedly lower. Also, it is not clear whether a sequencing process was performed for each individual egg or whether these 30 eggs were previously mixed to get sufficient material to ensure a successful extraction. Our wide expertise tells us that to ensure a successful DNA sequencing of a single egg by using standard extraction, amplification, and sequencing methods as those described for these ancient eggs (542), the egg should previously be experimentally embryonated. This was, of course, impossible with such ancient eggs. *Fasciola hepatica* eggs indeed degenerate quite rapidly and lose their viability in a relatively short time, and it becomes impossible to embryonate eggs that are more than two years old, even if conserved under optimal conditions. It is therefore logical to conclude that the aforementioned eggs were mixed to get sufficient initial material. This further suggests that at least several of the mixed eggs could have included developing or fully developed miracidia. Indeed, another ancient egg noted to be of the same age and from the same archeological site illustrated in another article (see Fig. 3 in reference 541 [p. 1527]) and whose photograph appears again identically reproduced later by other authors (see Fig. 2C in reference 543 [p. 691]) also shows a shell in perfect status and with a closed operculum. Unfortunately, because of the inappropriate microscopic focus, the inner content of this egg is not clear enough to conclude whether it is empty or degenerated or whether there is some material remaining.

The question arises whether one can assume that such ancient eggs have not degenerated so as to allow for the obtaining of a clean 417-bp *nad1* fragment and another clean 350-bp *cox1* fragment by means of primers specific for these markers following a standard direct sequencing method instead of using one of the specialized high-resolution modern methods now being applied to archeological materials allowing for the obtaining of aleatory sequence contigs from total DNA. It is therefore difficult to avoid thinking of a possible contamination of the egg initial pull material with *F. hepatica* recent eggs. *Fasciola hepatica* has already been found to infect the two native cervids in question, namely, the southern pudu (577, 578) and the huemul (579–581). The liver fluke also commonly infects the red deer *Cervus elaphus* in the same Patagonian region, a cervid that was introduced into Argentina and has been coexisting with livestock for more than 100 years (582). Livestock began to be introduced into Argentina by the Spanish in the early 16th century, thus transporting both *F. hepatica* and the highly susceptible vector species *Lymnaea neotropica* (Fig. 22) (101). Livestock multiplied at impressive rates in South America (Fig. 23) and reached millions of feral cattle, horses, and goats in the Argentinian territory throughout the rest of the 16th century and subsequent 17th century (583, 584). Long-term field studies have already demonstrated that livestock play a role as an *F. hepatica* reservoir for both the present very numerous red deer populations and the sympatric 100% of populations of huemul (582). Livestock has also been verified to constitute a reservoir for *F. hepatica* infection in two other wild autochthonous cervids, the white-tailed deer *Odocoileus virginianus* and the taruca *Hippocamelus antisensis* in the mountains of Peru (585), as well as the Pampas deer or guarani *Ozotoceros bezoarticus* in Uruguay (586). Livestock should logically also be the liver fluke infection source for populations of native southern pudu.

Cervids are considered to have colonized South America twice. The first arrival would have been in the Early Pliocene around 4.9 to 3.4 mya, although this hypothesis still remains without fossil and paleobiogeographical support. The second colonization occurred around the Plio-Pleistocene boundary, about 2.5 mya, widely diversifying subsequently in South America (551). The absence of *F. magna* in South America, a fasciolid species with known

FIG 23 Taurine cattle selling in the Easter fair at the Main Square, in front of the Cabildo, in Chuquisaca, present-day Bolivia, in 1870. Livestock impressively multiplied in South America after its introduction by the Spanish conquerors. (Original picture available at the Museo del Tesoro, Sucre, Bolivia.)

wide geographic spreading power, specifically infecting cervids throughout North America, and using lymnaeid snail species as vectors (587, 588), does not support an early introduction of *F. hepatica* by cervids into the Neotropical region.

Present South American camelids evolved ~2 mya by derivation from ancient North American forms having migrated to South America during the Pliocene/Pleistocene transition ~3.3 mya (557). However, as highlighted above, their defecating behavior, the low viability of *F. hepatica* eggs from camelid isolates, their high pathogenicity, and their infection in areas of sympatry with infected livestock (105) suggest that South American camelids neither maintained *F. hepatica* transmission in an area by themselves without the presence of livestock, nor were they the mammals responsible for its introduction via the long route from North America down to the Patagonia.

It is unlikely that there was an intervention of bovids in such a potential past colonization of South America by *F. hepatica*, since this ruminant group never crossed the Panama isthmus. Indeed, this is the main reason underlying the evolutionary success of cervids in the Neotropical region (551).

A detailed analysis of the nucleotide positions of the 417-bp *nad1* fragment (GenBank accession number MN207488) and another clean 350-bp *cox1* fragment (GenBank accession number MN207487) (542) in the corresponding alignments with the complete 903-bp *nad1* gene sequence and the complete 1,533-bp *cox*1 gene sequence shows the ancient Patagonian eggs to be base-to-base identical to the same fragments previously found in *F. hepatica* infecting livestock from Spain and other South American countries. Compared to other haplotypes, only a very few single nucleotide positions (SNPs) appear linked to the well-known intraspecific variability in these mtDNA markers (1, 32, 101). This result further contradicts an origin for these ancient eggs potentially different from the *F. hepatica* introduction with livestock by the Spanish around 500 years ago. If there were another origin for these ancient eggs by means of infected humans through Beringia and afterwards the Panama isthmus up to such a southern latitude as the Patagonia several thousands of years ago or by animals following the same long way several millions of years ago, the original mutations would be expected to appear in such fast-evolving mtDNA gene sequences (589). The detection of DNA polymorphisms in the monoxenous ageohelminth nematode *Enterobius vermicularis* in the Americas supporting a pre-Columbian existence in the New World is a good example (590).

The three aforementioned reports in a Peruvian mummy, a coprolite of a domestic camelid in northern Peru, and in deer coprolites of Patagonia refer for the first time to the potential introduction of a nonmonoxenous helminth, i.e., a two-host life cycle parasite able to infect humans, into South America during the pre-Columbian period (569, 573). Although it could be argued that there are American autochthonous lymnaeid snail vector species geographically covering all New World latitudes from (i) North America with *L. bulimoides* and *L. humilis*, through (ii) Central America and the circum-Caribbean region with *L. cubensis* and *Pseudosuccinea columella*, and (iii) northern and mid South America with *L. cousini*, *L. neotropica* and also *P. columella*, down to the (iv) Southern Cone with *L. viator*, we know today that the geographical distributions of these lymnaeid species have completely been modified by human activities (livestock movements, commercial plant trade, etc.) in recent times following the European colonization. There is consequently no way to know or assess which was the real geographical distribution of these native lymnaeids in the pre-Columbian period as to allow the transit for such a lymnaeid-transmitted helminth from North America down to the Patagonia through the Panama isthmus and along such a very long way. The absence of *F. magna* in South America, a fasciolid of high spreading capacity owing to its relatively low snail vector specificity allowing it to use different snail species of the same lymnaeid groups for its transmission, evidently does not support such an ancient introduction by geographically jumping from one lymnaeid species to another along this very great distance.

Fasciola hepatica Baseline Evolutionary Scenario for South America

Summing up, the evoked potential existence of *F. hepatica* in the Americas before the European colonization poses too many unresolved questions. Moreover, there is no verified argument to explain how it could arrive to the New World and how its life cycle could be maintained in ancient pre-Columbus times, both before and after the arrival of the first humans through Beringia. In addition, there are many verified arguments suggesting the impossibility of such an old introduction along different geological epochs. So, unless new, undeniable archeological findings counting on incontestable evidence appear, there is no reason to change the evolutionary picture of *F. hepatica* in the Americas, including: (i) the first arrival of *F. hepatica* into the New World with livestock transported by the Spanish conquistadores at the end of the 15th century and beginning of the 16th century; (ii) the initial spread of *F. hepatica* throughout the different territories of North America, Central America, the Caribbean islands, and South America with livestock movements guided by the initial European colonizers whether by marine or terrestrial routes; (iii) the mixing of *F. hepatica* populations from one territory or country to another inside the Americas by importations/exportations according to temporary local needs during the subsequent centuries, especially important inside South America (Fig. 22); (iv) the additional introductions of *F. hepatica* from other continents (Europe, Africa, Asia, and Australia) into different American countries, mainly South American countries but also in the United States and Mexico, above all with the aim of improving animal breeds but also for the importation of buffaloes which were not initially introduced by the Spanish ‘conquistadores’; and (v) the more recent livestock exportations from Latin American big livestock producers such as Uruguay and Argentina to other continents, mainly Asia (1, 4, 32, 101, 102, 124).

Future research may continue considering the same baseline, including the first *F. hepatica* introduction into the Americas during the European colonization started at the end of the 15th century and the beginning of the 16th century and the aforementioned subsequent evolution for the last 500 years (1), a scenario in which all the results of different multidisciplinary studies have thus far always proved to fit well. See, for instance, the results of local molecular studies (123, 591–594), just to refer to examples in one discipline, but without forgetting results of studies in other fields of fascioliasis, such as disease transmission, epidemiology, pathology, clinics, diagnostics, treatment, or control, among many others.

Finally, two aspects are of crucial importance in this scenario of fascioliasis in the New World. The first concerns the reports of *F. gigantica*-sized flukes in three American countries, namely, the United States (119), Mexico (120, 121), and Ecuador (122). Such findings in the United States were already analyzed and attributed to the introduction of *F. gigantica* with past livestock importation and subsequent hybridization events with the autochthonous *F. hepatica* in several states of this large country (1). In Mexico, phenotypic studies on the morphometry of the adult stage demonstrated that the biggest size found in the country did in fact enter within the intraspecific variability range of *F. hepatica*, and the molecular studies confirmed this conclusion (124).

Fortunately, the recent finding of *F. gigantica*-sized flukes infecting sheep in Ecuador has allowed for the appropriate morphometric phenotyping by computer image analysis system (CIAS) and DNA multimarker genotyping in this Andean country (32). A large comparison of these sheep flukes with *F. hepatica* populations naturally infecting sheep in Peru, Bolivia, and Spain, and experimentally infecting sheep in Spain, as well as *F. gigantica* populations naturally infecting sheep in Egypt and experimentally infecting sheep from Egypt and Vietnam, demonstrated that the Ecuadorian flukes pronouncedly and significantly surpassed the maxima and means in the different size parameters of *F. hepatica*. It should be considered here that the growth of the fasciolid adult stage is not unlimited but "damped" and cannot exceed certain characteristic maxima. Indeed, the growth of the adult stage starts from the moment it arrives to a biliary duct and begins the onset of egg production, along which it follows a logistic "saturated phase" which leads to a gradually stationary growth that differs according to the host species (77, 595). That is why phenotypic analyses on adult-stage morphometry should be carried out between populations naturally and/or experimentally infecting the same host species. These results justified previous published and also unpublished reports on *F. gigantica* in Ecuador, since in fact size is the most used feature to differentiate between *F. hepatica* and *F. gigantica*. Such a feature is only known in intermediate fasciolid forms in areas of Africa and Asia where the two species and their specific lymnaeid snail vectors overlap (32). Documented information about livestock importation indicated that *F. gigantica* infecting cattle imported from Pakistan in 1974 could be at the origin of local hybridizations, leading to a fluke lineage having kept its intermediate form characteristics until the present day, although another importation of Brown Swiss cattle from the United States potentially transporting intermediate forms in 1986 was not totally ruled out.

Interestingly, however, the complete sequences of the rDNA ITS-2 and ITS-1 and of the mtDNA *cox1* and *nad1* coding genes showed typical characteristics of *F. hepatica*, despite the detection of several haplotypes indicating additional importations from Europe and other South but also Central America. Neither heterozygotic ITS positions differentiating the two species nor introgressed fragments and heteroplasmic positions in the mtDNA were found. This suggested that the posthybridization period elapsed after the arrival of the imported animals, of ~47 years, should have been sufficiently long for rDNA concerted evolution to complete homogenization and mtDNA to return to homoplasmy. The corresponding analysis suggested that the absence of lymnaeids of the *Radix* group, that is, of snails specific for *F. gigantica* transmission, should have played an important evolutionary driving force. In fact, the presence of lymnaeids belonging to the *Galba*/*Fossaria* group and the absence of *Radix* representatives constitutes a timely stable evolutionary bottleneck always acting in the same sense (32). This situation differs from areas of Africa and Asia, where (i) coexistence of *Galba*/*Fossaria* and *Radix* in the same local transmission focus offers daily alternating development filtering toward one or other fasciolid species to an evolving lineage, or (ii) zonal overlapping presence of lymnaeid species of the two groups offering similar alternating seasonal filtering forces, such as, for instance, in cases of altitudinal transhumance (1, 596). Such gigantic *F. hepatica* specimens, maintaining the large phenotype at least in the midterm of several decades, were considered to be the consequence of concerted evolution not acting on the operon copy number, which could influence the growth and development of the organisms that are related to rRNA transcription. An rRNA unit number in the specimens of the intermediate-form lineage higher than that in genetically pure *F. hepatica* would thus provide a higher transcription capacity allowing for increased growth. From a health point of view, it is of great interest to consider that these results suggest that *F. gigantica*, experimentally demonstrated to be more pathogenic than *F. hepatica* (95), does not seem to be capable of successfully colonizing the Americas, because the absence of *Radix* lymnaeids represents an unsurmountable barrier (32).

The second aspect concerns the way to get correct interpretations of the results. Researchers have to consider that the worldwide spread of fascioliasis has occurred only throughout the last 12,000 years thanks to human activities of livestock management and that the scenario in the Americas only concerns the last 500 years of this already very short 12,000-year period. Molecular studies should therefore consider the evolutionary speed of the DNA markers they use (589). From an evolutionary point of view, 12,000 years is no more than a "millisecond," and therefore sequence similarities between different regions geographically very distant from one another and which appear highlighted in given articles, such as between South American countries and, for instance, Egypt, China, or Australia in fact constitute absolutely no surprise when working with the standard DNA markers commonly used today. Moreover, the numerous evolutionarily recent anthropogenic livestock movements leading to the mixing of liver fluke populations all over and the geographical overlap of chronologically different introductory waves along time in the same area (Fig. 22), and more modern mixing by intercountry import/export (32), together with the intra- and interspecific hybridization capacity of *F. hepatica* and *F. gigantica* despite their hermaphroditism, further contribute to generate highly complex local genetic puzzles whose heterogeneous origin may be difficult—and in many cases impossible—to elucidate with unbiased certainty. In addition, specific reports on livestock movements and importation/exportation events carried out in ancient times and which could sometimes be helpful in such assessments are unfortunately not available, not even when considering large-scale movements, and obviously almost never when dealing with small private owner initiatives performed before the arrival of the present international laws.

Motivated by the aforementioned report on the detection of eggs of *F. hepatica* in deer coprolites in Patagonia dated back to 2,300 years BP (540–542), an additional opinion article has recently appeared in which a completely different evolving scenario of fasciolids for the pre- and postdomestication periods, with emphasis on the Americas, is hypothesized (544). In this analysis, none of the above highlighted several question marks posed by the trematode eggs found in the archeological site in Patagonia is analyzed, nor are the many known problems posed by archeological findings of parasites in the Americas (569, 573) considered. Moreover, the DNA sequences of these supposedly old eggs are not analyzed in that article (544). Regarding the paleobiogeographical origins of *F. gigantica* and *F. hepatica*, an Asian origin for both species is suggested to have occurred after the introduction of old fasciolid ancestors by African elephants (Proboscidea) into Asia around 10 mya, *F. hepatica* to have split from *F. gigantica* in bovids in Asia, and a subsequent reintroduction of both species into Africa (544), resulting in a hypothesis which lacks solid paleontological support and ignores information from the wild fauna.

Nonetheless, the recent DNA multimarker characterization of *F. nyanzae*, its direct relationships with *F. gigantica* and *F. hepatica*, and the corresponding molecular clock dating estimates clearly indicate that the evolutionary focus should be given not to the elephants but to the hippopotamuses and that a southeastern African origin for *F. gigantica* and a Near East origin for *F. hepatica* continue to fit all available multidisciplinary information (139). Although an introduction of *Galba* lymnaeids into the Americas by humans or birds is not ruled out in the hypothesis of these authors (544), the efforts of this article focus on the hypothetical introduction of *F. hepatica* with old bovids from Asia into North America through Beringia occurred between 5 and 2 mya. However, neither the minimum development temperature threshold of *F. hepatica*, the low temperatures in such extreme latitudes during the period evoked, the absence of trematodes, and the peculiar characteristics of the malacofauna in this region, nor the absence of *F. magna* and old bovids in South America are considered. As already emphasized previously, only during the Miocene Climatic Optimum, from the early to the middle Miocene, there was warm climate in Beringia, but this was chronologically pronouncedly

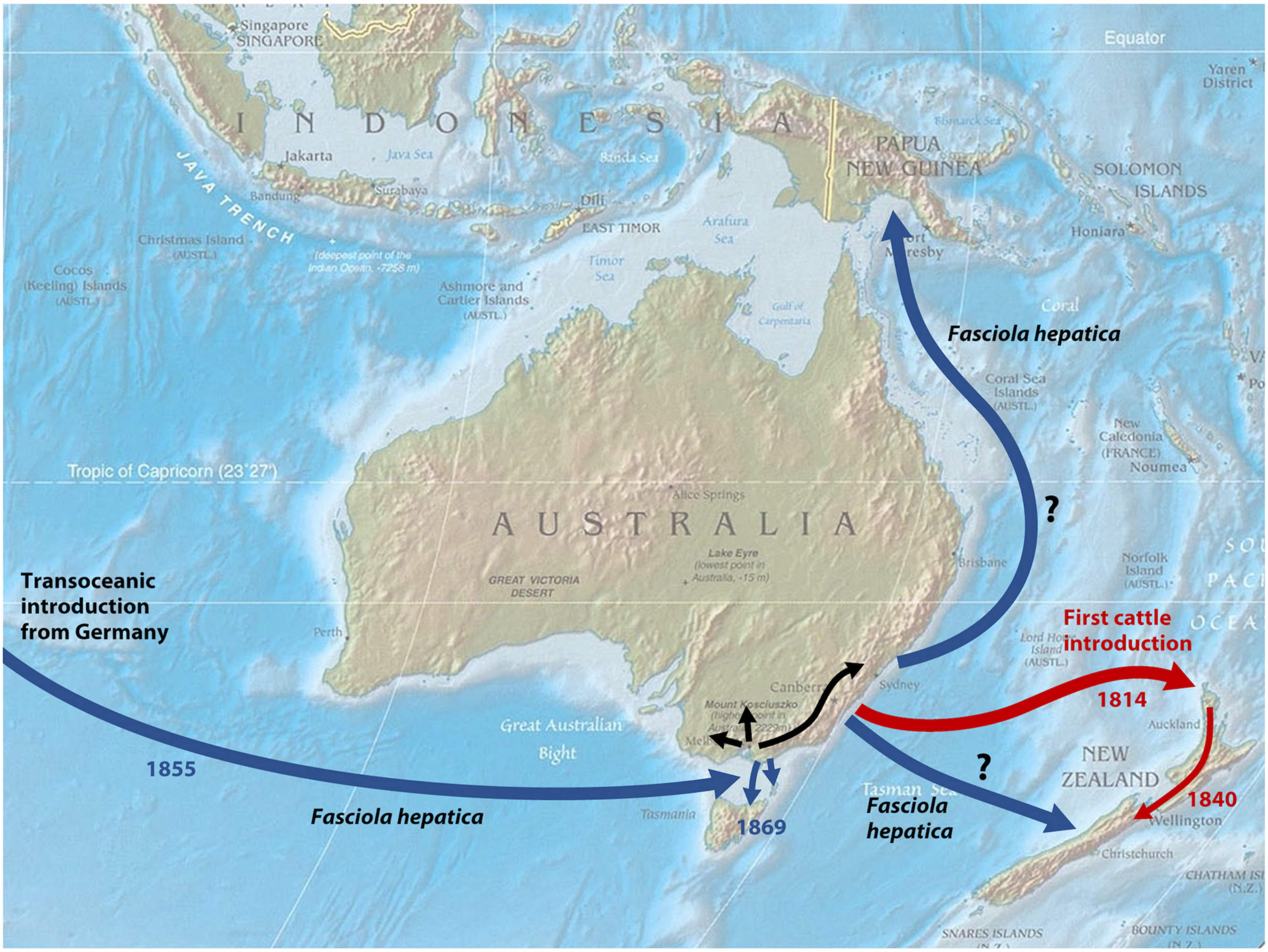

FIG 24 *Fasciola hepatica* was imported with a ram from Germany to Victoria in 1855 and later terrestrially spread throughout Victoria, New South Wales and southern Queensland, and reached Tasmania around 1869. Black arrows indicate terrestrial spread within southern Australia. Blue arrows indicate the spread by maritime routes. Dark red arrows indicate livestock introduction into New Zealand (the "?" indicates that although livestock arrived earlier, the date of the *F. hepatica* introduction into New Zealand remains still unknown, as is the date of its arrival to Papua New Guinea).

before the origins of *F. gigantica* and *F. hepatica*. Furthermore, nothing is mentioned about which mammals could have played the role of reservoirs maintaining the *F. hepatica* life cycle in the Americas during the pre-Columbus period. Indeed, in contrast to the animal hosts known to be able to maintain the transmission of *F. gigantica* in Africa and *F. hepatica* in the Near-Middle East in Asia, there are no sylvatic mammals having demonstrated this reservoir capacity in South America. Infection of all South American native mammal host species in fact proves that European-introduced livestock is the source for their liver fluke infection. In addition, in this article it is hypothesized that the ancient *F. hepatica* genotypes would have disappeared in the Americas after eventual genetic restructuration caused by introductions with European livestock, although no molecular mechanism explaining such a disappearance is suggested (544).

Summing up, verified research evidences appeared in recent years do not support the need for any drastic modification of the basic background lines on the evolutionary scenario already proposed for the Americas a few years ago (1, 102).

FASCIOLA HEPATICA SPREAD INTO OCEANIA

The introduction of *F. hepatica* into Australia has been said to have occurred in 1855 by imported rams sent from Germany to Victoria (see reference 22 [p. 152]). It jumped shortly thereafter to the close neighboring island of Tasmania (Fig. 24), where it was reported in

1869 (597). The first human infection was reported only a few years later from three patients with liver abscess in the Melbourne Hospital in the period from 1877 to 1881, namely, seven flukes in one patient and only one specimen in each of the two others (598, 599).

This liver fluke poses veterinary problems in sheep and cattle and is today restricted to the southeastern part of the country, including Victoria, New South Wales and southern Queensland, as well as Tasmania (Fig. 24) (600), which furnishes an idea about its geographically spreading capacity during the period elapsed of 166 years. It should be noted that *F. hepatica* has been observed to also infect native animals, such as free-ranging marsupials (601). In Tasmania, it originally colonized the eastern part of the island, but it was already observed in the northwestern part of the island in 1979, introduced by domestic herbivores because this coastal area is used for livestock agistment (602).

Many different lymnaeid species, including *L. tomentosa*, *L. lessoni*, *P. columella*, *L. viridis*, *L. rubiginosa*, and *L. peregra*, have been reported in Australia. Only *L. tomentosa* and *L. lessoni* may be considered species endemic to Oceania, whereas all of the others are exotic snails introduced with aquatic plants (603). The genus ascription for all of these species, except *P. columella*, has been very recently reassessed by DNA-based phylogenies and the aforementioned species included in different genera (452, 453), although, unfortunately, the bootstrap values supporting nodes appear to be too much lower than a minimum of 70% to consider the clades statistically significant and the phylogeny congruent. Otherwise said, the systematic-taxonomic assignment of these species still remains open.

The autochthonous species *L. tomentosa* proved to be a more suitable intermediate host for *F. hepatica* than any of the introduced exotic species, although *L. columella* and *L. viridis* were highly susceptible to the Australian strain of *F. hepatica* (603). In Australia, the most important intermediate host in nature is the indigenous freshwater snail *L. tomentosa*. The introduced North American snail *P. columella* and the introduced snail from the Pacific area *L. viridis*, found in defined locations of the coast of New South Wales, have also been identified as additional intermediate hosts (600).

Three lineages could be molecularly distinguished in the native *L. tomentosa*, distributed in south Australia, Tasmania, and eastern Australia. In addition, the New Zealand populations of *A. tomentosa* proved to be a very distinct lineage from any of the Australian populations attributed to this species (604). This speaks about about an old evolution of endemic snails. If the genetic differences are considered sufficient, *L. tomentosa* is the taxon to be ascribed to the snails of New Zealand because it was originally described there, which means that other taxa should be used, such as *L. huonensis* for the snails of Tasmania and *L. brazieri* and *L. papyracea* for the snails of Australia, as the oldest available names (3). Similar conclusions have been reached by Australian experts (605).

Recent DNA marker sequencing of triclabendazole-susceptible and -resistant fasciolid specimens from Australia allowed for the detection of 18 distinct haplotypes for the *nad1* gene and six haplotypes for the *cox1* gene, resulting in haplotype diversity levels comparable to that observed for mtDNA in European samples (606). All in all, this scenario of Australia recalls the introduction of *F. hepatica* into the Americas, where it was able to adapt to native species of the *Galba/Fossaria* group (1), whereas *F. gigantica* never succeeded because of the absence of appropriate lymnaeid vector species (32). It should also be emphasized that *F. gigantica* has never been reported from Oceania, despite the wide presence of this fasciolid in Southeast Asia and Indonesia, including the islands of Sumatra (607), Sulawesi (459), and Java (460), where it has even been found infecting native deer (608). Papua New Guinea is the only island in which *F. hepatica* is present, infecting domestic ruminants in some areas (609). Indeed, *F. hepatica* is believed to have been brought into Papua New Guinea (Fig. 24), with sheep imported from eastern Australia, and to became established in those parts of the highlands inhabited by the snail *L. viridis* to which it adapted (610, 611).

In New Zealand, the first dairy Shorthorns cows were introduced in 1814 by the missionary Samuel Marsden for mission stations in the Bay of Islands. These cows came from the New South Wales Crown herd, and were a gift from Governor Lachlan Macquarie. Shorthorn herds were established by the early 1840s, and for a long time Shorthorns were New Zealand's most popular cattle breed (Fig. 24). The South Island's tussock lands carried large numbers of sheep, while in the North Island sheep and cows were preferred because of its better-suited climate (612, 613). Sheep appear to be more important than cattle in maintaining *Fasciola* infections from year to year (614).

A total of five species of Lymnaeidae have been reported from New Zealand: the endemic native *L. tomentosa* and four exotic introduced species. The great pond snail *Lymnaea stagnalis*, which does not transmit fascioliasis, is known in both islands since long ago (615). The vector species *G. truncatula* has been also reported a long time ago (616), and its rarity in the country indicates that it never adapted well, perhaps also due to habitat competition with the native *L. tomentosa*. The species *R. auricularia* was also detected (617), although it is a lymnaeid nonsusceptible to *F. hepatica*. *Pseudosuccinea columella* has been the last lymnaeid detected and unknowingly introduced into New Zealand at least as early as the 1940s (618). This invasive species has successfully adapted to New Zealand temperatures and has become more reproductively active (614, 619), so that it is today found over the summer months throughout the whole of the North Island in permanently wet gullies and in several parts of the South Island. It has therefore become a key transmitter of the disease (620).

CONCLUDING REMARKS

The *Fasciola* origins and worldwide evolving scenario comprise (i) the long predomestication period, including the paleobiogeographical origins of *F. gigantica* in southeastern Africa around the mid-Miocene and of *F. hepatica* in the Near-Middle East of Asia around the latest Miocene to Early Pliocene; (ii) the evolutionary very short postdomestication period, including the worldwide spread of fascioliasis by human-guided movements of livestock in the last 12,000 years; and (iii) the more recent transoceanic anthropogenic introductions of *F. hepatica* into the Americas and Oceania and of *F. gigantica* into several large islands of the Pacific, with ships transporting livestock in the most recent 500 years.

The present multidisciplinary detailed analysis of new valuable information from well-contrasted results published in recent years has allowed for a more detailed redefinition of given evolutionary steps. It should be highlighted that we did not find a single local, zonal, or regional situation of fasciolids and lymnaeids that does not fit the historical records of the place in question. This does not mean, however, that in a few world zones the available knowledge on the local history, on the fasciolid-lymnaeid presence, or on fascioliasis transmission, appears to be insufficient. In addition, intercountry and even intercontinental livestock import-export events by means of modern vehicles (ships, flights, trucks, and also trains) have been numerous in recent times and mask the original situation, with the problem that information on such livestock import-export events are unfortunately not always obtainable.

Research on fascioliasis in a local area should therefore consider that several chronologically different fasciolid introduction waves may have occurred in the area in question and consequently that the present picture may show an overlap of fasciolids arrived at different times and from different geographical origins. Three good examples are (i) the present situation of extreme variation of haplotypes of *F. hepatica* and *F. gigantica* both inside livestock populations and inside the same individual animal host in Near and Middle East countries of Asia, (ii) the highly mixed *F. hepatica* haplotypes in countries of South America, and (iii) the coexistence of *F. gigantica* with hybrid forms resulting from hybridization with *F. hepatica* recently introduced with imported livestock in southeast Asian countries.

Confronting such situations has led to suggest the possibility of cryptic speciation in *Fasciola* (131). However, the extensive genetic and phenotypic variation both within and between populations is in fact a consequence of human-induced disturbances of the evolution of fasciolid species whose genome is not allowed to follow a normal evolution depending on natural selection by biogeographical constraints. This recalls the widely analyzed, similar phenomenon in populations of the wild peridomestic house mouse *Mus musculus*, from the moment of initial studies detecting species-level differences between populations inhabiting neighboring valleys (621) up to modern genetic studies going deep into the elucidation of this model (622, 623). As is well known, research into the domestication of livestock and companion animals sheds light both on their "evolution" and human history (624). The high polymorphism levels found in the very large 1.3-GB genome of *F. hepatica* infecting sheep in the United Kingdom (625) and the even markedly higher repeat content in *F. hepatica* from sheep of North America (626) undoubtedly underlie this human-altered evolution.

Summing up, we may define the two *Fasciola* species as parasites having suffered a domestication process along human history and judiciously call them "peridomestic endoparasites."

Researchers should consider that areas of endemicity pronouncedly differ depending on (i) the *Fasciola* species present; (ii) the presence of only one *Fasciola* species or the two species, whether in local or zonal overlap situations; and (iii) the existence of hybrid intermediate forms originated by the crossbreeding capacity of these hermaphroditic trematodes. The local situations do, moreover, differ depending (iv) on the presence of only one, two, or more lymnaeid vector species; (v) on their anthropophilic or zoophilic behavior which underlies a more or less important role in the transmission to humans or animals, respectively; and (vi) on the seasonality of their population dynamics defining the timely risk of infection. In addition, (vii) differences in species and breeds of animal reservoirs should also be considered given their different transmission capacity, such as, for instance, pigs, which are absent in Muslim countries, resistant in Europe, and highly efficient reservoirs in South America. The implications regarding crucial aspects of the disease, such as epidemiology, transmission risk, prevention and control, as well as on pathology, clinics, and symptomatology, and on diagnostics and treatment, are evident.

The present baseline update should therefore be taken into account in future research studies, and the fascioliasis dynamics according to different recently ongoing changes at local and/or large scales, such as livestock movements and climate change induced modifications of fascioliasis transmission, should also be considered. Let us hope the global picture furnished here will be as useful as the previous one has proved to be (1) and that new research results coming in the next years will again permit an appropriate tuning-up of our knowledge on fascioliasis situations in given areas.

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