Post-Miocene expansion, colonization, and host switching drove speciation among extant nematodes of the archaic genus *Trichinella*

D. S. Zarlenga[†‡§], B. M. Rosenthal[‡], G. La Rosa[¶], E. Pozio[¶], and E. P. Hoberg[‡]

[†]Bovine Functional Genomics Laboratory and [‡]Animal Parasitic Diseases Laboratory and U.S. National Parasite Collection, U.S. Department of Agriculture, Beltsville, MD 20705; and [¶]Department of Infectious, Parasitic, and Immunomediated Diseases, Istituto Superiore di Sanità, 00161 Rome, Italy

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Parasitic nematodes of the genus *Trichinella* cause significant food-borne illness and occupy a unique evolutionary position at the base of the phylum Nematoda, unlike the free-living nematode *Caenorhabditis elegans*. Although the forthcoming genome sequence of *Trichinella spiralis* can provide invaluable comparative information about nematode biology, a basic framework for understanding the history of the genus *Trichinella* is needed to maximize its utility. We therefore developed the first robust and comprehensive analysis of the phylogeny and biogeographic history of *Trichinella* using the variation in three genes (nuclear small-subunit rDNA, and second internal transcribed spacer, mitochondrial large-subunit rDNA, and cytochrome oxidase I DNA) from all 11 recognized taxa. We conclude that (*i*) although Trichinellidae may have diverged from their closest extant relatives during the Paleozoic, all contemporary species of *Trichinella* diversified within the last 20 million years through geographic colonization and pervasive host switching among foraging guilds of obligate carnivores; (*ii*) mammalian carnivores disseminated encapsulated forms from Eurasia to Africa during the late Miocene and Pliocene, and to the Nearctic across the Bering Land Bridge during the Pliocene and Pleistocene, when crown species ultimately diversified; (*iii*) the greatest risk to human health is posed by those species retaining an ancestral capacity to parasitize a wide range of hosts; and (*iv*) early hominids may have first acquired *Trichinella* on the African savannah several million years before swine domestication as their diets shifted from herbivory to facultative carnivory.

biogeography | mitochondrial DNA | phylogeny | ribosomal DNA

Exceptional biological diversity among nematodes is exemplified by certain attributes of the parasite *Trichinella spiralis.* Organization of its mitochondrial genome more closely resembles that of coelomate metazoans than that of its presumed closest relatives, the secernentean nematodes (1). In addition, *T. spiralis* (Dorylaimia) shares a similar proportion (45%) of its ESTs with the nematode *Caenorhabditis elegans* (Rhabditina) as it does with the fruit fly *Drosophila melanogaster* (Arthropoda: Drosophilidae) (2). Thus, many ESTs common to *T. spiralis* and *C. elegans* are not necessarily specific to nematodes but may be conserved among diverse taxonomic groups of invertebrates. *C. elegans* is often thought of as a prototypical nematode because of its acceptance as a model for studying biological processes; however, genomic variation among nematodes is extensive and commensurate with their phylogenetic and ecological diversity. Therefore, the forthcoming genome sequence of *T. spiralis* will contribute substantially to our understanding of nematode biology and the origins of parasitism.

In 1998, Blaxter *et al.* (3) used genetic data to delineate the phylum Nematoda into supertaxa consisting of five clades. *Trichinella*, a parasite of vertebrates, occupies a basal lineage (clade I) consisting of free-living Mononchida, plant parasitic Dorylaimida, and entomophagous Mermithida, with which it shares features of early embryogenesis (4) and small-subunit (SSU) rDNA sequences (3). Other clades show similar levels of diversity in host–parasite associations, suggesting that parasites of vertebrates arose independent of one another during the long evolutionary history for nematodes (3). Thus, understanding the origins and persistence of vertebrate parasitism requires phylogenetic and historic information from a broad spectrum of taxa. Among the Trichocephalida, parasitism may have arisen as early as the Paleozoic in the ancestor of the Trichinellidae and the Trichuridae; however, temporal origins of extant species of *Trichinella* remain unexplored.

The genus *Trichinella* comprises a monophyletic lineage in the Trichinellidae, the putative sister to the Trichuridae (Capillarinae, Trichurinae, and Trichosomoidinae). The superfamily Trichinelloidea to which *Trichinella* belongs is phylogenetically diagnosed by the stichosome, a region of the glandular esophagus, and the bacillary bands, an assembly of structural characters otherwise unknown among the nematodes. Trichinellidae is further diagnosed by a unique life cycle where worms undergo complete development within a single vertebrate host (autohexeroxeny) and by first-stage larvae, which localize within skeletal muscle cells as infectious organisms. Although eating raw or undercooked pork infected with *T. spiralis* accounts for most human cases of trichinellosis, zoonotic transmission also occurs from species that circulate among wildlife. Eleven distinct taxa are now recognized in mammals, birds, crocodilians, and saurians (5), characterized by either a thick (encapsulated) or a very thin (nonencapsulated) collagen membrane enveloping the parasite-infected muscle cell.

Despite extensive development of biochemical and genetic methods to distinguish taxa (6), evolutionary relationships among genotypes of extant *Trichinella* remain enigmatic. Delineating the phylogeny and biogeography of *Trichinella* will substantially increase the comparative value of its genome sequence and help elucidate the determinants of zoonotic risk among species. On a broader scale, this information will provide us an opportunity to investigate ecological and evolutionary forces that promote diversification in complex host–parasite systems.

Three central questions fueled our curiosity about the evolution of *Trichinella*. (*i*) Can prolonged coevolution with distinct tetrapod groups explain why encapsulated species of *Trichinella* are restricted to eutherian mammals and nonencapsulated species also infect saurian, crocodilian, avian, eutherian, and metatherian hosts (5, 7), or have more recent ecological and biogeographic events influenced current host associations? (*ii*) Why are most species and genotypes with broad host ranges

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Abbreviations: SSU, small-subunit; LSU, large-subunit; ITS-2, second internal transcribed spacer; ML, maximum likelihood; MY, million years; COI, cytochrome oxidase I.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AY851256–AY851277 and DQ007890–DQ007900).

[§]To whom correspondence should be addressed at: U.S. Department of Agriculture, Agricultural Research Service, Bovine Functional Genomics Laboratory, B1180 Beltsville Agricultural Research Center East, Beltsville, MD 20705. E-mail: zarlenga@anri.barc.usda.gov.

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Table 1. Characteristics of sequence alignments and their evolutionary models

	No. of bases†						Base frequencies					Clock§	
Data set*	Total	Var.	Inf.	Model type‡	C:T/Tv	G:A/Tv	A	C	G	I	Γ shape	ΔlnL	df > *P*
SSU	1,929	717	417	TrNefΓ	2.80	2.06	0.250	0.250	0.250	0	0.408	20.3	14 > 0.12
11mit	1,193	276	194	HKY Γ I	15.60	15.60	0.299	0.170	0.174	0.49	0.492	9.84	9 > 0.35
3gene	1,273	204	78	HKY Γ	3.21	3.21	0.277	0.165	0.200	0	0.156	1.86	6 > 0.93

*Data sets are as follows: SSU, 10 of 11 *Trichinella* genotypes including outgroup taxa *Trichuris muris, Trichuris suis, Adnoncholaimus* sp., *Pirapulus caudatus, Mormis nigrescens,* and *Mylonchulus arenicolu; 11mit,* mitochondrial LSU rDNA and COI from all 11 Trichinella taxa; *3gene,* mitochondrial LSU rDNA, COI, and ITS-2 from all eight encapsulated species and genotypes of *Trichinella.*

†Total, variable (Var.), and informative (Inf.) number of bases under the parsimony criterion.

‡TrNef, a model consisting of a single transversion rate but two distinct transition rates. HKY models correspond to that of Hasegawa *et al.* (8) in assuming unequal base frequencies and rates of transition and transversion substitutions. Γ, variation in the substitution across nucleotide sites modeled as a gamma distribution with an estimated shape parameter; I, the estimated proportion of invariant sites; Tv, transversion.

§Difference in log likelihood (ΔlnL) trees evaluated with and without the enforcement of a molecular clock. df = (number of taxa) −2. *P* represents statistical significance in the reduction of likelihood owing to enforcement of a molecular clock.

restricted to well defined geographical localities? (*iii*) Did humans become infected with *Trichinella* in the Neolithic before domesticating pigs, or did adaptation after pig domestication cause certain species of *Trichinella* to threaten human health? To explore these and related questions, we generated the first robust hypothesis for the phylogeny of *Trichinella* based on multilocus DNA sequence information from all ecologically and genetically recognized species and genotypes. We then interpreted these results with reference to the evolutionary history and biogeography of their hosts.

Results

The general features of the three comparative data sets are summarized in Table 1. Significant variation in the rate of evolution across sites was identified in each model. In no case did enforcing a molecular clock significantly worsen the likelihood of the data.

An initial analysis of the SSU rDNA variation served to evaluate *Trichinella* phylogeny within the broader context of nematode evolution. In this regard, the SSU rDNA sequences reflect a minimally differentiated, monophyletic assemblage (Fig. 1) where the maximum likelihood (ML) pairwise distance never exceeded 0.0052 among encapsulated species or 0.0135 among all species. These values are substantially less than the ML distance (0.2) separating *Trichuris muris* from *Trichuris suis*. If the locus evolved in each lineage at $\approx 0.4 \times 10^{-9}$ per site per year (4% over 100 MY), which typifies many metazoan phyla (9), then extant species of *Trichinella* shared a common ancestor ≈16 million years (MY) ago, and encapsulated taxa first began diversifying within the last 9 MY (Table 2). Only by postulating an implausible 18-fold rate decrease in divergence rate could the extant diversity among *Trichinella* species date to the origins of distinct tetrapod host groups that occurred some 300 MY B.P. Although species of *Trichinella* are poorly differentiated from one another at the SSU rDNA, this locus markedly distinguishes them from species of *Trichuris.* The extant pairwise difference between *Trichinella* and *Trichuris* exceeds 16%, and ML estimates of their genetic distance exceed 22%. Assuming a per lineage rate of 4% over 100 MY for SSU rDNA evolution, the most recent common ancestor of *Trichinella* and *Trichuris* may have existed 275 MY B.P.

Diverse tree-building approaches yielded consistent and nearly complete phylogenetic resolution among all 11 *Trichinella* congeners when compared at the two mtDNA genes (Fig. 1), or among all eight encapsulated species when the second internal transcribed spacer (ITS-2) sequences were also included (data

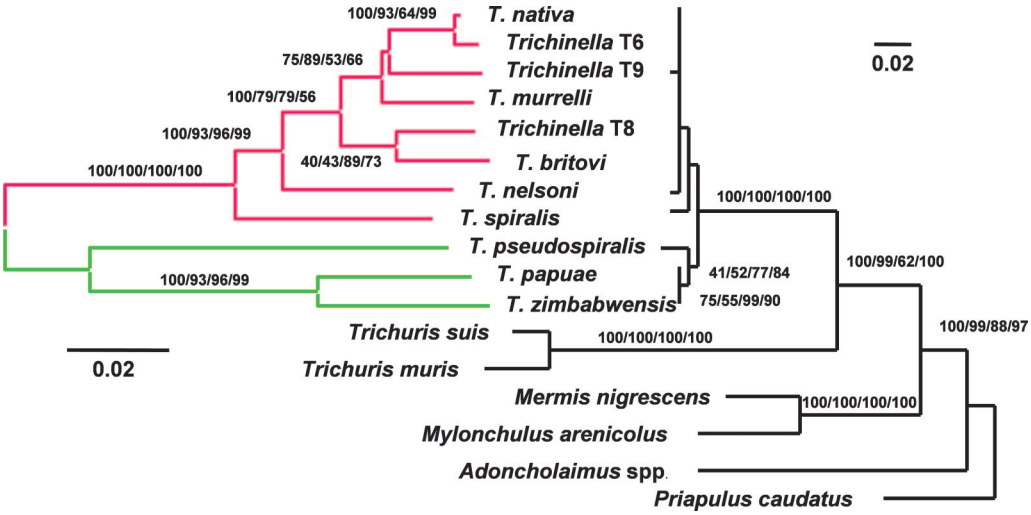

Fig. 1. Midpoint rooted minimum evolution trees reconstructed from all known encapsulated (red) and nonencapsulated (green) species and genotypes of *Trichinella* based on the variation in mitochondrial LSU and COI DNA (on the left) and SSU rDNA (on the right). Topological support is indicated by Bayesian posterior probabilities and by ML, minimum evolution (using ML distances), and parsimony bootstrap replicate analyses (B/ML/ME/P). Bootstrap support was reconstructed from 100-bp replicates.

EVOLUTION

Table 2. Estimates of divergence dates (MY B.P.)

	SSU rDNA		Mt DNA	
Genotypes	Obs*	Model*	Obs†	Model‡
All	16.1	16.8	7.0	11.1
T1 vs. T2	6.8	9.3	3.1	2.7
T6 vs. T2	0	0	0.32	0.14

Assumes that observed (Obs) or ML (Model) pairwise distances accrue at 4% per 100 MY (*), 2% per MY (†), or 5.2% per MY (‡).

not shown). Data suggest that the earliest diversifications among extant encapsulated species gave rise to *T. spiralis* and then to *Trichinella nelsoni*. The next diversification event would have separated the remaining encapsulated forms from an ancestor unique to *Trichinella* T8 and *Trichinella britovi*. This relationship is inferred by 98% and 77% bootstrap replicates in the distance and parsimony analyses of the mtDNA data, respectively, and by 75% of the ML bootstrap replicates and 78% of the Bayesian posterior distribution when ITS-2 data were included. Whether *Trichinella* T9 diverged from the common ancestor of *Trichinella nativa* and *Trichinella* T6 (weakly preferred by all analyses of mtDNA data alone) or from the ancestor of *Trichinella murrelli* (as indicated when ITS-2 variation is considered) remained unresolved. Each hypothesis received equivalent statistical support and required equivalent numbers of parsimony-informative changes. Thus, of >2 million rooted, bifurcating trees, only five are demonstrably compatible with the variation in our multilocus data set; on these trees we base our subsequent discussion of *Trichinella* evolution.

Discussion

The phylogenetic hypothesis generated herein affords us an opportunity to explore the history of host–parasite coevolution and biogeography for species of *Trichinella*. A vast interval, lasting perhaps 275 MY, separated the divergence between Trichinellidae and Trichuridae from the radiation of extant species of *Trichinella* (Fig. 1). The 11 recognized taxa are genetically and biologically delineated into two distinct clades, predicated in part on the presence or absence of a well defined, intramuscular collagen cyst. Ancestral lineages of *Trichinella* that lacked such capsules evidently infected tetrapods (Fig. 2).

A deep coevolutionary association had been proposed to explain the unique ability of nonencapsulated species to infect saurians, crocodilians, other nonavian archosaurs (*Trichinella papuae* and *Trichinella zimbabwensis*), and birds (*Trichinella pseudospiralis*), in contrast to the restriction of encapsulated species to synapsid and mammalian hosts (10, 11). However, our data undermine the view that these two parasite groups coevolved exclusively with distinct tetrapod host groups. Instead, ancestral *Trichinella* likely infected a guild of eutherian omnivores and carnivores including Suidae, Hyaenidae, and Felidae (Fig. 2). Divergence of the primary subclades would have followed in Eurasia during the late Tertiary. Encapsulated forms subsequently diversified through geographic colonization, host switching, and isolation in temperate, boreal, and Arctic habitats across the Holarctic, whereas nonencapsulated forms retained the capacity to infect eutherian mammals and became secondarily associated with some Metatheria, Sauria, Crocodilia, and Aves (Fig. 2). Although resolving the history of host colonization by nonencapsulated species will require additional data, the shallow divergence among *T. papuae* and *T. zimbabwensis* suggests that these species were only recently acquired by crocodilians and are not relicts of an ancient coevolutionary association. Thus, extant host distributions became established only since the Miocene.

Encapsulated forms of *Trichinella* evolved entirely with eutherian hosts (Fig. 2). Among these species, *T. spiralis* has been globally translocated by an array of domesticated and synanthropic mammals and is now perpetuated largely through a synanthropic cycle involving swine and a sylvatic cycle involving both swine and carnivores (5). Their Eurasian origins are suggested by tree topology, the geography of extant species (Figs. 2 and 3), and elevated genetic diversity among geographically localized isolates from East Asia (G.L.R., unpublished data).

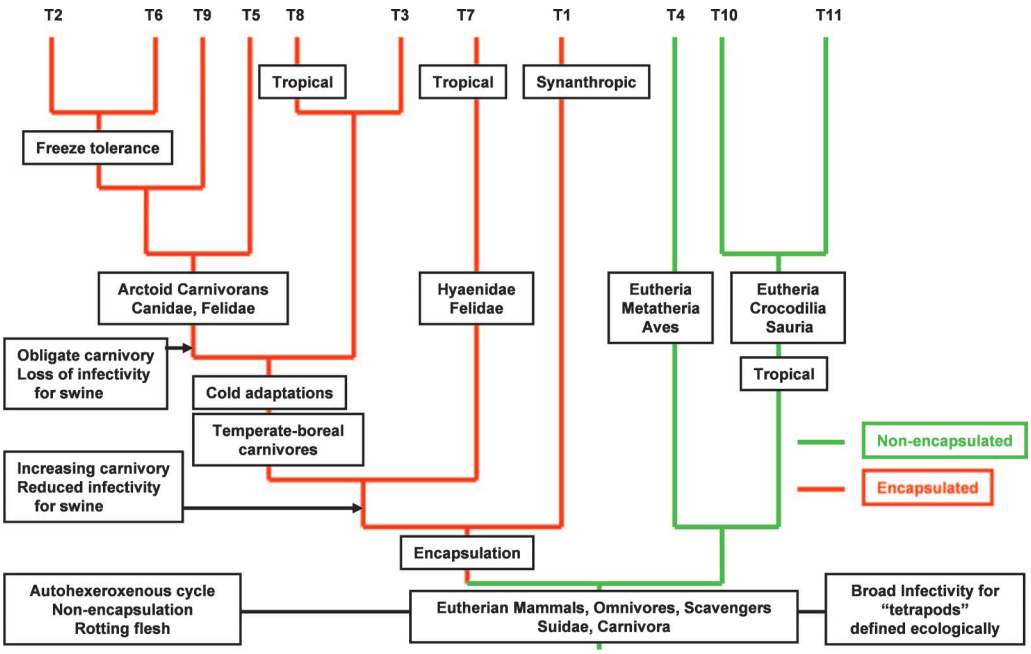

Fig. 2. Host associations and primary traits for the life history of *Trichinella* during diversification. Emphasized are shifts in patterns of transmission from omnivorous to carnivorous hosts, adaptations to changes in environmental temperatures, and retention of plesiomorphic traits among members of the nonencapsulated clade (green). Genotype abbreviations are consistent with Table 3.

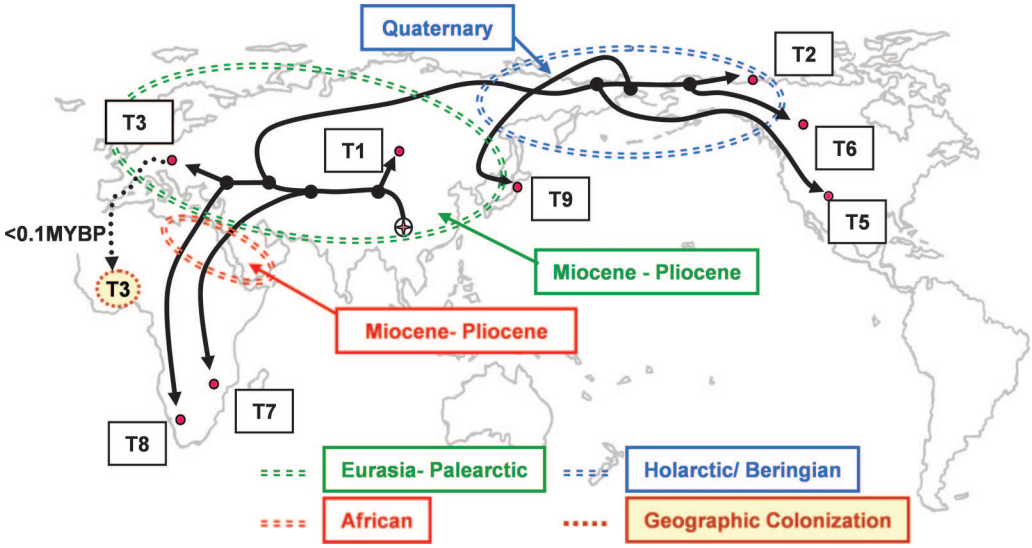

Fig. 3. Historical biogeography for species of the encapsulated clade of *Trichinella*. The cladogram for species of *Trichinella* has been mapped onto a global projection for range expansion. Subsequent to origins in Eurasia, *Trichinella* became distributed with its carnivorous hosts in Africa, broadly in the Palearctic, and later into the Nearctic. Emphasized is the role for independent events of biotic expansion and geographic colonization into Africa (Miocene/Pliocene/Pleistocene) and through Beringia into the Nearctic (Quaternary) as drivers for speciation. Genotype abbreviations are consistent with Table 3.

This finding implies that encapsulated species may have originated in Eurasia, where the Suidae and numerous carnivorous groups diversified as well (12, 13).

It is unlikely that encapsulated lineages colonized Africa before the breakup of Gondwana, because endemic forms are absent from South America, Australia, New Zealand, and much of Australasia. Such biogeographic evidence corroborates our belief that extant species radiated much later than (*i*) the diversification of basal eutherians (near the termination of the Cretaceous), (*ii*) the extinction of an archaic carnivorous fauna (Creodonta and Hyaenodonta in the Late Oligocene), or (*iii*) the divergence between Eutheria and Metatheria (14–16). Instead, encapsulated and nonencapsulated clades probably separated during the mid-Miocene as temperate ecosystems changed, followed by the diversification of extant species within the last 15–20 MY. The hosts of *T. spiralis*, *T. nelsoni*, *Trichinella* T8, and *T. britovi* all suggest an ancestral association with swine and carnivores (Fig. 2) (5). Interestingly, Suidae also completed their divergence from the Tayassuidae in the Lower Miocene (15–20 MY B.P.) (12, 13).

Transmission of *Trichinella* has depended on omnivory, carnivory, and scavenging of carrion among Eurasian and African paleoguilds (17). Omnivory continues to contribute to the transmission of the basal encapsulated lineages, *T. spiralis* and *T. nelsoni*. In contrast, a transition to facultative and finally to obligate carnivory characterizes *T. britovi* and the crown species of the encapsulated clade, respectively (Fig. 2). Encapsulated forms radiated through host switching within guild assemblages among Mustelidae, Ursidae, Canidae, Felidae, and Hyaenidae in regional settings ecologically isolated by stadial climate cycles in the late Pliocene and Pleistocene.

Trichinella genotypes now endemic to Africa and those distributed in the amphiberingian region appear to have resulted from episodic periods of biotic expansion, isolation, and host switching consistent with a "taxon pulse" model of biological diversification (18, 19). Three independent expansion events from Eurasia explain the occurrence of *T. nelsoni*, *Trichinella* T8, and *T. britovi* (20) in Africa (Fig. 3). These were arguably facilitated by the formation of land connections to Eurasia and Europe during the middle to upper Miocene, Pliocene, and Pleistocene and by incursions of Eurasian carnivores and omnivores into Africa (12, 14, 21, 22). *T. nelsoni* and *Trichinella* T8 have been reported only in sub-Saharan Africa, where they circulate among felids, hyaenids, and to a lesser degree among suids (5, 20). Carnivores, including felids, hyaenids, and viverrids (Feloidea) and canids, ursids, and mustelids (Arctoidea) radiated in Eurasia and subsequently disseminated to Africa from the Lower Miocene to the Quaternary. A more recent expansion of *T. britovi* into northern and western Africa from the Palearctic, during or since the terminal glaciation of the Pleistocene, is suggested by the genetic uniformity among isolates from these geographical regions (20) (Fig. 3).

Carnivorans, including ursids, canids, and felids, are principally responsible for the dispersal of Holarctic species (5), which likely expanded across Western Europe, through Beringia, and into North America (Fig. 3 and Table 2). Isolation across Beringia appears not to have commenced with the initial opening of Bering Strait 4.8–5.5 MY B.P. (23), but in association with cyclical and episodic glacial–interglacial stages during the Pleistocene. Ursids, mustelids, canids, and felids, all important hosts for Nearctic species of *Trichinella*, dispersed and became established in the Nearctic during the late Pliocene and Quaternary (24, 25). Increasingly cold and insular conditions in the Arctic during the late Miocene and Pliocene favored the development of cold-adapted forms at a time when Beringia may have represented a filter bridge for host–parasite assemblages limited by arctic and subarctic conditions.

Like other taxa (26, 27), *Trichinella* expanded through Beringia during the Pliocene and Quaternary and subsequently experienced isolation events that promoted speciation during stadial–interstadial cycles. Isolation during the Quaternary across the Bering Land Bridge, and north and south of the Laurentide and Cordilleran ice, likely drove the divergence of *T. murrelli* and crown taxa including *Trichinella* T9, *T. nativa*, and *Trichinella* T6 (Fig. 3). *Trichinella* T9 may represent a peripheral isolate on the islands of Japan derived from a widespread ancestral population that occupied a Holarctic distribution. The divergence of *Trichinella* T6 from *T. nativa* may have occurred during a subsequent episode of allopatry in Beringia and periglacial habitats south of the Cordilleran and Laurentide glaciers (28). If so, the contemporary Holarctic range for *T. nativa* would have arisen from a secondary geographic colonization of the

high-latitude Holarctic (−5°C January isotherm) during the Wisconsin or terminal Pleistocene. Indeed, secondary contact zones in western North America have developed for some hosts tracking the retreat of glaciers (29). Populations of *T. nativa* may have expanded into high arctic habitats of Canada and Greenland during the Holocene, as has been postulated for other Holarctic parasitic nematodes and their mammalian hosts (26, 30, 31). Alternatively, assuming that peripheral isolation played a role in diversifying these crown species, *T. nativa* may instead represent an ancestral and persistent high-latitude population in the Holarctic.

To date, no endemic species of *Trichinella* has been identified in the Neotropics even though felids, procyonids, canids, and mustelids colonized South America after formation of the Panamanian Isthmus during the Pliocene/Pleistocene ≈3.0 MY B.P. (32). In South America only *T. spiralis* has been found, presumably the result of anthropogenic translocation. Among sylvatic *Trichinella* adapted for survival in harsh, cold, and xeric environments, historical constraints and life history canalization may have precluded *Trichinella* from expanding into the Neotropics across the Panamanian Isthmus.

Human Trichinellosis

Did the divergence of *T. spiralis* coincide with the domestication of pigs? If so, its independent history would have commenced no earlier than the Neolithic, and a shallow genetic divergence would be evident between the parasites of wild and domesticated suids. Instead, *T. spiralis* appears to have commenced its independent evolutionary trajectory several MY before *Sus scrofa* was first domesticated (33) because none of the enzootic species share an especially close relationship with *T. spiralis.* Thus, the ecological setting in which hominids first acquired trichinellosis may parallel that of *Taenia* tapeworms (34) and lice (35). Hunting prey also pursued by felids, canids, and hyaenids may have exposed early humans to a suite of parasites already well established on the savannahs of Africa during the late Tertiary and Quaternary. Accordingly, hominids would have first acquired *Trichinella* when their diets shifted from herbivory to scavenging and facultative carnivory during the Pliocene or early Pleistocene, (17, 36–38).

Recent host adaptations may have enhanced the risk to human health posed by certain species of *Trichinella*; however, a broad host range characterizes basal lineages and therefore represents the ancestral condition. The capacity of nonencapsulated forms to infect a broad array of terrestrial vertebrates suggests they pose a significant zoonotic risk as well (11, 38, 39). By contrast, the capacity to infect swine is diminished among more recently radiated species (*T. britovi* and *T. nelsoni*) and is nonexistent among the crown species (*T. nativa*, *T. murrelli*, *Trichinella* T6, and *Trichinella* T9) (5).

Conclusions

Although the Trichinellidae and the Trichuridae apparently diverged as early as the Paleozoic, contemporary species of *Trichinella* diversified during the age of modern Eutheria. The following observations support this line of reasoning: (*i*) the genetic diversity among extant species of *Trichinella* accumulated within the last 15–20 MY; (*ii*) all extant species infect Eutherian carnivores or rodents; and (*iii*) infections in saurians, crocodilians, metatherians, and birds appear secondary rather than relictual. If, as we contend, extant diversity originated in Eurasia and disseminated to both Africa and the Nearctic during the late Tertiary, *Trichinella* should resemble other parasites for which host switching rather than strict cospeciation was a major diversifying force (40). These patterns underscore the role of episodic expansion, isolation, and host switching in promoting *Trichinella* diversification and highlight the exceptional dissemination potential conferred to *T. spiralis* by its association with domesticated swine and synanthropic rodents.

Table 3. *Trichinella* strains used in the analysis

Parasite	Genotype	ISS code
T. spiralis	T1	ISS003
T. nativa	T2	ISS010
T. britovi	T3	ISS005
T. murrelli	T5	ISS035
Trichinella T6*	T6	ISS040
T. nelsoni	T7	ISS029
Trichinella T8*	T8	ISS124
Trichinella T9*	T9	ISS409
T. pseudospiralis†	T4	ISS013
T. papuae‡	T10	ISS572
T. zimbabwensis‡	T11	ISS1029

ISS, Istituto Superiore di Sanità. Additional information can be obtained from www.iss.it/site/trichinella/.

*Genetically distinct but taxonomically undefined taxa.

†Nonencapsulated species infectious for birds in addition to mammals.

‡Nonencapsulated species infectious for reptiles in addition to mammals.

Materials and Methods

All species and genotypes of *Trichinella* (Table 3) were maintained in Swiss–Webster mice. Eviscerated mouse carcasses were digested with pepsin (1%) and HCl (1%), and the released muscle larvae were collected and washed through multiple exchanges in water. Genomic DNA was isolated by proteinase K and SDS digestion and treated with RNase. At least three full-length clones of PCR-amplified SSU rDNA were sequenced from each species and genotype by using *Trichinella*-specific primers (GenBank accession no. U60231). PCR-amplified mitochondrial large-subunit (LSU) rDNA and cytochrome oxidase I (COI) genes were directly sequenced. Three multiple sequence alignments were constructed by using CLUSTAL-X and visually confirmed. The first data set (*18S*) aligned the SSU rDNA of *Trichinella* species to homologues from several outgroup taxa (Table 1). To obtain better resolution of intrageneric relationships, we analyzed a second data set (*11-mit*) incorporating two mtDNA loci (COI and LSU rDNA) from all 11 species and genotypes of *Trichinella*. Inasmuch as the ITS-2 sequences of the eight encapsulated *Trichinella* taxa could not be aligned reliably with those of the nonencapsulated species, only the interrelationships among encapsulated forms were further explored by using a third data set (*3-gene*) by combining the ITS-2, COI, and mitochondrial LSU rDNA gene sequences. Likelihood ratio tests were used to identify which of 56 nucleotide substitution models best fit each of these three data sets by using MODEL TEST 3.0.4 (41) (Table 1) and to evaluate the compatibility of each data set with a molecular clock (42).

Phylogenies were reconstructed from bootstrap replicates of these alignments under the criteria of minimum evolution and ML by using the optimized model as implemented by PAUP* (43). Additionally, a Bayesian approach was used to simultaneously optimize tree topology and nucleotide substitution parameters, including six independent nucleotide substitution rates, the proportion of invariant sites, the shape parameter of the gamma distribution, and the frequency of each nucleotide, by using MR. BAYES 3.0 (44). Of a total 10 million generations, 1 of every 1,000 was sampled after discarding a "burn in" period of 100,000 generations. ML searches performed under topological constraints were also used to evaluate the relative statistical support of competing topologies.

Genetic distances were used to estimate the ages of divergence among species and genotypes of *Trichinella*. Several approaches were considered for each data set to derive a temporal range. For SSU rDNA, a per-lineage calibration rate of 0.4×10^{-9} per site per year was assumed based on data generally supported for metazoan phyla (9). For mtDNA, data

were initially assumed to diverge at a rate of 0.01×10^{-6} per site per year per lineage (i.e., 2% uncorrected pairwise divergence per MY) (45, 46). A second approach used distance and rate estimates derived from ML models, assuming an instantaneous substitution rate of 0.0259×10^{-6} per site per year (47, 48). Alternatively, mtDNA evolution rates (1.1–1.4% uncorrected and 1.4–1.7% ML distance per pair per MY) were derived by assuming that the initial diversification of *T. murrelli* resulted from isolation south of the Laurentide glaciers after 2.5–3.0 MY B.P.

Host associations and historical biogeography were examined in relation to the phylogenetic hypothesis for *Trichinella*. Primary hosts, patterns of life history, and geographical distribution (5) were mapped and optimized onto the phylogenetic tree by using MCCLADE 4.0 (49). The implications of accepting more disparate rate calibrations were also considered during this examination process.

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