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Benjamin Rosenthal
United States Department of Agriculture, Benjamin.Rosenthal@ars.usda.gov

Giuseppe LaRosa
Istituto Superiore di Sanita

Dante Zarlenga
United States Department of Agriculture

Detiger Dunams
United States Department of Agriculture

Yao Chunyu
Jilin University

See next page for additional authors

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Human dispersal of *Trichinella spiralis* in domesticated pigs

Benjamin M. Rosenthal [a,*], Giuseppe LaRosa [b], Dante Zarlenga [a], Detiger Dunams [a], Yao Chunyu [c], Liu Mingyuan [c], Edoardo Pozio [b]

[a] *Animal Parastic Diseases Laboratory, Agricultural Research Service, United States Department of Agriculture, Building 1180, Beltsville, MD 20705, USA*
[b] *Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, viale Regina Elena, 299-00161 Rome, Italy*
[c] *Key Laboratory of Zoonoses, Ministry of Education, Institute of Zoonoses, Jilin University, 5333 Xian Road, 130062 Changchun, PR China*

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ABSTRACT

To investigate the human impact on the evolutionary ecology of animal pathogens, we compared genetic diversity of severe foodborne parasites contracted by eating infected pork or wild game. In particular, we characterized *Trichinella* spp. from twenty-eight countries and four continents by genotyping nine microsatellite loci and sequencing one-fifth of the mitochondrial genome. All specimens of *Trichinella spiralis*, a swine parasite that can infect many species of wildlife, were remarkably uniform across Europe, North Africa, and the Americas. Far greater diversity characterized a comparable sample of *Trichinella britovi*, which parasitizes various sylvatic mammals endemic to Eurasia and North-Western Africa. A limited sample of *T. spiralis* in Asia, where swine were first domesticated, encompassed greater genetic variability than those in the West, as did small samples of *Trichinella nativa* and *Trichinella murrelli*, which parasitize wildlife hosts. We conclude that European lineages of *T. spiralis* originated several thousand years ago, approximately when pigs were first domesticated there. These data also imply that Europeans inadvertently introduced *T. spiralis* to the Americas via infected pigs and/or rats. Despite evidence that early hominid hunters ingested foodborne parasites by hunting wild game millions of years earlier, swine husbandry has governed the subsequent transmission, dissemination, and evolutionary diversification of *T. spiralis*. Where viable parasites have been eliminated from their diet, the residual risk posed to swine by exposure to wildlife or rats should be more precisely defined because breaking the cycle of transmission would confer enduring economic and health benefits.

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1. Introduction

Humans may be "the world's greatest evolutionary force" (Palumbi, 2001), inducing long-lasting change in other species. Animals and plants first domesticated ~9000 years ago still define our diet and landscape (Clutton-Brock, 1999; Diamond, 2002). Although early hominids first contracted certain animal parasites millions of years ago (Hoberg et al., 2001), efficient human transmission of other animal-derived pathogens was a consequence of intensive husbandry of animals. Diseases derived from Eurasian livestock, such as smallpox and measles, facilitated European colonial expansion by devastating previously unexposed human populations (Crosby, 1976, 1986). As present concerns over the pandemic potential of Avian Influenza attest, public health continues to be threatened when intensive agriculture exposes people to communicable animal pathogens (Horimoto and Kawaoka, 2005). Have humans engendered evolutionary change only in those pathogens transmitted from person to person, or also in those we contract from other animals? Herein, we examined whether or not domesticating swine helped establish and disseminate *Trichinella spiralis*, a highly pathogenic foodborne parasite (Dupouy-Camet et al., 2002).

To investigate the human impact on the evolutionary ecology of animal pathogens, we compared the genetic diversity of *T. spiralis* obtained from wildlife and domesticated pigs. To this end, we compared the diversity of parasite populations in Asia, where pigs were first domesticated in the Neolithic from several lineages of wild boar, and in Europe, where pigs were subsequently domesticated from distinct, genetically limited ancestors (Larson et al., 2005, 2007). In particular, we sought to understand whether the older and more genetically diverse pigs of Asia might harbor correspondingly more diverse parasite populations.

Nematodes in the genus *Trichinella* are acquired by ingesting uncooked meat (striated muscle) in which larval parasites have

* Corresponding author. Tel.: +1 301 504 5408; fax: +1 301 504 8979.
E-mail address: benjamin.rosenthal@ars.usda.gov (B.M. Rosenthal).

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Table 1
Specimen origins and genotypes

Isolate	Country	Host	Microsatellite genotype (base pairs) 1		2		3		4		5		6		7		8		9		Reference++
T_pseudospiralis_EU_*	Russia	Raccoon	199	199	226	226	185	189	330	330	214	221	150	163	227	227	232	232	192	192	13
T_nelsoni_AF_104	Kenya	Spotted hyena	196	200	231	231	204	204	320	320	225	225	161	161	220	220	229	229	183	192	29
T_nelsoni_AF_105	Tanzania	Warthog	196	200	231	231	204	204	320	320	225	225	161	161	220	220	229	229	181	192	37
T_britovi_EU_053	Italy	Pig	184	184	225	225	206	214	331	331	228	228	163	163	?	?	220	220	?	?	163
T_nativa_NA_096	Canada	Canadian fox	196	196	233	233	201	201	325	328	227	227	163	163	236	236	240	240	181	187	45
T_nativa_NA_095	Canada	Polar bear	196	196	233	233	201	201	328	331	227	227	163	163	234	236	240	240	181	188	43
T_nativa_AS_094	China	Domestic cat	196	196	223	223	201	201	325	325	237	248	163	163	238	248	237	237	192	196	532
T_nativa_EU_093	Finland	Raccoon dog	196	196	220	220	201	204	331	331	227	227	163	163	250	250	237	237	192	192	568
T_nativa_AS_091	China	Domestic dog	196	196	222	222	201	204	325	328	228	228	163	163	238	238	234	241	188	188	531
T_nativa_AS_092	Arctic	Polar bear	196	196	223	223	201	201	325	328	227	227	163	163	234	234	237	237	181	188	410
T_nativa_EU_090	Russia	Wolf	196	196	220	222	201	201	328	328	230	230	163	163	234	234	238	240	189	194	70
T_murrelli_NA_101	United States	Coyote	192	204	222	222	218	221	322	322	230	230	168	168	250	250	234	234	204	210	unpub. WI
T_murrelli_NA_100	United States	Raccoon	196	204	222	222	212	229	322	331	230	230	159	165	256	256	233	233	207	211	1708
T_murrelli_NA_099	United States	Raccoon	204	212	222	222	212	218	331	331	228	228	161	161	256	256	234	234	207	207	225
T_murrelli_NA_098	United States	Raccoon	204	204	222	222	218	218	331	331	228	228	167	167	256	256	234	234	205	211	227
T_murrelli_NA_097	United States	Raccoon	204	212	222	222	218	229	322	331	230	230	159	165	250	256	234	234	199	207	unpub. WI
T_britovi_EU_064	France	Red fox	204	204	222	225	221	221	331	331	228	228	152	163	242	242	220	244	192	203	325
T_britovi_EU_060	Slovakia	Red fox	184	200	221	225	214	223	331	331	228	228	157	163	238	251	234	234	194	199	360
T_britovi_EU_081	Switzerland	Red fox	184	200	225	225	204	214	331	331	228	228	157	157	232	248	219	240	194	194	384
T_britovi_EU_055	Italy	Red fox	200	204	225	225	201	214	328	328	228	228	157	165	?	?	219	219	194	200	2
T6_NA_102	United States	Cougar	200	200	226	226	201	201	320	328	228	228	152	152	236	236	220	220	194	194	40
T_britovi_EU_086	Spain	Wolf	200	204	225	225	201	214	328	328	228	228	157	165	232	232	219	219	193	200	11
T_britovi_EU_058	Bulgaria	Wild boar	200	204	225	225	201	214	328	328	228	228	157	165	232	242	219	219	195	195	1215
T_britovi_EU_089	Italy	Red fox	200	204	225	225	201	214	328	328	228	228	157	165	242	250	220	220	194	201	23
T_britovi_EU_070	Bulgaria	Wild boar	200	204	225	225	201	214	328	328	228	228	157	165	242	242	219	219	193	200	1214
T_britovi_EU_052	Bulgaria	Wild boar	200	204	225	225	201	214	328	328	228	228	157	165	232	242	219	219	194	200	1217
T_britovi_EU_076	Croatia	Wild boar	204	204	222	222	204	214	331	331	228	228	157	161	232	248	219	219	192	207	142
T_britovi_EU_051	Spain	Wild boar	200	204	222	225	204	204	331	331	228	228	157	157	238	244	219	219	194	200	1569
T_britovi*_EU_050	France	Red fox	200	204	225	225	204	214	331	331	228	228	157	157	242	242	220	241	199	199	138
T_britovi_EU_059	Italy	Stray dog	184	204	225	225	214	214	331	331	228	228	157	157	232	232	219	219	193	193	241
T_britovi_EU_074	Italy	Red fox	184	200	225	225	201	214	331	331	230	230	163	163	232	232	219	219	194	194	61
T_britovi_EU_067	Sweden	Red fox	184	200	225	225	214	214	334	334	228	228	157	159	232	244	220	220	196	196	277
T_britovi_EU_063	Estonia	Brown rat	184	184	222	225	?	?	331	334	228	228	157	157	232	236	220	220	192	199	359
T_britovi_EU_071	Estonia	Blue fox	184	184	221	221	204	214	331	334	228	228	157	157	232	236	219	219	192	199	354
T_britovi_EU_062	Estonia	Pig	184	184	221	221	204	214	331	334	228	228	157	157	232	232	219	219	?	?	333
T_britovi_EU_054	Slovak Republic	Brown bear	184	184	221	221	214	214	331	331	228	228	157	161	232	242	219	219	194	194	234
T_britovi_EU_080	Kazakhstan	Wild cat	192	204	222	225	204	223	331	331	228	228	163	163	240	240	219	223	192	192	133
T_britovi_EU_083	France	Red fox	200	204	225	225	218	218	328	331	228	228	159	165	240	240	219	219	183	192	351
T_britovi_EU_068	Spain	Wild boar	184	204	222	222	214	221	331	331	228	228	161	161	240	240	219	219	193	193	320
T_britovi_EU_077	Kazakhstan	Wild cat	184	204	222	225	201	214	331	331	228	228	157	157	240	240	219	219	192	192	177
T_britovi_EU_069	Italy	Red fox	184	204	222	225	201	214	331	331	228	228	157	171	240	240	219	219	194	194	107
T_britovi_EU_084	France	Red fox	184	204	222	222	206	214	331	331	228	228	163	163	242	242	220	220	192	192	326
T_britovi_EU_088	Italy	Red fox	184	200	222	225	204	214	331	331	228	228	157	165	246	246	220	220	183	189	198
T_britovi_EU_066	Spain	Wild boar	184	204	225	225	204	214	331	331	228	228	157	169	248	248	219	219	183	195	1555
T8_AF_103	South Africa	Spotted hyena	196	204	222	222	197	197	325	334	228	228	171	171	234	234	225	225	181	181	124
T_britovi_EU_078	Kazakhstan	Golden jackal	184	204	222	222	204	210	331	331	228	228	163	163	240	240	219	219	170	194	179
T_britovi_EU_082	France	Red fox	204	204	222	222	214	214	331	331	228	228	165	165	242	250	220	220	173	173	348
T_britovi_EU_073	Italy	Red fox	184	200	222	225	206	206	328	331	228	228	163	163	242	242	220	220	169	183	54
T_britovi_EU_057	Italy	Red fox	184	204	222	225	204	210	331	331	228	228	163	163	240	240	219	219	?	?	200
T_britovi_EU_085	France	Red fox	184	184	222	222	206	221	331	331	228	228	159	163	242	242	220	220	192	192	323
T_britovi_EU_065	France	Red fox	184	184	225	225	214	214	331	331	228	228	167	167	242	242	220	220	190	194	244
T_britovi_EU_061	Macedonia	Pig	184	184	222	222	214	214	331	331	228	228	165	165	240	240	219	219	192	192	236
T_britovi_EU_072	Italy	Red fox	184	184	222	225	210	210	331	334	228	228	157	157	242	242	220	220	193	193	57
T_britovi_EU_079	Kazakhstan	Golden jackal	184	184	222	225	201	214	331	331	228	228	157	165	240	240	219	219	194	194	128
T_britovi_EU_087	Norway	Red fox	184	184	223	225	204	210	328	331	228	228	157	165	240	248	219	219	194	194	392
T_britovi_EU_075	Italy	Domestic cat	184	184	222	225	204	204	331	334	230	230	157	157	244	244	219	219	194	194	18
T_britovi_EU_056	Italy	Red fox	184	184	222	225	204	214	331	331	228	228	163	163	244	244	222	222	194	194	119
T_britovi*_EU_049	Estonia	Raccoon dog	184	204	222	222	214	214	328	331	228	228	157	165	240	240	219	232	186	199	268
T_britovi*_EU_048	Estonia	Wild boar	184	204	222	222	221	221	331	331	228	228	163	163	234	234	219	219	186	196	274
T_spiralis_NA_026	United States	Raccoon	188	188	238	244	223	223	331	331	214	221	152	152	203	203	234	234	192	192	Racc1 (Murrell et al 1985)
T_spiralis_NA_039	United States	Pig	188	188	238	244	223	223	331	331	214	221	152	152	203	203	234	234	192	192	Beltsville
T_spiralis_NA_030	United States	Pig	188	188	238	244	223	223	331	331	214	221	152	152	203	203	233	233	192	192	Maine pig (Murrell et al 1985)
T_spiralis_NA_029	United States	Bobcat	188	188	244	244	223	223	331	331	214	221	152	152	203	205	233	233	192	192	41
T_spiralis_EU_016	Yugoslavia	Pig	188	188	244	244	223	223	328	331	214	221	152	152	205	205	233	233	192	192	161
T_spiralis*_EU_012	Italy	Red fox	188	188	241	244	223	223	328	328	214	221	152	152	203	205	233	233	192	192	151
T_spiralis_EU_004	Spain	Pig	188	188	244	244	223	223	331	331	214	221	152	152	203	203	233	233	192	192	206
T_spiralis_NA_032	United States	Skunk	188	188	244	244	223	223	331	331	221	221	152	152	203	203	234	234	192	192	Skunk-1(Murrell et al 1985)
T_spiralis_EU_025	Spain	Wild boar	188	188	247	247	223	223	331	331	221	221	152	152	203	203	233	246	192	192	192
T_spiralis_EU_017	Bulgaria	Pig	188	188	244	244	223	223	328	331	221	221	152	152	203	203	243	243	192	192	1222
T_spiralis_NA_031	United States	Pig	188	188	244	247	223	223	331	331	221	221	152	152	203	205	233	233	192	192	SC pig (Dame et al. 1987)
T_spiralis_EU_007	Spain	Wild boar	188	188	244	244	223	223	331	331	221	221	152	152	203	203	233	233	192	192	132
T_spiralis_EU_011	Spain	Wild boar	188	188	244	244	223	223	331	331	221	221	152	152	203	203	233	233	192	192	222
T_spiralis_NA_027	United States	Opossum	188	188	244	244	223	223	331	331	221	221	152	152	203	203	233	233	192	192	Opossum1 (Murrell et al 1985)
T_spiralis_NA_028	United States	Raccoon	188	188	244	244	223	223	331	331	221	221	?	?	203	203	233	233	192	192	Racc2 (Murrell et al 1985)
T_spiralis_NA_033	United States	Pig	188	188	241	244	223	223	331	331	221	221	152	152	203	203	233	233	192	192	III-1 (Gamble and Murrell, 1986)
T_spiralis_EU_022	Poland	Pig	188	188	241	244	223	223	331	331	221	221	152	152	203	203	234	234	192	192	168
T_spiralis_EU_015	Austria	Wild boar	188	188	241	241	223	223	331	331	221	221	152	152	203	203	234	234	192	192	102
T_spiralis_EU_001	Spain	Wild boar	188	188	241	247	223	223	331	331	221	221	152	152	203	203	233	233	192	192	207
T_spiralis_SA_110	Argentina	Pig	188	188	244	247	223	223	331	331	214	221	152	152	203	203	233	233	192	192	405
T_spiralis_NA_040	United States	Pig	188	188	244	244	223	223	331	331	214	221	152	152	203	203	234	234	192	192	195
T_spiralis_NA_041	United States	Red fox	188	188	244	247	223	223	331	331	214	221	152	152	203	203	234	234	192	192	229
T_spiralis_EU_020	Spain	Wild boar	188	188	244	247	223	223	331	331	214	221	152	152	203	203	234	234	192	192	252
T_spiralis*_EU_014	Spain	Wild boar	188	188	244	247	178	223	331	331	214	221	152	152	203	203	234	234	192	192	248
T_spiralis_NA_038	United States	Wild boar	188	188	244	244	223	223	331	331	214	214	152	152	203	203	234	234	192	192	WB-133 (Zarlenga and Barta, 1990)
T_spiralis_NA_035	United States	Wild boar	188	188	244	244	223	223	331	331	214	214	152	152	203	203	234	234	192	192	unpub. NH
T_spiralis_NA_037	United States	Wild boar	188	188	244	244	223	223	331	331	214	214	152	152	203	203	233	233	192	192	unpub. NH
T_spiralis_NA_036	United States	Wild boar	188	188	244	244	223	223	331	331	214	214	152	152	203	203	233	233	192	192	unpub. NH
T_spiralis_SA_109	Argentina	Pig	188	188	244	244	223	223	331	331	214	214	152	152	193	209	233	233	192	192	404
T_spiralis_NA_034	Canada	Polar bear	188	188	245	245	223	223	331	331	214	214	152	152	205	205	234	234	192	192	PB4 (Dame et al, 1987)
T_spiralis_EU_024	Denmark	Pig	188	188	245	245	223	223	331	331	214	214	152	152	205	205	233	233	192	192	26
T_spiralis*_EU_013	Slovak Republic	Red fox	188	188	247	247	223	223	331	331	214	221	152	152	203	203	234	234	192	192	174
T_spiralis_EU_019	Spain	Pig	188	188	247	247	223	223	331	331	214	221	152	152	203	203	233	233	192	192	212
T_spiralis_EU_010	Sweden	Pig	188	188	247	247	223	223	328	328	214	214	152	152	203	203	229	229	192	192	328
T_spiralis*_EU_023	Spain	Wild boar	188	188	244	247	223	223	331	331	214	221	152	152	188	203	234	234	192	192	204
T_spiralis_EU_018	Romania	Horse	188	188	241	250	223	223	331	331	214	214	152	152	188	203	233	233	192	192	482
T_spiralis_EU_002	Spain	Pig	188	188	247	247	223	223	331	331	214	221	152	152	188	203	233	233	192	192	208
T_spiralis_EU_021	Spain	Pig	188	188	241	244	223	223	331	331	214	221	152	152	203	203	233	233	192	192	214
T_spiralis_EU_009	Spain	Wild boar	188	188	241	241	223	223	331	331	214	221	152	152	203	203	233	233	192	192	88
T_spiralis_EU_003	Serbia	Horse	188	188	241	241	223	223	331	331	214	221	151	151	203	203	233	233	192	192	599
T_spiralis_EU_006	Finland	Pig	188	188	234	244	223	223	331	331	214	214	152	152	203	203	233	233	192	192	559
T_spiralis_AF_005	Egypt	Pig	188	188	238	238	223	223	331	331	214	214	152	152	203	203	234	234	192	192	154
T_spiralis_EU_008	France	Wild boar	188	188	226	244	223	223	322	331	214	221	152	152	203	203	233	233	192	192	186
T_spiralis_AS_047	Thailand	Pig	188	192	226	226	223	238	322	331	214	221	150	163	200	200	231	237	186	190	Thai pig (Dame et al, 1987)
T_spiralis_AS_044	South Korea	Badger	192	192	227	227	229	229	322	322	214	214	157	157	201	205	237	237	192	192	623
T_spiralis_AS_043	China	Pig	184	192	227	229	218	223	322	328	214	221	152	163	200	200	231	231	178	199	82
T_spiralis_AS_110	China	Pig	184	192	229	229	223	229	322	322	214	221	159	163	200	200	231	231	178	192	81
T_spiralis_AS_109	China	Pig	184	192	229	229	223	229	322	322	214	221	163	163	200	200	231	231	178	192	80
T_spiralis_AS_042	China	Pig	192	192	227	229	223	229	322	322	214	221	163	163	200	200	231	231	178	192	79
T_spiralis_AS_045	China	Pig	192	192	227	232	223	229	331	331	214	214	165	169	205	205	234	244	192	192	unpub.
T_spiralis_AS_046	China	Pig	184	192	230	230	178	218	328	328	214	214	?	?	201	201	234	243	192	192	unpub.

*Isolates previously assigned to other species. ++Accession number in the International Trichinellosis Reference Center (http://www.iss.it/site/Trichinella/) or specified publication. See Refs. (Dame et al., 1987; Gamble and Murrell, 1986; Murrell et al., 1985; Zarlenga and Barta, 1990).

encysted. Most species of *Trichinella* occupy well-defined geographic ranges (Zarlenga et al., 2006). *Trichinella britovi* parasitizes various sylvatic mammals (canidae, felidae, mustilidae, ursidae, viverridae and suidae) endemic to Eurasia and North-Western Africa, whereas *Trichinella murrelli* is restricted to North American wildlife (Kapel, 2000; Pozio and Murrell, 2006). Domesticated swine may become infected with *T. spiralis* if they are permitted to eat uncooked meat (Pozio and Murrell, 2006). Although swine represent the most significant source of human exposure to *T. spiralis*, this parasite is capable of infecting a wide range of other mammals (Kapel, 2000; Pozio, 2000; Pozio and Murrell, 2006).

To compare the extent and pattern of genetic variability in ecologically distinct species of *Trichinella*, we defined the variability of nine nuclear microsatellite loci and sequenced approximately one-fifth of the mitochondrial genome from the broadest population sample yet attempted for species of *Trichinella*, involving twenty-eight countries on four continents. We discovered that *T. spiralis* in domesticated pigs throughout the Western world share strikingly uniform genotypes. By implicating permissive agricultural practices as responsible for the parasite's historical dissemination, these data motivate renewed interest in understanding whether such practices remain necessary for this parasite's continued transmission.

2. Methods

2.1. Specimens

Decades of sampling contributed to our broad survey of specimens, most of which were derived from the International Trichinella Reference Centre in Rome, Italy (http://www.iss.it/site/Trichinella). Each specimen and its origin is specified in Table 1. Our study emphasized a comparison between *T. spiralis* and *T. britovi*, including additional specimens of other available species and genotypes for added context.

2.2. DNA extraction

DNA was extracted from small pools (~15) of larvae that were isolated from fresh tissue or from long-term liquid nitrogen storage, using Proteinase K digestion and adsorption to magnetic beads using the DNA-IQ system and the Tissue and Hair extraction kit (Promega).

2.3. PCR amplification

The primers for each locus, and the repeat motif they flank, are specified in Table 2. To identify microsatellite loci, we screened the draft *T. spiralis* genome project *in silico* for simple repeat motifs. Candidate loci were screened via PCR for their ability to amplify robust products under a range of annealing temperatures. We confirmed the target locus using agarose gel electrophoresis and bi-directional fluorescent sequencing using BigDye v.3.1 chemistries. Thereafter, microsatellite genotyping was accomplished via capillary electrophoresis of products labelled with 6-FAM on an ABI 3730 DNA sequencer. Alleles were called using Genemapper software (Applied Biosystems).

Table 3
Mitochondrial sequencing primers

Forward	Reverse
CACATGATTCACAATCACCT	GAAGCTTAAAATGTCTTCTC
GCAGTAAGAAACCCATCAGA	TAAGTAAGATTTCAATGGCG
GGAGTAACCAAAAATCTAGATCCAA	AAATCTTAAGTACTCGTAGTTTA
CTAGAATGAAAGGAGCAAAG	AGGTTGTGATTATTAGTTTCTAGGG
CCACAATTACCTTACTAATCAC	CCACAATTACCTTACTAATCAC
ACACACCATTAGGATGAATA	AGGAATACACCTACGATTAA
GCATGTCTAAGACTAATTGCATCA	CCTAGTCAGGAGGAGTTGGG

Approximately 3100 bp of contiguous mitochondrial DNA (spanning cytb, tRNA-Ser, SSU rDNA, tRNA-Val, LSU rDNA, atp6, and cox3 genes) were sequenced from each of 14 Western and 8 Asian isolates of *T. spiralis*. Primers Trichi-cob-F1 and Trichi-cox3-R1 (Lavrov and Brown, 2001) were used in conjunction with a series of internal primers designed from a full-length mitochondrial genome sequence AF293969 (Table 3).

Lyophilized DNAs were reconstituted in 50 μl water prior to use. Polymerase chain reactions (20 μl) were comprised of 2 μl DNA, 0.2 mM dNTPs, 0.5 μM of each primer, and either 0.5 U of Platinum® High Fidelity Taq polymerase (Invitrogen) in 0.6 mM $MgSO_4$ 1× buffer (Invitrogen), or 0.5 U Native Taq polymerase (Fisher Scientific) in 0.6 mM $MgCl_2$ 1× PCR buffer. Negative control reactions were included in each experiment. Each PCR commenced with 3 min denaturation at 94 °C and culminated with a final 10 min extension at 72°. Microsatellite loci were amplified using 35–40 cycles @ 94° for 30 s, 53° for 45 s, and 72° for 90 s, using 6-FAM labelled forward primers; mitochondrial loci were amplified using 35 cycles @ 94° for 30 s, 55° for 30 s, and 72° for 2 min.

2.4. Microsatellite length determination

Amplified products were diluted 50-fold in water and mixed 1:10 in Hi-Di™ Formamide containing 0.75% GeneScan™ 500 LIZ® Size Standard. Samples were electrophoresed on an Applied Biosystems 3730 DNA Analyzer and genotyped using Genemapper® v.3.7.

2.5. Sequencing

To prepare PCR products for dual-directional sequencing, excess primers and dNTPs were removed by adding 0.8 μl of ExoSap-IT® (USB Corp.) to 2 μl of the PCR product. After successive 15 min incubations at 37° and 80°, sequencing reactions were completed by adding 1 μl of BigDye® Terminator v.3.1, 2 μl of 5× Big Dye®

Table 2
Microsatellite primers and repeat motifs

Locus	Alias	Forward	Reverse	Repeat
1	TP1	GCGCGATTACGACACTACAA	ATTCGCCACTGTCACTTTCC	TTAA
2	TP5	TACATGGCCCACAGCAAAT	GATGGCCACCAGGTAAGAAA	TTA
3	TP19	AGGAAGATCAAGCGGCAATA	CACGAGTTTGCCTGATGAAA	CAA
4	TP26	GACGTTCAAGAAACGAATGCT	GGATAACCCTCGGCGTATTT	AAC
5	TP28	TCGTTTTTCGTGCTTGATTG	CGGACTTGGTTGCTAGTTGA	TTAAAA
6	TP32	GCGGGTGAAAAATTTCTCTTT	TCAGTCGAAGCAAACCAAAA	TG
7	TP43	TACAGGCGTTCGACACAATC	AGCGCTGAGGTGTCTTTCAT	TA
8	TP47	GAACAGCTTCGGTAGGATGC	TGAATGGCGTGTTTGACAAT	TA
9	TP53	TTGCACAAGTGCGAAAACTC	TGGGTGTGATAGCAACCAGT	TG

terminator buffer (Applied Biosystems) and 1 μl 3.2 pmol primer. These underwent 25 cycles @ 92° for 15 s, 50° for 15 s, 60° for 4 min. Unincorporated fluorescent dNTPs were then removed using gel cartridge columns (Edge Biosystems) prior to electrophoresis on an ABI 3100 sequencer. Sequence chromatograms were edited using Sequencher® v.4.6 (Genecodes Corp.). Sequence data were aligned in Vector NTI Advance v.10 (Invitrogen Corp.).

Mitochondrial gene trees were reconstructed under the criterion of minimum evolution from Kimura 2-parameter distances using MEGA 4.2 (Tamura et al., 2007).

2.6. Genotype-based assignment of individuals to populations

Structure 2.2 was used to assess the statistical confidence with which each specimen could be assigned to one (or more) population subdivisions. Each of ten replicate analyses sampled 1 million generations after discarding a 'burn-in' period of 100,000 generations, under the assumption of seven populations. The statistical plausibility and population composition of alternative scenarios, assuming less or more population subdivision, was also evaluated. These resulted in qualitatively similar outcomes: whereas parasites of wildlife hosts could be further subdivided, Western isolates of *T. spiralis* could not (data not shown).

2.7. Divergence time estimation

The mean difference in microsatellite length, $(\partial\mu)^2$ provides an accurate estimate of divergence time irrespective of changes in population size when this estimator has not yet asymptotically approached its maximum value (Goldstein et al., 1995). Its application to the divergence between Western and Asian populations of *T. spiralis* appears justified, since its value (40.59) is less than one-sixth the value as when Western *T. spiralis* and *T. britovi* are compared (257.57).

2.8. Estimating parasite generation length

Indirect estimates of parasite longevity were derived by considering the life history of their typical hosts. Traditionally, piglets were weaned at 2–3 months of age, at which point they could first acquire infection. Subsequent transmission could commence soon thereafter (when many such animals were slaughtered) or within 2 or 3 years (their typical maximum age) (Mason, 1986). Parasite longevity would be further reduced in swine dying before their intended slaughter, in swine whose immunity allowed them to outlive their parasites, and in synanthropic rats where transmission would cycle more rapidly.

3. Results

One group of *T. spiralis* isolates share remarkably uniform genotypes defined by nine autosomal microsatellite markers (Fig. 1A). This "Western" group, which included every isolate of *T. spiralis* sampled from Europe, the Americas, and Egypt ($n = 43$), harbored fewer alleles and multilocus genotypes than either *T. britovi* from European wildlife ($n = 44$) or *T. spiralis* from Asia

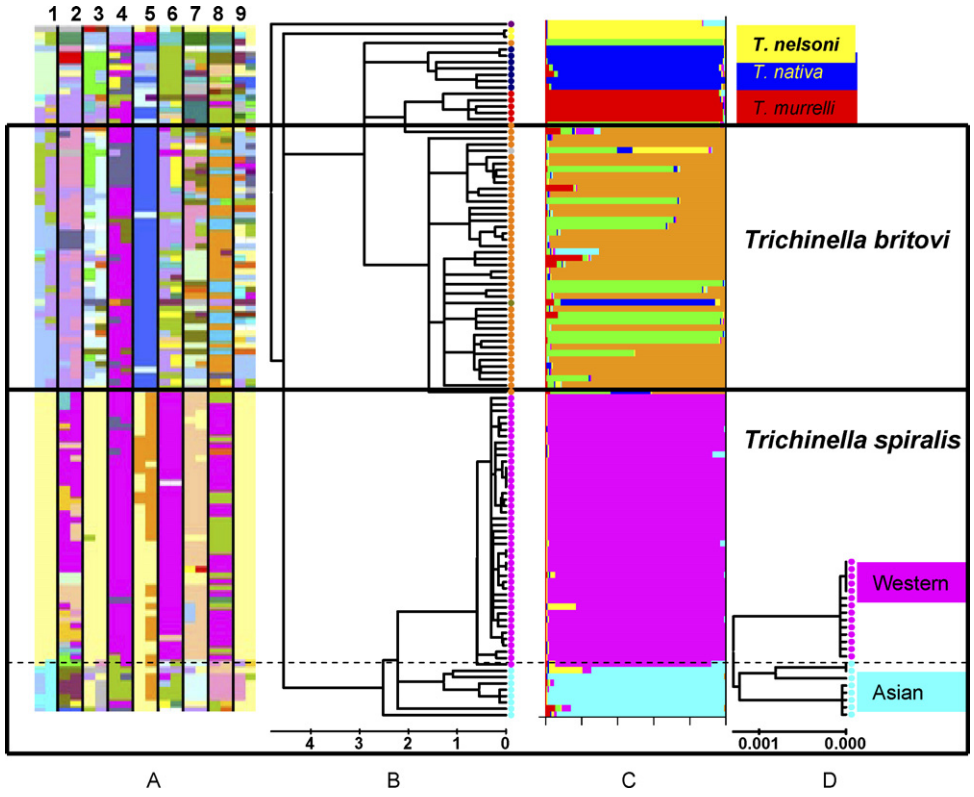

Fig. 1. Exceptional genetic uniformity among Western isolates of *T. spiralis*. The vertical order of specimens is conserved in A–C. (A) Diploid genotypes of 110 isolates at each of 9 microsatellite loci. Each allele is represented as a color. Western *T. spiralis* isolates are absolutely fixed at loci 1 and 9 (yellow) and nearly fixed at loci 3, 4, and 6. Greater diversity occurs in Asian *T. spiralis* (below dotted line) and wildlife parasites (above bold line). (B) Neighbor-joining tree reconstructed from the D_{sw} distances (Shriver et al., 1995) of the microsatellite data. Western isolates of *T. spiralis* (●) define an especially shallow clade. The tree is rooted with *T. pseudospiralis* (●) and includes individual specimens of T6 (●) and T8 (●). (C) Western isolates of *T. spiralis* are assigned to a single, especially homogeneous population (pink) by a Bayesian statistical procedure (PritchardStephens and Donnelly, 2000) applied to microsatellite data. Greater population subdivision is evident among *T. britovi* isolated from European wildlife. (D) Significantly deeper divergence is observed among Asian isolates of *T. spiralis* than among Western isolates in a Minimum Evolution tree reconstructed from 3100 bp of mt DNA.

($n = 8$). Although more than five times as many "Western" than Asian isolates were genotyped, Western isolates harbored more alleles at only one of nine microsatellite loci. The most frequent allele never exceeded 67% in Asian isolates, but did so in six of nine microsatellite loci in Western isolates. At eight of nine loci, the predominant allele in Western isolates was more frequent than the predominant allele in Asia ($p < .005$, Wilcoxan signed-rank test). Among polymorphic loci, half as many alleles occur in *T. spiralis* as in *T. britovi*. Despite their derivation from four continents and several host species (including domestic pigs wild boars, red foxes, opossums, raccoons, and horses), the isolates of the Western group in our entire sample set are genetically less variable than those European specimens of *T. britovi* obtained from a single wildlife species (red foxes, $n = 14$). They are also less variable than our modest sample of either *T. murrelli* or *T. nativa*, parasites endemic to wildlife hosts of temperate North America and the Arctic, respectively.

A tree reconstructed from inter-individual differences in microsatellite length confirms that all isolates of *T. spiralis* are monophyletic and that Western isolates share an especially recent common origin (Fig. 1B). Far greater microsatellite divergences are evident among isolates of *T. spiralis* in Asia, and among isolates of *T. britovi* and other taxa of *Trichinella* in wildlife. These data reaffirm the earlier conclusion (Zarlenga et al., 2006) that *T. spiralis* and a species endemic to African carnivores, *Trichinella nelsoni*, diverged prior to the lineage of Holarctic species comprised of *T. britovi*, *Trichinella nativa*, and *T. murrelli*, but point to a recent dissemination of *T. spiralis* in the West.

Using a Bayesian statistical procedure (Pritchard et al., 2000) that assigns individuals to subpopulations based solely on their microsatellite genotypes without reference to *a priori* taxonomic or geographic designations, each Western *T. spiralis* isolate was unambiguously assigned to one population, whereas Asian isolates of *T. spiralis* and isolates of *T. britovi* were assigned to other, more variable population subdivisions (Fig. 1C). Comparatively deeper branching in clades defined by *T. nativa* and *T. murrelli* again attests to their apparently greater degree of genetic variability.

Assuming that each microsatellite locus sustained an average of 5.6×10^{-4} mutations (Goldstein et al., 1995) during parasite generations averaging six months, Western and Asian lineages of *T. spiralis* would have diverged ~18,000 years ago. Greater precision in this estimate would be possible if the mutation and transmission rates were better known. To further test whether the geographically widespread Western isolates of *T. spiralis* share an especially recent common ancestry, we characterized the extent of variation evident in a substantial portion of the maternally inherited mitochondrial genome. Each specimen of Western *T. spiralis* had one of only two haplotypes differing at only one of 3100 base pairs ($d = 0.00032$). By contrast, among only eight sampled Asian isolates, three mitochondrial haplotypes were identified that differed by as much as eight times that amount (Fig. 1D; Fig. 2). If mitochondrial lineage pairs accumulated differences at a roughly constant rate of ~2% per million years (Brown et al., 1979), Western *T. spiralis* matrilineages would have undergone a population bottleneck within the last 16,000 years. That bottleneck might have occurred only 6000 years ago if mitochondrial substitutions actually accumulated 2.6 times faster, as implied by models that account for substantial variation in the substitution rate among sites in mitochondrial genes (Arbogast et al., 2002).

4. Discussion

Because the evolutionary ecology of *Trichinella* spp. has been insufficiently examined (Tibayrenc, 2001), we undertook a global survey of population genetic variation in parasites that exploit wildlife hosts and domesticated swine. Developing a suite of markers which show promise in elucidating the history and diversity of other species of *Trichinella*, we discovered that a nearly uniform lineage of *T. spiralis* now occupies an exceptionally broad, trans-Atlantic distribution. More limited geographic ranges typify species of *Trichinella* restricted to wildlife hosts. The absence of regional differentiation between far-flung Western populations underscores the hypothesis that *T. spiralis* disseminated there only recently. Genealogies reconstructed from both the microsatellite and mitochondrial data indicate that the earliest diversifications in *T. spiralis* occurred in Asia. More intensive sampling in Asia might establish with greater precision where and when the Western lineage of *T. spiralis* originated. Stochastic sampling error renders molecular clock estimates imprecise even when rate calibrations are well-supported (Ayala, 1997; Hillis and Moritz, 1996), but extant Western isolates of *T. spiralis* share more recent common ancestry than do lineages of *Trichinella* evidently separated during the Pleistocene (i.e. *T. nativa* and the T6 genotype) (Zarlenga et al., 2006). Neither domesticated pigs nor their feral descendants inhabited the Americas before the Colonial Era. Our genetic data suggest that the same is true of *T. spiralis*.

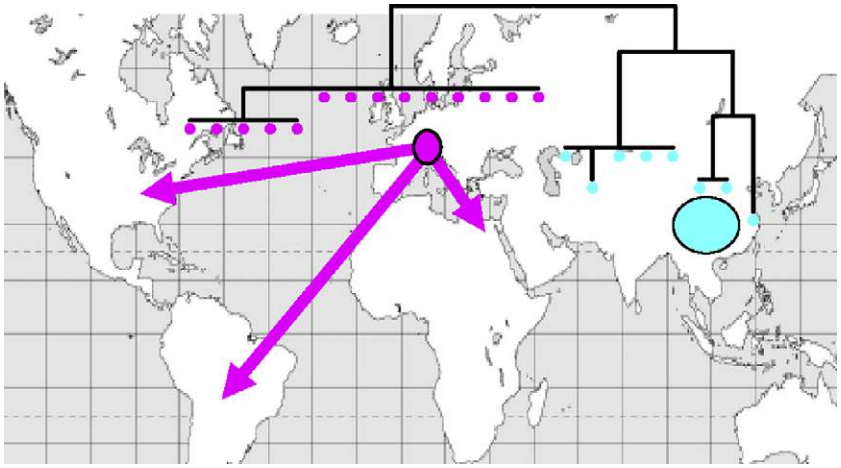

Fig. 2. Limited mitochondrial diversity in Western isolates of *T. spiralis*. A Minimum Evolution tree was reconstructed from Kimura 2-parameter distances in 3100 bp of mitochondrial DNA. Western isolates of *T. spiralis* differ by at most one substitution (0.00032). Isolates derived from Asia differ to a much greater extent. Colors as in Fig. 1B and C.

Human beings may have first consumed meats infected with *Trichinella* and other parasites millions of years before the advent of agriculture (Owen et al., 2005; Zarlenga et al., 2006). *T. spiralis* diverged early in the history of the genus; its distant relationship to other species of *Trichinella* therefore renders its ultimate origins enigmatic (Zarlenga et al., 2006). Evidence from other parasites ingested in meat suggest that *Trichinella* could have infected human beings millions of years before the domestication of pigs. For example, swine and cattle probably first became infected with tapeworms (of the genus *Taenia*) by Neolithic farmers, whose remote ancestors first became infected while consuming the traditional prey of hyenas (Hoberg et al., 2001). If such early hominid hunters were simultaneously exposed to infection with *Trichinella*, then the extant genetic variation in *T. spiralis* reflects only its most recent transmission and dispersal history.

The extant ecology and epidemiology of foodborne parasites has been profoundly influenced by livestock domestication. As may also be true for pork tapeworms (Campbell et al., 2006; Nakao et al., 2002), *T. spiralis* in the West originated in domesticated pigs only within the last several thousand years and was disseminated by European colonists only within the last several hundred years. Interestingly, the diversity in *T. spiralis* appears to be greater where pigs were first domesticated, in East Asia. There, the true diversity of *T. spiralis* undoubtedly exceeds that evident in the current, admittedly limited sample. By contrast, additional sampling of isolates in the West would seem unlikely to identify heretofore uncharacterized variants.

Although *T. spiralis* remains a parasite of animals, its abundance, distribution, and diversity were profoundly influenced by human activity. This distinguishes *T. spiralis* from other pathogens engendered by Eurasian agriculturalists and disseminated by their seafaring descendants (i.e. smallpox and measles viruses, which lost their dependency on animal reservoirs) and from other zoonoses requiring animal reservoirs (which, like anthrax and rabies, generally originated in the tropics prior to the advent of agriculture) (Wolfe et al., 2007). Thus, a distinct evolutionary ecology may characterize zoonotic pathogens of domesticated livestock.

The expansion of agriculture was evidently responsible for the historical dissemination of *T. spiralis*, but the requirements for ongoing transmission remain uncertain. In particular, it is unclear how extensive a risk wildlife or rats pose to swine where transmission among pigs has been prevented by eliminating, from their diet, meats harboring viable parasites. The species of *Trichinella* that most commonly infect wildlife pose negligible risk to the safety of pork (Kapel and Gamble, 2000). Nonetheless, the risks to swine posed by suspected wildlife reservoirs of *T. spiralis* should be evaluated in the various agro-ecological settings where swine are raised, because measures intended to safeguard food safety require substantial effort and cost. Does *T. spiralis* thrive primarily where poor management facilitates its transmission among swine (Kapel, 2000; Pozio, 2000; Pozio and Murrell, 2006), or has the parasite instead become established in self-sustaining cycles among wildlife (Rafter et al., 2005)? If eliminating *T. spiralis* from swine herds irrevocably breaks the cycle of transmission necessary for its local persistence, such interventions would provide enduring benefits to the economy and to public health.

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