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Mitochondrial Diversity Supports Multiple Origins for Invasive Pigs

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Population Ecology

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ABSTRACT Our objective was to identify and evaluate mitochondrial diversity of wild pigs in the United States (U.S.). We obtained tissue samples from 81 individual pigs in 30 U.S. states and amplified a 403 base-pair region of mitochondrial DNA. We then downloaded overlapping sequences ($n = 904$) from public repositories to create a global reference. We used parsimony and Bayesian techniques to evaluate phylogenetic relationships, and we used origins of published sequences from Eurasian wild boar to establish a phylogeographic reference. We then compared gene and nucleotide diversity measures for introduced pigs in North America with those of domestic swine, Eurasian wild boar, and feral pigs within broad-scale geographic groupings. We identified 15 haplotypes for introduced pigs, representing wild and domestic animals from >30 countries spanning the indigenous range of *S. scrofa*. Mitochondrial diversity measures and phylogenetic relationships indicated a strong association between introduced pigs and European domestic breeds, reflecting the known history of human colonization, trade, and settlement in the United States. Based on the geographic distribution of haplotypes in North America, we found that range expansion is a product of translocation from historical populations and introduction from new genetic sources. Finally, haplotype network analyses provided evidence of past demographic expansions within lineages, contributing to observed mtDNA variation among introduced pigs in North America. © 2014 The Wildlife Society.

KEY WORDS diversity, feral, genetics, mitochondrial DNA, pig, *Sus scrofa*, United States.

Invasive species present one of the greatest threats to native ecosystems globally. In the United States (U.S.), tens of thousands of nonnative species have been introduced, posing serious risk to natural systems and accounting for >$100 billion in damages annually (Pimentel et al. 2005). Although not all nonnative species are invasive, some are particularly adept at colonizing new environments (Kolar and Lodge 2001). In some cases, close association with humans aids dispersal. This is especially true for pigs (*Sus scrofa*; Mayer and Brisbin 1991).

Pigs were first domesticated ≥9,000 years ago (Giuffra et al. 2000, Larson et al. 2007, Vigne et al. 2009) and domestication has occurred subsequently in multiple locations throughout Eurasia (Larson et al. 2005, 2010; Luetkemeier et al. 2010). Pigs have been repeatedly transported to new locations and released under free-range livestock conditions, often leading to establishment of new wild populations. With the advent of

world travel and trade in the 16th century, this situation was exacerbated. Though native to portions of Europe, Asia, and Africa, wild pigs can now be found on many islands and all continents except Antarctica (Oliver and Brisbin 1993).

Among U.S. states, Hawaii was the first to be affected by introduced pigs. Polynesian settlers are thought to have released pigs on the islands ≥1,000 years before present (Mayer and Brisbin 1991). However, introduction of European domestic stock in the 1700s and subsequent introduction of a variety of domestic breeds continuing to modern times is thought to have caused interbreeding among feral island populations (Mayer and Brisbin 1991). The extent to which these ancient and recent introductions have contributed to feral pig distributions on the islands today is unclear.

Domestic pigs were first brought to North America during explorations of the 1500s (Towne and Wentworth 1950, Mayer and Brisbin 1991). Because of free-range livestock practices, escape, or release, feral populations were commonly established around colonies (Mayer and Brisbin 1991). As the interior of the continent was settled, pigs were introduced to many locations, with some populations persisting and others perishing. By the late 1800s, established

feral populations were present in at least 13 U.S. states in the southern tier of North America (Mayer and Brisbin 1991). At this time, an increased interest in pig hunting prompted the importation and release of Eurasian wild boar. The term Eurasian wild boar here refers to all wild *S. scrofa* L (i.e., undomesticated pigs) and the term hybrid represents any level of crossing between domestic pigs and Eurasian wild boar or feral pigs and Eurasian wild boar (Mayer and Brisbin 1991). Eurasian wild boar bred freely with feral pigs wherever populations came into contact, leading to hybrid animals with a range of intermediate phenotypic characteristics (Mayer and Brisbin 1991). Since the introduction of the wild boar, trade and translocation of pigs throughout the U.S. for hunting purposes became commonplace and hybrid animals possessing wild boar phenotypic characters were highly sought after for establishment of new populations or improvement of existing herds (Mayer and Brisbin 1991, Waithman et al. 1999). This is an important consideration, because the type of pigs colonizing areas may affect the course of establishment and the rate of range expansion (Waithman et al. 1999).

During the last 30 years, anthropogenic factors have been thought to be the leading cause of range expansion in the U.S. (Gipson et al. 1998, Waithman et al. 1999). Since 1980, the invasive range of pigs has increased steadily from 17 to 44 U.S. states (Mayer and Brisbin 1991, 2009, Gipson et al. 1998). Although recorded accounts provide some insights, it is unclear if stock from historical introductions persist or if they have been replaced by subsequent invasions. The secretive nature of recent anthropogenic dispersal has added to this uncertainty, and calls into question the accuracy of introduction records. However, molecular techniques may provide an opportunity for understanding the current diversity of wild pigs in the United States.

Mitochondrial DNA (mtDNA) has been used successfully for phylogeographic studies of wild boar and domestic breeds throughout Eurasia (Larson et al. 2005, Scandura et al. 2008, Luetkemeier et al. 2010) and has helped identify putative geographic and breed origins for feral populations in New Zealand and Australia (Gongora et al. 2004). Furthermore, mtDNA phylogenies have elucidated patterns of transcontinental human dispersal of pigs and associated breed development in Asia and Europe (Giuffra et al. 2000, Fang and Andersson 2006, Luetkemeier et al. 2010), and mtDNA has helped identify hybridization events between domestic pigs and Eurasian wild boar (Fang et al. 2006, Scandura et al. 2008). Finally, the vast amount of published sequence makes mtDNA a particularly valuable genetic marker for global analysis of introduced pigs (Giuffra et al. 2000).

Although mtDNA presents many positive attributes for global phylogenetics, the history of U.S. wild pigs presents challenges for molecular investigation. Genetic relationships may be confounded by human redistribution of the species, the short duration of inhabitation in North America, and the introduction of both domestic pigs and Eurasian wild boar to wild-living populations. All would preclude the effective use of divergence-based analyses for examining wild pig dispersal

subsequent to introduction, because we cannot expect DNA variation to reflect geographic distribution for this invasive species (Spencer and Hampton 2005). Alternately, animal invasions have been tracked with mtDNA by linking haplotype occurrences between historical and newly invaded areas (Evans et al. 2003, Tooman et al. 2011). However, important limitations intrinsic to mtDNA and published sequence must be considered when assessing both global and national molecular genetic relationships for pigs. For instance, mtDNA is a single haploid marker that is prone to stochastic variation. Therefore, genetic drift, founder effect, and bottlenecks may limit mtDNA haplotypes shared between sampled locations. Also, published sequence may be incorrectly identified to species or breed and may contain nucleotide errors, affecting phylogenetic analysis and interpretation (Wesche et al. 2004, Nilsson et al. 2006). Additionally, mtDNA pseudogenes (NUMTs) can possibly be amplified, leading to erroneous phylogenetic results (Parr et al. 2006, Goios et al. 2009). Though NUMTs have been identified for domestic cats and humans (Lopez et al. 1994, Bensasson et al. 2003), we could find no reference to rates of occurrence for NUMTs in pigs. To address the possibility of NUMTs biasing phylogenetic analysis, we used recently compiled genomic data (Archibald et al. 2010) to search for duplication events (see methods).

Despite these challenges, careful assessment of mtDNA relationships can provide insights to the multiple origins of introduced pigs and the genetic diversity of disparate populations. We investigated phylogenetic relationships of wild pigs in the U.S. in the context of published mtDNA sequence for domestic pigs and Eurasian wild boar. Our objective was to identify and evaluate mitochondrial diversity of wild pigs in the United States. We expected to find relatively high genetic diversity for introduced pigs and shared ancestry with both domestic swine and Eurasian wild boar, as indicated by historical accounts of the invasion.

STUDY AREA

We sampled pigs occurring on private and public lands from 61 counties in 30 U.S. states during the period July 2007–May 2010. We included locations representative of both long-established populations in the southern tier of North America and recently occupied sites in the upper Midwest and elsewhere (Fig. 1). We also included published sequence of wild and domestic pigs reported from island locations (Giuffra et al. 2000, Watanobe et al. 2001, Larson et al. 2005, Tanaka et al. 2008) and 4 other continents: Africa (Larson et al. 2005), Asia (Giuffra et al. 2000, Kijas and Andersson 2001, Watanobe et al. 2001, Kim et al. 2002, Randi et al. 2002, Alves et al. 2003, Yang et al. 2003, Larson et al. 2005, Fang and Andersson 2006, Fang et al. 2006, Wu et al. 2007, Ishiguro et al. 2008, Tanaka et al. 2008, Ramayo et al. 2011), Australia (Kim et al. 2002, Larson et al. 2005), and Europe (Ursing and Arnason 1998, Lin et al. 1999, Giuffra et al. 2000, Kijas and Andersson 2001, Watanobe et al. 2001, Kim et al. 2002, Randi et al. 2002, Alves et al. 2003, Gongora et al. 2003, Yang et al. 2003, Larson
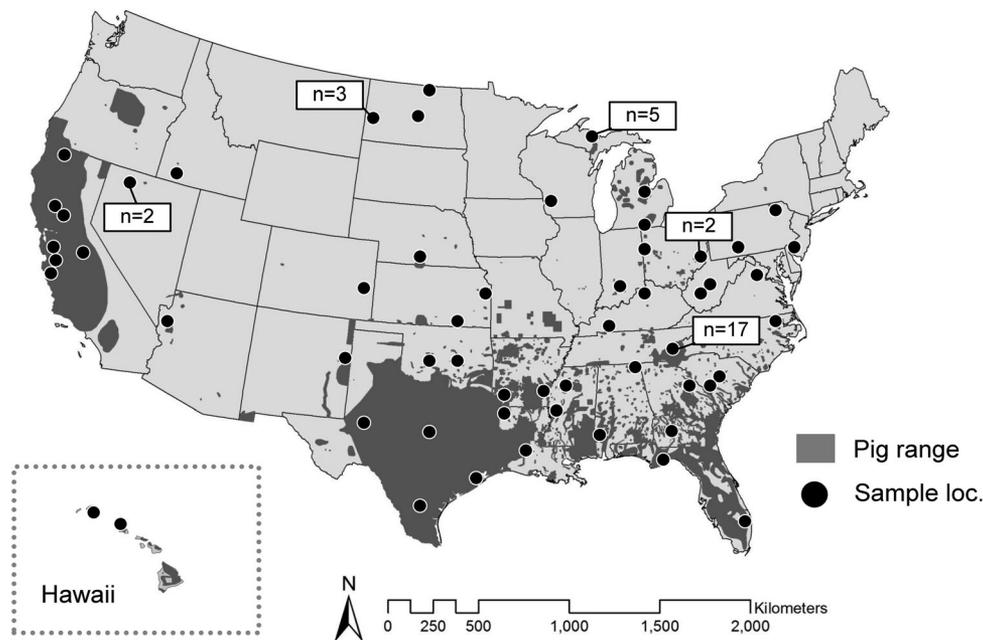
Figure 1. Distribution of 81 samples from wild pigs (*Sus scrofa*) collected from 30 U.S. states during the period July 2007–May 2010. Dark regions of map indicate areas of established pig populations as denoted by state and federal agencies (Southeastern Cooperative Wildlife Disease Study 2010). Each dot represents the location (loc.) of an individual sample, unless otherwise noted on map.

et al. 2005, Fang and Andersson 2006, Fang et al. 2006, Scandura et al. 2008; Fig. 2).

METHODS

Sampling Techniques

We cooperated with United States Department of Agriculture Wildlife Services, National Park Service, state agencies, and private organizations involved in sanctioned pig control, eradication, or research programs to obtain samples. When a pig was destroyed, field personnel collected blood or other somatic tissue (e.g., skeletal muscle, skin) and recorded the sample location. Blood samples were stored on FTA cards (Whatman Inc., Florham Park, NJ), allowed to air dry, and sent to the University of North Dakota (UND) where we stored them at room temperature. Other somatic tissues were frozen and shipped overnight to UND and stored at −20° C upon arrival. We obtained 81 samples for mtDNA analyses (Fig. 1). We collected all samples secondarily from management actions authorized by state and federal agencies required to adhere to welfare protocols for handling of mammalian species. Therefore, this research was deemed exempt by the UND institutional animal care and use committee.

We established a global dataset by obtaining published mtDNA control region sequence ($n = 904$) from National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/) representing wild, domestic, and feral pigs from around the world. Among these, we included sequence representing 114 haplotypes of the control region identified by Scandura et al. (2008) and incorporated breed and geographic information referenced therein. We also searched NCBI for sequence from entries submitted after the Scandura et al. (2008) publication and for samples from new geographic areas or those representing additional breeds (Table S1 available online at www.onlinelibrary.wiley.com).

Laboratory Methods

For total DNA extraction, we followed standard protocols for dried blood (Whatman Inc.) and tissue with the DNEasy blood and tissue kit (Qaigen, Santa Clarita, CA). We quantified genetic concentrations using an ND-1000 spectrophotometer and software V3.1.0 (Nanodrop Technologies, Inc., Wilmington, DE), and diluted with distilled water as necessary for the polymerase chain reaction (PCR). We amplified an approximately 550 base-pair segment of the mtDNA control region using forward primer PigF (5′-ACTCTGGT CTTGTAAACC-3′) and reverse primer PigR (5′-TAAGGGGAAAGACTGGGC-3′; Okumura et al. 1996, Loggins 2007). We conducted PCR with the Ex Taq kit (Takara Biotechnology, Ltd., Shiga, Japan) using standard procedures in an Eppendorf thermocycler (Eppendorf, Hamburg, Germany; Simmons and Scheffer 2004). We then checked product for presence and size of DNA fragments on a 2% agarose gel containing 0.1 µg/ml of ethidium bromide, and visualized gels with an AutoChemi ultraviolet transilluminator and Labworks 4.6 computer software (UVP Bio-Imaging Systems, Cambridge, United Kingdom). We cleaned PCR products for sequencing using a Qiaquick PCR purification kit (Qiagen).

We performed sequencing reactions with a Big Dye Terminator Version 3.1 sequencing kit (Applied Biosystems, Foster City, CA) and the forward and reverse primers described above. We used an ABI 3100 system (Applied Biosystems) to visualize and record the sequence and BIOEDIT 5.0.6 (Hall 2001) for alignment and assembly
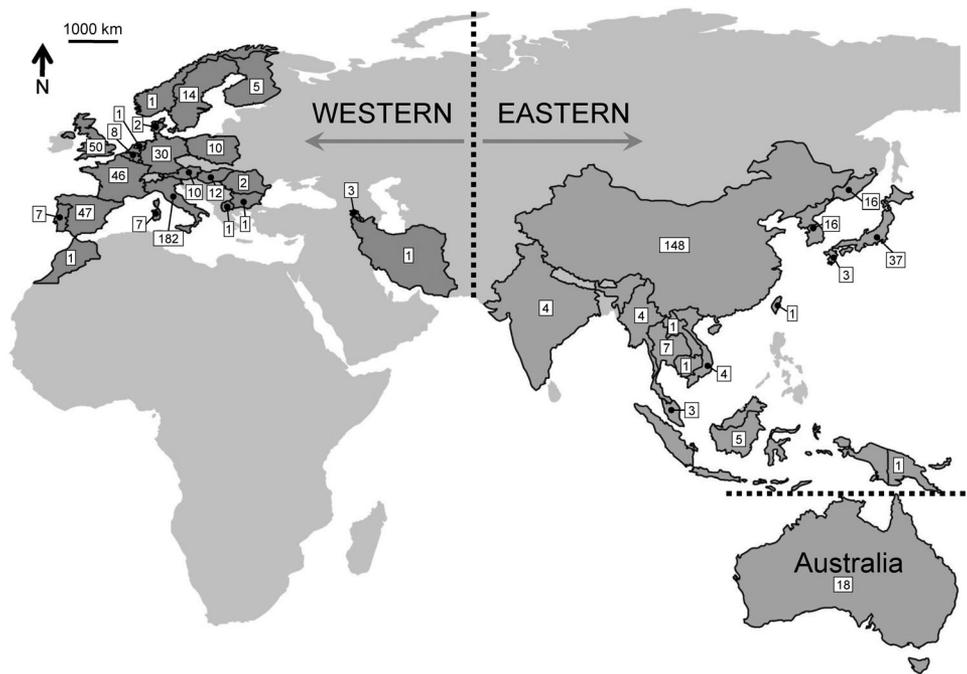
Figure 2. Distribution of reported geographic locations (by country of origin) for published sequence of *S. Scrofa* in the global dataset; we report number of sequences for each country. Dotted lines depict geographic divisions established to delineate Western, Eastern, and Australian groupings of sequences representing domestic pigs, Eurasian wild boar, and introduced pigs used in molecular diversity analyses. Note: Hawaii ($n = 2$), Iceland ($n = 1$), Mexico ($n = 1$), South Carolina USA ($n = 1$), the Cook Islands ($n = 1$), the Mariana Islands ($n = 1$), and Vanuatu ($n = 1$) are not represented because of map constraints. Ambiguous locations within Asia ($n = 17$), Europe ($n = 128$), Siberia ($n = 1$), the Iberian Peninsula ($n = 17$), and those of unknown or unclear origin ($n = 23$), also are not depicted.

of consensus sequences. Finally, we trimmed all sequences to minimize potential errors in nucleotide identification and missing data in our matrix, resulting in a 403 base-pair alignment of the control region for analysis. Sequences are available in online holdings at NCBI (JF701989-JF702002, JF702006, JF702009-JF702012, JF702017, JF702023-JF702037, JF702040, JF702046, JF702049, JF702054, JF702056-JF702078, JF702081, JF702087-JF702093, and JF702105-JF702115).

To avoid errors associated with NUMT contamination, we re-processed any samples where we observed ambiguities within the 403 base-pair region in chromatograms. Further, we conducted a Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990) search using our mtDNA sequence to probe the pig genome (Sscrofa 10; http://www.ncbi.nlm.nih.gov/genome/guide/pig/) for NUMTs. We downloaded all BLAST results with >90% coverage and identity for our mtDNA matrix. We then compared the nuclear sequence with our mtDNA matrix to ensure that none matched mtDNA consensus sequences or polymorphic nucleotide positions defining haplotypes.

Phylogenetic Analyses

We used TCS 1.21 (Clement et al. 2000) with gaps set as a fifth character state to determine number of haplotypes and construct a haplotype network. We constructed phylogenetic trees from our total haplotype matrix using MRBAYES 3.1.2 (Ronquist and Huelsenbeck 2003) with 10,000,000 Markov Chain Monte Carlo generations using the GTR +

I + G model as determined by jModeltest (Guindon and Gascuel 2003, Posada 2008). To root trees, we included sequences from other *Sus* species occurring in Southeast Asia, where mtDNA evidence suggests divergence of *S. scrofa* between 5 and 1 million years ago (Randi et al. 1996, Mona et al. 2007) followed by a radiation of the species across Eurasia during the last 500 thousand years (Giuffra et al. 2000; Larson et al. 2005, 2010; Lucchini et al. 2005). Outgroup sequences included: *S. barbatus* Muller (bearded pig; $n = 3$), *S. verrucosus* Muller (Javan pig; $n = 4$), *S. celebensis* Muller and Schlegel (Celebese or Sulawesi warty pig; $n = 2$), *S. cebifrons* Heude (Visayan warty pig; $n = 2$), and *S. philippensis* Nehring (Philippine warty pig; $n = 2$) published at NCBI (Table S1). We then used recorded collection locations from published Eurasian wild boar sequences for phylogeographic interpretation of tree topology. Finally, we prepared a haplotype network including sequences from U.S. pigs and all closely associated haplotypes in our Bayesian phylogenetic tree ($n = 120$ haplotypes, total).

We used ARLEQUIN 3.5 (Excoffier et al. 2005) to compare mtDNA diversity of pigs in North America with that of introduced pigs in Australia and wild and domestic pigs from the Eastern Hemisphere represented in our global dataset. Based on the findings of Groves and Grubb (1993), we categorized published sequences from North Africa, Europe, and parts of Central Asia as Western, and we categorized all sequences published from points east of Central Asia generally as Eastern (Fig. 2). Using this

methodology, we organized the dataset into 6 broad-scale groupings corresponding roughly to geography and animal type: North America, introduced ($n = 79$); Australia, introduced ($n = 18$); Western, wild boar ($n = 253$); Western, domestic ($n = 334$); Eastern, domestic ($n = 161$); and Eastern, wild boar ($n = 101$). We excluded 32 sequences from this analysis because of insufficient information regarding geographic location or type of pig represented. We excluded 7 sequences representing feral pigs from the Mariana Islands ($n = 1$), Papua-New Guinea ($n = 1$), Vanuatu ($n = 1$), and the Hawaiian Islands ($n = 4$) because of their remote location and small number of representatives. However, we used sequences from these island locations for subsequent ad hoc comparisons of haplotypes present among invaded areas. In ARLEQUIN, we generated descriptive statistics and calculated diversity and genetic distance measures within and between groups (Tajima 1983, Nei 1987). We then identified specific haplotypes shared between invaded areas in our global sample.

RESULTS

We identified 148 haplotypes for the 403 base-pair mtDNA alignment of global sequences of *S. scrofa*, delineated by 12 insertions or deletions, 38 transitions, and 1 transversion (substitutions per nucleotide = 0.097). Our sample of 81 wild pigs included 14 haplotypes, and 1 published sequence from Hawaii (AY884613; Larson et al. 2005) provided a fifteenth haplotype for consideration in our analyses of introduced pigs in the United States. Based on reported geographic information and pig types, 11 of these haplotypes corresponded cumulatively to >70 domestic breeds in 15 countries, Eurasian wild boar from 20 countries, and animals of all types from >30 countries (Appendix A). Four mtDNA haplotypes identified in our U.S. sample were unique, having no identical match to published sequence (Appendix A).

Phylogenetic Relationships

Bayesian analysis revealed tree topology corresponding roughly with geographic locations for published sequences of outgroups and Eurasian wild boar, which we used as a basis for phylogeographic reference (Fig. 3). Most haplotypes representing wild pigs collected in the United States were associated with 2 large polytomies, one of Asian phylogeographic origin and one of European phylogeographic origin (Fig. 3). One haplotype (h84), from Hawaii, was part of a monophyletic group composed of feral pigs and *S. verrucosus* (Fig. 3, Fig. S1 available online at www.onlinelibrary.wiley.com).

Network analysis generally supported Bayesian tree topology and revealed substructure elucidating the importance of select haplotypes in domestication of pigs (Fig. 4). Starburst features associated with haplotypes representing both Eurasian wild boar and domestic breeds suggest rapid diversification and provide evidence of past demographic expansions within lineages, including processes leading to haplotypes 145–148 that were identified only in North America (Fig. 4). Most haplotypes found in the U.S. were

among those shared by domestic pigs and Eurasian wild boar and were widely distributed globally (Fig. 4; Appendix A).

Mitochondrial Diversity

Gene diversity was highest among wild boar groupings, followed by domestic groups, and then introduced pigs in North America and Australia (Table 1). Alternately, nucleotide diversity was highest for Western domestic pigs, followed by wild boar groupings, introduced pigs in North America, Eastern domestic pigs, and then introduced pigs in Australia (Table 1). Our North American sample was most closely related to Western domestic and Western wild boar groupings (Table 2). Mitochondrial haplotypes were shared between all groups, but Western domestic pigs and Western wild boar groupings had the most haplotypes in common with introduced pigs in North America (Table 3). Feral pigs from Hawaii also shared haplotypes with all of the above groups, but this relationship was driven by the occurrence of 2 very common haplotypes in Hawaii (h19 and h13; Appendix A). However, a single haplotype (h84) was exclusive to feral animals reported from Hawaii, the Mariana Islands, Papua-New Guinea, and Vanuatu, as well as *S. verrucosus* from Indonesia (Appendix A). Otherwise, mitochondrial haplotypes associated with Eastern groupings were present at relatively low frequency in all invaded areas (Table 3).

We found 8 mitochondrial haplotypes in more than 1 state and some (e.g., h17, h19, and h37) were distributed throughout the continental United States (Fig. 5, Appendix A). Haplotype variation was highest in Tennessee, North Carolina, Michigan, and North Dakota, though number of haplotypes observed was positively correlated (Spearman $r = 0.876$, $P < 0.001$) with the number of samples collected in states (Fig. 5). Patterns in haplotype distribution potentially useful for tracking dispersal include: 1) the eastern distribution of h7 from South Carolina to Michigan; 2) the identification of h2 only in Michigan and Idaho; 3) the discovery of h20 only in North Dakota and South Carolina; and 4) the occurrence of h39 in Florida, Ohio, and Michigan (Fig. 5). Unique haplotype 148 was found in both Indiana and Kansas, whereas unique haplotypes 145–147 were found only in Great Smoky Mountains National Park, bordering Tennessee and North Carolina (Figs. 1 and 5).

DISCUSSION

The genetic variation identified here using only a haploid marker and a modest sample dataset supports multiple origins for introduced pigs into the United States (Mayer and Brisbin 1991). Though the 15 mtDNA haplotypes found in the U.S. are a fraction of those identified for the global dataset, they are a diverse molecular subset (Table 1) and represent a wide array of pig types from throughout the native range of *S. scrofa* (Fig. 3, Appendix A). This finding suggests that the nationwide population possesses intrinsic adaptive potential through genetic variability.

The importance of genetic variation for species survival is well documented (Johnson and Dunn 2006, Wright et al. 2008). In populations founded by only a few
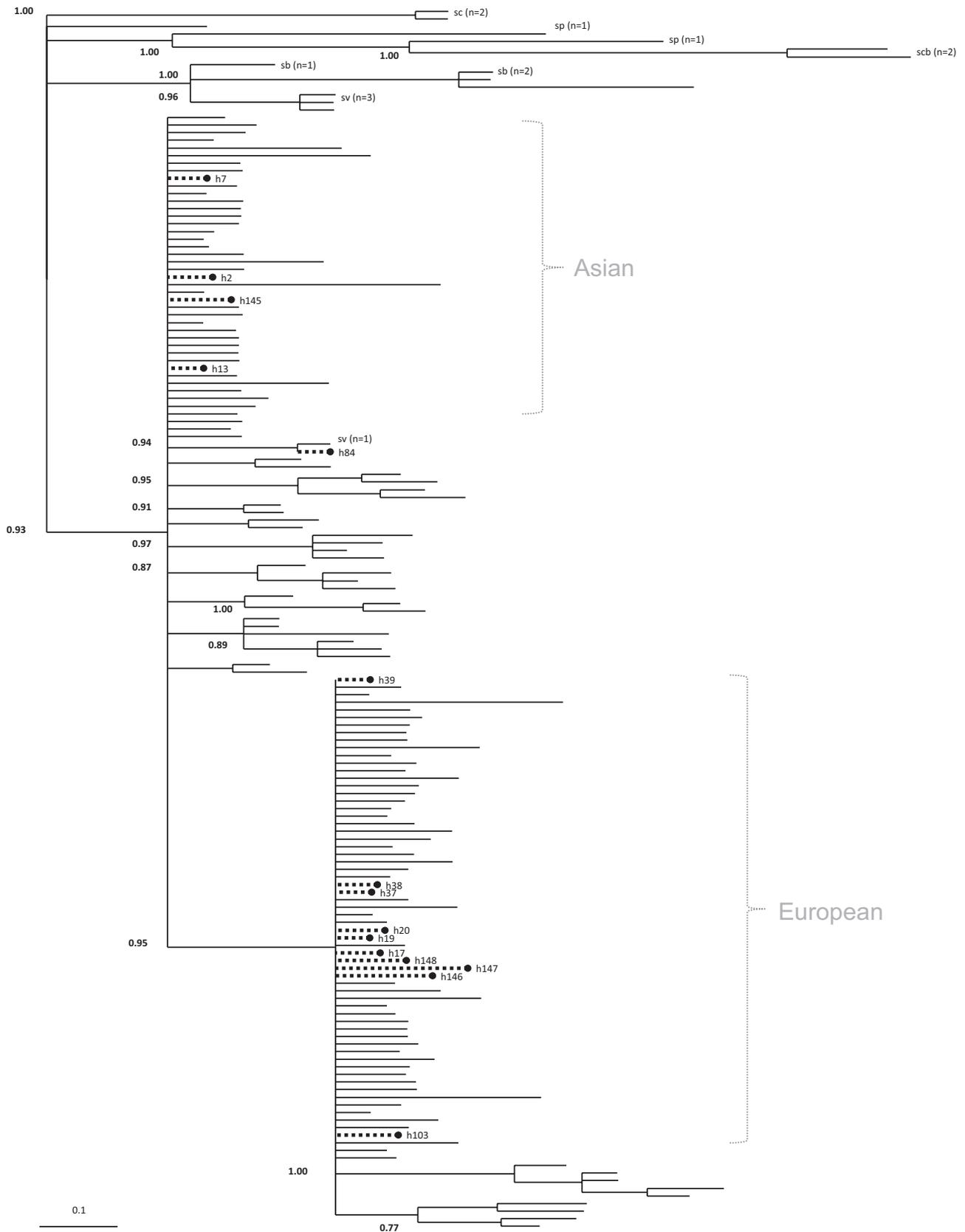
Figure 3. Rooted phylogram of 148 *Sus scrofa* haplotypes and 13 sequences from 5 other *Sus* species: *S. barbatus* (sb; *n* = 3), *S. verrucosus* (sv; *n* = 4), *S. celebensis* (sc; *n* = 2), *S. cebifrons* (scb; *n* = 2), and *S. philippensis* (sp; *n* = 2). Dotted lines in tree indicate haplotypes identified for pigs collected in the United States. We provide Asian and European phylogeographic associations, based on geographic origin of Eurasian wild boar sequences, to the right of the tree. Numbers at nodes indicate mean posterior probabilities (≥75% shown) and scale bar (bottom left) indicates genetic distance.
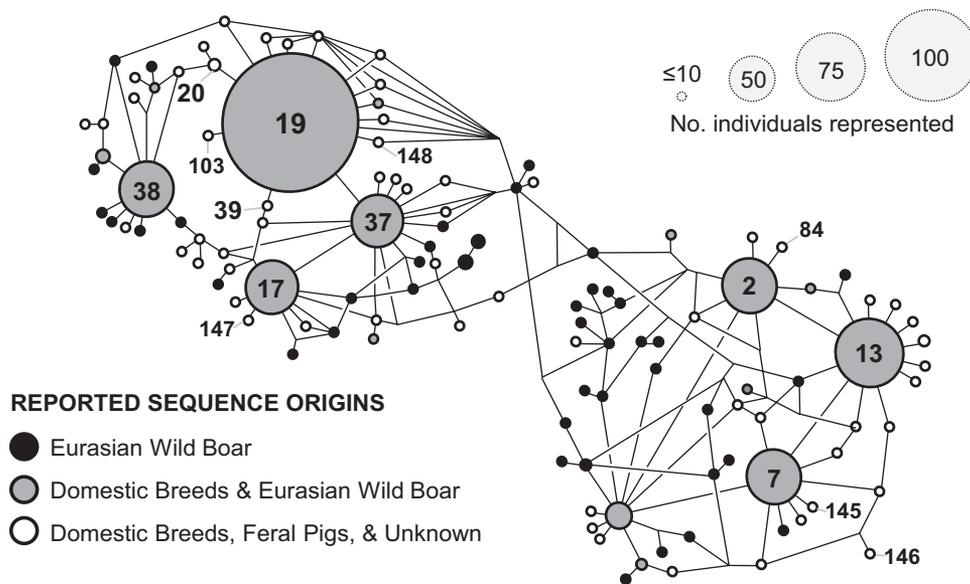
Figure 4. Network of 120 haplotypes representing introduced pigs from the United States, domestic swine, and Eurasian wild boar that were associated in large polytomies in the Bayesian phylogenetic tree. Frequency of haplotypes in the dataset is represented by relative size of nodes (as indicated at top right). Pig types inferred from metadata associated with accessions are denoted by black (Eurasian wild boar), gray (shared between domestic and Eurasian wild boar), and white (domestic, feral, unknown) shades. Haplotypes found in the United States are numbered within or next to nodes. Intermediate nodes, mutational steps, and genetic distance are not represented.

individuals, genetic variation may be insufficient for reproductive health and ongoing adaptation to environmental fluctuations, diseases, and other ecological stressors (Wright et al. 2008). Therefore, low genetic diversity may have limited the invasiveness of the first pig populations established in the United States and this may explain the decline and limited range of the species in some locations prior to 1900 (Mayer and Brisbin 1991). However, genetic variation does not appear to be limiting wild pig populations in the United States today. Instead, the various genetic backgrounds filtering into wild pig populations are likely contributing to their invasion of novel habitats.

The high genetic diversity detected for introduced pigs is an important challenge from a management perspective. Translocation and release of pigs has undoubtedly augmented genetic diversity of disparate populations in the past and will likely continue to do so in the future (Gipson et al. 1998, Mayer and Brisbin 2009). This frequent movement of animals across great geographic distances has allowed invasive pigs to repeatedly bridge geographic barriers that might otherwise restrict gene flow. Thus, spatially structured

emigration and immigration patterns will be relevant for management, so that we may identify the extent of population units and determine sources for new introductions (Robertson and Gemmell 2004, Helgen et al. 2008, Ross and Shoemaker 2008). Therefore, questions of ancestry, diversity, and gene flow (i.e., dispersal patterns) will be increasingly important for population control strategies. Clearly, molecular techniques can be wielded to inform management in this regard (Gongora et al. 2004, Hampton et al. 2004, Spencer and Hampton 2005). However, in the case of mtDNA, we must proceed cautiously and consider the limitations of this molecular marker in light of prior research and trends in our current dataset.

Global Relationships Affecting Genetic Diversity of Introduced Pigs

The phylogenetic patterns identified through our Bayesian analysis generally agree with prior research on swine ancestry that divides mtDNA lineages into European and Asian clades (Alves et al. 2003, Gongora et al. 2004, Larson et al. 2005). From previous studies, we also know that

Table 1. Mitochondrial variation among 6 arbitrarily assigned groupings of swine based on geographic location (North America [N.A], eastern or western Eurasia, and Australia) and pig type (introduced, domestic, or wild boar). We examined 403 base pairs (bp) in the analysis; usable bp were those having less than 5% missing data within groups. Numbers in parentheses below gene and nucleotide diversity figures are standard deviations of the estimate.

Molecular characteristic	N.A. introduced	Eastern domestic	Eastern wild boar	Western domestic	Western wild boar	Australia introduced
No. individual sequences	80	161	101	334	253	18
No. haplotypes	13	29	46	42	38	5
No. usable bp	398	381	381	380	381	393
No. polymorphic sites	20	36	48	37	39	16
Gene diversity	0.825 (±0.023)	0.903 (±0.010)	0.969 (±0.007)	0.897 (±0.011)	0.926 (±0.007)	0.549 (±0.127)
Nucleotide diversity	0.012 (±0.007)	0.007 (±0.004)	0.016 (±0.009)	0.017 (±0.009)	0.014 (±0.007)	0.006 (±0.004)

Table 2. Genetic distance measures for 6 arbitrarily assigned groupings of swine based on geographic location (North America [N.A], eastern or western Eurasia, and Australia) and pig type (introduced, domestic, or wild boar). Average pairwise nucleotide difference within and between populations are provided on and above the diagonal, respectively. Population pairwise F_{st} values are below the diagonal. Asterisks indicate significance ($P < 0.01$) from permutation tests, excluding figures on the diagonal.

Group	N.A. introduced	Eastern domestic	Eastern wild boar	Western domestic	Western wild boar	Australia introduced
N.A. introduced	4.899	11.118*	11.293*	5.784	5.132	10.561*
Eastern domestic	0.691*	2.536	4.977*	10.725*	12.410*	2.693
Eastern wild boar	0.507*	0.140*	6.151	11.157*	12.458*	4.859
Western domestic	0.020*	0.548*	0.434*	6.408	6.153	10.126*
Western wild boar	0.045*	0.671*	0.552*	0.053*	5.235	11.517*
Australia introduced	0.612*	0.119*	0.099*	0.480*	0.606*	2.157

domestic pig breeds have arisen independently in both world-geographic regions (Wu et al. 2007, Larson et al. 2010, Luetkemeier et al. 2010) and that European and Asian cross-breeding has occurred since the late 1700s, with a predominance of Asian mtDNA introgression into Europe (Darwin 1868, Kim et al. 2002, Fang and Andersson 2006). This history has direct implications for mtDNA diversity of wild pigs in the United States, because select haplotypes (e.g., h2, h7, h13, h17, h19, h37, h38) of Asian and European phylogeographic origins were apparently propagated in domestic breeds and distributed globally by European exploration, colonization, and trade (Gongora et al. 2004, Larson et al. 2005; Appendix A). This assertion is supported by the abundance of these select haplotypes in our dataset, their relationship with both domestic pigs and Eurasian wild boar, and the large number of closely related haplotypes that have diverged from these sources (Fig. 4).

Given prior evidence of Asian mtDNA introgression into European breeds, the high level of nucleotide diversity observed for our Western domestic pig grouping is logical (Fang and Andersson 2006; Table 1). The relatively high diversity of our North American sample follows, considering its similarity to Western pig groupings and the fact that most domestic lines historically propagated in the United States were of European origin (Table 3; Jones 1998). However, some caution should be exercised when evaluating gene and nucleotide diversity of artificial populations, as both

measures are affected by the composition of assigned groups (i.e., group size and abundance of common haplotypes), our efforts to include widely divergent sequences in the dataset, and sparse sampling among geographic locations. Regardless, raw comparisons of haplotypes shared between regions generally support extensive anthropogenic dispersal of pig breeds carrying both European and Asian mtDNA lineages (Table 3). However, Asian mtDNA transfer via European stock cannot be differentiated from direct introductions of Asian domestic breeds to wild-living populations of swine in the United States and elsewhere (Gongora et al. 2004).

National Relationships Relevant to Management

Though multiple breed associations for haplotypes prevent effective identification of specific sources for introductions, useful insights on invasion processes can be gained by evaluating haplotype distributions. The high frequency and wide distribution of haplotypes h17, h19, and h37 suggest that these maternal lineages have been present in wild pig populations in North America for a long period of time and that historical populations are playing an important role in recent range expansion in midwestern and northern States (Fig. 5). Similar patterns for h7, h20, and h39 in both southern and northern states may indicate dispersal of animals from long-established populations in the southern tier of the continent (Fig. 5; Appendix A). Alternately, the presence of h2 only in recently invaded areas (i.e., Michigan

Table 3. Shared mtDNA haplotypes between Hawaii and 6 arbitrarily assigned groupings of swine based on geographic location (North America [N.A], eastern or western Eurasia, and Australia) and pig type (introduced, domestic, or wild boar). Number of shared haplotypes are on the top diagonal matrix, and a visual aid with size of darkened circles proportional to number of shared haplotypes is on the bottom diagonal matrix. Published sequences from feral animals in Papua-New Guinea, the Mariana Islands, and Vanuatu shared haplotypes only with Hawaii and are not represented.

Group	N.A. introduced	Eastern domestic	Eastern wild boar	Western domestic	Western wild boar	Australia introduced	Hawaii introduced
N.A. introduced		3	2	9	5	1	1
Eastern domestic	●		7	7	4	2	1
Eastern wild boar	●	●		6	3	2	1
Western domestic	●	●	●		11	3	2
Western wild boar	●	●	●	●		1	2
Australia introduced	●	●	●	●	●		1
Hawaii introduced	●	●	●	●	●	●	
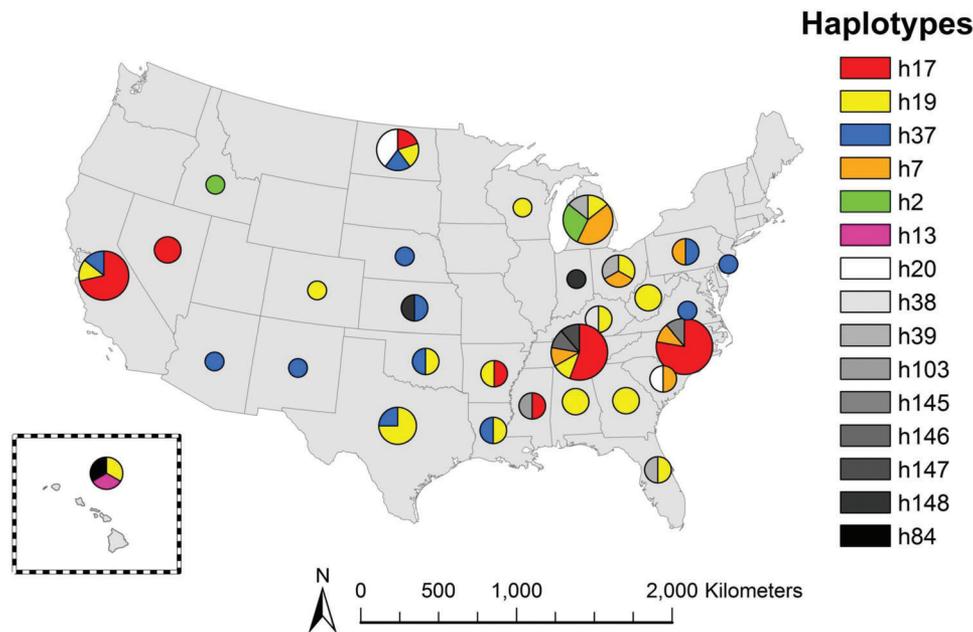
Figure 5. Geographic distribution and haplotype richness within states for 15 mitochondrial DNA haplotypes identified from introduced pigs in the United States.

and Idaho) suggests that this lineage represents a new wave of introductions (Fig. 5). Therefore, we infer that wild pig range expansion in North America is a product of both anthropogenic dispersal of animals from existing populations and introduction of swine from new genetic sources.

Unique haplotypes 145–148 present an interesting challenge for evaluating their possible origins. We propose several plausible explanations for the presence of these haplotypes in the United States and their absence from published sequence databases: 1) unique haplotypes could represent pigs from early introductions whose lineages are no longer propagated in modern domestic breeds, 2) they might represent Eurasian wild boar from geographic areas not included in public sequence databases, and 3) they may be the product of divergence from common haplotypes subsequent to introduction. However, network relationships generally support their divergence from common haplotypes (Fig. 4), and close geographic proximity (e.g., h17 with h147 and h7 with h145 and h146) indicates that spatial context necessary for divergence has occurred (Fig. 5). Therefore, genetic mutations may be contributing to the mtDNA diversity of wild pigs in North America.

In Hawaii, feral pigs have a long history of establishment, and successive waves of introduction are apparent from molecular relationships. For instance, haplotypes h13 and h19 probably represent swine introduced after European settlement because of their association with many modern domestic breeds (Appendix A). The third mtDNA haplotype identified on the islands (h84) likely represents the earliest introductions to Hawaii because of its exclusive occurrence among wild-living pigs on other South Pacific islands with human populations arising from Polynesian dispersal (Allen et al. 2001, Larson et al. 2005; Appendix A).

The positive relationship between sample size within areas and haplotypes detected in our study suggests that additional sampling would elucidate even more genetic variation among wild pigs in the United States. An expanded sampling regime might also provide insights as to the broader geographic distribution of unique haplotypes and their relevance in the history of pig invasion. Although mtDNA has provided useful insights toward the objectives of this study, mitochondrial lineages will be of limited utility for tracking gene flow. Ongoing research utilizing multilocus nuclear DNA markers or genes linked to morphological traits will be necessary to provide additional insights on the origins and dispersal pathways of invasive pigs in the United States (Hampton et al. 2004, Spencer and Hampton 2005, Koutsogiannouli et al. 2010, Scandura et al. 2011, McCann 2012).

MANAGEMENT IMPLICATIONS

Our identification of new genetic sources for expanding wild pig populations should be of particular concern to managers, because ongoing exchange of animals between domestic and wild environs has implications for disease transmission. Further, new genetic stock will augment existing populations, potentially resulting in some level of hybrid vigor that benefits survival, exacerbating difficulties in controlling introduced pigs. The ongoing translocation of animals between geographic areas will have similar implications, where distantly related herds are mixed. Finally, global mtDNA relationships provide some unique insights for evaluating the ancestry of animals reported to be Eurasian wild boar, which are highly sought after for hunting purposes. Where pure Eurasian wild boar are sampled, the pure status of pigs may be refuted if they possess mtDNA haplotypes of Eastern phylogeographic origin, as we could

find no record of Eurasian wild boar from central or eastern Eurasia ever being introduced to North America (Mayer and Brisbin 1991).

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LITERATURE CITED

Allen, M. S., E. Matisoo-Smith, and A. Horsburgh. 2001. Pacific 'Babes': issues in the origins and dispersal of Pacific pigs and the potential of mitochondrial DNA analysis. International Journal of Osteoarchaeology 11:4–13.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403–410.

Alves, E., C. Ovilo, M. C. Rodriguez, and L. Silio. 2003. Mitochondrial DNA sequence variation and phylogenetic relationships among Iberian pigs and other domestic and wild pig populations. Animal Genetics 34:319–324.

Archibald, A. L., L. Bolund, C. Churcher, M. Fredholm, M. A. M. Groenen, B. Harlizius, K.-T. Lee, D. Milan, J. Rogers, M. F. Rothschild, H. Uenishi, J. Wang, and L. B. Schook, The Swine Genome Consortium. 2010. Pig genome sequence-analysis and publication strategy. BMC Genomics 11:438.

Bensasson, D., M. W. Feldman, and D. A. Petrov. 2003. Rates of DNA duplication and mitochondrial DNA insertion in the human genome. Journal of Molecular Evolution 57:343–354.

Clement, M., D. Posada, and K. S. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657–1660.

Darwin, C. 1868 The variation of animals and plants under domestication. John Murry, London, United Kingdom.

Evans, J. D., J. S. Pettis, W. M. Hood, and H. Shimanuki. 2003. Tracking an invasive honey bee pest: mitochondrial DNA variation in North American small hive beetles. Apidologie 34:103–109.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47–50.

Fang, M., and L. Andersson. 2006. Mitochondrial diversity in European and Chinese pigs is consistent with population expansions that occurred prior to domestication. Proceedings of the Royal Society 273:1803–1810.

Fang, M., F. Berg, A. Ducos, and L. Andersson. 2006. Mitochondrial haplotypes of European wild boars with 2n = 36 are closely related to those of European domestic pigs with 2n = 38. Animal Genetics 37:459–464.

Gipson, P. S., B. Hlavachick, and T. Berger. 1998. Range expansion by wild hogs across the central United States. Wildlife Society Bulletin 26:274–278.

Giuffra, E., J. M. H. Kijas, V. Amarger, O. Carlborg, J.-T. Jeon, and L. Andersson. 2000. The origin of the domestic pig: Independent domestication and subsequent introgression. Genetics 154:1785–1791.

Goios, A., A. Carvalho, and A. Amorim. 2009. Identifying NUMT contamination in mtDNA analyses. Forensic Science International: Genetics Supplement Series 2:278–280.

Gongora, J., O. Peltoniemi, I. Tammen, H. Raadsma, and C. Moran. 2003. Analyses of possible domestic pig contribution in two populations of Finnish farmed wild boar. Acta Agriculturae Scandinavica, Section A. Animal Sciences 53:161–165.

Gongora, J., P. Fleming, P. B. S. Spencer, R. Mason, O. Garkavenko, J.-N. Meyer, C. Droegemueller, J. H. Lee, and C. Moran. 2004. Phylogenetic relationships of Australian and New Zealand feral pigs assessed by mitochondrial control region sequence and nuclear GPIP genotype. Molecular Phylogenetics and Evolution 33:339–348.

Groves, C. P., and P. Grubb. 1993. The Eurasian suids *Sus* and *Babyrousa*. Pages 167–175 *in* W. L. R., Oliver, editor. Pigs, peccaries, and hippos: status survey and conservation action plan of the IUCN. World Conservation Union, Gland, Switzerland.

Guindon, S., and O. Gascuel. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Systematic Biology 52:696–704.

Hall, T. 2001. Bioedit version 5.0.6. Department of Microbiology, North Carolina, State University. <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>. Accessed Nov 1, 2010.

Hampton, J. O., P. B. S. Spencer, D. L. Alpers, L. E. Twigg, A. P. Woolnough, J. Doust, T. Higgs, and J. Pluske. 2004. Molecular techniques, wildlife management and the importance of genetic population structure and dispersal: a case study with feral pigs. Journal of Applied Ecology 41:735–743.

Helgen, K. M., J. E. Maldonado, D. E. Wilson, and S. D. Buckner. 2008. Molecular confirmation of the origin and invasive status of West Indian raccoons. Journal of Mammalogy 89:282–291.

Ishiguro, N., M. Sasaki, M. Iwasa, N. Shigehara, H. Hongo, T. Anezaki, V. T. Long, D. T. B. Lan, and P. T. Long. 2008. mtDNA variation in Vietnamese pigs, with particular emphasis on the genetic relationship between wild boars from Vietnam and the Ryukyu Islands. Mammal Study 33:51–58.

Johnson, J. A., and P. O. Dunn. 2006. Low genetic variation in the Heath Hen prior to extinction and implications for the conservation of prairie-chicken populations. Conservation Genetics 7:37–48.

Jones, G. F. 1998. Genetic aspects of domestication, common breeds and their origin. Pages 17–50 *in* M. F., Rothschild, and A., Ruvinsky, editors. The genetics of the pig. CAB International, Wallingford, Oxfordshire, United Kingdom.

Kijas, J. M., and L. Andersson. 2001. A phylogenetic study of the origin of the domestic pig estimated from the near-complete mtDNA genome. Journal of Molecular Evolution 52:302–308.

Kim, K.-I., J.-H. Lee, K. Li, Y.-P. Zhang, S.-S. Lee, J. Gongora, and C. Moran. 2002. Phylogenetic relationships of Asian and European pig breeds determined by mitochondrial DNA D-loop sequence polymorphism. Animal Genetics 33:19–25.

Kolar, C. S., and D. M. Lodge. 2001. Progress in invasion biology predicting invaders. Trends in Ecology and Evolution 16:199–204.

Koutsogiannouli, E. A., K. A. Moutou, T. Sarafidou, C. Stamatis, and Z. Mamuris. 2010. Detection of hybrids between wild boars (*Sus scrofa scrofa*) and domestic pigs (*Sus scrofa f. domestica*) in Greece, using the PCR-RFLP method on melanocortin-1 receptor (MC1R) mutations. Mammalian Biology 75:69–73.

Larson, G., U. Albarella, K. Dobney, P. Rowley-Conwy, J. Schibler, A. Tresset, J. D. Vigne, C. J. Edwards, A. Schlumbaum, A. Dinu, A. Balacsescu, G. Dolman, A. Tagliacozzo, N. Manaseryan, P. Miracle, L. Van Wijngaarden-Bakker, M. Masseti, D. G. Bradley, and A. Cooper. 2007. Ancient DNA, pig domestication, and the spread of the Neolithic into Europe. Proceedings of the National Academy of Science 104:15276–15281.

Larson, G., K. Dobney, U. Albarella, M. Fang, E. Matsoo-Smith, J. Robins, S. Lowden, H. Finlayson, T. Brand, E. Willerslev, P. Rowley-Conwy, L. Andersson, and A. Cooper. 2005. Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. Science 307:1618–1621.

Larson, G., R. Liu, X. Zhao, J. Yuan, D. Fuller, L. Barton, K. Dobney, Q. Fan, Z. Gu, X.-H. Liu, Y. Luo, P. Lv, L. Andersson, and N. Li. 2010. Patterns of East Asian pig domestication, migration, and turnover revealed by modern and ancient DNA. Proceedings of the National Academy of Science 107:7686–7691.

Lin, C.-S., Y.-L. Sun, C.-Y. Liu, P.-C. Yang, L.-C. Chang, I.-C. Cheng, S. J. T. Mao, and M.-C. Huang. 1999. Complete nucleotide sequence of pig (Sus scrofa) mitochondrial genome and dating evolutionary divergence within Artiodactyla. Genetics 236:107–114.

Loggins, R. E. 2007. Historic origin, range expansion, and habitat suitability of introduced wild pigs (Sus scrofa) in California. Dissertation, University of North Dakota, Grand Forks, North Dakota, USA.

Lopez, J. V., N. Yuhki, R. Masuda, W. Modi, and S. J. Obrien. 1994. Numt, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. Journal of Molecular Evolution 39:174–190.

Lucchini, V., E. Meijaard, C. H. Diong, C. P. Groves, and E. Randi. 2005. New phylogenetic perspectives among species of South-east Asian wild pig (Sus sp) based on mtDNA sequences and morphometric data. Journal of the Zoological Society of London 266:25–35.

Luetkemeier, E. S., M. Sodhi, L. B. Schook, and R. S. Malhi. 2010. Multiple Asian pig origins revealed through genomic analyses. Moleclar Phylogenetics and Evolution 54:680–686.

Mayer, J. J., and I. L. Brisbin. 1991. Wild pigs in the United States: their history, comparative morphology, and current status. University of Georgia Press, Athens, Georgia, USA.

Mayer, J. J., and I. L. Brisbin, Jr. 2009. Wild pigs: biology, damage, control techniques and management. Savannah River National Laboratory, Aiken, South Carolina, USA.

McCann, B. E. 2012. Genetic relationships of wild pigs (Sus scrofa) in the United States: geographic origins and genotypic distribution of the species with implications for management. Dissertation, University of North Dakota, Grand Forks, North Dakota, USA.

Mona, S., E. Randi, and M. Tommaseo-Ponzetta. 2007. Evolutionary history of the genus Sus inferred from cytochrome b sequences. Molecular Phylogenetics and Evolution 45:757–762.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, New York, USA.

Nilsson, R. H., M. Ryberg, E. Kristiansson, K. Abarenkov, K.-H. Larsson, and U. Koljalg. 2006. Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. PLoS ONE 1(1), e59. DOI: 10.1371/journal.pone.000059

Okumura, N., N. Ishiguro, M. Nakano, K. Hirai, A. Matsui, and M. Sahara. 1996. Geographic population structure and sequence divergence in the mitochondrial control region of the Japanese wild boar (Sus scrofa leucomystax), with reference to those of domestic pigs. Biochemical Genetics 34:179–189.

Oliver, W. L. R., and I. L. Brisbin, Jr. 1993. Introduced and feral pigs: problems, policy, and priorities. Pages 175–189 in and W. L. R., Oliver, editor. Pigs, peccaries, and hippos: status survey and conservation action plan of the IUCN. World Conservation Union, Gland, Switzerland.

Parr, R. L., J. Maki, B. Reguly, G. D. Dakubo, A. Aguirre, R. Wittock, K. Robinson, J. P. Jakupciak, and R. E. Thayer. 2006. The pseudo-mitochondrial genome influences mistakes in heteroplasmy interpretation. BMC Genomics 7:185.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52:273–288.

Posada, D. 2008. jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25:1253–1256.

Ramayo, Y., I. N. Shemeret'eva, and M. Perez-Enciso. 2011. Mitochondrial DNA diversity in wild boar from the Primorsky Krai Region (East Russia). Animal Genetics 42:96–99.

Randi, E., J. P. d'Huart, V. Lucchini, and R. Aman. 2002. Evidence of two genetically deeply divergent species of warthog Phacochoerus africanus and P. aethiopicus (Artiodactyla: Suiformes) in East Africa. Mammalian Biology 67:91–96.

Randi, E., V. Lucchini, and C. H. Diong. 1996. Evolutionary genetics of the suiformes as reconstructed using mtDNA sequencing. Journal of Mammalian Evolution 3:163–193.

Robertson, B. C., and N. J. Gemmell. 2004. Defining eradication units to control invasive pests. Journal of Applied Ecology 6:1042–1048.

Ronquist, F., and J. P. Huelsenbeck. 2003. MRBAYES3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Ross, K. G., and D. D. Shoemaker. 2008. Estimation of the number of founders of an invasive pest insect population: the fire ant Solenopsis invicta in the USA. Proceedings of the Royal Society of Britain 275:2231–2240.

Scandura, M., L. Iacolina, and M. Apollonio. 2011. Genetic diversity in the European wild boar Sus scrofa: phylogeography, population structure and wild x domestic hybridization. Mammal Review 41:125–137.

Scandura, M., L. Iacolina, G. Crestanello, E. Pecchili, M. F. Di Benedetto, V. Russo, R. Davoli, M. Apollonio, and G. Bertorelle. 2008. Ancient vs. recent processes as factors shaping the genetic variation of the European wild boar: are the effects of the last glaciation still detectable? Molecular Ecology 17:1745–1762.

Simmons, R. B., and S. J. Scheffer. 2004. Evidence of cryptic species within the pest Copitarsia decolora (Guenée) (Lepidoptera: Noctuidae). Annals of the Entomological Society of America 97:675–680.

Southeastern Cooperative Wildlife Disease Study. 2010. 2010 National Feral Swine Mapping System. Southeastern Cooperative Wildlife Disease, Study, University of Georgia <www.feralswinemap.org>. Accessed 1 Sep 2010.

Spencer, P. B. S., and J. O. Hampton. 2005. Illegal translocation and genetic structure of feral pigs in Western Australia. Journal of Wildlife Management 69:377–384.

Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics 105:437–460.

Tanaka, K., Y. Iwaki, T. Takizawa, G. Dorji, G. Tshering, Y. Kurosawa, Y. Maeda, H. Mannen, K. Nomura, V. B. Dang, L. Chhum-Phith, B. Bouahom, Y. Yamamoto, T. Daing, and T. Namikawa. 2008. Mitochondrial diversity of native pigs in the mainland South and South-east Asian countries and its relationships between local wild boars. Animal Science Journal 79:417–434.

Tooman, L. K., C. J. Rose, C. Carraher, D. M. Suckling, S. R. Paquette, L. A. Ledezma, T. M. Gilligan, M. Epstein, N. B. Barr, and R. D. Newcomb. 2011. Patterns of mitochondrial haplotype diversity in the invasive pest Epiphyas postvittana (Lepidoptera: Tortricidae). Journal of Economic Entomology 104:920–932.

Towne, C. W., and E. N. Wentworth. 1950. Pigs: from cave to cornbelt. University of Oklahoma Press, Norman, Oklahoma, USA.

Ursing, B. M., and U. Arnason. 1998. The complete mitochondrial DNA sequence of the pig (Sus scrofa). Journal of Molecular Evolution 47:302–306.

Vigne, J.-D., A. Zazzo, J.-F. Saliege, F. Poplin, J. Guilaine, and A. Simmons. 2009. Pre-Neolithic wild boar management and introduction to Cyprus more than 11,400 years ago. Proceedings of the National Academy of Science 106:16135–16138.

Waithman, J. D., R. A. Sweitzer, D. Van Vuren, J. D. Drew, A. J. Brinkhaus, I. A. Gardner, and W. M. Boyce. 1999. Range expansion, population sizes, and management of wild pigs in California. Journal of Wildlife Management 63:298–308.

Watanobe, T., N. Ishiguro, N. Okumura, M. Nakano, A. Matsui, H. Hongo, and H. Ushiro. 2001. Ancient mitochondrial DNA reveals the origin of Sus scrofa from Rebun Island, Japan. Journal of Molecular Evolution 52:281–289.

Wesche, P. L., D. J. Gaffney, and P. D. Keightley. 2004. DNA sequence error rates in Genbank records estimated using the mouse genome as a reference. DNA Sequence 15:362–364.

Wright, L. I., T. Tregenza, and D. J. Hosken. 2008. Inbreeding, inbreeding depression, and extinction. Conservation Genetics 9:833–843.

Wu, G. S., Y. G. Yao, K. X. Qu, Z. L. Ding, H. Li, M. G. Palanichamy, Z. Y. Duan, N. Li, Y. S. Chen, and Y. P. Zhang. 2007. Population phylogenomic analysis of mitochondrial DNA in wild boars and domestic pigs revealed multiple domestication events in East Asia. Genome Biology 8(11), R245.

Yang, J., J. Wang, J. Kijas, B. Liu, H. Han, M. Yu, H. Yang, S. Zhao, and K. Li. 2003. Genetic diversity present within the near-complete mtDNA genome of 17 breeds of indigenous Chinese pigs. Journal of Heredity 94:381–385.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Appendix A. Global and national relationships for 15 mitochondrial DNA haplotypes representing introduced pigs in the United States (U.S.). The geographic distribution and frequency of haplotypes found among 81 introduced pigs in United States are compared to published sequence from around the world ($n = 483$) obtained from National Center for Biotechnology Information (http://www. ncbi.nlm.nih.gov/). We inferred phylogeographic relationships for haplotypes through Bayesian analysis of sequences from Eurasian wild boar with known collection sites. Geographic origin of sequence and type of pigs represented are denoted for each haplotype; GRSM, Great Smoky Mountains National Park; EWB, Eurasian wild boar. Note: the 81 U.S. pigs analyzed during the current study are represented (No. U.S.) with 1 published sequence (h13 Hawaii).

Haplotype	Phylogeographic origin	No. domestic	No. EWB	No. feral	No. other	No. U.S.	Total	National distribution	Global distribution	Domestic breed associations
2[a]	Asian	40	3	12	3	3	61	ID, MI	Australia, China, France, Germany, Japan, Sweden, Taiwan, Thailand, UK	>20 Asian and European domestic breeds
7[b,c]	Asian	42	9	1	1	8	61	MI, NC, OH, PA, SC, TN	Belgium, China, France, Italy, Japan, Malaysia, Mariana Islands, South Carolina USA, Russia, Spain, Thailand, UK	~20 Asian and European breeds
13[d]	Asian	64	5	3	3	1	76	HI	China, UK, Korea, Germany, Spain, Japan, Hawaii USA, Australia, Thailand, Italy	~16 Asian and European breeds
17[b]	European	25	12			22	59	AR, CA, MS, NC, ND, NV, TN,	Belgium, Germany, Iceland, Italy, Macedonia, Norway, Spain, UK	12 European domestic breeds
19[b]	European	91	32		8	22	153	AL, AR, CA, CO, FL, GA, HI, KY, LA, MI, ND, OH, OK, TN, TX, WI, WV	Corsica, Finland, France, Germany, Hungary, Holland, Italy, Portugal, Sweden, UK	>20 European domestic breeds
20[b]	European	10				3	13	ND, SC	UK	Duroc, Large White, Tamworth
37[b]	European	22	19		5	12	58	AZ, CA, KS, LA, ND, NJ, NE, NM, OK, PA, TX, VA	Austria, Bulgaria, Germany, Hungary, Indonesia, Italy, Korea, Morocco, Poland, Portugal, Spain, UK	12 European domestic breeds, 1 Asian type "Korean Native"
38	European	24	34		2	1	61	KY	Austria, Corsica, Denmark, France, Germany, Hungary, Italy, Portugal, Sardinia, UK	Bisaro, Duroc, Gloucester Old Spot, Hampshire, Hungarian Mangalica, Iberian Red, Landrace,
39[b]	European	7				3	10	FL, MI, OH	Germany, Sweden	Angler sattle-schwein, German Angler, Hampshire
84	Unknown			3		1	4	HI	Papua-New Guinea, Vanuatu	Feral animals and *S. verrucosus*
103	European	2				1	3	MS	Europe	Landrace
145[e]	Asian					1	1	NC	None	Unique haplotype
146[e]	European					1	1	TN	None	Unique haplotype
147[e]	European					1	1	TN	None	Unique haplotype
148[a,e]	European					2	2	IN, KS	None	Unique haplotype

[a] Found only in recently occupied U.S. states (i.e., those invaded during the last 30 years).
[b] Distributed between historical and recently occupied states.
[c] Haplotype reported for published sequence of "Ossabaw Hog" breed from South Carolina, USA (AY884715; Larson et al. 2005); reference material with this published sequence did not specify domestic or feral origins.
[d] Haplotype reported only for published sequence of a feral pig from Hawaii, USA (AY884613; Larson et al. 2005).
[e] Unique haplotype with no exact matches to published sequence.