Biol Invasions (2013) 15:1201–1207
DOI 10.1007/s10530-012-0375-x

INVASION NOTE

Narrow phylogeographic origin of five introduced populations of the Siberian chipmunk *Tamias* (*Eutamias*) *sibiricus* (Laxmann, 1769) (Rodentia: Sciuridae) established in France

B. Pisanu · E. V. Obolenskaya · E. Baudry · A. A. Lissovsky · J.-L. Chapuis

Received: 14 December 2011 / Accepted: 20 November 2012 / Published online: 29 November 2012

Abstract Large native geographical range and number of introduction events are often invoked to explain the successful establishment of alien mammal species. To infer the native geographic range of the Siberian chipmunks *Tamias* (*Eutamias*) *sibiricus* (Lax.) invasion in France, we identified the subspecies that individuals sampled in 5 out of the 11 established populations belonged. Based on craniometrical measures, variation in the mtDNA sequence of the cytochrome b, and alarm call sonogram shape, all French specimens were members of *T.* (*E.*) *s. barberi* which has the smallest geographical range (Korean peninsula) of the three recognized subspecies. An intense pet trade between European countries and Korea until the 1980's best explains the success in establishment of this small ground-squirrel in France, where it was released mainly by private owners. Size of the native geographical range should be interpreted with caution in explaining the establishment of an invasive species, at least without a precise knowledge of its introduction pathways and taxonomic status.

Keywords Siberian chipmunk · *Tamias* (*Eutamias*) *sibiricus* · Introduced species · Invasive pet · Phylogeography · Species range · Taxonomy

B. Pisanu · J.-L. Chapuis (✉)
Département Ecologie et Gestion de la Biodiversité, Muséum National d'Histoire Naturelle, UMR 7204 CERSP, 61 rue Buffon, CP 53, 75231 Paris Cedex 05, France
e-mail: chapuis@mnhn.fr

E. V. Obolenskaya · A. A. Lissovsky
Zoological Museum of Moscow State University, Moscow, Russia

E. Baudry
University of Paris 11, ESE, Paris, France

Introduction

For invasive vertebrate species, recent quantitative studies presenting comparisons between successful and unsuccessful introductions of non-indigenous species allowed the recognition of major factors associated with the early invasion process (Kolar and Lodge 2001; Duncan et al. 2003). For non-indigenous mammals introduced to mainland Australia, such analyses identified two factors explaining the invasiveness of non-indigenous species: (1) a high number of introduction events and (2) a large overseas range (Forsyth et al. 2004).

The Siberian chipmunk *Tamias* (*Eutamias*) *sibiricus* (Laxmann, 1769) is naturalized in western European countries, and 11 of the 22 surveyed populations occur in France (Chapuis 2005; Chapuis et al. 2011). This species was originally imported from eastern Asia to be sold in European pet shops in the late 1960s, and most introduction events began with releases by owners in the 1970s. To a lesser extent, these introductions also resulted from escapes from pet farms or zoos (Chapuis et al. 2011). The Siberian chipmunk has been recognised as an invasive species of concern (Chapuis 2009) because it can act as a reservoir for agents of the Lyme borreliosis *Borrelia burgdorferi* sensu lato (Vourc'h et al. 2007; Marsot et al. 2011). More specifically, these introduced chipmunks show a greater susceptibility to infestation by local hard ticks, *Ixodes ricinus,* than the indigenous bank voles *Myodes glareolus* (Pisanu et al. 2010) which is one of the best-known native rodent reservoirs for Lyme disease in western European (Vourc'h et al. 2007). This finding highlights the potential for re-transmission of vector borne disease by introduced chipmunks to the native host community, thereby locally increasing the risk of transmission to humans (Marsot et al. 2011).

A recent review supports to the existence of three sub-species of Palaearctic chipmunks *Tamias* (*Eutamias*) *sibiricus* (Laxmann, 1769) (Obolenskaya et al. 2009). One of these sub-species, *T.* (*E.*) *s. sibiricus* (Lax.), has a broad range covering approximately half of the northern part of the Eurasiatic continent (i.e., 40°–60°N, 40°–160°E), including Russia, extreme northeast of the Korean peninsula, Mongolia, Japan, and northeast China. The two other taxa have much smaller ranges (i.e., 20°–40°N, 100°–140°E): *T.* (*E.*) *s. senescens* (Miller, 1898) inhabiting only central China to the south Liaoning province, and *T.* (*E.*) *s. barberi* (Johnson and Jones, 1955) the Korean peninsula. Moreover, recent re-evaluations of the current taxonomy within Palaearctic chipmunks strongly suggest that *T.* (*E.*) *s. barberi* may be soon elevated to the species level (Lee et al. 2008; Koh et al. 2009, 2010; Obolenskaya et al. 2009).

In this paper, our main aim was to identify the sub-species of Siberian chipmunks established in France. We wanted to confirm that these squirrels originated from Korea (Chapuis et al. 2011), a small subset of the native range of this species, which would indicate that size of native range had only a minor role in explaining their successful establishment of this species, compared to the trade of this species increasing the probability of numerous introduction events.

Materials and method

Study species and populations

The Siberian chipmunk is a diurnal ground squirrel, with solitary (Kawamichi et al. 1987; Kawamichi 1999) and sedentary habits (Marmet et al. 2009). In its native range, northern populations of *T.* (*E*) *s. sibiricus* occupy various habitats, including coniferous and deciduous forests, and even sandy or rocky soils which can be covered by shrubs or tall herbs (Obolenskaya 2008). Adults are not sex-dimorphic (Obolenskaya et al. 2009), and weigh on average 100 g (Chapuis et al. 2011). This species reproduces and hibernates in a burrow (Kawamichi 1989, 1996; Chapuis et al. 2011). In Russia and Japan, breeding takes place once a year depending on the duration of snow cover (Kawamichi and Kawamichi 1993), and two times per year in France (Marmet 2008; Chapuis et al. 2011).

In France, 8 of the 11 surveyed populations are localised in the Ile-de-France region, within a 30 km radius around Paris (Fig. 1). These populations are found in suburban deciduous forests or urban parks (Table 1; reviewed in Chapuis et al. 2011). The 3 other populations are found in deciduous forests, two approximately 40 km north of Paris, and one in the Baie de Somme approximately 120 km north.

Biometrics

Seventeen measurements were taken (accuracy 0.1 mm; see Obolenskaya et al. 2009) for each of 17 adult chipmunk skulls collected by trapping in two French localities (Fig. 1; Table 1): the Bois de Verneuil-sur-Seine in 2007 (n = 7), and the Forêt de Sénart between 2004 and 2008 (n = 10). These individuals were compared to a database of the same measurements from 29 individuals collected in 4 localities in central and southern China corresponding to *T.* (*E.*) *s. senescens*, 12 individuals collected in 3 localities in South Korea corresponding to *T.* (*E.*) *s. barberi*, and 664 individuals from 216 localities

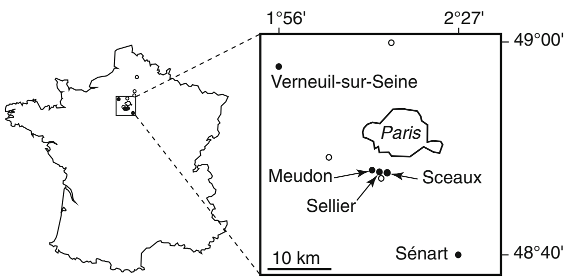


Fig. 1 Distribution of the populations of Siberian chipmunks near Paris (Ile-de-France region). Sampled populations with *filled circles*

Table 1 Characteristics of the five populations of Siberian chipmunks sampled in Ile-de-France (Taken from Chapuis et al. 2011)

Populations	Localisation	Area (ha)	Abundance
Forêt de Sénart	48°40′N, 2°27′E	3,200	<20,000
Forêt de Meudon	48°47′N, 2°14′E	1,100	<1,000
Bois de Verneuil-sur-Seine	48°59′N, 1°56′E	230	<1,000
Parc Henry Sellier	48°46′N, 2°15′E	26	<100
Parc de Sceaux	48°46′N, 2°18′E	180	<50

from Russia, Kazakhstan, Japan, Mongolia, northeastern China and Korea corresponding to *T.* (*E.*) *s. sibiricus* (Obolenskaya et al. 2009). Cluster analysis was performed on the basis of Mahalonobis distances using the unweighted pair group method with arithmetic mean, and biases due to age, sex or variation in sample size were checked (Obolenskaya et al. 2009). Data were processed using standard algorithms implemented in STATISTICA 8.0 (statsoft.com) and several customs written by one of us (A. A. L.) under Statistica Visual Basics.

DNA extraction, amplification and sequencing

Ear punch samples were obtained from 10 individuals in each of four populations, Verneuil-sur-Seine, Henri Sellier, Sceaux and Sénart (Fig. 1; Table 1). DNA was extracted from the ear punches with a Macherey–Nagel Nucleospin tissue kit, following manufacturer protocol. Extracted DNA was resuspended in 100 ml elution buffer. Mitochondrial DNA variation was assayed by the amplification of a fragment of the cytochrome B (cytB) gene. The fragment was amplified by PCR using the primers CytB376-F ACAGC ATTTATAGGCTATGT, designed using a published cytochrome b sequence of *T. sibiricus* (GenBank Accession no. EU050999.1) and H15906 GGTTTACAAGACCAGAGTAAT (Zheng et al. 2003). Each PCR reaction was run in a 20 µl volume containing 1 µl of DNA solution, 400 µM of each dNTP, 1.75 µM of Mg^{++}, 1 µM of each primer and 1.25 unit of Taq polymerase. Thermocycle conditions were 94 °C for 30 s, 48 °C for 30 s, and 72 °C for one min, for a total of 35 cycles. Purified template DNA was sequenced on both strands with the PCR primers, using standard sequencing techniques. We thus obtained sequences of a 758 bp cytB fragment for 40 French Siberian chipmunks. Phylogenetic reconstructions were performed using the maximum likelihood (ML) algorithm. The mtDNA of French specimens were compare to a set of 36 sampled individuals from South Korea, and 80 individuals from various part of Russia, one from Japan (Obihiro, Hokkaido), nine from northern China (Heilongjiang province; Mandchuria: see Obolenskaya et al. 2009), and one with unknown origin from a Taiwanese petshop (Obolenskaya et al. 2009; Lee et al. 2008). Only one sequence from each set of identical sequences was retained in the analysis. All calculations were performed using Treefinder (Jobb 2008). Bootstrap support values were calculated on the basis of 500 data replicates.

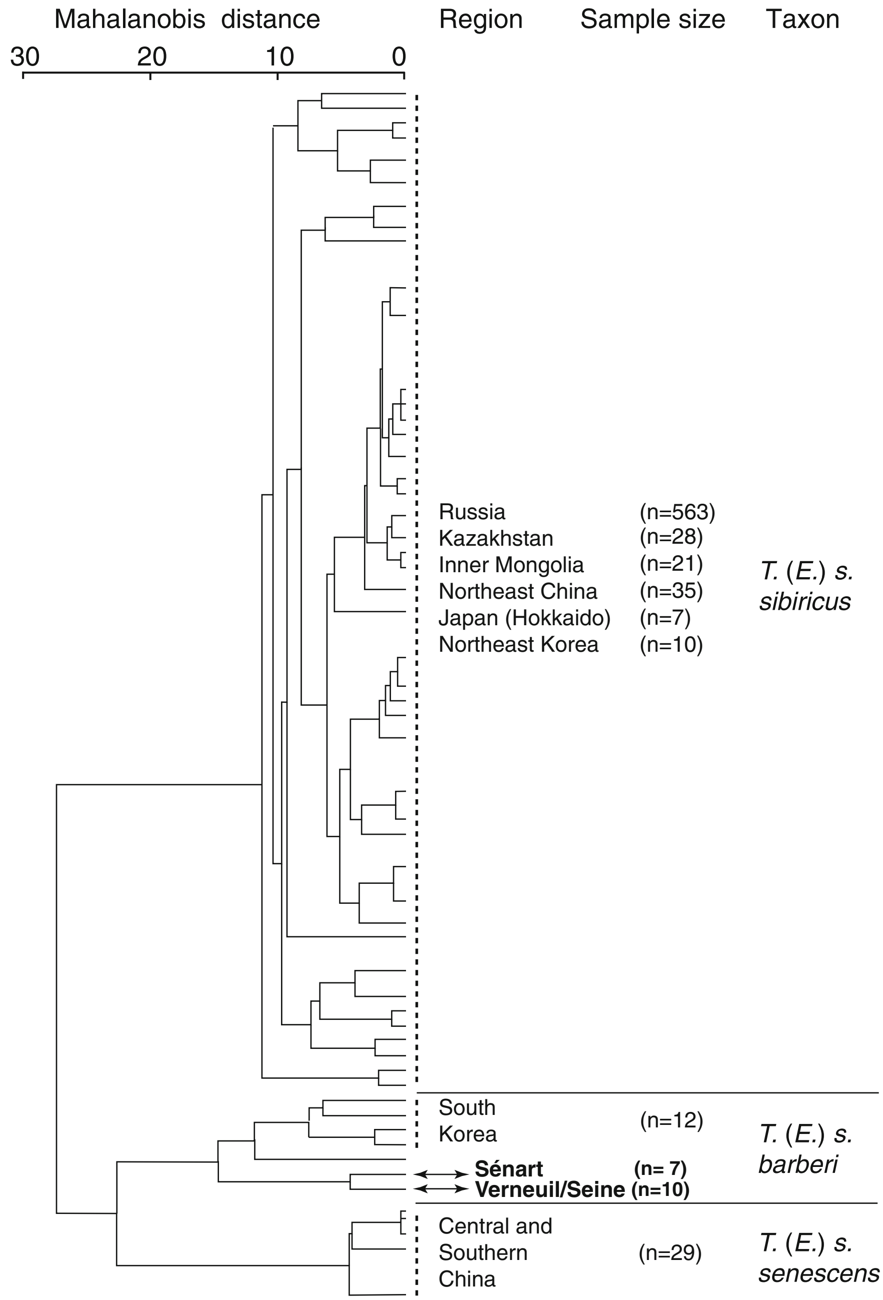
Acoustics

Two types of calls have been described in Siberian chipmunks: 'chips', and 'chucks', which respectively sound like whistles and gurgles (Lissovsky et al. 2006). 'Chips' are made of a single or a series of short calls when an animal is disturbed, and will be referred to hereafter as alarm calls. These calls were recorded and analyzed in Syrinx sound analysis program (John Burt, www.syrinxpc.com) according to Lissovsky et al. (2006). Recordings made by tape recorder were digitized with Sound Forge v 4.5 on EgoSys Wave Terminal v. 3.85 at 44.1 MHz. In total, we analyzed 198 chips from 36 individuals recorded on 19 localities of *T.* (*E.*) *s. sibiricus* (Lissovsky et al. 2006), and series of 53 chips of *T.* (*E.*) *s. barberi* from the Korean peninsula, as well as 31 chips from four individuals in one French locality in 2009 (Forêt de Meudon, Fig. 1; Table 1).

Results

Within the three broad geographic groupings delimited by the hierarchical clustering dendogram of craniometrical measures (Fig. 2), all individuals from the two French localities were clustered with the samples from southern and central Korea.

Fig. 2 Dendrogram for hierarchical cluster analysis of skull measurements separating the 3 taxa in *Tamias sibiricus* (Lax.) on its native range, and including those from the French populations of Sénart and Verneuil-sur-Seine



The maximum likelihood tree constructed from variation in the mtDNA of the cyt B sequence showed that the chipmunks sampled from four populations in Ile-de-France were closely related to those originating from the southern and central Korean peninsula (Fig. 3).

The sonograms of the alarm calls of *T.* (*E.*) *s. sibiricus* from Russia and in Inner Mongolia were V- or U-shaped, while the sonograms sampled in the Forest of Meudon were more similar to those recorded from two individuals in central Korea, which were L-shaped (Fig. 4).

Collectively, these results are consistent with the hypothesis that the chipmucks from the five French populations correspond to the Korean peninsula *T.* (*E.*) *s. barberi* sub-species.

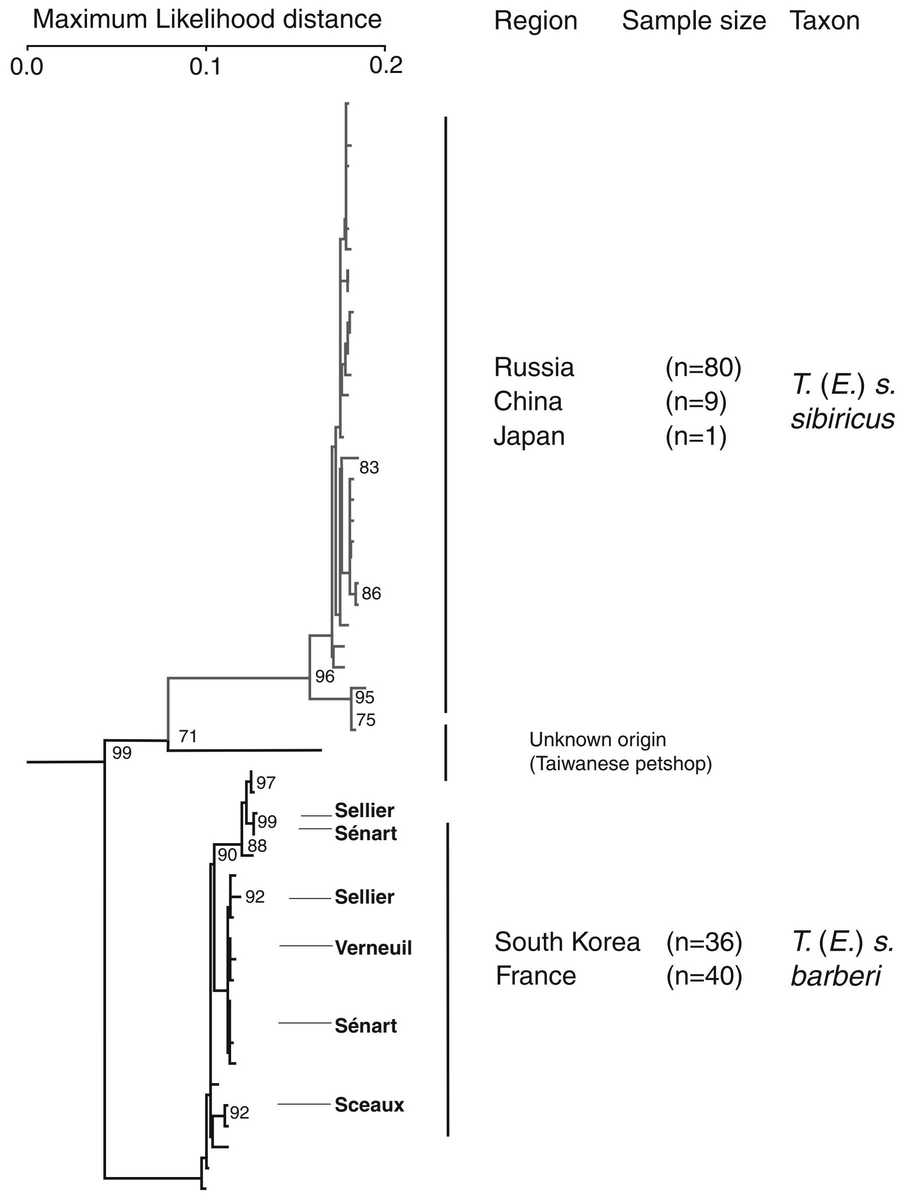


Fig. 3 Maximum likelihood tree separating the 2 taxa based on mtDNA sequence of the cyt B in *Tamias sibiricus* (Lax.) on its native range, and including those from the 4 French populations of Sceaux, Sellier, Sénart, Verneuil-sur-Seine

Discussion

Our results confirmed that individuals from five populations of Siberian chipmunks introduced on Ile-de-France, representing approximately one half of the known established populations, and three out of the four most abundant in France (Sénart, Verneuil-sur-Seine, Meudon: Chapuis et al. 2011), can be reasonably included into the *T.* (*E.*) *s. barberi* subspecies. This subspecies has the smallest geographical distribution, found only in the Korean peninsula (Obolenskaya et al. 2009; Lee et al. 2011). The narrow phylogeographical origin of Siberian chipmunk populations in Ile-de-France indicates that the size of the native geographical range does not appear to be an explanation of their successful establishment. This insight was gain only with precise knowledge of their taxonomic status.

An intense pet trade between Europe and Korea occurred between the 1960s and the 1980s, with probably hundreds of thousands of chipmunks commercially imported yearly during that period (Han SH,

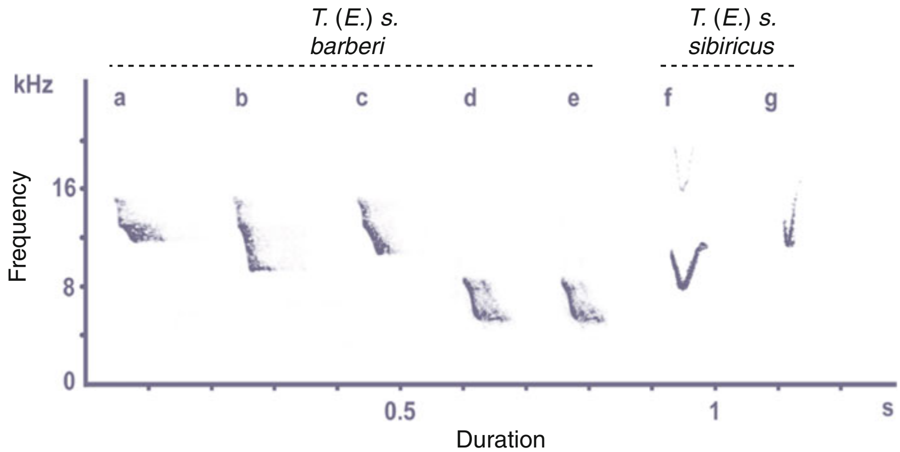


Fig. 4 Shapes of the digitalized alarm calls of Palaearctic chipmunks on their native range and from French specimens; *a*, *b*, *c*: Forest of Meudon (France); *T. s. barberi d*, *e*: Central Korea; *T. s. sibiricus f*: China, Inner Mongolia; *g*: Russia, Primorskiy Territory

personal communication, in Chapuis et al. 2011). Therefore, the frequency of introduction events, and the number of founders per such event, is a more important explanation of the successful establishment of chipmunks compared to the role of native geographical distribution.

Several life-history traits of introduced mammal species are known to increase their ability to spread once established (Forsyth et al. 2004). There is little information on the biology and ecology of wild populations of chipmunks in Korea. In France, however, *T.* (*E.*) *s. barberi* was found to be solitary and sedentary with a high level of site fidelity in adults and a low juvenile dispersal distance (Marmet et al. 2009, 2011). Females may become fertile at less than a year old, can breed twice per year, and produce on average 4 pups per litter (Chapuis et al. 2011). Moreover, the average lifespan is short (i.e., approximately 2–3 years), and the diet consists primarily of seeds from dominant deciduous trees during the autumn and spring but also includes insects during the summer (Chapuis et al. 2011). Overall, these life-history traits are found in populations on the native range, and correspond to those assumed to increase the ability of a mammalian species, once established, to spread within the introduced range (Forsyth et al. 2004).

The taxonomic analysis in the present study confirms that the intensity of the pet trade in exotic squirrels is primarily responsible for the successful establishment of Palaearctic chipmunks in Ile-de-France. The importance of pet trade in explaining establishment of exotic squirrels agrees with the analysis provided by Bertolino (2009). Such results serve as a reminder that the geographical range and the taxonomic status of a species are intimately related (see for example Dissanayake and Oshida 2012). The process of invasion, which is suspected to be primarily driven by 'natural' events, can in fact be blurred by anthropogenic activities (Gaubert et al. 2009; Daümer et al. 2012).

Acknowledgments The Region Ile-de-France, the Conseil Général des Hauts-de-Seine and the Office National des Forêts funded this study. Françoise Cardou kindly commented on this paper and improved the English language.

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