Mammal Research
https://doi.org/10.1007/s13364-021-00569-4

SHORT COMMUNICATION

Where do Chip and Dale come from? Origins of invasive populations of the Siberian chipmunk in Europe

Luca Nerva[1,2] · Alessio Iannucci[3] · Mattia Menchetti[3,4] · Alley Andreoni[5] · Walter Chitarra[1,2] · Matilde Martini[6] · Nadine Mueller[7] · Theo M. J. Peeters[8] · Elias Pesenti[9] · Goedele Verbeylen[10] · Rudy Zozzoli[5] · Emiliano Mori[11]

Received: 10 January 2021 / Accepted: 8 April 2021

Abstract

Among invasive squirrels in Europe, the Siberian chipmunk *Eutamias sibiricus*, native to North-East Asia, shows the highest number of free-ranging populations in European countries, due to the intense pet trade it underwent between the 1960s and the 1980s. We describe 628-bp cytochrome b sequences from the Netherlands ($N = 3$), Belgium ($N = 4$) and Switzerland ($N = 1$), and through phylogenetic analysis show that they belong to the Korean subspecies. This confirms previous findings that the Korean subspecies (Eutamias sibiricus barberi) has been the most commonly introduced to Europe. Another subspecies (the Northern subspecies, Eutamias sibiricus sibiricus) has only been reported previously in an Italian free-living population. If the splitting of the Korean taxon as a proper species (*Eutamias barberi*) will be confirmed by analysis of nuclear markers, a revision of the European Regulation 1143/2014 should be conducted to explicitly ban the trade of newly identified taxa.

Keywords Alien invasive species · Cytochrome-*b* · *Eutamias sibiricus* · Mitochondrial DNA · Species splitting · Taxonomy

Introduction

Origins of introduced species, particularly those listed within the EU Regulation 1143/2014, need to be assessed (Lawson Handley et al. 2011; Mazzamuto et al. 2016; Iannucci et al. 2018, 2019; Mori et al. 2019a; Fratini et al. 2020), to prevent further invasions and to regulate/ban their trade. Among those, the Siberian chipmunk *Eutamias sibiricus* is a small-sized squirrel species, naturally distributed in Northern and Eastern Asia, with free-ranging populations occurring in Europe, particularly in France, Belgium, the Netherlands, Switzerland and Italy (Mori et al. 2018a; www.waarnemingen.be). Besides isolated observations in several other European countries (i.e. Sweden, the UK, and Austria), reproduction in Denmark (Teglgårdsparken) and Greece (Ymittos mountain) has not been confirmed in recent years

Communicated by: Jeremy Herman

✉ Alessio Iannucci
alessio.iannucci@unifi.it

[1] CREA Research Centre for Viticulture and Enology, Via XXVIII Aprile 26, 31015 Conegliano, (Treviso), Italy

[2] Consiglio Nazionale delle Ricerche, Istituto per la Protezione Sostenibile delle Piante, Strada delle Cacce 73, 10135 Torino, Italy

[3] Department of Biology, University of Florence, Via Madonna del Piano 6, 50019 Sesto Fiorentino, (FI), Italy

[4] Institut de Biologia Evolutiva (CSIC-UPF), Passeig Maritim de la Barceloneta 37/49, 08003 Barcelona, Spain

[5] Dipartimento di Scienze della Vita, Università degli Studi di Siena, Via P.A. Mattioli 4, 53100 Siena, Italy

[6] Department of Biology, University of Pisa, Via Alessandro Volta 4, 56126 Pisa, Italy

[7] Natuurmuseum Brabant, Spoorlaan 434, 5038, CH Tilburg, The Netherlands

[8] Bachlaan 752, 5011, BR Tilburg, The Netherlands

[9] Service des Forêts et de la Nature, Direction des Institutions de l'Agriculture et des Forêts, Ruelle de Notre-Dame 2, 1700 Fribourg, Switzerland

[10] Natuurpunt Studie (Mammal Working Group), Michiel Coxiestraat 11, 2800 Mechelen, Belgium

[11] Consiglio Nazionale delle Ricerche, Istituto di Ricerca sugli Ecosistemi Terrestri, Via Madonna del Piano 10, 50019 Sesto Fiorentino, (FI), Italy

Published online: 15 April 2021

and local nuclei may have gone extinct. Similarly, in Germany, the Siberian chipmunk has not been able to establish reproductive populations, with nearly all individuals vanished in a few years. Only in North Rhine-Westphalia, two very small nuclei (i.e. south of Essen and south of Bonn) were present since the 1970s up to 2 years ago, with no recent confirmation (Grimmberger 2014; Vierhaus 2020).

Although nine morphological subspecies have been described for *Eutamias sibiricus* (Thorington et al. 2012), molecular mitochondrial and skull morphology confirmed the existence of three major lineages of Palearctic chipmunks: the Korean subspecies (*Eutamias sibiricus barberi*), the Central Chinese subspecies (*Eutamias sibiricus senescens*) and the Northern subspecies (Russia, Mongolia, Northern China, North Korea and Japan: *Eutamias sibiricus sibiricus*: Obolenskaya et al. 2009; Lissovsky et al. 2017; Mori et al. 2019a).

Most of the free-ranging populations of Siberian chipmunks in Italy and France belonged to the Korean subspecies (Pisanu et al. 2013; Mori et al. 2018b), most likely as a result of an intense pet trade between Korea and Europe in the 1960–1980s (Bertolino 2009; Pisanu et al. 2013; Mori et al. 2019a). As to Italy, some chipmunks of the Northern subspecies have also been released (Mori et al. 2018b).

In the rest of Europe, Siberian chipmunks have been poorly studied and their origin never assessed (Schmidt 2003; Verbeylen 2003; Peeters 2012). Thus, in our work, we aimed at identifying the origin of Siberian chipmunks with established alien populations in Europe (Mori et al. 2018a). We predicted that most of them belong to the Korean subspecies, given the intense trade which has occurred between Korea and Europe when most populations established, and in line with previous studies including those on French (Pisanu et al. 2013) and Italian (Mori et al. 2018b) populations.

Materials and methods

We contacted local Natural History Museums as well as local rodent experts throughout Europe to ask for chipmunk samples from European populations, but we obtained little feedback. Therefore, we analysed hair and tissue samples from individuals directly sampled by the authors. We were not able to collect any samples from Germany, as the local population density of Siberian chipmunks is very low (Table 1).

DNA was extracted from samples (i.e. ears/tongues from dead specimens; hairs from living and museum-preserved individuals) of nine Siberian chipmunk specimens collected in 1983–2018 from the Netherlands ($N = 3$), Belgium ($N = 4$) and Switzerland ($N = 1$: Figure S1 in Supplementary Material 1), and from one more specimen from Siberia (Russia). We used the PureLink™ DNA Mini Kit (Invitrogen, by Thermo Fisher Scientific ®) and we followed the manufacturer's instructions. The reliability of DNA extraction was controlled through a negative control (i.e. no tissue added) and the DNA content determined through an Eppendorf Ultraviolet Spectrophotometer (AG Eppendorf ®). Sequences of the mtDNA gene cytochrome-*b* show significant divergence among the three subspecies of the Siberian chipmunk (Pisanu et al. 2013; Mori et al. 2018b), thus allowing researchers to infer the geographical origin of populations of this species. We used species-specific primers (Pisanu et al. 2013) to sequence a 628 bp fragment of the mtDNA cytochrome-*b*, so to compare our results with previous studies (Lee et al. 2008; Pisanu et al. 2013; Mori et al. 2018b). Obtained sequences were aligned using MUSCLE (Edgar 2004). Conserved regions at both ends were used to design custom primers (Bio-Fab Research, Rome, Italy) with high melting temperature (i.e. > 60°C) following Nerva et al. (2016). PCR reactions were run through a 2720 Thermal Cycler (Applied Biosystems), using about 35 ng/μl of each DNA template and following this profile: 3 min 94°C, 30 cycles of 35 s at 94°C, 45 s at 62°C and 3 min at 72°C, followed by 7 min at 72°C.

We downloaded 153 sequences of cytochrome-*b* from GenBank from throughout the range (Piaggio and Spicer 2001; Lee et al. 2008; Obolenskaya et al. 2009; Pisanu et al. 2013; Lissovsky et al. 2017; Mori et al. 2018b: Table S1 in Appendix 1); three more sequences from France were provided to us by Pisanu et al. (2013). We excluded from our analyses sequences of Siberian chipmunks from pet shops (Chang 2008; Lee et al. 2011) and voucher samples whose native origin cannot be assessed in detail (Reid et al. 2012), as well as cytochrome-*b* fragments smaller than 400 bp (Kim et al. 2018). Sequences of North American chipmunks (Piaggio and Spicer 2001) and of *Tamiops maclellandii* (Li et al. 2008) were used as outgroups. We used ClustalW to perform pairwise alignments (Higgins and Sharp 1988). The best fitting evolutionary model was selected using the algorithm implemented in MEGA7 (Kumar et al. 2016).

Phylogenetic relationships were inferred through the maximum likelihood method based on the Tamura-Nei model, as well as through the neighbour-joining method (1000 bootstrap replicates: Figure S2 in Supplementary Material 1). A haplotype network of *E. sibiricus* was built with the program TCS 1.21 (Clement et al. 2000) and then edited with tcsBU (dos Santos et al. 2016) and Adobe Illustrator CC 2019.

Results

We obtained a total of eight new sequences of Siberian chipmunk in Europe, from two Belgian (and two additional samples from Flanders without exact locations), the largest Dutch

Table 1 Estimated population sizes of chipmunks *Eutamias sibiricus* in Europe (Verbeylen 2003; Peeters 2012; Andreoni 2020)

Country	Population counts	N individuals
Belgium	7,500 in 1998 (Sonian Forest), 2 other populations: possibly extinct	7,500
the Netherlands	About 100 (1 large population, Tilburg)	100
Switzerland	About 100 (1 population)	100
Italy	4–6 (Valbelluna), less than 10 (Rome), 45 (Sigurtà Garden Park)	60
France	About 10,000 (22 populations)	10,000
Germany	No more than 20 individuals (max. 2 populations)	20
Total		17,780

Populations of *Tamias striatus* in Wuppertal (North Rhine-Westphalia) and of *Tamiops swinhoei* at Neuss (North Rhine-Westphalia) seem to be extinct

and the only Swiss populations (Accession numbers in Table S1 and Figure S2, Supplementary Material 1: Fig. 1). A new sequence was also obtained from a Russian specimen. Furthermore, we added sequences of French specimens previously analysed by Pisanu et al. (2013) to online databanks (Table S1 in Supplementary Material 1).

European sequences belonged to 14 haplotypes (Belgium, $N = 1$; Italy, $N = 7$; France, $N = 3$; the Netherlands, $N = 2$; Switzerland, $N = 1$). All individuals from Europe, except for one from Italy (Rome), were clustered with the samples from South Korea (Fig. 1; phylogenetic trees: Figure S2 in Supplementary Material 1). Only one individual from Rome (Villa Ada) clustered within the Northern clade (Fig. 1). Our phylogenetic reconstruction confirmed that Japan hosts both native populations of the Northern subspecies and introduced populations of the Korean subspecies (Fig. 1).

Discussion

Our new results for the Netherlands, Belgium and Switzerland confirm that Siberian chipmunks introduced into Europe are typically of the Korean subspecies. Similarly, in Japan, the Northern subspecies is considered to be native at least in Hokkaido, but several Korean individuals have been released, therefore threatening the genetic purity of the native population of Siberian chipmunks (Mori et al. 2019a; Morishima et al. 2020). The Korean chipmunk has the smallest native range within the *Eutamias sibiricus* complex, thus providing a strong confirmation that size of native range is not a good predictor of invasion success (Pisanu et al. 2013; Mori et al. 2019a). Pisanu et al. (2013) reported evidence of an intense pet trade between Korea and other countries, including European ones (cf. Chang 2008; Lee et al. 2011), peaking between the 1960s and 1980s. The great trade effort may have

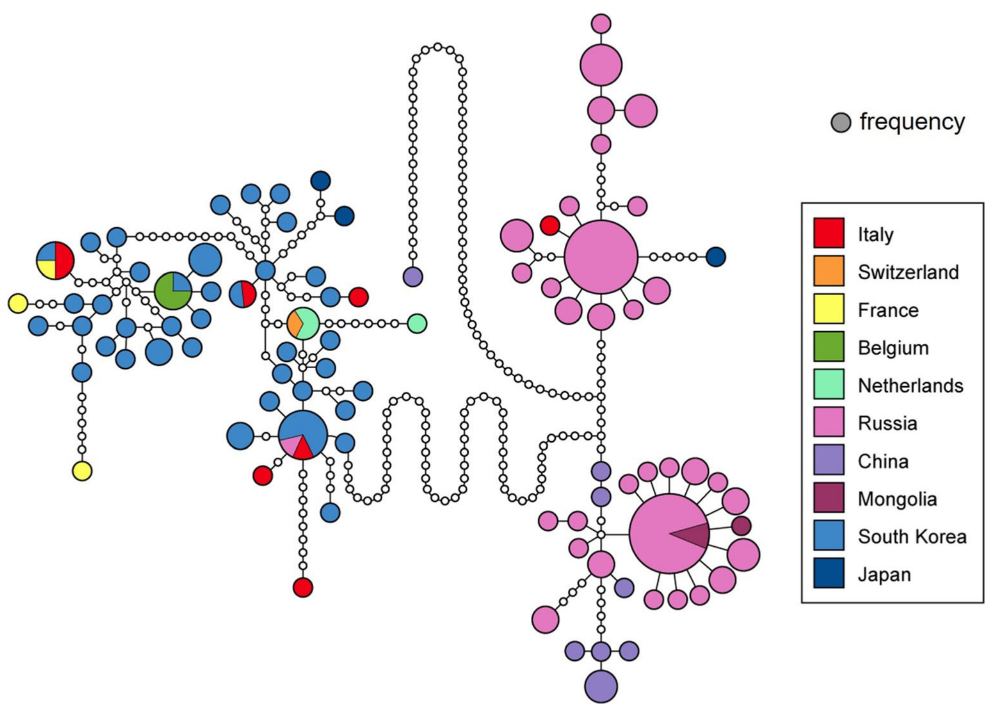

Fig. 1 Haplotype network based on all available sequences of Siberian chipmunks. Different colours refer to different countries of origin. Size of each circle is proportional to the frequency of each haplotype in our sample. Median vectors, which represent either extant unsampled or extinct ancestral sequences, are indicated by white circles

resulted in a high propagule pressure for European populations, also supported by the observation of several isolated individuals and by several unestablished nuclei (Mori et al. 2018b). Our pan-European analysis supports the importance of the pet trade as a factor positively influencing the establishment of alien squirrels (Bertolino 2009). Accordingly, the recent EU Regulation 1143/2014 on Invasive Alien Species has listed several species of squirrels, including *Eutamias sibiricus*, banning their trade and promoting (i) prevention of further introductions, (ii) early detection and rapid removal of new nuclei and (iii) management (e.g. numerical control) of established populations. The Siberian chipmunk was a very common pet before this law (Chang 2008; Bertolino 2009; Lee et al. 2011).

Nuclear markers are required to assess whether the Northern and Korean taxa may in fact be distinct species and nuclear markers should be deployed rapidly to determine whether this is the case (Mori et al. 2019b; Ottenburghs 2020). If the Korean chipmunk were confirmed as a proper species (*Eutamias barberi*) and accepted by the global community (e.g. the IUCN), given its impacts in a highly environmentally suitable Europe (Mori et al. 2019a), a rapid risk assessment should be carried out to avoid the newly designated species would be sold again in European pet markets.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13364-021-00569-4.

Acknowledgements Authors would like to thank all the people who helped in data collection (in alphabetical order): Giovanni Amori, Sam Bennekens, Sandro Bertolino, Mauro Bon, Dario Capizzi, Family Camurri-Piloni, Michele Cassol, Fabio Dartora, Maxime Fajgenblat, Peter Lurz, Roberto Mattinzioli, Giuseppe Mazza, Valeria Mazza, Holger Meinig, Andrea Monaco, Pieter Moysons, Benoit Pisanu, Conte Giuseppe Inga Sigurtà, Arianna Spada, Giuseppe Tormen, Andrea Vendramin and Koen Verschoore. Two anonymous reviewers and the Associate Editor kindly provided us with useful comments. E. Basset (University of Birmingham) revised the first version of our manuscript and carried out a professional language polishing. Support to MM for this research (grant LCF/BQ/DR20/11790020) was provided by 'La Caixa' Foundation (ID 100010434).

References

Andreoni A (2020) Il tamia siberiano *Eutamias sibiricus* (Laxman, 1769) nel Parco Giardino Sigurtà (Italia Settentrionale): studio sull'abbondanza, filogeografia e analisi dei ritmi di attività di una specie invasiva. In: Msc Thesis in Scienze e Gestione delle Risorse Faunistico-Ambientali. Università di Firenze, Firenze

Bertolino S (2009) Animal trade and non-indigenous species introduction: the world-wide spread of squirrels. Divers Distrib 15:701–708

Chang SW (2008) Identification of chipmunks sold in pet shops in Taiwan. Teyou Shengwu Yanjiu 10:25–34

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659

dos Santos AM, Cabezas MP, Tavares AI, Xavier R, Branco M (2016) tcsBU: a tool to extend TCS network layout and visualization. Bioinformatics 32(4):627–628

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797

Fratini S, Natali C, Zanet S, Iannucci A, Capizzi D, Sinibaldi I, Sposimo P, Ciofi C (2020) Assessment of rodenticide resistance, eradication units, and pathogen prevalence in black rat populations from a Mediterranean biodiversity hotspot (Pontine Archipelago). Biol Invasions 22:1379–1395

Grimmberger E (2014) Die Säugetiere Deutschlands. Quelle & Meyer, Wiebelsheim, pp 214–216

Higgins DG, Sharp PM (1988) CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. Gene 73:237–244

Iannucci A, Natali C, Ciofi C, Sposimo P, Fratini F (2018) Population genetic analysis of invasive black rats: defining eradication units in the Tuscan Archipelago National Park. Hystrix 29:67–73

Iannucci A, Baratti M, Gotti C, Baccetti N, Giannini F (2019) A genetic analysis of the European hedgehog (*Erinaceus europaeus*): an applicative case study to support its eradication from Pianosa Island (Tuscan Archipelago). Conserv Genet 20:395–402

Kim TW, Lee HJ, Kim YK, Oh HS, Han SH (2018) Genetic identification of prey species from teeth in faeces from the Endangered leopard cat *Prionailurus bengalensis* using mitochondrial cytochrome *b* gene sequence. Mitochondrial DNA A 29:170–174

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Lawson Handley LJ, Estoup A, Evans DM, Thomas CE, Lombaert E, Facon B, Aebi A, Roy HE (2011) Ecological genetics of invasive alien species. Biocontrol 56:409–428

Lee MY, Lissovsky AA, Park SK, Obolenskaya EV, Dokuchaev NE, Zhang Y, Yu L, Kim YJ, Voloshina I, Myslenkov A, Choi TY, Min MS, Lee H (2008) Mitochondrial cytochrome *b* sequence variations and populations structure of Siberian chipmunk (*Tamias sibiricus*) in Northeastern Asia and population substructure in South Korea. Mol Cell 26:566–575

Lee SJ, Jung G, Min MS, Kim CK, Lee H, Kim CB, Lee MY (2011) Genetic origin identification of Siberian chipmunks (*Tamias sibiricus*) in pet shops of South Korea. Anim Cells Syst 15:161–168

Li S, Yu F, Yang S, Wang Y, Jiang X, McGuire PM, Feng Q, Yang J (2008) Molecular phylogeny of five species of *Dremomys* (Rodentia: Sciuridae), inferred from cytochrome *b* gene sequences. Zool Scr 37:349–354

Lissovsky AA, Obolenskaya EV, Ge D, Yang Q (2017) Phylogeny and distribution of Palearctic chipmunks *Eutamias* (Rodentia: Sciuridae). Hystrix 28:107–109

Mazzamuto MV, Galimberti A, Cremonesi G, Pisanu B, Chapuis JL, Stuyck J, Amori G, Su H, Aloise G, Preatoni DG, Wauters LA, Casiraghi M, Martinoli A (2016) Preventing species invasion: A role for integrative taxonomy? Integr Zool 11:214–228

Mori E, Zozzoli R, Menchetti M (2018a) Global distribution and status of introduced Siberian chipmunks *Eutamias sibiricus*. Mammal Rev 48:139–152

Mori E, Milanesi P, Menchetti M, Zozzoli R, Capizzi D, Monaco A, Nerva L (2018b) Genetics reveals that free-ranging chipmunks introduced to Italy have multiple origins. Hystrix 29:239–242

Mori E, Menchetti M, Zozzoli R, Milanesi P (2019a) The importance of taxonomy in species distribution models at a global scale: the case of an overlooked alien squirrel facing taxonomic revision. J Zool 307:43–52

Mori E, Nerva L, Lovari S (2019b) Reclassification of the serows and the gorals: the end of a neverending story? Mammal Rev 49:256–262

Morishima K, Nakano T, Aizawa M (2020) Sika deer presence affects the host-parasite interface of a Japanese land leech. Ecol Evol 10:6030–6038

Nerva L, Ciuffo M, Vallino M, Margaria P, Varese GC, Gnavi G, Turina M (2016) Multiple approaches for the detection and characterization of viral and plasmid symbionts from a collection of marine fungi. Virus Res 219:22–38

Obolenskaya EV, Lee MY, Dokuchaev NE, Oshida T, Lee MS, Lee H, Lissovsky AA (2009) Diversity of Palaearctic chipmunks (*Tamias*, Sciuridae). Mammalia 73:281–298

Ottenburghs J (2020) Ghost introgression: spooky gene flow in the distant past. Bioessays 42:2000012

Peeters TMJ (2012) Een prachtige exoot: de Siberische grondeekhoorn. In: Peeters T, van Eck A, Cramer T (eds) Natuurstudie in De Kaaistoep. Verslag 2012, 18e onderzoeksjaar. TWM Gronden BV. Natuurmuseum Brabant & KNNV-afdeling, Tilburg, pp 107–114

Piaggio AJ, Spicer GS (2001) Molecular phylogeny of the chipmunks inferred from mitochondrial cytochrome *b* and cytochrome oxidase II gene sequences. Mol Phylogenet Evol 20:335–350

Pisanu B, Obolenskaya EV, Baudry E, Lissovsky AA, Chapuis JL (2013) Narrow phylogeographic origin of five introduced populations of the Siberian chipmunk *Tamias* (*Eutamias*) *sibiricus* (Laxmann, 1769) (Rodentia: Sciuridae) established in France. Biol Invasions 15:1201–1207

Reid N, Demboski JR, Sullivan J (2012) Phylogeny estimation of the radiation of western North American chipmunks (*Tamias*) in the face of introgression using reproductive protein genes. Syst Biol 61:44–62

Schmidt O (2003) Neue Arten in Bayern. Bereicherung oder Bedrohung für unsere Wälder? LWF Aktuell, Freising

Thorington RWJ, Koprowski JL, Steele MA, Whatton JF (2012) Squirrels of the World. The John Hopkins University Press, Baltimore, Maryland

Verbeylen G (2003) Aziatische grondeekhoorn. In: Verkem S, De Maeseneer J, Vandendriessche B, Verbeylen G, Yskout S (eds) Zoogdieren in Vlaanderen. Ecologie en verspreiding van 1987 tot 2002. Natuurpunt Studie & JNM-Zoogdierenwerkgroep, Mechelen & Gent, pp 284–289

Vierhaus H (2020) Burunduk (Sibirisches Streifenhörnchen) (*Tamias sibiricus*). AG Säugetierkunde NRW — Online, Atlas der Säugetiere Nordrhein-Westfalens Downloaded from saeugeratlas-nrw.lwl.org on 30.09.2020