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Are red squirrels a red herring?

S Souguel, T Oueslati, G Grine, M Drancourt

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17 **ABSTRACT**

18 In the United Kingdom, declining population of the red squirrel, the acknowledged
19 historical reservoir for zoonotic *Mycobacterium leprae* leprosy, is thought to have
20 been behind the decline in leprosy. However, remaining red squirrels are mainly
21 plagued by *Mycobacterium lepromatosis*, questioning their historically role in the
22 dynamics of zoonotic *M. leprae* leprosy. To resolve this issue, forthcoming
23 paleomicrobiology studies will address the *M. leprae* complex as a whole;
24 investigating the remains of squirrels buried for eight centuries, and burrowing
25 animals imported in the United Kingdom from the British Empire countries between
26 the 16th and 19th centuries; also looking at the situation in continental Europe, to gain
27 a more comprehensive view of the historical zoonotic dynamics of leprosy in the
28 United Kingdom and Europe. The results may indicate that the declining red squirrel
29 population is no more than a red herring, obscuring a broader decline in leprosy.

30

31

32 PUZZLING LEPROSY DYNAMICS

33 Leprosy became a notifiable disease in England and Wales in 1951, and statistics
34 subsequently indicated its decreasing prevalence, with all cases being imported from
35 leprosy-endemic countries parts of the British Empire. Rare cases of indigenous
36 transmission were recorded, although these were ultimately traced to such imported
37 cases.¹ In one illustrative, fatal case, the patient had, for six months, slept in the
38 same bed as his infected brother who had returned from India.² The dynamics of
39 leprosy is more puzzling when it involves data prior to the 20th century. The
40 decreasing prevalence observed between the 19th and 21st centuries appears to have
41 started in the 16th century, when the United Kingdom first colonised parts of North
42 America, before expanding around the globe, occasioning extensive exchanges of
43 human, animal, and plant populations, and possibly accompanying microbes. This is
44 reflected, for example, in the records of the Natural History Museum in London, which
45 began registering exotic animals in the 17th century (**Figure 1**). Before modern times,
46 leprosy peaked between the 9th and 11th centuries, possibly fuelled by crusades and
47 historically attested to by pictorial representations, texts, and a large number of leper
48 colonies. Accordingly, populations in mediaeval and modern times recognised leprosy
49 featuring pathognomonic leonine facies and distal amputations, due to neurotrophic
50 bone deformations (also visible in buried skeletons). They addressed the endemicity
51 of leprosy, reflected in social distancing, and referred to in numerous texts and
52 pictorial representations. In the Roman world (delimited in the United Kingdom by
53 Hadrian's wall), there is no paleopathological or paleomicrobiological evidence that
54 leprosy circulated along military and trade routes from putative Egyptian and Near
55 East foci to southern Britain.³ Circulating strains of the causative, yet axenically
56 uncultured *Mycobacterium leprae* (*M. leprae*) are known only for mediaeval times,

57 after *M. leprae* whole genome sequences (and mycolic acids) were recovered from
58 bone tissues and dental calculus in the remains of eighteen individuals, buried from
59 the 7th century onwards, which indicated five *M. leprae* lineages (lineages 0, 1, 2, 3,
60 and 4)³⁻⁹ (**Figure 2**). Nothing is known regarding causative organisms after 1268-
61 1283 in the United Kingdom,⁵ leaving it unknown which *M. leprae* lineages were
62 infecting United Kingdom populations after colonisation of North America and the
63 expansion of the British Empire. It is also unknown whether *Mycobacterium*
64 *lepromatosis* (*M. lepromatosis*), another leprosy pathogen, played any role in the
65 indigenous dynamics of leprosy in the United Kingdom.

66 **RED SQUIRRELS: THE HISTORICAL RESERVOIR FOR ZONOTIC LEPROSY**

67 In the United Kingdom, *M. leprae* has been documented not only in the remains of
68 individuals from mediaeval times, but also in some red squirrel populations (*Sciurus*
69 *vulgaris*). Almost a decade ago, Meredith *et al.* published a groundbreaking study
70 providing the first concrete evidence of *M. leprae* in red squirrels in the United
71 Kingdom.¹⁰ Combining molecular techniques, including PCR and DNA sequencing
72 with serology, *M. leprae* was documented in a total of 25 red squirrels, all from
73 Brownsea Island, and including eight clinically diseased squirrels. Histopathology and
74 immunohistochemistry confirmed granulomatous lesions which were consistent with
75 leprosy in infected squirrels. Infecting *M. leprae* strains yielded genomic sequences
76 most closely related to those retrieved from buried individuals, in 13th-century
77 England and 14th-century Denmark. Similar sequences were also collected from the
78 remains of one red squirrel from mediaeval Winchester, not far from Brownsea
79 Island.¹¹ This discovery suggests that *M. leprae* may have persisted in the United
80 Kingdom for centuries, even after human cases of leprosy had dramatically declined,
81 and supports the hypothesis that the red squirrel was the reservoir for zoonotic

82 leprosy.¹⁰ The fact that the *M. leprae* strain found in red squirrels was related to a
83 medieval European strain suggested that *M. leprae* had a longer evolutionary history
84 in Europe than previously thought. This could mean that leprosy in humans may have
85 been sustained, in part, by animal reservoirs such as red squirrels, long after human-
86 to-human transmission had diminished. Although the identification of *M. leprae* in red
87 squirrels provided compelling evidence, the potential for zoonotic transmission
88 (transmission from animals to humans) remains a subject of ongoing research. Unlike
89 armadillos, which are known to transmit *M. leprae* to humans in certain regions of the
90 Americas,¹² there is currently no direct evidence that red squirrels have transmitted
91 leprosy to humans.

92

93 **THE DECLINE IN RED SQUIRRELS MIRRORS THE DECLINE IN LEPROSY**

94 The red squirrel is now an endangered species in the United Kingdom, and its
95 population has declined to around 140 000 animals. After the introduction of the grey
96 squirrel (*Sciurus carolinensis*) from North American deciduous forests in 1876, red
97 squirrel populations declined in all regions, while the grey squirrel population rapidly
98 increased to more than 2.5 million animals.¹³ The grey squirrel is better able to exploit
99 resources in deciduous and mixed (deciduous and coniferous) forests than the red
100 squirrel. In England, studies comparing sites inhabited by red squirrels with sites
101 inhabited by both red and grey squirrels showed that while their spring breeding rate
102 was similar, the red squirrels' summer breeding rate was much lower in mixed sites
103 (respectively about 20% and 50%). This reduction prevents females from producing
104 two litters per year, probably due to lower female body weight in late spring and early
105 summer.¹⁴ Squirrel Poxvirus (SQPV) only appears in red squirrel populations in areas

106 already colonised by grey squirrels, and causes a deadly infection in red squirrels
107 (although a small proportion of red squirrels, particularly juveniles, may survive
108 exposure to the virus by developing an immune response), while grey squirrels are
109 spared.¹⁵ Furthermore, SQPV outbreaks in Scotland show spatial and temporal
110 distributions predictive of a significant reduction in red squirrel populations within the
111 next 25 years.¹⁶ Incidentally, no evidence of leprosy has been detected in the United
112 Kingdom's grey squirrel population, either using anti-PGL-I serology or DNA
113 detection.¹⁰ In sum, the grey squirrel's success combines ecological and pathogenic
114 factors that disadvantage the red squirrel. After the red squirrel was hypothesised to
115 be the reservoir for zoonotic leprosy, the decline in the red squirrel population was
116 hypothesised to be behind the decline in leprosy.⁷

117 **CHALLENGING THE RED SQUIRREL HYPOTHESIS**

118 Opportunities for humans to come into contact with red squirrels are now limited to
119 leisure activities in parks and forests. This was undoubtedly not the situation in
120 mediaeval times, when young red squirrels were captured as companion animals for
121 the wealthy (**Supplementary Figure 1**), and the fur of adult squirrels was used for
122 clothing and shoes (perhaps the slipper that Cinderella lost when making her escape
123 from the ball was made from squirrel fur).¹⁷ However, *M. leprae*-infected red squirrels
124 exhibit skin lesions and swelling similar to those seen in leprosy patients, and such
125 lesions may have discouraged hunters from catching visibly diseased animals.¹⁰ One
126 alternative hypothesis is that both red squirrels and human populations were
127 contaminated from a common source. It may be relevant to consider that red
128 squirrels are burrowing mammals (as are *M. leprae*-infected armadillos in America),
129 and a few reports have mentioned *M. leprae* in the soil.¹⁸ Organic soil compartments
130 and rhizospheres may be the source of mycobacteria closely related to the *M. leprae*

131 complex, such as *Mycobacterium ulcerans*.¹⁹ The recent discovery that not only *M.*
132 *leprae*, but also *M. lepromatosis* was infecting red squirrels, clearly poses a challenge
133 to the hypothesis of the red squirrel, as only *M. leprae* and not *M. lepromatosis* has
134 been documented to date in human remains from mediaeval times. In the 2016 study
135 by Avanzi *et al.*, *M. lepromatosis* was found in nine red squirrels in the Isle of Wight,
136 southern England, Ireland, and Wales, and no codetection with *M. leprae* was
137 observed. This followed the seminal discovery of *M. lepromatosis* in red squirrels by
138 the same research group.¹⁰ These pioneering observations were then confirmed, and
139 it was found that *M. lepromatosis* is detected 25 times more frequently in red
140 squirrels than *M. leprae*.²⁰ Finally, some asynchronisms suggest that the red squirrel
141 hypothesis may not tell the story of the decline of leprosy in the United Kingdom. The
142 rapid decline in the red squirrel populations following the 1876 introduction of the
143 grey squirrel to the United Kingdom, postdates by at least two centuries the decline of
144 leprosy in the country, suggesting another, preceding, driver event between the 16th
145 and 18th centuries.

146

147 **ALTERNATIVE WORKING HYPOTHESES**

148 We propose that the replacement of *M. leprae* by *M. lepromatosis* was a major driver
149 for the progressive decline in leprosy in the United Kingdom. In this scenario, *M.*
150 *lepromatosis* was imported into the United Kingdom following the progressive
151 expansion of the British Empire, starting from the 16th century expansion into Canada
152 through to the 19th expansion into the Middle East and New Zealand (**Figure 1**). A
153 review of historical charts held by the Natural History Museum in London
154 [<https://data.nhm.ac.uk/search>] indicates that at least eight burrowing mammals
155 (including the nine-banded armadillo (*Dasypus novemcinctus*)) were imported into

156 the United Kingdom during these centuries, contemporaneously with the decline of
157 leprosy (**Supplementary Table 1**). While there is no documentation of *M.*
158 *lepromatosis* in humans or animals predating its seminal description in 2008,²¹ *M.*
159 *lepromatosis* has been further reported in red squirrels in a geographical belt limited
160 by the 55th parallel north (in Scotland) and the 25th parallel south (in Paraguay),
161 giving an indication regarding the geographical source of its introduction. Combining
162 this geographical indication with the time scale indicative of a 16th to 20th century
163 event, we tentatively propose that that event was related to the introduction of *M.*
164 *lepromatosis*-contaminated animals, most probably imported from British colonies for
165 their fur. In this scenario, the recent decline in the red squirrel population was no
166 more than a tangential, albeit important, event. We argue that the current lack of data
167 regarding the dynamics of leprosy prevents us from concluding that the red squirrel
168 was a pivotal factor in the declining reservoir for leprosy, in the United Kingdom, and
169 possibly continental Europe. *M. leprae* and *M. lepromatosis* have never been
170 detected in contemporary squirrels in continental Europe. A limited study carried out
171 by our Institute of 251 Pallas's squirrels (*Callosciurus erythraeus*) caught by the
172 French Biodiversity Agency in 2023 and 2024, found no evidence of *M. leprae* using
173 qPCR-based detection (Hamza Makoudi, personal data) (**Figure 2C**). These
174 observations indicate that ancient burrowing mammals, including squirrels, should be
175 investigated if we are to resolve the puzzling dynamics of zoonotic leprosy in
176 continental Europe and the United Kingdom. Testing the slipping pathogen
177 hypothesis would rely on cumulative observations regarding the distribution of *M.*
178 *leprae* and *M. lepromatosis* on a time scale from the 16th to the 20th centuries. Such a
179 study should be conducted on a geographical scale including the United Kingdom
180 and continental Europe and on a diversity scale including, as a priority and non-

181 exclusively, burrowing mammals potentially imported from colonised territories.
182 Ancient materials exist in continental Europe, with red squirrels having been
183 documented archaeologically, including in a 15th century hospital in northern France²²
184 and accumulations of pelt processing waste in the 11th century site at Dieue-sur-
185 Meuse (Meuse) which uncovered 417 individuals.²³ This reveals the potential of
186 archaeology to document the presence of red squirrels. Nevertheless, at the majority
187 of sites, finds of squirrel remains are rare. An experimental approach could rely on
188 rapid high throughput screening for *M. leprae* complex aDNA in collections, on sites,
189 in museums, and in osteological collections. While there is no longer any doubt
190 regarding the fact that both the human and red squirrel populations suffered from
191 leprosy over recent millennia in northern Europe, the trends of prevalence in both
192 populations remain unknown against the backdrop of an increasing human
193 population and a decreasing red squirrel population. This situation has led to at least
194 three non-contradictory hypotheses that humans infected red squirrels
195 (anthroponosis), that red squirrels infected humans (zoonosis), and that both humans
196 and red squirrels were infected from a common, third source of infection. Particularly
197 in a situation where the modes and routes of *M. leprae* and *M. lepromatosis*
198 contamination remain unclear, the third hypothesis should not be discounted but
199 rather investigated by challenging the presence of *M. leprae* and *M. lepromatosis*
200 DNA in both ancient and modern environmental samples comprising animals, chiefly
201 squirrels, and also food sources which are common to squirrels and humans. The
202 detection of *M. leprae* itself (not only *M. leprae* DNA) in faeces collected from a
203 leprosy patient also suffering from *Entamoeba coli* enteritis in Burkina Faso brought
204 into question, although it did not prove, the possibility that *M. leprae* may colonise the
205 digestive tract from certain food sources.²⁴ It was recently established that red

206 squirrels consume potatoes, fungi, and fungi rhizomes in addition to well-known
207 fruits, nuts, berries, tree seeds as part of their burrowing activities. This new
208 information should be taken into account when investigating the possible overlooked
209 ecosystems in which *M. leprae* and *M. lepromatosis* may persist live in biotic
210 environments. Finally, as red squirrels are widespread across Europe and parts of
211 Asia, this could, theoretically, contribute to the transmission of leprosy outside the
212 United Kingdom. Thus far, our findings have been restricted to the United Kingdom,
213 while investigations of red squirrels in continental Europe have failed to document
214 leprosy.^{25,26}

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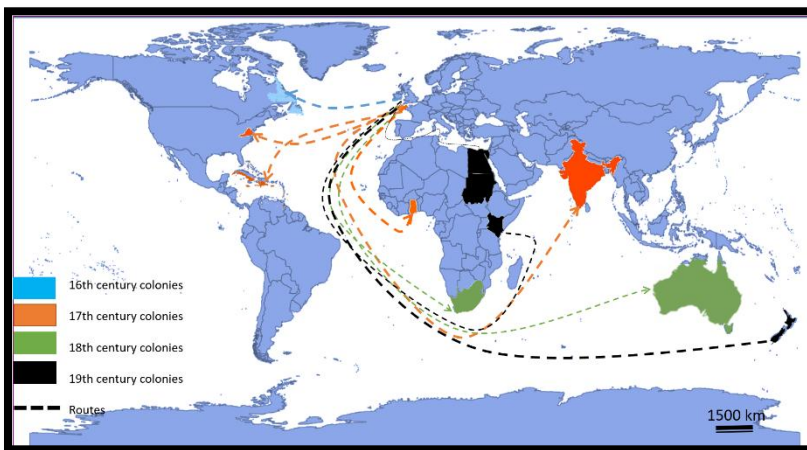
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417 **FIGURES**

418 **Figure 1. Territorial expansion of the British Empire between the 16th and the**
419 **19th centuries opened up opportunities for the importation of various exotic**
420 **animals, which were possibly reservoirs for the leprosy pathogens, as depicted**
421 **in records held at the Natural History Museum in London**
422 **(<https://data.nhm.ac.uk/search>).** Here, light blue ◆ indicates territories occupied
423 during the 16th century, orange ◆ indicates territories occupied during the 17th
424 century, green ◆ indicates territories occupied during the 18th century and black ◆
425 indicates territories occupied during the 19th century.



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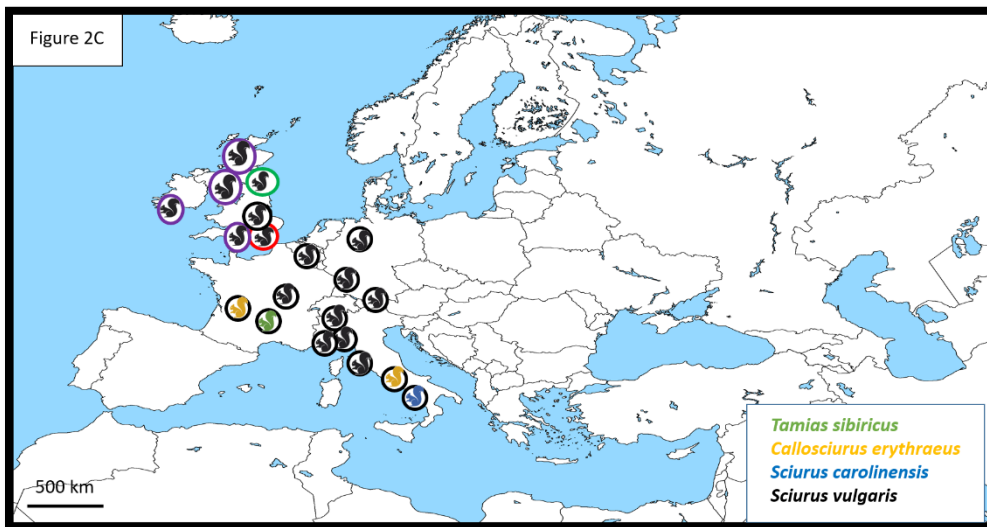
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447 **Panel C** features a series of DNA-based evidence for *M. leprae* (red circle), *M.*
 448 *lepromatosis* (purple circle) and negative detection (black circle) in red squirrels (with
 449 clinical signs of leprosy, green circle) in the 21st century. Colour-coded squirrel icons:
 450 green icons for *Tamias sibiricus*, yellow for *Callosciurus erythraeus*, blue for *Sciurus*
 451 *carolinensis*, and black for *Sciurus vulgaris*.



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454 **SUPPLEMENTARY MATERIALS**

455 **Supplementary Figure 1. A British woman playing with a companion red**

456 **squirrel, 14th century.** The illustration is taken from a collection of English hymns

457 from the start of the 14th century, known as the Luttrell Psalter. (Photo: Council of the

458 British Library).

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464 **Supplementary Table 1.** List of burrowing mammals imported from a wide range of
465 countries in the British Empire into the United Kingdom, 18th-19th centuries. Source:
466 Natural History Museum, United Kingdom (<https://data.nhm.ac.uk/search>).

467 **Burrowers and Ground Diggers:**

468 1. **Giant Anteater (South America)**

469 Latin Name: *Myrmecophaga tridactyla*

- 470 ○ Digs to find insects, especially termites and ants.

471 2. **Nine-Banded Armadillo**

472 Latin Name: *Dasybus novemcinctus*

- 473 ○ Well-known for digging burrows for shelter and searching for food.

474 3. **Warthog (Kenya)**

475 Latin Name: *Phacochoerus africanus*

- 476 ○ Uses its snout to dig up roots and tubers, and its tusks to enlarge holes
477 in the ground.

478 4. **African White-Bellied Pangolin (Central and West Africa)**

479 Latin Name: *Phataginus tricuspis*

- 480 ○ Digs to access ant and termite nests.

481 5. **Chinese Pangolin**

482 Latin Name: *Manis pentadactyla*

- 483 ○ Also digs to access underground insect nests.

484 6. **North American Beaver**

485 Latin Name: *Castor canadensis*

- 486 • Although better known for building dams, it can dig into riverbanks to create
487 lodges.

488 • **Grey Squirrel (United States of America) (*Sciurus carolinensis*)**: While it
489 may dig to bury seeds, it is not a typical burrower.

490 ○

491

492 • **Platypus (*Ornithorhynchus anatinus*)**: Not a terrestrial digger but can
493 burrow into riverbanks to create shelters.

494 • **Java Porcupine (*Hystrix javanica*)**: Digs simple shelters but this is not its
495 primary habitat.

496 • **Raccoon (*Procyon lotor*) and European Otter (*Lutra lutra*)**: Does not dig
497 but often uses abandoned burrows made by other animals.

498