

# PCR Characterization Suggests that an Unusual Range of *Bartonella* Species Infect the Striped Field Mouse (*Apodemus agrarius*) in Central Europe

Joanna Hildebrand,<sup>a</sup> Anna Paziewska-Harris,<sup>b</sup> Grzegorz Zaleśny,<sup>c</sup> Philip D. Harris<sup>b</sup>

Department of Parasitology, Institute of Genetics and Microbiology, Wrocław University, Wrocław, Poland<sup>a</sup>; Natural History Museum, University of Oslo, Blindern, Oslo, Norway<sup>b</sup>; Institute of Biology, Department of Invertebrate Systematics and Ecology, Wrocław University of Environmental and Life Sciences, Wrocław, Poland<sup>c</sup>

**Blood samples from *Apodemus agrarius* from Poland yielded PCR amplicons of *Bartonella* species. These included *B. grahamii*, *B. taylorii*, and *B. birtlesii*, as is typical of European *Apodemus*, as well as *B. elizabethae*-like forms and a recombinant strain of *B. taylorii*, most closely related to an American isolate from *Tamiasciurus hudsonicus*.**

The alphaproteobacterium *Bartonella* is associated with emerging infection in humans. Between 30 and 40 species have been described (1), primarily from rodents; of 1,400 deposited citrate synthase (*gltA*) sequences, over 530 are from rodents (2). In Europe, studies on *Bartonella* in rodents have focused on *Apodemus flavicollis* and *Apodemus sylvaticus* and the *Myodes* and *Microtus* voles (2–5). These have a characteristic *Bartonella* microbiota, dominated by *B. grahamii*, several clades of *B. taylorii*, *B. doshiae*, and *B. birtlesii*. *Apodemus agrarius*, also a common rodent in this region, has been almost ignored. This mouse recently migrated into Europe (6) from Central Asia and can therefore give insights into the evolution and phylogeography of zoonotic pathogens within Eurasia.

*Apodemus agrarius* mice were captured at Wrocław Mokry Dwor (WMD; 51°04'57"N, 17°06'14"E) and Ruda Milicka (RM; 51°31'56"N, 17°20'12"E) during spring and autumn of 2009 to 2011, and whole blood collected into 0.001 M EDTA. Infection with *Bartonella* spp. was detected by PCR amplification of *gltA* (7). Other housekeeping genes, including *ribC*, *groEL*, and the *virB5* locus, were amplified as described previously (2, 8). PCR products were sequenced, and phylogenetic analysis carried out using MEGA 5.10 (9). Of 94 *A. agrarius* mice (77 from RM and 17 from WMD), 36 (38.3%) were infected with *Bartonella*. The prevalence was higher in WMD than in RM (52.9% and 35.1%, respectively).

Six *Bartonella* sequences were analyzed in detail (Fig. 1). Based on *gltA* (Fig. 1A), 2 were identical to the *B. grahamii*-type isolate (accession number Z70016) and were not studied further. Three belonged to *B. taylorii* clades: 54-WMD and 34-RM showed 100% homology to sequences with GenBank accession numbers GU338950 and JQ694004, and 40-RM to sequences with accession numbers JQ694005 and GU338962. One (78-RM) was identical to *Bartonella* from *A. agrarius* in Slovakia (accession number DQ155392) and created a sister clade to *Bartonella elizabethae*, which normally infects rats.

Sequencing of *virB5* revealed 5 variants (Fig. 1B). One (39-WMD) was typical for *B. grahamii* (8, 10), and two (40-RM and 36-WMD) were most similar to *B. birtlesii* (98.9% and 100% homology to the sequences with accession numbers AKIP01000002 and AIMC01000045). The 78-RM *Bartonella* sequence, most similar to *B. elizabethae* at *gltA*, also grouped with this species at *virB5* but with low homology (88.3% to the sequences with accession numbers AIMF01000048 and AILW01000028). *groEL* was also most similar to *B. elizabethae* but again with low homology

(92.7%). Sequences 54-WMD and 34-RM, which by *gltA* were *B. taylorii*, were identical at *virB5*, forming a sister group to AR 15-3 (GenBank accession number FN645479), isolated from an American squirrel, *Tamiasciurus hudsonicus*, imported into Japan (11). The *ribC* gene of these strains also grouped with AR 15-3.

The *Bartonella* species from *A. agrarius* therefore include *B. taylorii* and *B. grahamii*, the typical species infecting rodents in this region. However, strains resembling *B. elizabethae* were also collected, while two others had *ribC* and *virB5* sequences that were closest to a *Bartonella* from the American squirrel *Tamiasciurus hudsonicus*. *Bartonella elizabethae* is a worldwide rat pathogen, and the presence of a *B. elizabethae*-like *Bartonella* from *A. agrarius* could be explained by cross-infection from a rat-infecting form. However, this genotype is 5% different from *B. elizabethae* at the loci sequenced, and an identical isolate has been collected elsewhere from *A. agrarius* (GenBank accession number DQ155392). We therefore hypothesize that this clade is specific to *A. agrarius*, brought into Europe by the westward expansion of the host. Similarly, sequences most similar to those from a *Bartonella* species infecting American squirrels can best be explained by the westward migration of *A. agrarius*, bringing Asian *Bartonella* clades into western Europe. These clades clearly recombine freely, and the form most similar to isolate AR 15-3 is strongly introgressed with *B. taylorii*, having an AR 15-3-like *virB5* and *ribC* and a *B. taylorii* *gltA* sequence. Recombination has strongly influenced other collected strains. One with *B. grahamii* *gltA* had *virB5* identical to that of *B. birtlesii*, while another with *B. taylorii* *gltA* had *virB5* very similar and *groEL* identical to those of *B. birtlesii*. This combination has been noted previously from *Apodemus flavicollis* in Northeast Poland (2).

We would interpret these results as evidence that *A. agrarius* brought a central Asian *Bartonella* microbiota with it during its expansion into Western Europe and that traces of this microbiota can still be discerned, despite widespread introgression into *B.*

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Address correspondence to Joanna Hildebrand, joanna.hildebrand@microb.uni.wroc.pl.

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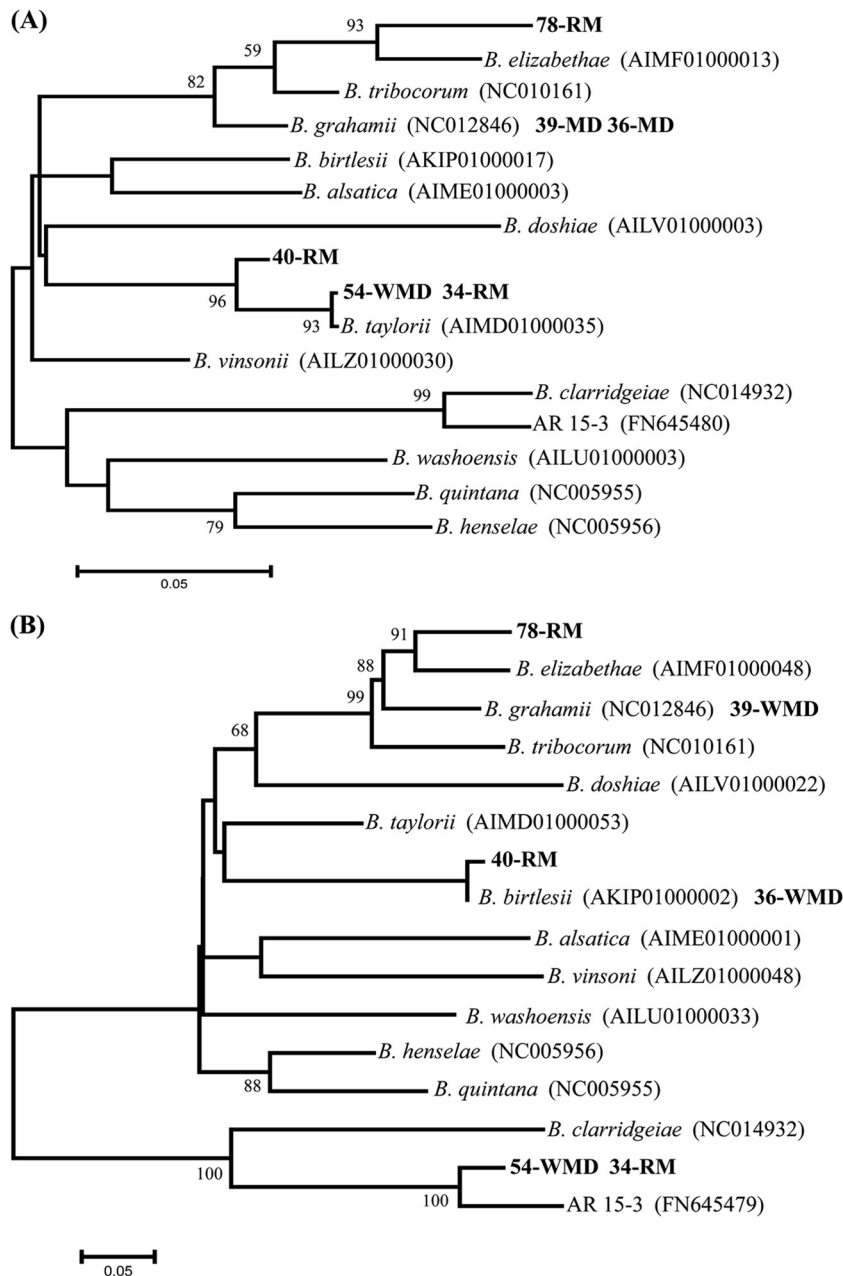


FIG 1 Maximum likelihood trees of (A) *gltA* gene fragment (Tamura 3-parameter model with gamma distribution) and (B) *virB5* gene (general time reversible model with gamma distribution) for analyzed strains of *Bartonella* (boldface). Accession numbers of reference sequences obtained from GenBank are shown.

*taylorii* and *B. grahamii*. Key questions are the extent of host specificity shown by *Bartonella* clades and the barriers which may exist to recombination and the generation of novel variants. Numerous studies (e.g., references 1, 2, and 12) have led to disparate conclusions. On one hand, a strong association between specific *Bartonella* strains and certain rodents occurs, including *B. elizabethae* in rats or *B. washoensis* in squirrels (13, 14). On the other hand, *B. grahamii* has been isolated from rodents that include *Apodemus*, *Microtus*, and *Myodes* species (15, 16). A key element in host specificity appears to be the *virB5* infectivity gene, which tracks host identity much more closely than other housekeeping genes (8). It is interesting to note that the *A. agrarius*-infecting strains can best

be differentiated by their distinct *virB5* genes, while other housekeeping genes have become introgressed with those of *B. grahamii* and *B. taylorii*. A further point to note is that the greatest diversity of *Bartonella* clades and of recombinant strains can be found within open, regenerating grassland habitats, the preferred habitat of *A. agrarius*, rather than within forest (2).

The expansion of *A. agrarius* west from Central Asia represents one of the most dramatic range extensions of a small mammal without human assistance; the expansion is complex and may have been made up of several phases (17), but most European records are less than 10,000 years before present (BP) (18), and the species did not occur in Poland before ca. 1,000 years BP (6). The

role of this rodent as a link between rodent pathogen communities of the east and west Palaearctic region has been ignored by epidemiologists and evolutionary biologists, despite the potential importance of this species in the appearance of novel recombinant *Bartonella* clades within Western Europe.

**Nucleotide sequence accession numbers.** *virB5* gene sequences obtained in this study were deposited in the GenBank database under accession numbers [KF051532](#) (40-RM), [KF051533](#) (54-WMD), and [KF051534](#) (78-RM).

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