

BIODIVERSITY, MAMMALIAN EVOLUTION, AND DISEASE SURVEILLANCE IN CENTRAL ASIA

P. A. Larsen¹, H. N. Meeks¹, J. P. Carrera¹, V. J. Swier¹, B. J. Briggs², J. Hay², R. Hewson³, A. T. Junushov⁴, O. N. Gavrilova⁵, I. Breining⁵, C. J. Phillips¹, and R. J. Baker¹

¹Department of Biological Sciences, Texas Tech University, Lubbock TX; ²Department of Microbiology and Witebsky Center for Microbial Pathogenesis and Immunology, University at Buffalo, Buffalo, New York; ³Public Health Affairs, Centre for Applied Microbiology and Research, Health Protection Agency, Porton Down, Salisbury, UK; ⁴Biotechnology Institute, National Academy of Sciences of the Kyrgyz Republic, Bishkek, Kyrgyz Republic; ⁵Republican Center of Quarantine and Especially Dangerous Infections, Ministry of Healthcare of the Kyrgyz Republic, Bishkek, Kyrgyz Republic

Introduction

The Central Asian country of the Kyrgyz Republic is located in the mountainous regions northwest of China, north of Tajikistan, and south of Kazakhstan (Fig 1). Although approximately 100 species of mammals are listed as having distributions in the Kyrgyz Republic (Vorobeev and van der Ven 2003 and Wilson and Reeder 2005), little is known about the geographic and ecological ranges of these species. Furthermore, the evolutionary and biogeographic histories of mammalian populations and their associated zoonotic viruses within the Kyrgyz Republic are poorly understood. We conducted a field trip in June, 2007 to collect species of small mammals distributed throughout the country and to survey for zoonotic viruses. This effort was also designed to establish a collaborative relationship with the government of the Kyrgyz Republic and to train Kyrgyz scientists in classical field methods and in the creation and maintenance of appropriate databases. During the three-week expedition voucher specimens, ectoparasites, and tissue samples were prepared from 185 specimens of rodents and shrews representing approximately 11 species (Table 1). Four main collecting localities were sampled (Fig 1). Subsequent to this collecting effort, we molecularly identified species by analyzing DNA sequence data (see Phylogenetics and Phylogeography section).

Table 1. Species collected during the 2007 Sowell Expedition to the Kyrgyz Republic.

<i>Alticola argentatus</i>	2
<i>Apodemus pallipes</i>	79
<i>Apodemus agrarius</i>	11
<i>Crocicidura siberica</i>	10
<i>Crocicidura leucodon</i>	1
<i>Dryomys nitedula</i>	12
<i>Microtus cf. juldaschi</i>	39
<i>Mus musculus</i>	3
<i>Myodes sp.</i>	1
<i>Rattus pyctoris</i>	26
<i>Rattus norvegicus</i>	1
~11 species	185



Apodemus pallipes



Crocicidura siberica



Irina Breining and Carleton J. Phillips



Microtus cf. juldaschi



Field party of the 2007 Sowell/CRDF Expedition to the Kyrgyz Republic: Sary Chelek



Asankadyr T. Junushov and Juan P. Carrera

From 18 to 29 June 2007 a Sowell/CRDF Expedition to the Kyrgyz Republic (Kyrgyzstan) was conducted by Texas Tech University, SUNY Buffalo School of Medicine, Ministry of Defense: Porton Down of the United Kingdom, the National Academy of Sciences of the Kyrgyz Republic, and the Republican Center for Quarantine and Especially Dangerous Diseases of the Kyrgyz Republic. Goals of this expedition were to: 1) generate preliminary data on species of small mammals distributed throughout the country and 2) survey collected mammals for zoonotic viruses. We prepared vouchers and collected tissues from 185 specimens of rodents and shrews from 4 collecting localities. Prior to this collecting effort, only 8 specimens of mammals from the Kyrgyz Republic were listed in the MaNIS database as archived in accredited museums in the United States. We present phylogenetic and phylogeographic results from the rodent genera *Alticola*, *Apodemus*, *Microtus*, *Myodes*, and *Rattus* as well as the sorcid genus *Crocicidura*. Results indicate that the mammalian fauna of the Kyrgyz Republic has complex biogeographic connections with East Asia, South Asia, and East Europe.

Kyrgyz Republic

The Kyrgyz Republic borders northern Tajikistan, northwestern China, eastern Uzbekistan, and southern Kazakhstan in Central Asia. The country covers an area of approximately 200,000 km² and 75% of it is 2,000 m above sea level (Vorobeev and van der Ven 2003). The Kyrgyz Republic is ecologically complex and ranges from semi-arid environments in the Tian Shan Mountains to coniferous forests at mid elevations and alpine meadows. Over 700,000 hectares of the country has been set aside for national parks, nature reserves, and hunting reserves. In 1998 and 2001 22% of the Kyrgyz Republic (4.3 million hectares) were declared a UNESCO Biosphere Reserve (Vorobeev and van der Ven 2003).

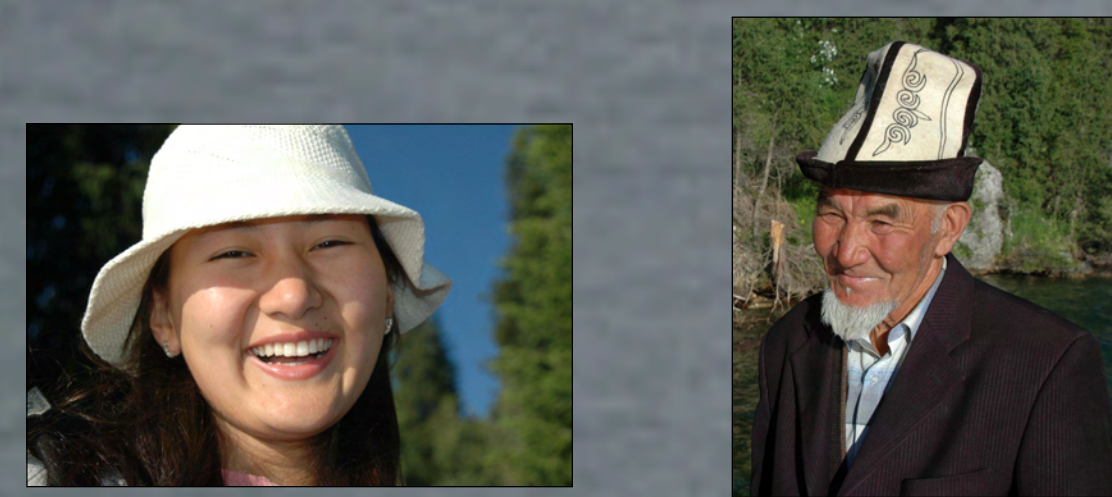


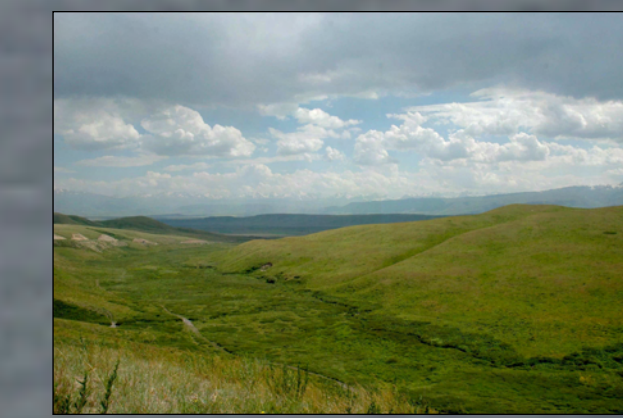
Figure 1. Map of the Kyrgyz Republic. Collecting sites of the 2007 Sowell Expedition are indicated by red triangles. Sites and collecting elevations were: 1 = Sary Chelek (1,897 m and 1,558 m), 2 = Cymbamp (2,270 m), 3 = Ala Archa (2,134m), and 4 = Tokmak (786 m).



Near Cymbamp



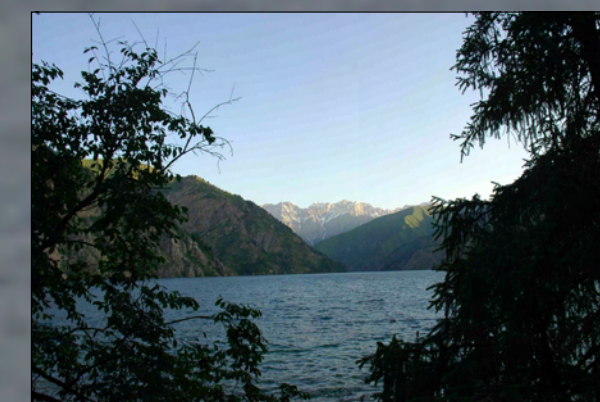
Ala Archa National Park



Cymbamp



Roger Hewson and John Hay



Sary Chelek



Olga N. Gavrilova and Carleton J. Phillips



Roger Hewson, John Hay, and Benjamin Briggs

Spotlight: *Apodemus agrarius*

Apodemus agrarius
(Striped Field Mouse)



Type Locality: Russia, Ulianovsk Obl.
Distribution: Palearctic and Oriental regions

Apodemus agrarius is widely distributed throughout Europe and Asia and is a reservoir of Hantaan and Dobrava viruses. Both of these viruses are within the genus *Hantavirus* and cause hemorrhagic fever with renal syndrome (HFRS) in humans. The geographic distribution of these viruses is similar to that of *A. agrarius*, with Hantaan virus occurring in northeast Asia and Dobrava virus in Eastern Europe and Asia. Hantaan and Dobrava viruses are closely related phylogenetically (Klempa et al. 2005). As such, knowledge of the biogeographic and evolutionary history of the reservoir species *A. agrarius* will provide valuable information regarding the distribution and evolutionary history of these zoonotic viruses. Our phylogenetic results indicate that specimens of *A. agrarius* collected in the Kyrgyz Republic are separated by a genetic distance of less than 2% from populations in South Korea and central China and less than 1% from populations in Estonia and the Czech Republic. Such molecular data in combination with voucher specimens and georeferenced collecting localities will provide essential information for future disease surveillance programs in the Kyrgyz Republic. For example, studies have shown a correlation between the distribution of *A. agrarius* and the incidence of HFRS in China (Figure 6; Yan et al. 2007).

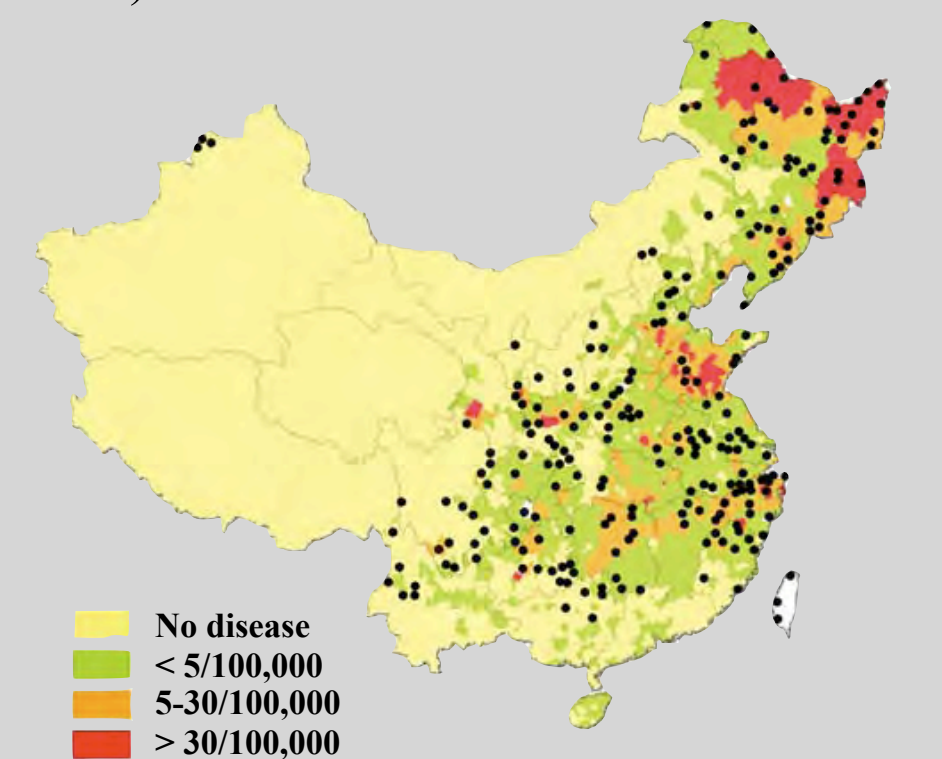


Figure 6. Reproduced from Yan et al. 2007. Incidence of hemorrhagic fever with renal syndrome in China. Black dots show collecting localities of *Apodemus agrarius*.

Karyotypes

We present karyotypes from species of *Microtus* and *Crocicidura*. Figure 2 depicts a karyotype from a specimen molecularly identified as *Microtus (Neodon) juldaschi*. This karyotype is composed of 54 acrocentric chromosomes, an acrocentric X, and a dot sized Y. The published karyotype of *M. juldaschi* has a diploid number of 54 and a fundamental number of 58 (including 6 metacentric chromosomes and a metacentric X) (Gileva et al. 1982). However, Mekada et al. (2002) reported three karyotypes for *M. carruthersi*, a species synonymized with *M. juldaschi* by Corbet (1978). One of the three karyotypes reported in Mekada et al. (2002) matches the karyotype of our specimen of *Microtus* from the Kyrgyz Republic. Given these preliminary results, further investigation into the taxonomic status of *carruthersi* is merited.

Figure 3 depicts a karyotype from a specimen of *Crocicidura siberica* collected in Sary Chelek (see Fig 1). This karyotype is composed of 38 autosomes (5 pairs of metacentrics and 14 pairs of acrocentrics), a metacentric X and acrocentric Y. Many *Crocicidura* species that have a diploid number of 40 also have a fundamental number of 50 (Biltueva et al. 2001). This differs from our karyotype of *C. siberica* that has a fundamental number of 48. Additional karyotypic studies are underway.



Figure 2. Karyotype of *Microtus juldaschi*.



Figure 3. Karyotype of *Crocicidura siberica*.

Phylogenetics and Phylogeography

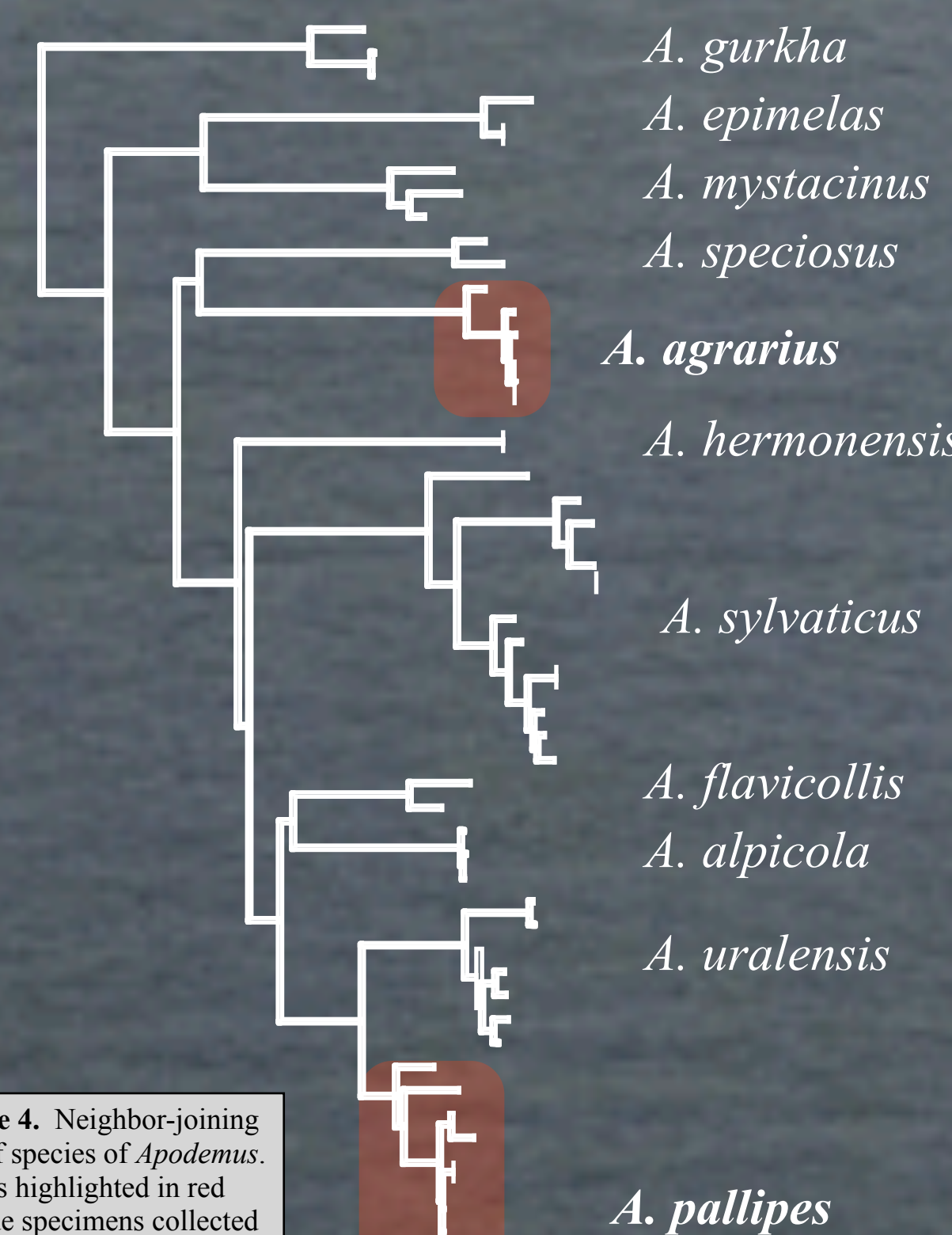


Figure 4. Neighbor-joining tree of species of *Apodemus*. Clades highlighted in red include specimens collected from the Kyrgyz Republic.

Phylogenetics and Phylogeography

To confirm field identifications of specimens collected in the Kyrgyz Republic, we analyzed sequence data from the mitochondrial cytochrome-b gene and compared these sequences to available GenBank sequences. Additionally, we examined the genetic diversity within conspecifics in the Kyrgyz Republic and, when possible, throughout the Palearctic and Oriental regions. We have generated sequence data from the rodent genera *Alticola*, *Apodemus*, *Microtus*, *Myodes*, and *Rattus* as well as the sorcid genus *Crocicidura*. Results indicate that rodent fauna of the Kyrgyz Republic is the result of recent invasions from East Asia, East and Central Europe, and South Asia. For example, specimens of *Apodemus agrarius* collected from northern regions of the Kyrgyz Republic are closely related to populations of *A. agrarius* from South Korea and southern China (genetic distance < 2%). The phylogenies of *Apodemus pallipes* and the shrew *Crocicidura siberica* also reflect recent invasions into the Kyrgyz Republic. The genetic distance within Kyrgyz populations of both species is less than 1%, despite the potential for geographic and ecological isolation (see Figs 4 and 5). Additionally, specimens molecularly identified as *A. pallipes* are ~2% in cytochrome-b gene variation from a specimen collected in Nepal. These data provide phylogeographic evidence of recent (< 20,000 ybp) colonization events. We hypothesize that zoonotic agents were introduced by colonization and will have affinities to pathogens at the geographic origin of colonizing mammals. Additional results for *Alticola*, *Microtus*, *Myodes*, and *Rattus* are below:

- Alticola*: Two specimens identified as *Alticola argentatus* collected from Sary Chelek and Ala Archa National Park (Fig 1) are separated by a genetic distance of 1.5% and are 8.5% from a single specimen identified as *A. argentatus* from Russia.
- Microtus*: We identified at least 2 species of *Microtus* in the Kyrgyz Republic, *Microtus gregalis* and *Microtus (Neodon) juldaschi*. (see discussion in Karyotypes section).
- Myodes*: a single specimen of *Myodes centralis* collected from Cymbamp (Fig 1) is separated by ~1% in cytochrome b gene variation from a specimen of *M. centralis* collected from southeastern Kazakhstan.
- Rattus*: our sample of *Rattus pyctoris* (collected from Sary Chelek; Fig 1) averaged a genetic distance ~6% from species of *Rattus* distributed in Southeast Asia (*Rattus tiomanicus* and *Rattus hoffmani*).

Crocicidura

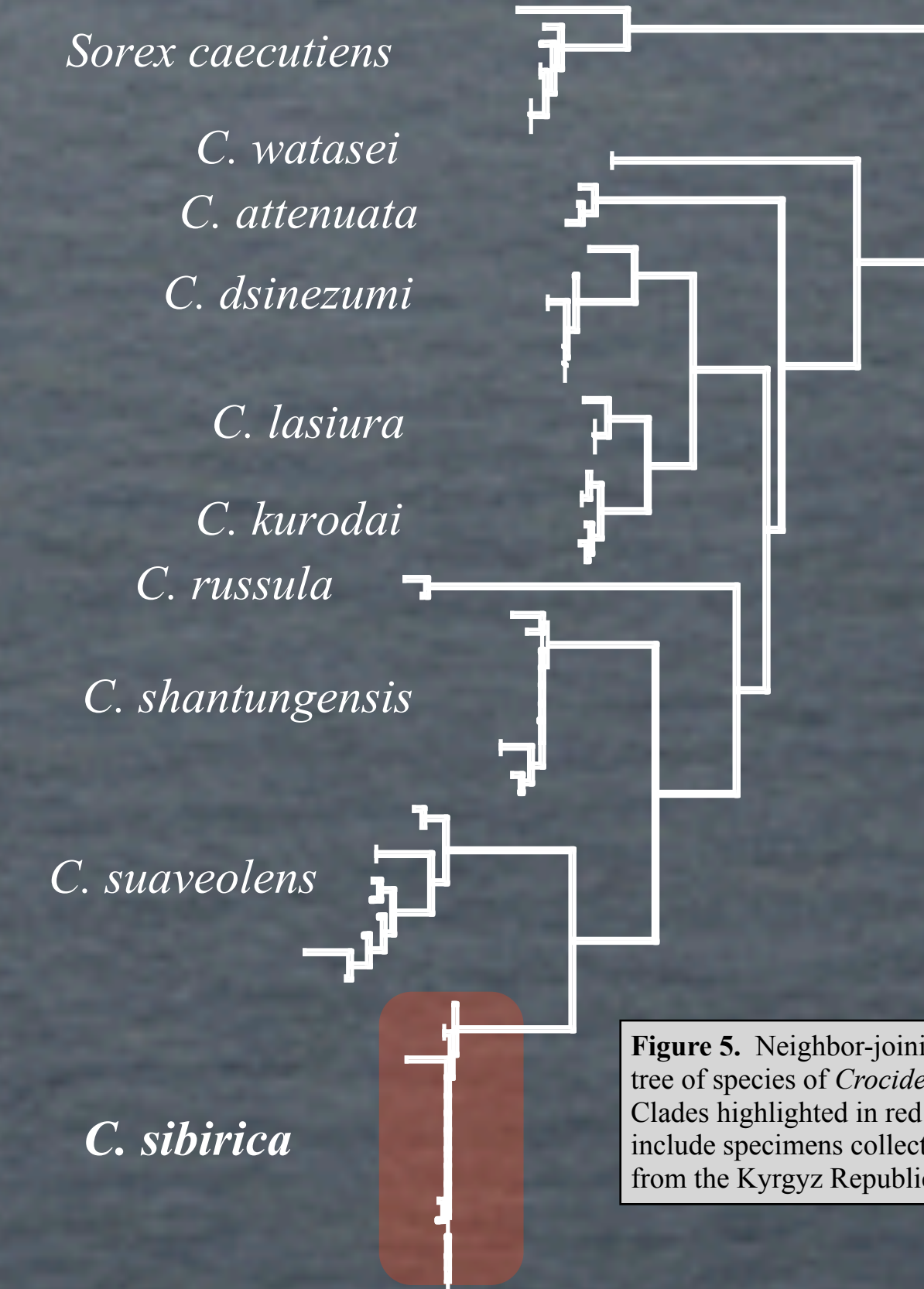


Figure 5. Neighbor-joining tree of species of *Crocicidura*. Clades highlighted in red include specimens collected from the Kyrgyz Republic.

Acknowledgements

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