**Data material for:**

**Bedbugs (Cimicidae) Evolved Before Their Bat Hosts**

**and Did Not Co-Speciate with Ancient Humans**

**Steffen Roth1,\*, Ondřej Balvín2, Michael T. Siva-Jothy3, Osvaldo Di Iorio4,\*\*, Petr Benda5, Omar Calva6, Eduardo I. Faundez7, Faisal Ali Anwarali Khan8, Mary McFadzen9, Margie P. Lehnert10, Richard Naylor11, Nikolay Simov12, Edward H. Morrow13, Endre Willassen1, Klaus Reinhardt3,14,\***

1University Museum of Bergen, PO Box 7800, N-5020 Bergen, Norway.

2Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Praha-Suchdol, Czech Republic.

3Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK.

4Entomología, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, 40 Piso, Pabellón II, Ciudad Universitaria C1428EHA, Buenos Aires, Argentina.

5Department of Zoology, National Museum (Natural History), Václavské nám. 68, 115 79 Praha 1, Czech Republic; Department of Zoology, Charles University, Viničná 7, 128 43 Praha 2, Czech Republic.

6Posgrado en Biociencias, Departamento de Investigaciones Científicas y Tecnológicas de la Universidad de Sonora, Luis Donaldo Colosio, 83000 Hermosillo, México.

7Laboratorio de Entomología, Instituto de la Patagonia, Universidad de Magallanes, Avenida Bulnes, 01855, Punta Arenas, Chile.

8Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia.

9Montana State University, Montana Institute on Ecosystems, Bozeman, 605 Leon Johnson Hall, MT 59717, USA.

10Department of Biology, Cuyahoga Community College, 1000 W Pleasant Valley Road, Parma, OH 44130, USA.

11CimexStore, Prior’s Loft, Coleford Road, Tidenham, Chepstow, Monmouthshire, NP16 7JD, UK.

12National Museum of Natural History, Bulgarian Academy of Sciences, 1 Tzar Osvoboditel Blvd, 1000 Sofia, Bulgaria.

13Evolution, Behaviour, and Environment Group, School of Life Sciences, University of Sussex, Brighton BN1 9QG, UK.

14Applied Zoology, Department of Biology, Technische Universität Dresden, Helmholtzstrasse 10, 01069 Dresden, Germany.

\* Correspondence. Steffen.Roth@uib.no (S.R.), Klaus.Reinhardt@tu-dresden.de (K.R.)

\*\* Deceased.

**a)**

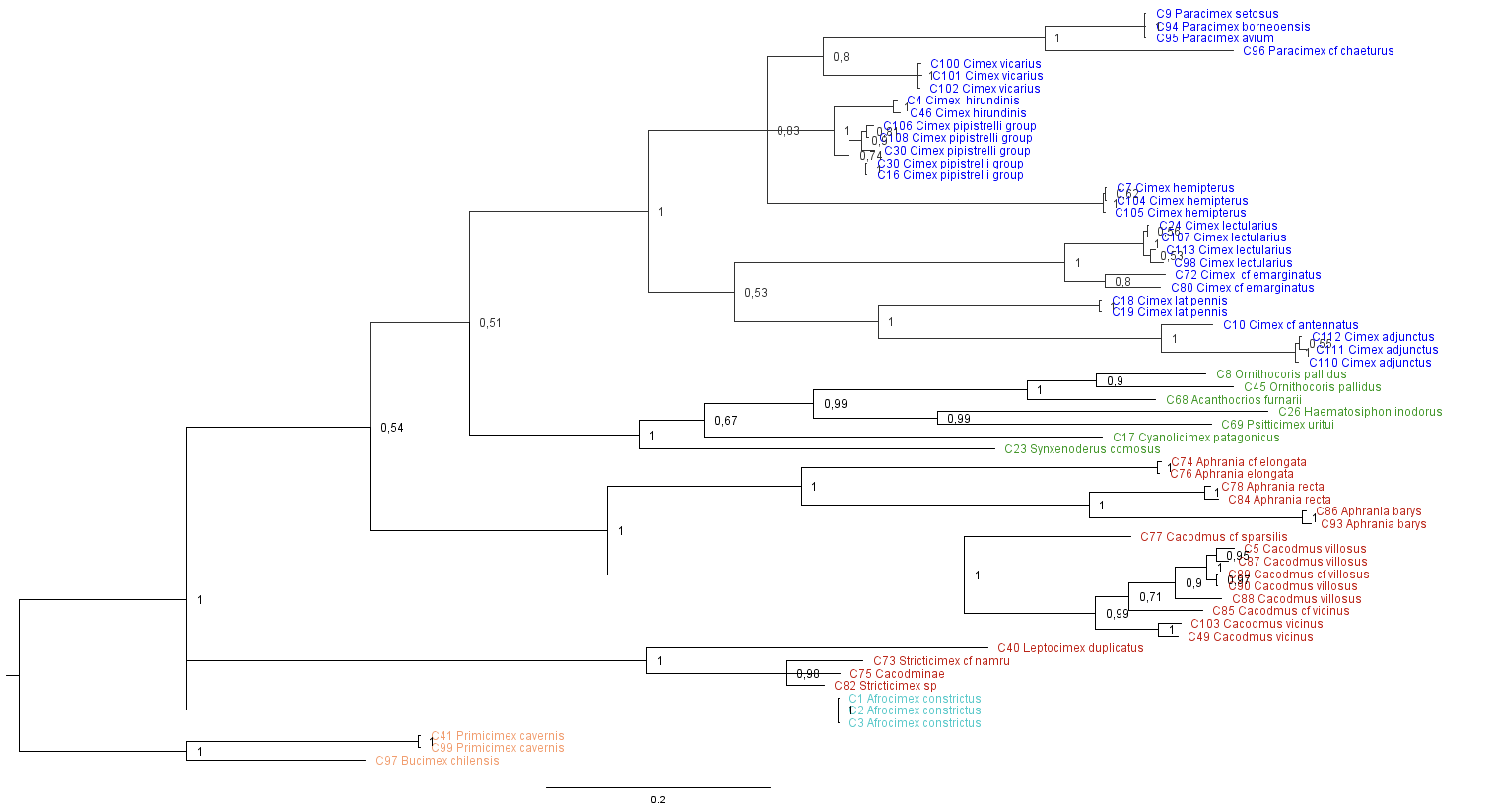


**b)**



**Roth *et al.* Figure: GBlock alignment tests for trees using strict and relaxed models. Related to Figure 1.** Neighbor Joining (NJ) tree for the combined data set with original alignment set and GBlocks data set with tree strict (a) and relaxed (b) model using default settings of Gblocks V.0.91b [48]. NJ analysis was performed in MEGA v.6 [47]. NJ analysis using strict (a) and relaxed GBlock alignments (b) of all molecular markers separately showed no significant effect of alignments and no need to eliminate poorly aligned positions and divergent regions, except some outgroup taxa. The original alignment data set was used for further analysis. Samples C41 and outgroup taxa *Curalium cronini* were removed from this analysis because of missing sequences.

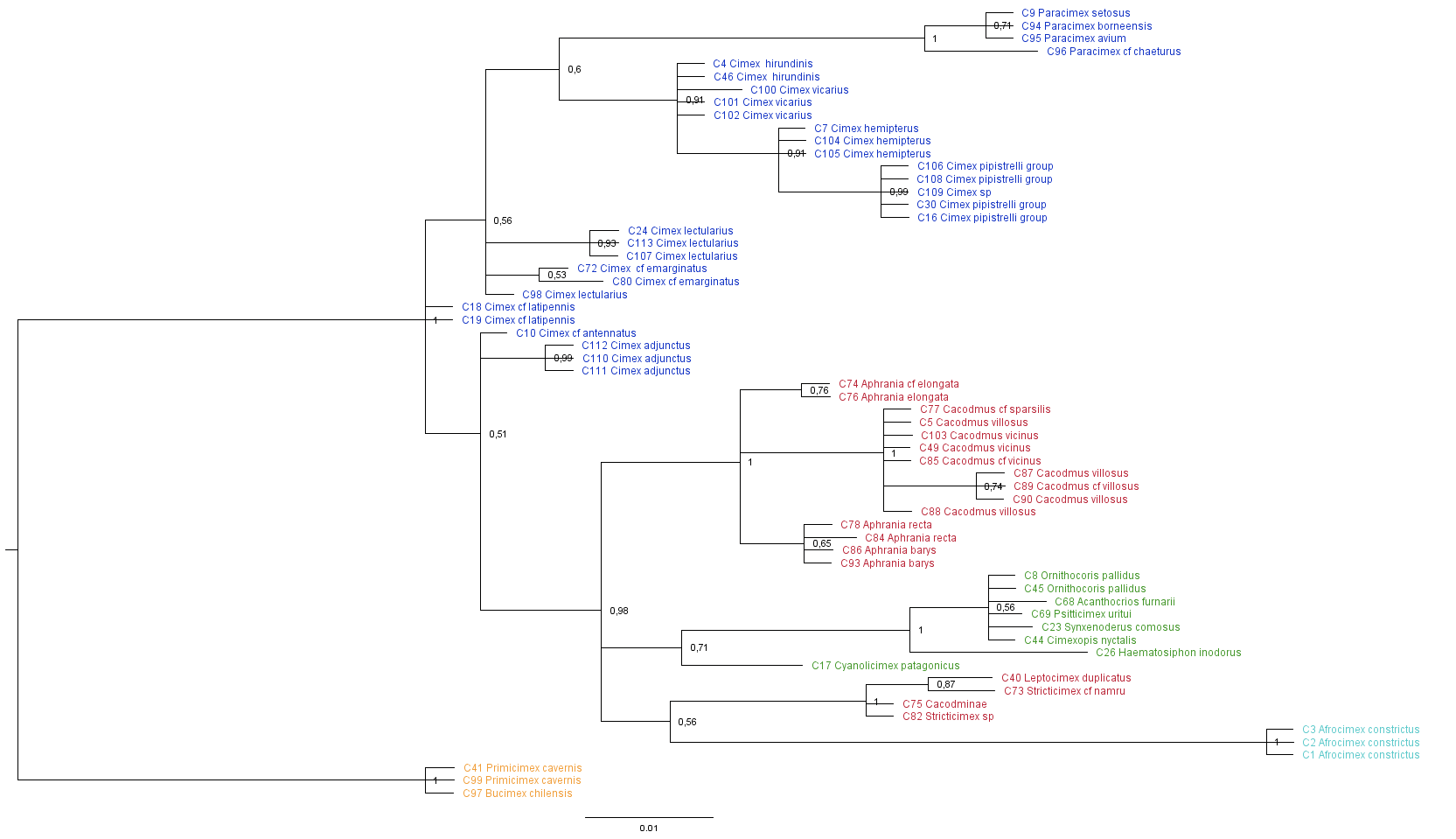
**a)**



**b)**



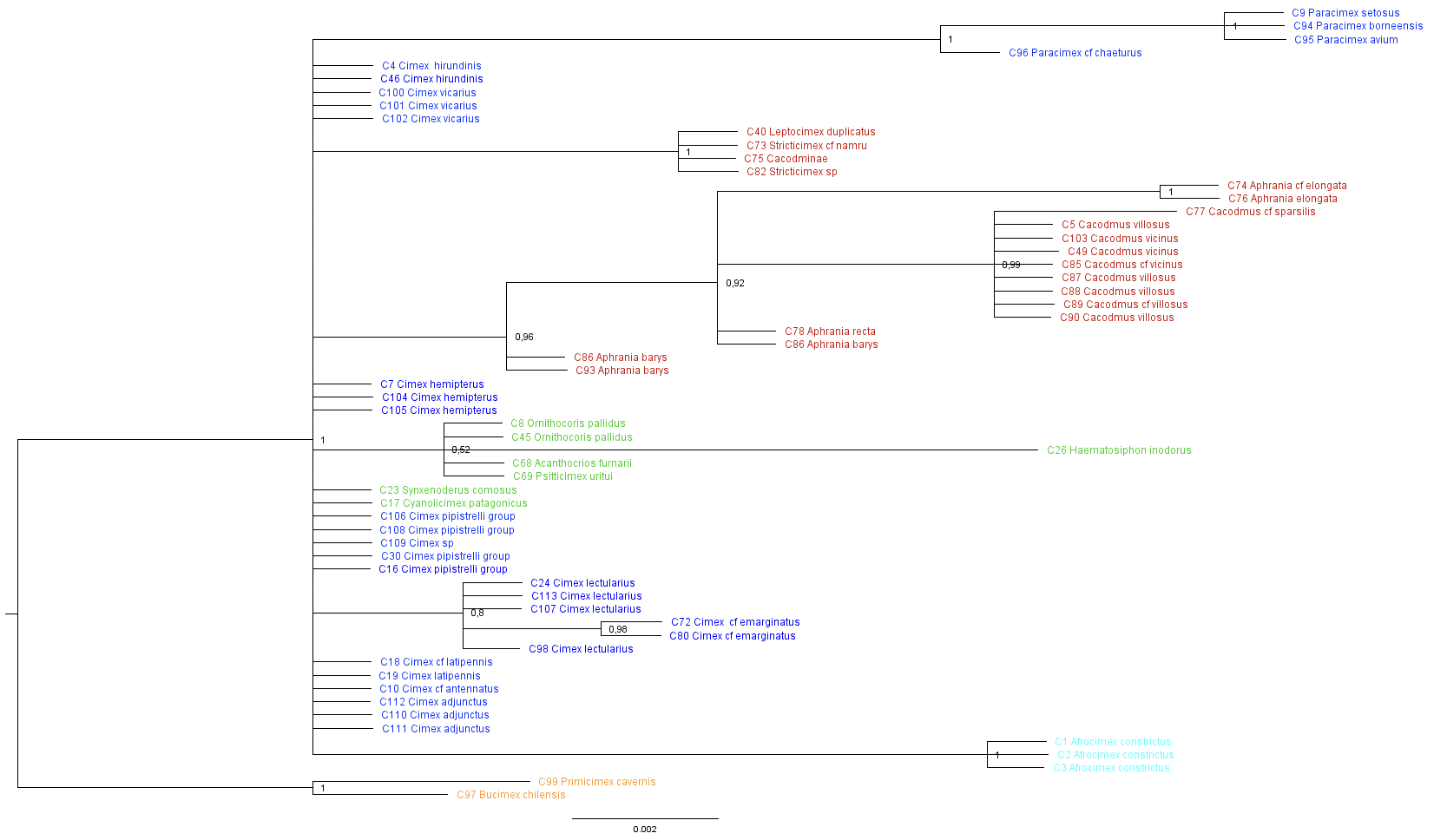
**c)**



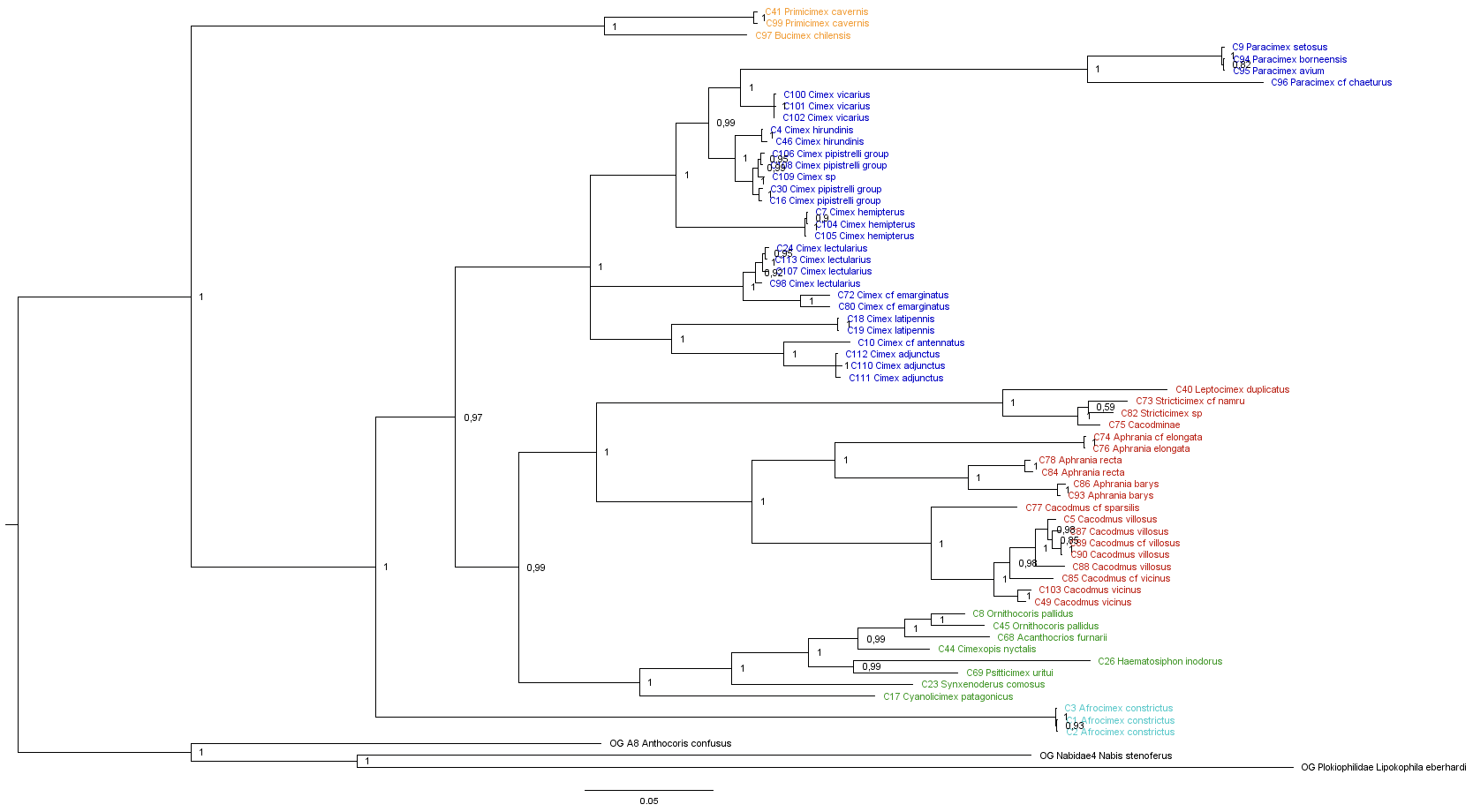
**d)**



**e)**



**Roth *et al.* Figure: Bayesian analysis (BA) of phylogenetic relationships of the Cimicidae inferred from individual genes. Related to Figure 1.** The analysis was carried out using MrBayes v.3.2.1 [44] for individual genes, substitution models were as chosen in the combined data set analysis (Table S3). Details for settings in MrBayes for single genes BA can be requested from the authors. Consensus trees inferred from the single gene fragments (18S rDNA part1 and part 2, COI, 16S rDNA, 28S D3 rDNA - Table S3) shows their different phylogenetic information but also that single gene analyses are unable to recover phylogenetic relationship.



**Roth *et al.* Figure: MrBayes consensus tree using one representative species of the closest phylogenetic taxa (Anthocoridae, Nabidae and Plokiophillidae) within our outgroup sampling. Related to Figure 1.** The tree is a Bayesian consensus tree based on four genes (see STAR Methods). Numbers beside the nodes indicate posterior probability values. Topology and support value of the Cimicidae clades did not change due to different outgroup sampling (see Figure 1).



**Roth *et al.* Figure: Maximum Likelihood analysis of the combined molecular data set. Related to Figure 1.** The Maximum Likelihood analysis confirmed the results of the Bayesian Analysis but the sister relationship of Cacodminae and Haematosiphoninae was not resolved. There was also low support for the node (*Leptocimex*+*Stricticimex*) + (*Aphrania*+*Cacodmus*).