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Short Report: Isolation and Phylogenetic Analysis of Batai Virus, Germany

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Abstract. A molecular survey including 16,057 mosquitoes captured in Southwest Germany during the summer of 2009 showed the presence of Batai virus (BATV) in *Anopheles maculipennis* sensu lato. Until this survey, there was no evidence for circulation of BATV in Germany. Analysis of partial S, M, and L segments showed that the sequences from all three segments were most closely related to BATV, indicating that the virus has not undergone reassortment. Phylogenetic analysis revealed a close relationship of the isolated BATV strain from Germany with strains from Slovakia, Ukraine, and Russia.

Batai virus (BATV) belongs to the genus *Orthobunyavirus* of the family *Bunyaviridae* and is an arthropod-borne (arbo) single-stranded RNA virus that is widely distributed in Africa, Asia, and Europe.[1] In Europe, BATV is transmitted by the zoophilic mosquitoes *Anopheles maculipennis* s.l. and *An. claviger* and less often, by *Ochlerotatus* spp.[2] Orthobunyaviruses are able to extend their genetic diversity by reassortment of genome segments during a mixed infection.[3] Ngari virus (NRIV) was shown to be a genetic reassortant with S and L RNA segments from Bunyamwera virus and an M RNA segment from BATV.[3,4] In contrast to BATV, which is the etiologic agent of rather mild febrile illness in humans and animals,[1] the reassortant NRIV was found to be associated with hemorrhagic fever outbreaks in East Africa.[5] Until now, there was no evidence for circulation of BATV in Germany.[6]

Mosquitoes were trapped during the summer of 2009 at three sites (Kühkopf: 49°49′N 8°24′E; Waghäusel: 49°15′N 8°31′E; Weinheim: 49°33′N 8°40′E) in Southwest Germany with CO_2-baited encephalitis vector surveillance traps (BioQuip, Compton, CA) and gravid traps (John W. Hook Company, Gainesville, FL). Mosquito identification (species and sex) as well as virus isolation and RNA extraction was performed as described recently.[7] Extracted RNA was analyzed by a newly designed BATV real-time reverse transcription polymerase chain reaction (RT-PCR) using the primers BATAI-Fwd (5-GCTGGAAGGTTACTGTATTTAATAC-3; nucleotide [nt] positions 268–292 according to GenBank accession number X73464) and BATAI-Rev (5-CAAGGAA TCCACTGAGTCTGTG-3; nt positions 345–366) and probe BATAI-P (5-FAM-AACAGTCCAGTTCCAGACGATGG TC-BHQ-1-3; nt positions 312–336 [FAM = 6-carboxyfluorescein; BHQ-1 = black hole quencher 1]). The target was a 99-nt-long region of the S segment. Real-time RT-PCR was performed with a Quanti-Tect probe RT-PCR kit according to the manufacturer's protocol (Qiagen, Hilden, Germany).

There were 16,057 female mosquitoes[7] captured and pooled according to species (25 mosquitoes per pool). BATV RNA was detected by real-time RT-PCR in 1 of 643 pools tested (pool number 52.3). This pool included mosquitoes of the species complex *An. maculipennis* s.l. Inoculation of Vero cells with pool 52.3 caused cytopathic effect (CPE) after 48 hours,

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Table 1
Primers used to amplify and sequence the partial BATV S, M, and L segments

Segment primer	Sequence (5′ to 3′)	Nucleotide position*	Amplicon size (nt)	Temp. (°C)
S segment primer				
BATAIS1F	TGGAATTCAATGATGTCGCTGCTAAC	79–104	344	60
BATAIS1R	TATAATCAATTTTTCCGGGTCACTCACTTT	393–422	344	60
BATAIS2F	TTGGGGGCTGGAAGGTTACTGT	262–283	363	58
BATAIS2R	TATATCTTTGGCGCATGGTCTTCTCC	599–624	363	60
BATAIS3F	CTGGGCAGATGGGGAGGAG	467–485	451	56
BATAIS3R	AAACTGCAATGCTTCAAAAACAAT	894–917	451	54
M segment primer				
BATAIM2F	TGTGGCCTAGCATATCACCCTTTCA	793–817	1,407	60
BATAIM2R	AGACCGGTGATGATGATCTGTAACCTCTA	2,171–2,199	1,407	60
BATAIM3F	CCTGGGGAAGCATTGTGATTACT	1,704–1,726	525	56
BATAIM3R	CTAGCCAGCGACTCTTGCCTTCC	2,206–2,228	525	60
BATAIM4F	GTCGCTGGCTAGTGCTACCTCTGG	2,217–2,240	510	60
BATAIM4R	CTGATTATTGTCGGATTTATTGGGAACCT	2,698–2,726	510	60
BATAIM5F	AAAGGTTCCCAATAAATCCGACAA	2,696–2,719	525	57
BATAIM5R	CAAATTCTTCACATCCCCAACGACTA	3,195–3,220	525	59
BATAIM6F	AGAATTTGGGTGCCTTGCTGTCA	3,213–3,235	874	59
BATAIM6R	AGATGTTTGGTCCCCTGTGCTTATTT	4,061–4,086	874	59
L segment primer				
BATAIL1F	GATGGCGATTTCCTGATTAT	283–302	711	48
BATAIL1R	TGACCCCAAGAGTTTCCTATTAT	971–993	711	51

*The nucleotide positions are given according to the numbering in the Batai virus reference strain MM2222 (GenBank accession numbers X73464, AB257763, or AY822469).

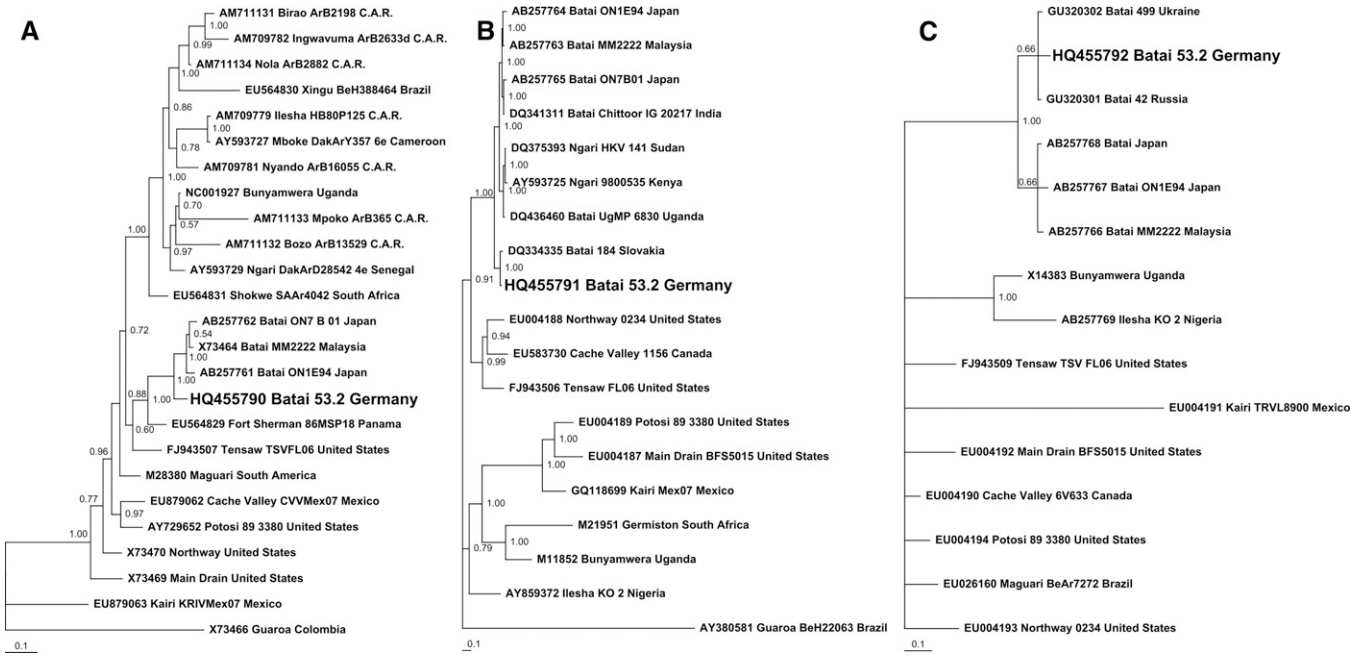

FIGURE 1. Bayesian phylogenetic tree of selected orthobunyaviruses based on partial S (**A**; length = 838 nucleotides), M (**B**; length = 3,152 nucleotides), and L segment (**C**; length = 200 nucleotides) sequences. Each sequence is identified by GenBank accession number, virus name, strain designation, and strain origin. Phylogenetic analysis was performed using MrBayes 3.0 program.[8] Three heated chains and a single cold chain were used in all Markov Chain Monte Carlo (MCMC) analyses, which were run for 1,000,000 generations, sampling one tree every 100 generations. Trees obtained before convergent and stable likelihood values were discarded (i.e., a 2,500 tree burn-in). Four independent runs, each started from different randomly chosen trees, were performed to assess convergence. Posterior probabilities for nodes were assembled from all post–burn-in trees (i.e., 30,004 trees per analysis). Posterior probabilities are shown on each branch. Scale bar indicates number of nucleotide substitutions per site.

and BATV-specific RNA was detected by real-time RT-PCR in the supernatant of the infected cell culture after five passages. Moreover, electron microscopy of the infected cell culture showed enveloped viral particles measuring ~90 nm in diameter. For phylogenetic analysis, partial S, M, and L segments (838, 3,152, and 200 nt, respectively) of the isolate (called BATV strain 53.2) were amplified by RT-PCR and sequenced using the primers listed in Table 1. The sequences from all three segments were most closely related to BATV (Figure 1A–C), indicating that the virus has not undergone reassortment. The nucleotide identity between the German BATV strain and BATV strains from other regions ranged from 91.9% to 92.1% in the S segment, from 84.8% to 96.2% in the M segment, and from 84.3% to 93.8% in the L segment. Phylogenetic analysis by Bayesian inference revealed a close relationship of BATV strain 53.2 from Germany with strain 184 from Slovakia (Figure 1B), strain 499 from Ukraine, and strain 42 from Russia (Figure 1C).

In conclusion, a molecular survey in mosquitoes showed the occurrence of BATV in the south of Germany. The BATV infection rate in the mosquito population seems to be low, because only 1 of 19 *An. maculipennis* s.l. pools tested positive. The infection rate was even lower when pools of other known BATV vectors, *An. claviger* and *Ochlerotatus* spp., were included in the analysis. BATV circulates in a mosquito to mammal cycle in agro-ecosystems.[1] The Waghäusel trapping site is indeed a typical agro-ecosystem, with pig, sheep, and horse farms. BATV may cause a mild illness among sheep, but also, stillbirth and congenital abnormalities have been reported in association with BATV infections.[9] In addition, BATV was isolated from a sentinel cattle herd in Okinawa, Japan.[3] Moreover, the virus may cause human disease with influenza-like symptoms.[10] Therefore, further studies have to be conducted to estimate the veterinary and medical importance of BATV in the affected area.

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