Genetic Diversity and Phylogeography of *Thottapalayam thottimivirus* (Hantaviridae) in Asian House Shrew (*Suncus murinus*) in Eurasia

Fuka Kikuchi, Keita Aoki, Satoshi D. Ohdachi, Kimiyuki Tsujiya, Masaharu Motokawa, Takamichi Jogahara, Nguyễn Trường Sơn, Saw Bawm, Kyaw San Lin, Thida Lay Thwe, Chandika D. Gamage, Marie Claudine Ranorosoa, Hasmahzaiti Omar, Ibnu Maryanto, Hitoshi Suzuki, Keiko Tanaka-Taya, Shigeru Morikawa, Tetsuya Mizutani, Motoi Suzuki, Richard Yanagihara and Satoru Arai

Mfur and cricetid rodents were previously believed to be the principal reservoir hosts of hantaviruses. Recently, however, multiple newfound hantaviruses have been discovered in shrews, moles, and bats, suggesting a complex evolutionary history. Little is known about the genetic diversity and geographic distribution of the prototype shrew-borne hantavirus, *Thottapalayam thottimivirus* (TPMV), carried by the Asian house shrew (*Suncus murinus*), which is widespread in Asia, Africa, and the Middle East. Comparison of TPMV genomic sequences from two Asian house shrews captured in Myanmar and Pakistan with TPMV strains in GenBank revealed that the Myanmar TPMV strain (H2763) was closely related to the prototype TPMV strain (VRC66412) from India. In the L-sector tree, on the other hand, the Pakistan TPMV strain (PK3629) appeared to be the most divergent, followed by TPMV strains from Nepal, then the Indian-Myanmar strains, and finally TPMV strains from China. The Myanmar strain of TPMV showed sequence similarity of 79.3–96.1% at the nucleotide level, but the deduced amino acid sequences showed a high degree of conservation of more than 94% with TPMV strains.
INTRODUCTION

More than four decades following the original isolation of *Thottapalayam thottimvirus* (TPMV) from an Asian house shrew (*Suncus murinus*) in southern India (Carey et al., 1971), other genetically distinct hantaviruses (family *Hantaviridae*) have been detected in shrews (order Eulipotyphla, family Soricidae) of multiple species in Europe, Asia, Africa, and North America. To date, these include Imjin thottimivirus in the Ussuri white-toothed shrew (*Crocidura lasiura*) (Song et al., 2009), Kilimanjaro thottimivirus in the Kilimanjaro mouse shrew (*Myosorex zinki*) (Kang et al., 2014), Uluguru thottimivirus in the geeta mouse shrew (*Myosorex geata*) (Kang et al., 2014), Tanganya orthohantavirus in the Therese's shrew (*Crocidura thersae*) (Klempa et al., 2007), Azagny orthohantavirus in the West African pygmy shrew (*Crocidura obscurior*) (Kang et al., 2011b), Jeju orthohantavirus in the Asian lesser white-toothed shrew (*Crocidura shantungensis*) (Arai et al., 2012), Bòwé orthohantavirus in the Doucet's musk shrew (*Crocidura doucettii*) (Gu et al., 2013), Cao Bàng orthohantavirus in the Chinese mole white shrew (*Anourusorex squamipes*) (Song et al., 2007c), Seewis orthohantavirus in the Eurasian shrew (*Sorex araneus*) (Song et al., 2007b), Ash River orthohantavirus in the masked shrew (*Sorex cinereus*) (Arai et al., 2008), Jemez Springs orthohantavirus in the dusky shrew (*Sorex monticolus*) (Arai et al., 2008), Kenkeme orthohantavirus in the flat-skulled shrew (*Sorex roboratus*) (Kang et al., 2010), Asikakala orthohantavirus in the Eurasian pygmy shrew (*Sorex minutus*) (Radosa et al., 2013), Yâkèshï orthohantavirus in the taiga shrew (*Sorex isodon*) (Guo et al., 2013), Quan Hu Shan orthohantavirus in the greater stripe-backed shrew (*Sorex cylindricauda*) (Zuo et al., 2014), Boginia orthohantavirus in the Eurasian water shrew (*Neomys fodiens*) (Gu et al., 2013), and Camp Ripley orthohantavirus in the northern short-tailed shrew (*Blarinna brevicauda*) (Arai et al., 2007). Data suggest that shrews, rather than rodents, may have served as the earlier mammalian hosts of hantaviruses (Song et al., 2007a; Kang et al., 2011a; Bennett et al., 2014; Yanagihara et al., 2014). That said, the evolutionary history of hantavirus expansion is still unclear. In particular, the genetic diversity and phylogeography of the prototype shrew-borne hantavirus (TPMV) warrants clarification.

Keywords: *Thottapalayam thottimvirus*, *Suncus murinus*, genetic diversity, phylogeography, shrew-borne hantavirus

MATERIALS AND METHODS

Ethics Statement

The guidelines of the American Society of Mammalogists (Kirkland, 1998; Sikes and Animal Care and Use Committee of the American Society of Mammalogists, 2016) were followed for trapping and euthanasia of shrews and for tissue collection and processing. And approvals were obtained from the Ministry of Agriculture and Rural Development in Vietnam and the Institutional Animal Care and Use Committee of the National Institute of Infectious Diseases to conduct the study (permission numbers: 108074, 111126, 112152, 115162, 118180).

Animals

Asian house shrews were collected, using Sherman live traps, during biological distribution surveys from 2011 to 2016 in Asia (Indonesia, Japan, Malaysia, Myanmar, Pakistan, Sri Lanka, Vietnam, Yemen) and Africa (Comoros, Madagascar, Tanzania) (Ohdachi et al., 2016) (Figure 1). Lung tissues, preserved in RNAlater® Stabilization Solution, were analyzed for hantavirus RNA by reverse transcription polymerase chain reaction (RT-PCR) (Arai et al., 2016a). Asian house shrews from Bangladesh (early-onset diabetes in *Suncus*: EDS) and Nepal (Kathmandu: KAT), which were being maintained for experimentation, were also used for host genetic analysis (Jogahara, 2016) (Supplementary Data S1).

RNA Extraction and cDNA Synthesis

Total RNA was extracted from RNAlater®-preserved lung tissues, using the GC series Magtration®-MagaZorb® RNA Common N Kit or MagDEA RNA 100 Kit (Precision System Science, Matsudo, Japan), and then reverse transcribed, using PrimeScript® II 1st strand cDNA Synthesis Kit (Takara Bio, Otsu, Japan) and oligonucleotide primer (OSM55F, 5′-TAGTAGTAGACTGC-3′), designed from the conserved 5′-ends of the S, M, and L segments of hantaviruses (Klempa et al., 2006, 2007; Song et al., 2007c; Arai et al., 2008, 2016a).

RT-PCR and DNA Sequencing

Nested primers for TPMV and other recently identified shrew-borne hantaviruses were used to initially screen tissues for hantavirus RNA (Song et al., 2009; Kang et al., 2011c; Gu...
et al., 2013). Thereafter, amplification of the full-length S-, M-, and L-genomic segments was attempted. Oligonucleotide primer sequences have been deposited as Supplementary Data S2. First- and second-round PCR was performed in 20-µL reaction mixtures, containing 250 µM dNTP, 2 mM MgCl₂, and 0.25 µM of each primer. LA Taq hot start version (Takara Bio) and AmpliTaq gold 360 DNA polymerase (Applied Biosystems, Foster City, CA, USA) were used at 1 U each for the first- and second-round PCR, respectively (Arai et al., 2016b). Initial denaturation at 94°C for 2 min was followed by two cycles each of denaturation at 94°C for 30 s, two-degree step-down annealing from 48 to 38°C for 40 s, and elongation at 68°C for 1 min, then 32 cycles of denaturation at 94°C for 40 s, annealing at 42°C for 40 s, and elongation at 68°C for 1 min, in a Veriti thermal cycler (Applied Biosystems) and Mastercycler X50 (Eppendorf, Hamburg, Germany) (Arai et al., 2008, 2012). Amplicons were treated with Exonuclease I and Shrimp Alkaline Phosphatase (New England Biolabs, Ipswich, MA, USA) for 30 min. DNA was sequenced directly using an ABI Prism 377XL Genetic Analyzer (Applied Biosystems) (Arai et al., 2007; Kang et al., 2011c).

**Genetic and Phylogenetic Analysis**

Partial S-, M-, and L-segment nucleotide and amino acid sequences, amplified from Asian house shrews, were aligned with available hantavirus sequences, using the ClustalW in BioEdit (Thompson et al., 1994). The degree of sequence homology was assessed by pair-wise comparisons (Kang et al., 2009, 2011a). Phylogenetic trees were constructed using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003), with the GTR+I+Γ model of evolution, as selected by using jModelTest version 2.1.7 (Darriba et al., 2012). Bayesian analysis consisted of 10 million Markov chain Monte Carlo generations to ensure convergence across two runs of six chains each, with average standard deviations of split frequencies <0.01 and effective sample sizes well over 100, resulting in consensus trees supported by posterior-node probabilities (Kang et al., 2009, 2011a). The co-evolutionary relationships between hantaviruses and their shrew and rodent reservoir hosts were analyzed by the comparative concordance between host and hantavirus cladograms in TreeMap 3b1243 (Charleston and Robertson, 2002; Kang et al., 2009; Arai et al., 2012).
mtDNA and Nuclear Genes Sequencing and Host Phylogeny

To verify the geographic diversity of Asian house shrews and to study their phylogenetic relationships, genomic DNA was extracted from lung tissue using the MagDEA® DNA 200 (GC) (Precision System Science). The entire 1,140-nucleotide cytochrome b (cyt b) gene of mitochondrial DNA (mtDNA), the 1,545-nucleotide cyt c oxidase subunit I (COI) gene and the recombinant activating gene 1 (RAG1) were amplified using the following primer sets: Cy-14724F (5′-TGAAAAAYCAYCGTTGT-3′) (Kodama et al., 2006) and RAG1-61F (5′-CTTTCCGCGGCTGCTCT-3′) (Kodama et al., 2009) and KOD multi-enzyme (Toyobo, Osaka, Japan), MammMt-5533F (5′-GCYTTGTSYTTYRATTACAGTYAA-3′)/MammMt-7159R (5′-GRRGTTTCAWW CCTYCCTYCTT-3′) (Arai et al., 2019) and Phusion enzyme (New England Biolabs), and newly designed primers RAG1-61F (5′-TCTGCACCYGATGAAATTCARCACC-3′)/RAG1-3139R (5′-CTCCCCATTGAAATCTTGGCTTCCC-3′) and KOD multi-enzyme, respectively. PCR was performed in 50-µL reaction mixtures, containing 200 µM dNTP and 1 U of KOD multi and Epi DNA polymerase or Phusion enzyme. Initial denaturation was at 95°C for 2 min, followed by two cycles each of denaturation at 95°C for 15 s, two-degree step-down annealing from 60 to 50°C for 30 s, and elongation at 68°C for 1 min 30 s, then 30 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 30 s, and elongation at 68°C for 1 min 30 s, in a Veriti thermal cycler (Arai et al., 2019). PCR products were purified by Mobispin S-400 (Molecular Biotechnology, Lotzzestrasse, Germany) and were sequenced directly (Arai et al., 2012, 2019). The models of host nucleotide evolution were selected under jModeltest version 2.1.7, the GTR+I+Γ model for host phylogenetic sequence set, the TrN+G for Cytb, TIM3+I for COI of and TPM2ulf +I models for RAG1 of Suncus sequence sets. The results of modtest were shown in Supplementary Data 3A–G. Host phylogenetic analysis also consisted of 10 million Markov chain Monte Carlo generations to ensure convergence across two runs of six chains each, with average standard deviations of split frequencies <0.01 and effective sample sizes well over 100, resulting in consensus trees supported by posterior-node probabilities.

RESULTS

Hantavirus Detection

In all but two of the 198 shrew lung tissue samples, multiple attempts to detect hantavirus RNA were unsuccessful (Table 1). The exceptions were one of 11 and one of three Asian house shrews from Pakistan (captured in Karachi: 24.947802 N, 67.122999 E) and Myanmar (captured near a cattle farm in Taung gyi, Shan state: 20.804169 N, 97.060360 E, detail in Supplementary Table 1), respectively, collected in 2013. Sequence analysis of the amplicons revealed TPMV. Amplification of the full-coding region of the S segment and the partial M and L segments was achieved for TPMV strain H2763 (Myanmar), while only partial L-segment sequences were obtained for TPMV strain PK3629 (Pakistan).

Nucleotide and Amino Acid Sequence Analysis

Analysis of the S-, M-, and L-segment sequences of TPMV strain H2763 from Myanmar indicated an overall genomic organization similar to prototype TPMV strain VRC66412 from India. The 1,506-nucleotide S-genomic segment encoded a nucleosacpid (N) protein of 435 amino acids, possibly starting at nucleotide position 68, and a 130-nucleotide 3′-non-coding region. The TPMV S-genomic segment, like that of other recently described hantaviruses in shrews, did not contain the hypothetical NSs open reading frame, typically found in hantaviruses harbored by cricetid rodents.

Hantavirus Phylogeny

TPMV strain H2763 from Myanmar appeared as one cluster in phylogenetic trees, based on the S-, M-, and L-segment sequences, using the Bayesian methods (Figure 2). The TPMV strain PK3629 from Pakistan also constructed one cluster in a tree based on the L segment. The phylogenetic trees suggested that the primordial strain of TPMV originated in northern India and surrounding countries, including Pakistan or Nepal (Figure 2).

Pair-Wise Alignment and Comparison

Pair-wise alignment and comparison of the S segment (1,506 nucleotides), M segment (2,382 nucleotides), and L segment (4,963 nucleotides) revealed that TPMV strain H2763 from Myanmar exhibited high sequence similarity to prototype TPMV strain VRC66412 from southern India. The TPMV strain PK3629 from Pakistan showed low nucleotide sequence similarity (79.3%) in the L segment, but the encoded amino acid sequences were highly conserved (94.1–99.2%) with TPMV strains from Nepal, India, Pakistan, and China (Table 2). Compared with representative hantaviruses from rodents, shrews, and bats, the TPMV strain from Myanmar differed by ~20–60% at the nucleotide and amino acid levels for each segment.
**FIGURE 2** | Phylogenetic trees generated by the Bayesian method, under the best-fit GTR+I+Γ model of evolution, based on the partial S-, M-, and L-genomic segments of TPMV strains H2763 (S: MT225396; M: MT225397; L: MT225398) in Myanmar and PK3629 in Pakistan (L: MT225399), and other representative hantaviruses. The phylogenetic positions of TPMV strains VC66412 (S: AY526097, M: EU001329, L: EU001330) in India; TPMV strains LongwanSm5 (S: JF784176; M: JF784177; L: JF784178), LongwanSm6 (S: JF784173; M: JF784174; L: JF784175), LongwanSm9 (S: JF784171; M: JF784172; L: JF784170), and other representative hantaviruses, respectively. The numbers at each node are posterior node probabilities based on 45,000 trees: two replicate Markov chain Monte Carlo runs consisting of six chains of two million generations each sampled every 100 generations with a burn-in of 7,500 (25%). The scale bar indicates nucleotide substitutions per site.

**A** S-segment, **B** M-segment, **C** L-segment phylogenetic trees. Color of TPMV strains: red (Pakistan); orange (China); green (Myanmar); light blue (Nepal); pink (India).
### Table 2: Nucleotide and amino acid sequence similarity (%) between TPMV strain H2763 and other TPMV strains, as well as representative rodent-, shrew-, and bat-borne hantaviruses.

| Hantavirus strain | S-segment | NP | M-segment | GP | L-segment | RdRp |
|-------------------|-----------|----|-----------|----|-----------|------|
| TPMV VRC66412     | 1308 nt   | 435 aa | 2188 nt   | 240 aa | 4963 nt   | 1654 aa |
|                   | 94.7%     | 98.4% | 96.1%     | 99.2% | 94.9%     | 98.4% |
| TPMV H0274/96     | 80.0%     | 98.2% | –         | –     | –         | –     |
| TPMV H0570/96     | –         | –     | 81.7%     | 94.8% | –         | –     |
| TPMV H1779/96     | 81.1%     | 97.8% | 81.7%     | 95.2% | 81.3%     | 96.7% |
| TPMV H1863/96     | 80.6%     | 97.7% | 81.5%     | 94.7% | 80.2%     | 95.9% |
| TPMV H3758/96     | –         | –     | –         | –     | 81.2%     | 96.4% |
| TPMV H4111/96     | 80.6%     | 98.0% | –         | –     | 80.5%     | 96.0% |
| TPMV H4116/96     | –         | –     | –         | –     | 80.4%     | 95.8% |
| TPMV Longwan53    | 84.0%     | 97.2% | 84.0%     | 96.4% | 82.2%     | 96.4% |
| TPMV Longwan450   | 84.0%     | 97.9% | 84.1%     | 95.9% | 82.7%     | 95.5% |
| TPMV Longwan465   | 84.5%     | 98.8% | 84.1%     | 96.2% | 83.2%     | 96.3% |
| TPMV Longwan505   | 84.4%     | 98.6% | 83.9%     | 95.7% | 83.0%     | 96.4% |
| TPMV Longwan561   | 84.3%     | 98.6% | 84.1%     | 95.3% | 82.5%     | 96.4% |
| TPMV Wencheng305  | 84.6%     | 98.4% | 84.3%     | 96.6% | –         | –     |
| TPMV Longwan412   | 84.5%     | 98.8% | 84.3%     | 96.6% | –         | –     |
| TPMV Yuhuan1101   | –         | –     | –         | –     | 80.4%     | 95.8% |
| TPMV PK3629       | –         | –     | –         | –     | 79.3%     | 94.1% |

**Note:** Blue color indicates AZGV, BOWV and JJUV in genus Orthohantavirus from crocidurine shrews, and Yellow color indicates TPMVs. The bolded numbers show nucleotides and amino acids.
Co-phylogenetic Analysis of Asian House Shrew and TPMV

As evidenced by co-phylogeny mapping, using a consensus tree based on L-segment sequences, TPMV strains segregated according to the geographic locations of the Asian house shrews (Figures 3B,C). The phylogenetic positions of TPMV strains based on the S and M segments mirrored the phylogenetic relationships of their Asian house shrews, except for the Pakistan strain in the L-segment tree. The Pakistan strain was mismatched between virus and host phylogeography (Figure 3A).

Phylogenetic Analysis of Asian House Shrew

The molecular identification of TPMV-infected shrews was confirmed as *S. murinus murinus* by amplification and sequencing of the cyt* b* and COI genes of mtDNA and *RAG1* gene of nuclear DNA. Phylogenetic analysis based on the cyt* b* gene indicated Asian house shrews and Etruscan shrews (*Suncus etruscus*) were clearly distinct (Figure 4). However, the relationships between Asian house shrews, Asian highland shrews (*Suncus montanus*) and some *S. murinus* subspecies (such as *S. m. murinus*, *S. m. kandianus*, and *S. m. caerulescens*) (Meegaskumbura et al., 2010) were less clear.

Segregation of Asian house shrews in Asia and Africa was demonstrated by co-phylogeny mapping, using consensus trees based on the cyt* b*, COI and *RAG1* genes (Figure 5). The phylogenetic positions of *RAG1* in nuclear DNA and cyt* b* and COI in mtDNA were not synchronized for each gene. These data suggest that *S. murinus* and *S. montanus* are hybrid species and comprise the *S. murinus*-*S. montanus* species complex.

DISCUSSION

The Asian house shrew, one of 18 species in the genus *Suncus*, is widely distributed throughout Asia and the Pacific,
Africa, and the Middle East (Figure 1). It is peridomestic, typically found within areas of human habitation, and has become dependent on discarded human food waste. Asian house shrews may have been intentionally introduced by humans, similar to Rattus rodents, into Africa (Egypt, Eritrea, Kenya, Republic of Djibouti, Rwanda, Sudan, and Tanzania), the Middle East (Iran, Iraq, Kingdom of Bahrain, Kuwait, Saudi Arabia, Sultanate of Oman, and Yemen), the islands within the Indian Ocean (Comoros, Republic of Madagascar, Republic of Mauritius, and Réunion), and Asia and the Pacific (Japan, Guam, and Philippines) (Kang et al., 2011c). Genetic analysis and treemap dendrograms of RAG1 and COI, RAG1 and cyt b, and COI and cyt b suggest that Asian house shrews may represent hybrids with the Asian highland shrew in Sri Lanka and some area of Eurasia (Figure 5).

The previously held conventional view that hantaviruses co-evolved with their reservoir hosts has been challenged recently by the conjecture that preferential host switching and local host-specific adaptation account for the congruent phylogenies of hantaviruses and their small mammal hosts (Ramsden et al., 2009). Multiple examples of host sharing are now known for hantaviruses hosted by rodents and shrews (Yanagihara et al., 2014). Whether or not TPMV exhibits such host sharing with evidence of carriage by other species of the genus Suncus requires future investigation.

Based on phylogenetic analysis of mtDNA and nuclear genes, as well as karyotype and morphological analysis, the taxonomy...
of the Asian house shrew is still unclear. Asian house shrews include at least two subspecies (S. murinus murinus, S. murinus kandianus, and S. murinus caerulescens), and the Asian highland shrew (S. montanus) is morphologically very similar. Our genetic analysis suggests that morphological based S. m. murinus, S. m. kadianus, S. m. caerulescens, and S. montanus represent hybrid species. Thus, a species complex has been proposed (Ohdachi et al., 2016).

Although genetically diverse strains of TPMV have been detected in Asian house shrews from Nepal (Kang et al., 2011c) and China (Guo et al., 2011), the geographic distribution and evolutionary origins of TPMV are still unclear. Our data
FIGURE 6 | Map of distribution of Asian house shrew and phylogenetic tree based on L segment of TPMV. (A) TPMV cluster based on L-segment in phylogenetic analysis. (B) Blue arrows in map were estimated expansion root based on TPMV phylogeny. Pakistan strain was captured at red star, Nepal strains (Kang et al., 2011c) were captured at light blue circle, Indian strain (Carey et al., 1971) was captured at pink circle, Myanmar strain was captured at green star and Chinese strains (Guo et al., 2011) were captured at red circles. Pakistan and Myanmar strains were collected in this study (star symbols). Pink area is shown Asian house shrew distribution (Ohdachi et al., 2016).
suggest the possibility that TPMV expanded from the Indian subcontinent (Figure 6). The evolutionary time scale of TPMV is faster than that of its host and the host is older than the ancient trade routes between the Middle East and China.

CONCLUSION

Disappointingly, TPMV was detected in lung tissues of only two Asian house shrews, one from Myanmar and one from Pakistan. The reasons for this are not entirely clear, but it might be the result of the focal nature of TPMV infection, as is typical of other hantaviruses. Future studies on the phylogeography of TPMV and the Asian house shrew should provide valuable insights into the geographic radiation.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found at: https://www.ncbi.nlm.nih.gov/, with the following accession numbers: MT225596—MT225599; MT344729—MT344941; MT363682—MT363701; MT364899.

ETHICS STATEMENT

The animal study was reviewed and approved by National Institute of Infectious Diseases (NIID), Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

FK, KA, SM, RY, and SA conceived the study and designed the experiments. SO, KT-T, MM, TJ, NS, SB, KL, TT, CG, MR, HO, IM, HS, and SA conducted the trapping and field collections. FK, KA, SM, RY, and SA analyzed the data. SO, KT-T, MM, TJ, and NS analyzed the host morphology. FK, KA, KT-T, SM, TM, MS, RY, and SA contributed reagents, materials, and analysis tools. FK, SM, TM, RY, and SA prepared the figures and draft manuscript. All authors contributed to the final manuscript.

FUNDING

This research was supported in part by a grant-in-aid from the Research Program on Emerging and Re-emerging Infectious Diseases, Japan Agency for Medical Research and Development (AMED) [JP15fk0108005, JP16fk0108117, JP17fk0108217, JP18fk0108017, JP19fk0108097, and JP20fk0108097]; a grant-in-aid for scientific research from the Japan Society for the Promotion of Science (KAKENHI) (24405045 and JP18H03602); a grant-in-aid from the program of developing basic sciences in Chemistry, Life sciences, Earth sciences and Marine sciences, Vietnam (KHCBSS.01/20-22); as well as a grant from the U.S. National Institutes of Health (P30GM114737).

ACKNOWLEDGMENTS

We thank the following individuals for supporting field investigations: Taher Ghadirian of the Persian Wildlife Heritage Foundation in Tehran, Iran; Razafindrakoto Tidisoa and Mamisoa Colette Vincentine of the Botanical and Zoological Garden of Tsimbazaza in Antananarivo, Madagascar; Atushi Nakamoto of Okayama University of Science in Okayama, Japan; Chihiro Tanaka of the Yagiya Zoological Park in Sendai, Japan; Shinichiro Kawada of the National Museum of Nature and Science in Tokyo, Japan; and Dai Fukui of the University of Tokyo Hokkaido Forests, the University of Tokyo in Furano, Japan. We also thank Shinichiro Kawada, Dai Fukui, and Takashiro Akitsu of the Tokyo University of Science in Tokyo, Japan, for helpful suggestions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2020.00438/full#supplementary-material

REFERENCES

Arai, S., Bennett, S. N., Sumibcay, L., Cook, J. A., Song, J.-W., Hope, A., et al. (2008). Phylogenetically distinct hantaviruses in the masked shrew (Sorex cinereus) and dusky shrew (Sorex monticolus) in the United States. Am. J. Trop. Med. Hyg. 78, 348–351. doi: 10.4269/ajtmh.2008.78.348
Arai, S., Gu, S. H., Baek, L. J., Tabara, K., Bennett, S. N., Oh, H. S., et al. (2012). Divergent ancestral lineages of newfound hantaviruses harbored by phylogenetically related crocidurine shrew species in Korea. Virology 424, 99–105. doi: 10.1016/j.virol.2011.11.013
Arai, S., Kang, H. J., Gu, S. H., Ohdachi, S. D., Cook, J. A., Yashina, L. N., et al. (2016a). Genetic diversity of Arybush virus in the Laxmann’s shrew (Sorex caecutiens). Vector Borne Zoonotic Dis. 16, 468–475. doi: 10.1089/vbz.2015.1903
Arai, S., Kikuchi, F., Bawm, S., Son, N. T., Lin, K. S., Tu, V. T., et al. (2019). Molecular phylogeny of mobaviruses (Hantaviridae) in Myanmar and Vietnam. Viruses 11, 228. doi: 10.3390/v11030228
Arai, S., Song, J.-W., Sumibcay, L., Bennett, S. N., Nerurkar, V. R., Parmenter, C., et al. (2007). Hantavirus in northern short-tailed shrew, United States. Emerg. Infect. Dis. 13, 1420–1423. doi: 10.3201/eid1309.070484
Arai, S., Taniguchi, S., Aoki, K., Yoshikawa, Y., Kyoza, S., Tanaka-Taya, K., et al. (2016b). Molecular phylogeny of a genetically divergent hantavirus harbored by the Geoffroy’s rousette (Rousettus amplexicaudatus), a frugivorous bat species in the Philippines. Infect. Genet. Evol. 45, 26–32. doi: 10.1016/j.meegid.2016.08.008
Bennett, S. N., Gu, S. H., Kang, H. J., Arai, S., and Yanagihara, R. (2014). Reconstructing the evolutionary origins and phylogeography of hantaviruses. Trends Microbiol. 22, 473–482. doi: 10.1016/j.tim.2014.04.008
Carey, D. E., Reuben, R., Panicker, K. N., Shope, R. E., and Myers, R. M. (1971). Thottapalayam virus: a presumptive arbovirus isolated from a shrew in India. Indian J. Med. Res. 59, 1758–1760.
Charleston, M. A., and Robertson, D. L. (2002). Preferential host switching by primate lentiviruses can account for phylogenetic similarity with the primate phylogeny. Syst. Biol. 51, 528–535. doi: 10.1080/10635150290069940
Darriba, D., Taboada, G. L., Doallo, R., and Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9:772. doi: 10.1038/nmeth.2109
Gu, S. H., Nicolas, V., Lalas, A., Sathirapongsasuti, N., and Yanagihara, R. (2013). Complete genome sequence and molecular phylogeny of a newfound
hantavirus harbored by the Doucet’s musk shrew (Crocidura douceti) in Guinea. Infect. Genet. Evol. 20, 118–123. doi: 10.1016/j.meegid.2013.08.016

Guo, W. P., Lin, X. D., Wang, W., Tian, J. H., Cong, M. L., Zhang, H. L., et al. (2013). Phylogeny and origins of hantaviruses harbored by bats, insectivores and rodents. PLoS Pathog. 9e1003159. doi: 10.1371/journal.ppat.1003159

Guo, W. P., Lin, X. D., Wang, W., Zhang, X. H., Chen, Y., Cao, J. H., et al. (2011). A new subtype of Thottapalayam virus carried by the Asian house shrew (Suncus murinus) in China. Infect. Genet. Evol. 11, 1862–1867. doi: 10.1016/j.meegid.2011.07.013

Jogahara, T. (2016). Animal bio-resources in the Department of Zoology, Faculty of Science, Okayama University of Science. Proc. Okayama Assoc. Lab. Anim. Sci. 32, 12–18. Available online at: http://ousar.lib.okayama-u.ac.jp/files/public/5/54401/201606827151010751151/poolas_032_012_018.pdf

Kang, H. J., Arai, S., Hope, A. G., Cook, J. A., and Yanagihara, R. (2010). Novel hantavirus in the flat-skulled shrew (Sorex roboratus). Vector Borne Zoonotic Dis. 10, 593–597. doi: 10.1089/vbz.2009.0159

Kang, H. J., Bennett, S. N., Hope, A. G., Cook, J. A., and Yanagihara, R. (2011a). Novel hantavirus harbored by cricetid rodents. J. Virol. 85, 7496–7503. doi: 10.1128/JVI.02450-10

Kang, H. J., Bennett, S. N., Sumibcay, L., Arai, S., Hope, A. G., Mocz, G., et al. (2009). Evolutionary insights from a genetically divergent hantavirus harbored by the European common mole (Talpa europaea). PLoS One 4e6149. doi: 10.1371/journal.pone.006149

Kang, H. J., Kadjo, B., Dudey, S., Jacquet, F., and Yanagihara, R. (2011b). Molecular evolution of Asaguya virus, a new hantavirus harbored by the West African pygmy shrew (Crocidura obscurior) in Cote d'Ivoire. Virol J. 8:373. doi: 10.1186/1743-422X-8-373

Kang, H. J., Kosoy, M. Y., Shrestha, S. K., Shrestha, M. P., Pavlin, J. A., Gibbons, R. V., et al. (2011c). Genetic diversity of Thottapalayam virus, a hantavirus harbored by the Asian house shrew (Suncus murinus) in Nepal. Am. J. Trop. Med. Hyg. 85, 540–545. doi: 10.4269/ajtmh.2011.11-0034

Kang, H. J., Stanley, W. T., Esselstyn, J. A., Gu, S. H., and Yanagihara, R. (2014). Expanded host diversity and geographic distribution of hantaviruses in sub-Saharan Africa. J. Virol. 88, 7663–7667. doi: 10.1128/JVI.00285-14

Kirkland, J. G. L. (1998). Guidelines for the capture, handling and care of mammals as approved by the American Society of Mammalogists. J. Mammal. 79, 1416–1431. doi: 10.2307/1383033

Klempa, B., Fichet-Calvet, E., Lecompte, E., Auster, B., Anisikin, V., Meisel, H., et al. (2006). Hantavirus in African wood mouse, Guinea. Emerg. Infect. Dis. 12, 838–840. doi: 10.3201/eid1205.051487

Klempa, B., Fichet-Calvet, E., Lecompte, E., Auster, B., Anisikin, V., Meisel, H., et al. (2007). Novel hantavirus sequences in shrew, Guinea. Emerg. Infect. Dis. 13, 529–532. doi: 10.3201/eid1303.061198

Meeaskumbura, S., Meeaskumbura, M., and Schneider, C. J. (2010). Systematic relationships and taxonomy of Suncus montanus and S. murinus from Sri Lanka. Mol. Phylogenet. Evol. 55, 473–487. doi: 10.1016/j.ympev.2010.01.031

Ohdachi, S. D., Kinoshita, G., Oda, S.-I., Motokawa, M., Jogahara, T., Arai, S., et al. (2016). Intraspécific phylogeny of the house shrews, Suncus murinus-S. montanus species complex, based on the mitochondrial cytochrome b gene. Mammal Study 41, 229–238. doi: 10.3106/041.041.0408

Radosa, L., Schlegel, M., Gebauer, P., Anisorge, H., Heroldová, M., Jánová, E., et al. (2013). Detection of shrew-borne hantavirus in Eurasian pygmy shrew (Sorex minutus) in Central Europe. Infect. Genet. Evol. 19, 403–410. doi: 10.1016/j.meegid.2013.04.008

Ramsden, C., Holmes, E. C., and Charleston, M. A. (2009). Hantavirus evolution in relation to its rodent and insectivore hosts: no evidence for codivergence. Mol. Biol. Evol. 26, 143–153. doi: 10.1093/molbev/msn234

Ronquist, F., and Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574. doi: 10.1093/bioinformatics btg180

Sikes, R. S., and Animal Care and Use Committee of the American Society of Mammalogists (2016). Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. J. Mammal. 97, 663–688. doi: 10.1093/jmammal/gwy078

Song, J.-W., Baek, L. J., Schmaljohn, C. S., and Yanagihara, R. (2007a). Thottapalayam virus, a prototype shrewborne hantavirus. Emerg. Infect. Dis. 13, 980–985. doi: 10.3201/eid1307.070031

Song, J.-W., Gu, S. H., Bennett, S. N., Arai, S., Puorger, M., Hilbe, M., et al. (2007b). Seewis virus, a genetically distinct hantavirus in the Eurasian common shrew (Sorex araneus). Virol J. 4:114. doi: 10.1186/1743-422X-4-114

Song, J.-W., Kang, H. J., Gu, S. H., Moon, S. S., Bennett, S. N., Song, K. J., et al. (2009). Characterization of Imjin virus, a newly isolated hantavirus from the Ussuri white-toothed shrew (Crocidura lusiiaria). J. Virol. 83, 6184–6191. doi: 10.1128/JVI.00371-09

Song, J.-W., Kang, H. J., Song, K. J., Truong, T. T., Bennett, S. N., Arai, S., et al. (2007c). Newfound hantavirus in Chinese mole shrew, Vietnam. Emerg. Infect. Dis. 13, 1784–1787. doi: 10.3201/eid1311.070492

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680. doi: 10.1093/nar/22.22.4673

Yanagihara, R., Gu, S. H., Arai, S., Kang, H. J., and Song, J. W. (2014). Hantaviruses: rediscovery and new beginnings. Virus Res. 187, 6–14. doi: 10.1016/j.viruses.2013.12.038

Zuo, S. Q., Gong, Z. D., Fang, L. Q., Jiang, J. F., Zhang, J. S., Zhao, Q. M., et al. (2014). A new hantavirus from the stripe-backed shrew (Sorex cylindricauda) in the People’s Republic of China. Virus Res. 184, 82–86. doi: 10.1016/j.viruses.2014.02.004

Conflict of Interest: KT, who is deceased, was previously employed by the Applied Biology Co., Ltd., in Tokyo, Japan.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Kikuchi, Aoki, Ohdachi, Tsuji, Motokawa, Jogahara, Sön, Bawon, Tswe, Gamage, Ranorosa, Omar, Maryanto, Suzuki, Tanaka-Taya, Morikawa, Mizutani, Suzuki, Yanagihara and Arai. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.