



# Discovery of Four Novel Circular Single-Stranded DNA Viruses in Fungus-Farming Termites

Mason Kerr,<sup>a</sup>  Karyna Rosario,<sup>a</sup> Christopher C. M. Baker,<sup>b\*</sup> Mya Breitbart<sup>a</sup>

<sup>a</sup>College of Marine Science, University of South Florida, Saint Petersburg, Florida, USA

<sup>b</sup>Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey, USA

**ABSTRACT** Here, we describe four novel circular single-stranded DNA viruses discovered in fungus-farming termites (*Odontotermes* sp.). The viruses, named termite-associated circular virus 1 (TaCV-1) through TaCV-4, are most similar to members of the family *Genomoviridae* and were widely detected in African termite mounds.

Fungus-farming termites from the genus *Odontotermes* build extensive mounds that can enhance ecosystem productivity (1). These social insects have symbiotic fungi and bacteria that digest foraged plant material and serve as nutritional sources for the termites (2–4). Although several studies have characterized bacterial and fungal communities associated with fungus-farming termites (5, 6), viruses associated with these unique agricultural systems have not been investigated. Here, we describe four novel circular single-stranded DNA (ssDNA) viruses discovered in *Odontotermes* sp. termites.

Termites were collected opportunistically from individual mounds located at the Mpala Research Center in Kenya and catalogued based on caste, mainly workers and soldiers, with nymphs collected less commonly. Castes from each mound were screened for the presence of ssDNA viral genomes encoding a replication-associated protein (Rep) following methods previously used for the discovery of circular ssDNA viruses in dragonflies (7). Briefly, virus-like particles were partially purified from each sample by homogenization and filtration through a 0.45- $\mu$ m Sterivex filter (Millipore). DNA was extracted from the filtrate using the MinElute virus spin kit (Qiagen) and amplified through rolling circle amplification (RCA). RCA products were then digested with restriction enzymes in separate reactions, and digestion products between 1 and 4 kb were cloned using the CloneJET PCR cloning kit (Thermo Scientific) and sequenced with vector primers. Sequences with significant matches to known circular, Rep-encoding ssDNA (CRESS DNA) viruses were used to design back-to-back primers to obtain complete genomes through inverse PCR.

Four CRESS DNA viruses, named termite-associated circular virus 1 (TaCV-1) through TaCV-4, were detected in workers. BLAST searches in GenBank and the genome organization of TaCVs indicate that these viruses belong to the family *Genomoviridae*. Similar to genomoviruses, the TaCV genomes range from 2,155 to 2,222 nucleotides (nt) in length and contain two major open reading frames (ORFs) encoding a Rep and a capsid protein. The Rep ORF for TaCV-2, TaCV-3, and TaCV-4 is interrupted by an intron. The four TaCVs share <70% genome-wide pairwise identity with each other and previously described genomoviruses, indicating that each TaCV represents a novel species within the family *Genomoviridae* (8). Based on the Rep ORF, TaCV-1, TaCV-2, and TaCV-4 are most closely related to genomoviruses identified in animal feces, insects, and sewage, respectively (9–11), while TaCV-3 is most similar to a plant-infecting mastrevirus (12).

PCR assays revealed the presence of one or more TaCVs in 23% of the 47 mounds tested, including mounds separated by 11 km. TaCV-1 was the most prevalent since it was detected in 8 of the 11 TaCV-positive mounds, compared to TaCV-2, TaCV-3, and

**Received** 13 March 2018 **Accepted** 22 March 2018 **Published** 26 April 2018

**Citation** Kerr M, Rosario K, Baker CCM, Breitbart M. 2018. Discovery of four novel circular single-stranded DNA viruses in fungus-farming termites. *Genome Announc* 6:e00318-18. <https://doi.org/10.1128/genomeA.00318-18>.

**Copyright** © 2018 Kerr et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Karyna Rosario, [krosari2@mail.usf.edu](mailto:krosari2@mail.usf.edu).

\* Present address: Christopher C. M. Baker, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, USA.

TaCV-4, which were found in 2, 3, and 1 mound, respectively. Notably, TaCVs were only detected in the worker caste. Since termite workers ingest fungi and maintain the fungal gardens (13, 14), and genomoviruses have been identified in both fungi and insects (15, 16), it is difficult to predict the host of the novel TaCVs described here. Nevertheless, the widespread detection of TaCVs in termite mounds suggests that these ssDNA viruses may play a role in the ecology of fungus-farming termite systems.

**Accession number(s).** The genome sequences of termite-associated circular virus 1 (TaCV-1) through TaCV-4 have been deposited in GenBank under the accession numbers [MG917674](#) to [MG917677](#).

## ACKNOWLEDGMENTS

This work was funded through NSF Assembling the Tree of Life Program grant DEB-1239976 to K.R. and M.B.

Termite samples were collected in the course of fieldwork funded by NSF grant DEB-1355122 to Corina Tarnita and Robert Pringle from Princeton University.

## REFERENCES

1. Pringle RM, Doak DF, Brody AK, Jocqué R, Palmer TM. 2010. Spatial pattern enhances ecosystem functioning in an African savanna. *PLoS Biol* 8:e1000377. <https://doi.org/10.1371/journal.pbio.1000377>.
2. Shinzato N, Muramatsu M, Watanabe Y, Matsui T. 2005. Termite-regulated fungal monoculture in fungus combs of a macrotermitine termite *Odontotermes formosanus*. *Zoolog Sci* 22:917–922. <https://doi.org/10.2108/zsj.22.917>.
3. Aanen DK, Eggleton P, Rouland-Lefevre C, Guldberg-Froslev T, Rosendahl S, Boomsma JJ. 2002. The evolution of fungus-growing termites and their mutualistic fungal symbionts. *Proc Natl Acad Sci U S A* 99: 14887–14892. <https://doi.org/10.1073/pnas.222313099>.
4. Poulsen M, Hu H, Li C, Chen Z, Xu L, Otani S, Nygaard S, Nobre T, Klaubauf S, Schindler PM, Hauser F, Pan H, Yang Z, Sonnenberg ASM, de Beer ZW, Zhang Y, Wingfield MJ, Grimmelikhuijzen CJP, de Vries RP, Korb J, Aanen DK, Wang J, Boomsma JJ, Zhang G. 2014. Complementary symbiont contributions to plant decomposition in a fungus-farming termite. *Proc Natl Acad Sci U S A* 111:14500–14505. <https://doi.org/10.1073/pnas.1319718111>.
5. Mathew GM, Ju Y-M, Lai C-Y, Mathew DC, Huang CC. 2012. Microbial community analysis in the termite gut and fungus comb of *Odontotermes formosanus*: the implication of *Bacillus* as mutualists. *FEMS Microbiol Lett* 329:504–517. <https://doi.org/10.1111/j.1574-6941.2011.01232.x>.
6. Otani S, Hansen LH, Sørensen SJ, Poulsen M. 2016. Bacterial communities in termite fungus combs are comprised of consistent gut deposits and contributions from the environment. *Microb Ecol* 71:207–220. <https://doi.org/10.1007/s00248-015-0692-6>.
7. Rosario K, Dayaram A, Marinov M, Ware J, Kraberger S, Stainton D, Breitbart M, Varsani A. 2012. Diverse circular ssDNA viruses discovered in dragonflies (Odonata: Epiprocta). *J Gen Virol* 93:2668–2681. <https://doi.org/10.1099/vir.0.045948-0>.
8. Varsani A, Krupovic M. 2017. Sequence-based taxonomic framework for the classification of uncultured single-stranded DNA viruses of the family *Genomoviridae*. *Virus Evol* 3:vev037. <https://doi.org/10.1093/ve/vev037>.
9. Steel O, Kraberger S, Sikorski A, Young LM, Catchpole RJ, Stevens AJ, Ladley JJ, Coray DS, Stainton D, Dayaram A, Julian L, van Bysterveldt K, Varsani A. 2016. Circular replication-associated protein encoding DNA viruses identified in the faecal matter of various animals in New Zealand. *Infect Genet Evol* 43:151–164. <https://doi.org/10.1016/j.meegid.2016.05.008>.
10. Kraberger S, Argüello-Astorga GR, Greenfield LG, Galilee C, Law D, Martin DP, Varsani A. 2015. Characterisation of a diverse range of circular replication-associated protein encoding DNA viruses recovered from a sewage treatment oxidation pond. *Infect Genet Evol* 31:73–86. <https://doi.org/10.1016/j.meegid.2015.01.001>.
11. Kraberger S, Polston JE, Capobianco HM, Alcalá-Briseño RI, Fontenele RS, Varsani A. 2017. Genomovirus genomes recovered from *Echinothrips americanus* sampled in Florida, USA. *Genome Announc* 5(21):e00445-17. <https://doi.org/10.1128/genomeA.00445-17>.
12. Hadfield J, Thomas JE, Schwinghamer MW, Kraberger S, Stainton D, Dayaram A, Parry JN, Pande D, Martin DP, Varsani A. 2012. Molecular characterisation of dicot-infecting mastreviruses from Australia. *Virus Res* 166:13–22. <https://doi.org/10.1016/j.virusres.2012.02.024>.
13. Hongoh Y, Ekpornprasit L, Inoue T, Moriya S, Trakulnaleamsai S, Ohkuma M, Noparatnaraporn N, Kudo T. 2006. Intracolony variation of bacterial gut microbiota among castes and ages in the fungus-growing termite *Macrotermes gilvus*. *Mol Ecol* 15:505–516. <https://doi.org/10.1111/j.1365-294X.2005.02795.x>.
14. Aanen DK. 2006. As you reap, so shall you sow: coupling of harvesting and inoculating stabilizes the mutualism between termites and fungi. *Biol Lett* 2:209–212. <https://doi.org/10.1098/rsbl.2005.0424>.
15. Krupovic M, Ghabrial SA, Jiang D, Varsani A. 2016. *Genomoviridae*: a new family of widespread single-stranded DNA viruses. *Arch Virol* 161: 2633–2643. <https://doi.org/10.1007/s00705-016-2943-3>.
16. Liu S, Xie J, Cheng J, Li B, Chen T, Fu Y, Li G, Wang M, Jin H, Wan H, Jiang D. 2016. Fungal DNA virus infects a mycophagous insect and utilizes it as a transmission vector. *Proc Natl Acad Sci U S A* 113:12803–12808. <https://doi.org/10.1073/pnas.1608013113>.