

# Human-associated Redondoviruses Infect the Oral Protozoan Entamoeba gingivalis

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Redondoviruses are circular Rep-encoding single-stranded DNA (CRESS) viruses of high prevalence in healthy humans. Redondovirus abundance is increased in oro-respiratory samples from individuals with periodontitis, acute illness, and severe COVID-19. We investigated potential host cells supporting redondovirus replication in oro-respiratory samples and uncovered the oral amoeba Entamoeba gingivalis as a likely host. Redondoviruses are closely related to viruses of Entamoeba and contain reduced GC nucleotide content, consistent with Entamoeba hosts. Redondovirus and E. gingivalis cooccur in metagenomic data from oral disease and healthy human cohorts. When grown in xenic cultures with feeder bacteria, E. gingivalis was robustly positive for redondovirus RNA and DNA. A DNA proximityligation assay (Hi-C) on xenic culture cells showed enriched cross-linking of redondovirus and Entamoeba DNA, supporting E. gingivalis as the redondovirus host. While bacteria are established hosts for bacteriophages within the human virome, this work shows that eukaryotic commensals also contribute an abundant human-associated virus.

dispersed globally, and associated with disease



Figure 1. Illustration of the discovery of redondoviruses

**Initial detections:** Metagenome of human oro-respiratory samples [1,2]

- Taxonomy: Within Cressdnaviricota phylum; contains a single genus, Torbevirus, and two species, Brisavirus and Vientovirus [2]
- Genome organization: Circular ssDNA, ~3.0-3.1 kb, encodes a capsid protein (Cap), a replicationassociated protein (Rep), and a functionally unknown protein (ORF3) [2]

COVID-19) [2,3,4,5,6]

Prevalence: Detected in the US, UK, Spain, Italy, Cameroon, Ethiopia, Vietnam, Botswana, Tanzania, and China, with prevalence ranging from 2-82% [2,3,4,5,6,7,8]

Meta-omic sequencing has enabled the detection of novel viruses without isolation in cell culture. However, the hosts of these viruses are often unknown given the many possible virus-host pairings in a single sample; up until this work, redondoviruses were one such virus.



Figure 2. Illustration of the host issue posed by meta-omic-based virus discovery.

<sup>-</sup>11 Cui. L., Wu. B., Zhu. X. (2017

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