

# What's that twinkle in your eye? Mischievous or a stellate opportunist?



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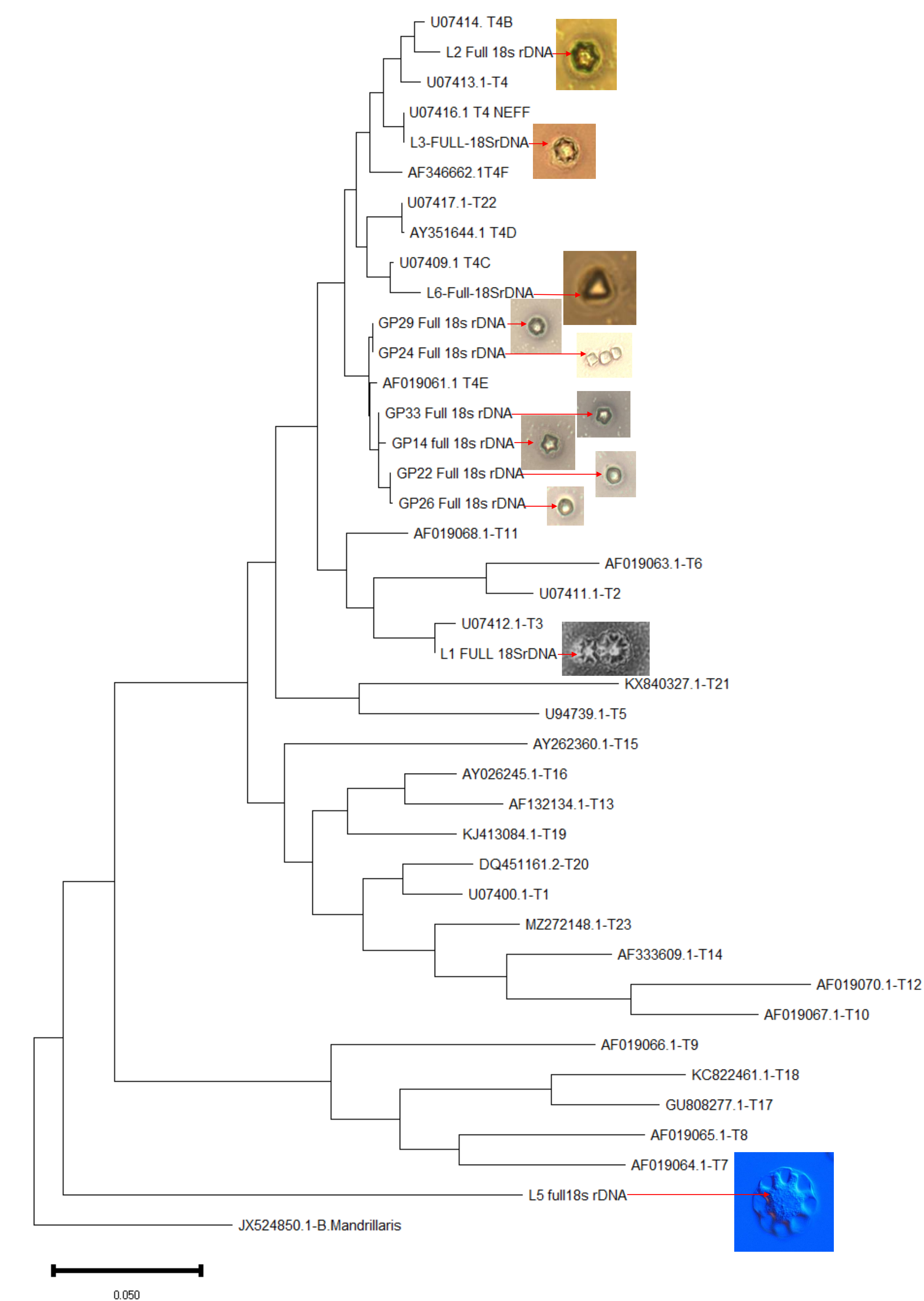
## Acanthawhatnow?

Acanthamoeba is a genus of free-living amoebae that opportunistically infect humans, most commonly as an infection of the cornea known as Acanthamoeba Keratitis (AK). AK is a very painful and destructive infection that, if untreated or insufficiently treated can cause total blindness in one or both of a patient's eyes. AK is difficult to treat because of the environmentally resistant cyst stage of its life cycle, which is also more tolerant to drug treatment.

The consensus clinical position is that there is no difference in treatment outcome related to the causative species in cases of AK. One of the aims of the Fight for Sight studentship is to test that hypothesis. With a large range of isolates that have also been well characterised with as much phenotypic and genotypic data as possible.

## Results

Figure 1: Maximum likelihood tree using full 18s rRNA gene



Built using the Ohio State University reference T-type sequences<sup>1</sup> aligned with MUSCLE, Tree created using MEGA11<sup>2</sup>. All sequences labelled with the prefix "GP" & "L" are isolates from the Diagnostic Parasitology Laboratory at LSHTM from UK patients with AK

## Key Conclusions

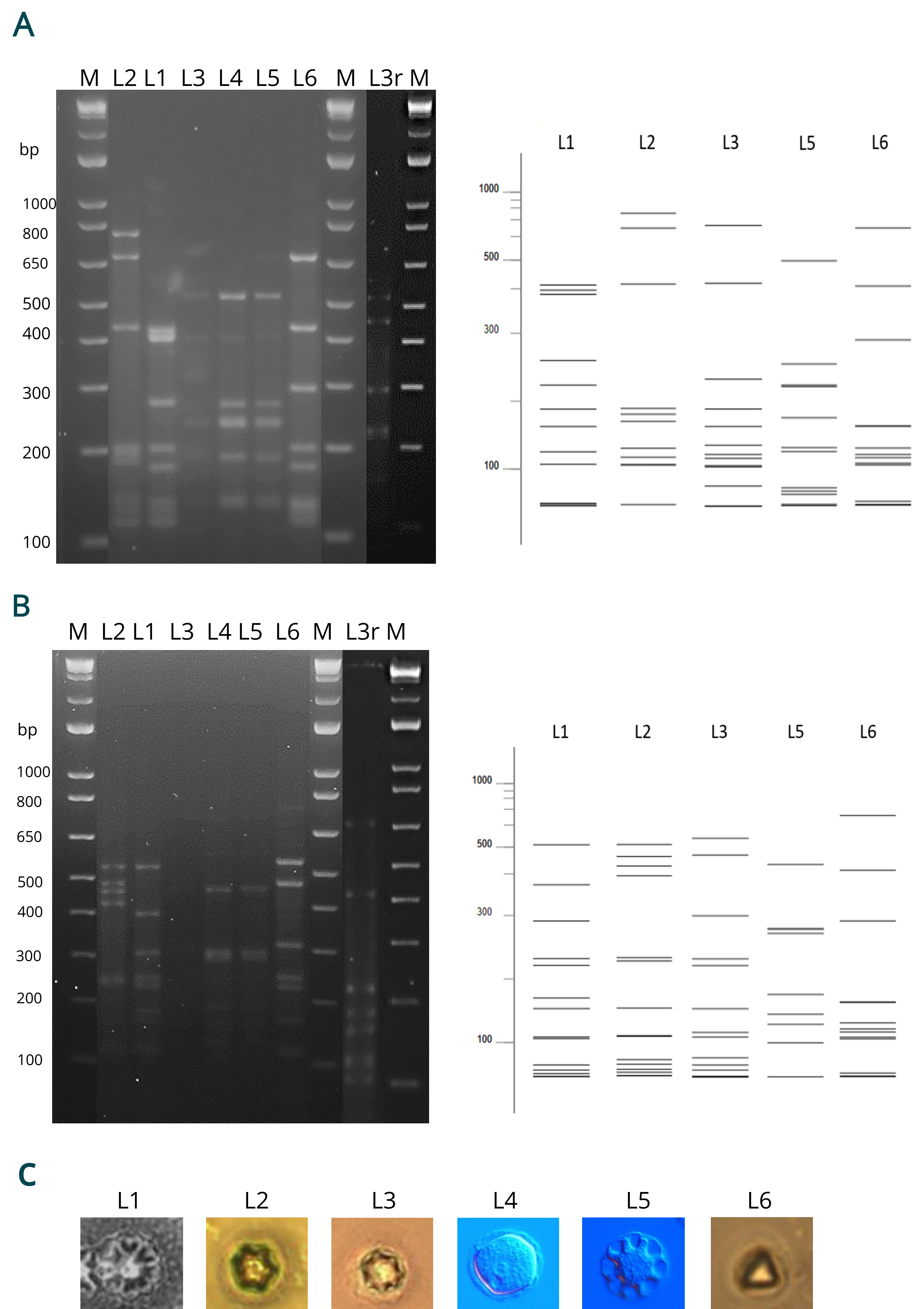
- Isolate L5 does not cluster closely with other T-types in this tree however it produces an indistinguishable RFLP pattern to isolate L4 which is likely a different species by microscopy and smaller fragment based phylogeny (L4 absent from tree due to lack of complete 18s rRNA data)

- All the other L samples produced different patterns backed up by their places in the phylogenetic tree and the morphological differences. Although, L3 has a discrepancy between the predicted HpaII digest fragments and the real gel, which warrants further investigation.

- The "GP" samples (all the 2021 samples sequenced so far), have clustered with the T4 isolates

Figure 2: RFLP Digests of full 18s rDNA amplicons, Figure 2 (A): digested with HaeIII. Figure 2 (B): digested with HpaII. Predicted gels created using NEBcutter<sup>®</sup> V3<sup>3</sup> with the same sequenced amplicons. Figure 2 (C): representative morphotypes of the L (Legacy) Samples (not sized to scale)

(NB L3r is a repeat digest of L3 which failed, on a different gel)



## So what now?

- Whole genome sequence analysis
- Multi locus sequence typing
- More isolates sequenced

1. <https://u.osu.edu/acanthamoeba/phylogenetic-relationship-among-t4-subgroups/>

2. Sudhir Kumar, Glen Stecher, Michael Li, Christina Nnyaz, and Koichiro Tamura (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35:1547-1549

3. <https://nc3.neb.com/NEBcutter/prj/>

