Amplification and sequencing of entire tick mitochondrial genomes for a phylogenomic analysis

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Background

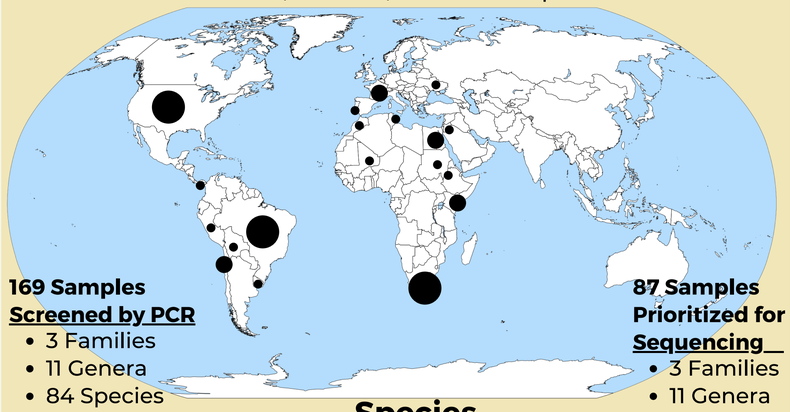
- Many tick species are important vectors of disease causing pathogens
- Tick bites can also cause non-infectious syndromes such as alpha-gal syndrome (red meat allergy) and tick toxicosis
- Identifying ticks to the species-level is critical for surveillance efforts and tracking geographic distributions
- Mitochondrial genome (mitogenome) sequencing has been crucial for tick species identification, taxonomy, systematics, and surveillance
- Current strategies for tick mitogenome sequencing include low-coverage genome skimming and multi-amplicon amplification and sequencing, both costly to scale
- Our strategy uses 2 full-length mitogenome PCRs with Oxford Nanopore Technologies (ONT) Mk1B MinION platform for cost-effective sequencing at scale

Objective

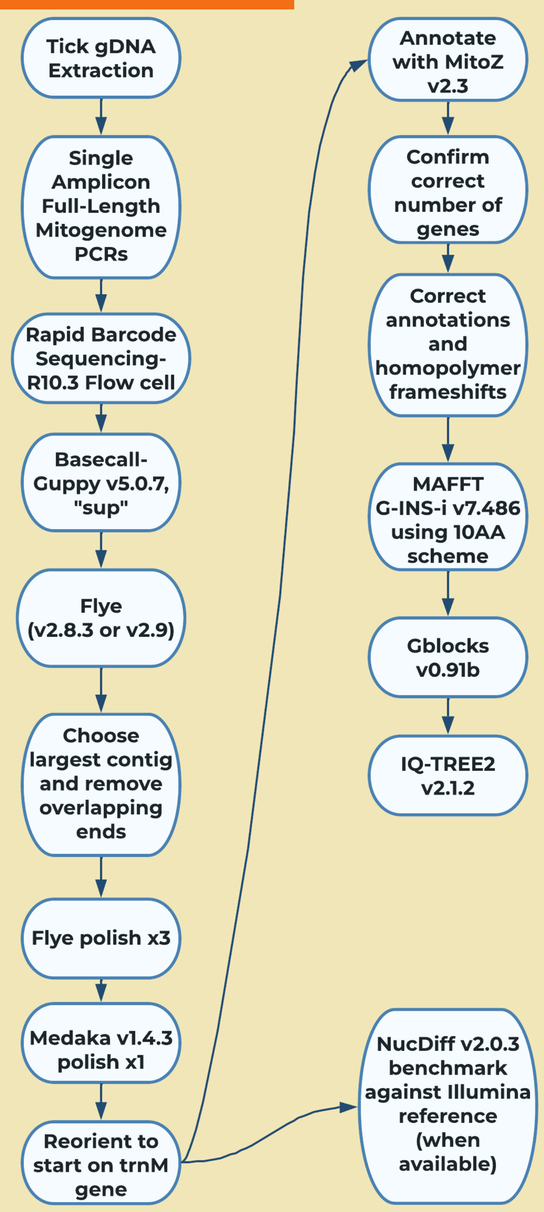
To develop a quick, cost-effective strategy to amplify and sequence complete tick mitogenomes with the MinION platform for phylogenomic analysis.

Tick Sample Set

- Tick samples were screened from North and South America, Africa, and Europe



Methodology

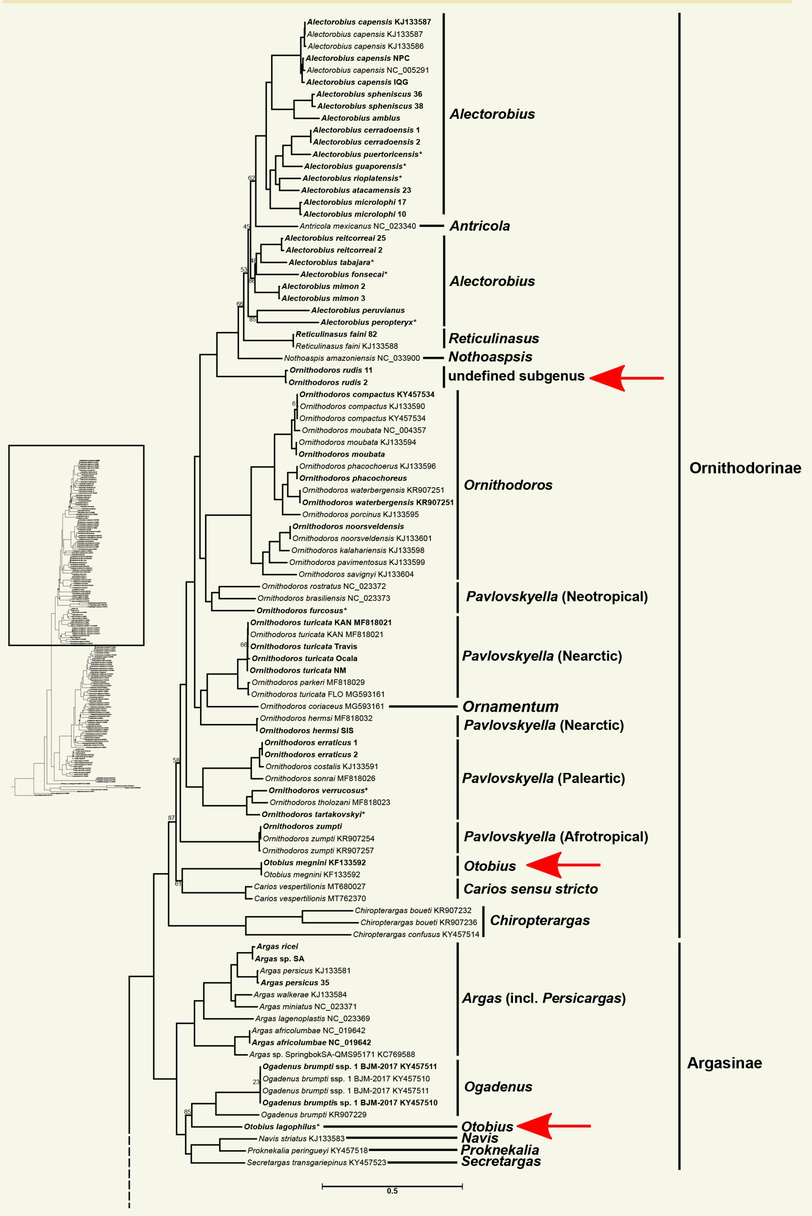


Sequencing Summary Stats

- Rapid barcode libraries had low pore occupancy on R10.3 flow cells (~15-20% of pores) and required ~16hr sequencing runs
- Mitogenomes for 85/87 samples were successfully assembled
- Three different multiplexed libraries were run SQK-RBK004 (6 samples), SQK-RBK110.96 (72 samples), and SQK-RBK110.96 (20 samples)
- Range for average read lengths per sample: 1.5kb to 4.1kb
- Average number of reads per sample: 2,186
- Average yield per sample: 6.84Mb
- Range for mean depth of coverage for each sample's mitogenome assembly: 96x-1,460x

Phylogenomic Analysis

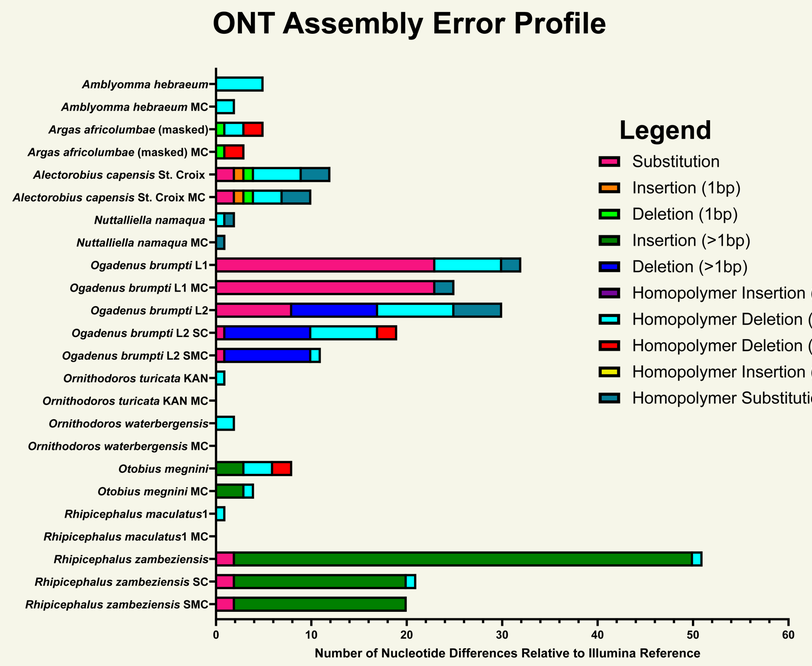
- Phylogenomic analysis was conducted using the 10AA scheme by Kelava et al.[1]
  - ATP6, ATP8, COX1, COX2, COX3, CYTB, NAD1, NAD2, NAD3, NAD4, NAD4L, ND5, ND6
- The Argasidae portion of the phylogeny is shown below. The full phylogeny can be seen via the QR code at the top of the poster.
  - The phylogeny is a maximum likelihood tree inferred using IQ-TREE2 with an edge-linked partitioned scheme
  - Numbers on branches indicate the percent of bootstrap support (100,000 ultrafast bootstrap replicates). Only branch supports with less than 90% support are shown
  - Subgenera are indicated to the right of the tips and the subfamilies are indicated to the right of the subgenera.

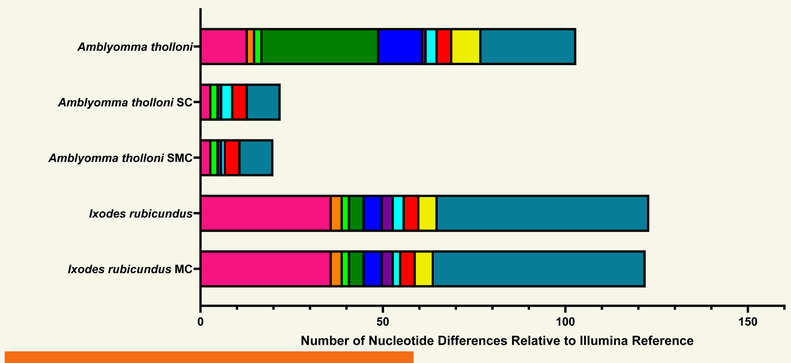


- New systematics findings are indicated on the phylogeny with red arrows:
  - The new *Otobius lagophilus* mitogenomes from this work indicated that the *Otobius* genus was polyphyletic
  - *Ornithodoros rudis*' unique morphological and behavioral characteristics were supported by molecular data, which place it in an undefined subgenus

Benchmarking Analysis

- 15 of the 85 samples we sequenced were previously sequenced by Mans et al.[2] by low-coverage Illumina genome sequencing
- The error profile of the ONT assemblies vs Illumina reference is seen in the figure below.
  - 2 ONT assemblies were identical to the Illumina assemblies.
    - Manual correction (MC) of homopolymers increased this to 5 assemblies
  - Three ONT assemblies had clusters of discordant sequence
    - Sanger sequencing of 5 selected areas showed 4/5 discordant areas agreed with ONT vs Illumina, referred to as SC (Sanger correction) in the figure below
    - SMC below refers to comparing manually corrected ONT assembly vs Sanger corrected Illumina reference after Sanger correction
- Note: *Ixodes rubicundus* was a low-coverage assembly from only one of the two amplicons





Conclusions

- Single-amplicon full-length mitogenome amplification was achieved across all three tick families
- These new mitogenome assemblies were phylogenomically informative and identified new systematic relationships
- Benchmarked assemblies were comparable or superior to a low-coverage Illumina genome skimming strategy (99.98% median concordance)
- Homopolymers were an issue but the ONT assemblies identified errors in the Illumina references
- Accurate tick mitogenome assembly is possible for ~$10 per sample using the R10.3 flow cell, rapid barcoding kit, and Guppy v5.0.7 sup basecalled amplicon data with minor manual correction
- This strategy is likely applicable to other organisms with circular mitogenomes

References

1. Kelava S, Mans BJ, Shao R, Moustafa MA, Matsuno K, Takano A, Kawabata H, Sato K, Fujita H, Ze C, Plantard O. Phylogenies from mitochondrial genomes of 120 species of ticks: Insights into the evolution of the families of ticks and of the genus Amblyomma. Ticks and tick-borne diseases. 2021 Jan 1;12(1):101577.
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