

The complete mitochondrial genome of Africa's largest freshwater copepod, *Lovenula raynerae*

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ABSTRACT

Lovenula raynerae is the largest known African freshwater copepod. To date, it has only been sampled from ephemeral freshwater ecosystems. This paper reports the complete mitochondrial genome of *L. raynerae*, which was found to be 14,365 bp long. Base composition of 33.5% base A, 19.3% base G, 34.6% base T, and 12.5% base C was found, with 13 protein-coding genes, 22 tRNAs, and 2 rRNAs. This paper contributes to an improved understanding of phylogenetic relationships in an important crustacean group.

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Copepods are a diverse group of aquatic crustaceans found in both marine and freshwater environments (Boxshall and Defaye 2008; Kim et al. 2013; Battuello et al. 2017). Of the 10 copepod orders (Boxshall and Defaye 2008), Calanoida is particularly important, as it is both species-rich (~2266 species have been described; Battuello et al. 2017) and very abundant (they may constitute up to 95% of marine plankton samples; Mauchline 1998) and thus plays an important role in trophic interactions as a link between primary producers and secondary consumers (Dalu et al. 2016; Battuello et al. 2017; Wasserman et al. 2018). Despite their ecological importance and abundance, only one complete mitogenome has been published for the order Calanoida (Kim et al. 2013).

In this study, we describe the second complete mitochondrial genome of a calanoid copepod, that of *Lovenula raynerae* (Suarez-Morales et al. 2015). This recently described freshwater copepod has so far been found exclusively in ephemeral ponds in the Eastern Cape of South Africa (Suarez-Morales et al. 2015). One individual was collected in an ephemeral pond near Grahamstown, South Africa (33.250705°S, 26.436940°E). DNA was extracted using the CTAB method (Doyle and Doyle 1987) and the sample was then sequenced as follows. The gDNA was sonicated to ~500 bp fragment size using the Covaris® Ultrasonicator (Covaris, Woburn, MA) and processed using the NEBNext Ultra DNA library prep kit for Illumina (New England Biolabs,

Ipswich, MA). The library was subsequently denatured and sequenced a MiSeq desktop sequencer (Illumina, San Diego, CA) at Monash University Malaysia. Mitogenome assembly was performed with MITObim version 1.8 (Hahn et al. 2013) using the 16S rRNA sequence of *Mastigaodiaptomus nevus* (Accession Number: EU582541.1) as the initial bait template. The mitogenome was manually re-circularized and re-oriented to the COI gene prior to submission to MITOS (Bernt et al. 2013) for annotation.

A sequence with a total length of 14,365 bp was generated whose base composition was 33.5% base A, 19.3% base G, 34.6% base T, and 12.5% base C. The sequence contained 13 protein-coding genes, 22 tRNAs and 2 rRNAs.

A phylogenetic tree was reconstructed using all 9 published mitogenomes from the subclass Copepoda. Due to extensive gene reshuffling in this group (Minxiao et al. 2011) the sequences were separated into the protein-coding genes, which were aligned separately via MAFFT (Katoh et al. 2017). A Bayesian phylogenetic tree was constructed using an RB substitution model (Bouckaert et al. 2013; Drummond and Bouckaert 2015) in BEAST v.2.5.0 (Bouckaert et al. 2014) with a chain length of one billion and a burn-in of 25%. The resulting tree was visualized in Figtree v.1.4.3 (Rambaut 2016). The phylogenetic tree (Figure 1) shows that *L. raynerae* is monophyletic with *Calanus hyperboreus* and that these two calanoid copepods have a sister taxon relationship with *Amphiascoides atopus*.

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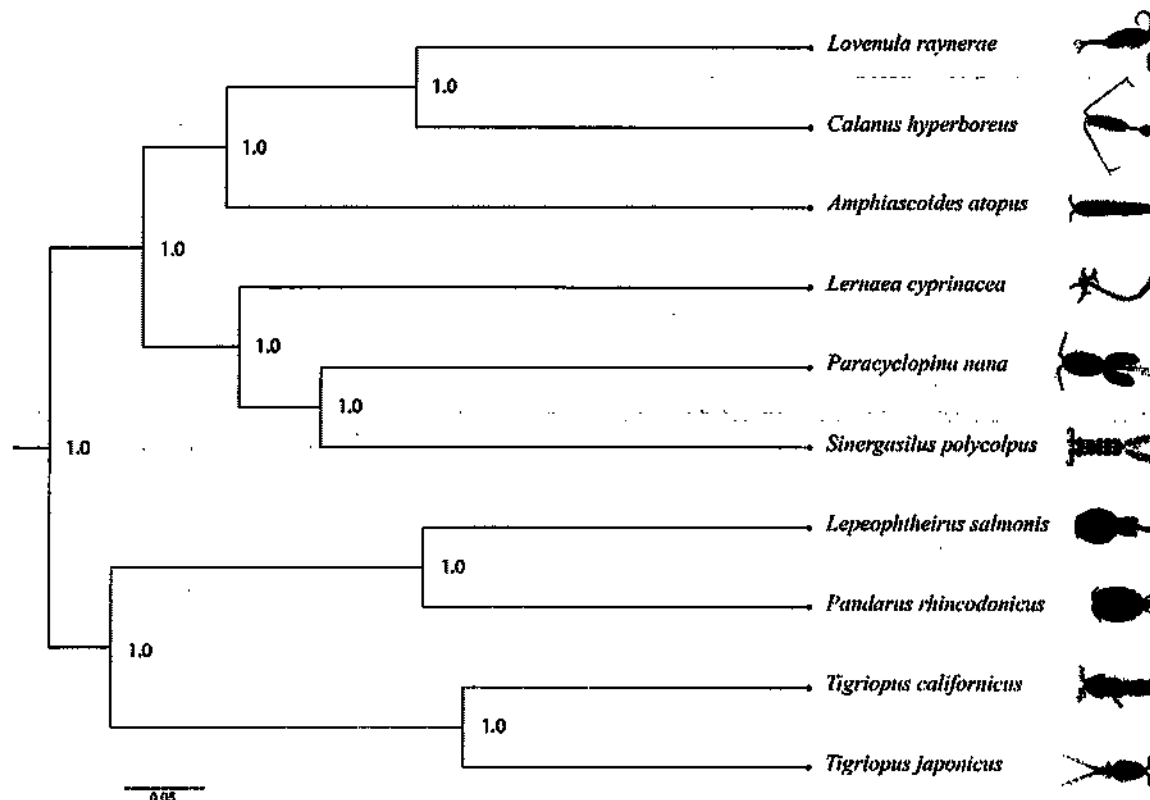


Figure 1. Bayesian phylogenetic tree of *Lovenula raynerae* and 9 other species. Numbers above the nodes indicate posterior probabilities. Accession numbers of all species are as follows: MH_710604 *Lovenula raynerae*, NC_019627 *Calanus hyperboreus*, NC_023783 *Amphiascoides atopus*, NC_025239 *Lernaea cyprinacea*, NC_012455 *Paracyclops nana*, NC_028085 *Sinergasilus polycolpus*, NC_007215 *Lepeophtheirus salmonis*, NC_024046 *Pandarus rhincodonicus*, NC_008831 *Tigriopus californicus*, NC_003979 *Tigriopus japonicus*.

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