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## The complete mitogenome of a South African cryptic species of tadpole shrimp within the *Triops granarius* (Lucas, 1864) species group

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### ABSTRACT

The complete mitochondrial genome of a cryptic species of tadpole shrimp from South Africa (Accession Number: MG770893) was recovered by low coverage shotgun sequencing. The mitogenome consists of 15,216 bp with a GC content of 30.95%. COX1 sequence alignment indicates that this specimen is the most closely related to *Triops granarius* with a pairwise nucleotide identity of less than 90% (86.6%). Phylogenetic analysis using whole mitogenome dataset supports its affiliation to the genus *Triops* with moderate support for its relatively basal position within the clade.

### ARTICLE HISTORY

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### KEYWORDS



Cryptic species; Illumina; mitogenome; phylogenetics; tadpole shrimp

Tadpole shrimps belong to the genus *Triops* Schrank, 1803 (Schranck 1803) and are considered as ‘living fossils’ due to their conserved morphology and fossil records dating back to 250 MYA (Gore and Traverse 1986; Garrouste et al. 2009). Attempts to elucidate the phylogenetic relationships among members of the genus *Triops*, to date, have met with mixed success. Discordance between phylogenetic species and morphological species seems common (Sunou-Uchi et al. 1997) and global genetic analysis of tadpole shrimps indicates high levels of cryptic diversity and unexpected relationships between major lineages (Vanschoenwinkel et al. 2012). Phylogeny inference using mitogenome sequences is emerging as a powerful tool to resolve shallow evolutionary relationships (Jacobsen et al. 2012; Gan et al. 2018). Despite the advent of next-generation sequencing platforms and reported successes of crustacean mitogenome assembly using established pipeline (Gan et al. 2014; Tan et al. 2015; Gan et al. 2016a), *Triops* mitogenomes remain scarce and lack representation from the African continent (Umetsu et al. 2002; Ryu and Hwang 2010; Horn and Cowley 2014; Gan et al. 2016b). We report the complete mitogenome of a cryptic species of tadpole shrimp collected from South Africa and assess its evolutionary relationships within the *Triops* genus.

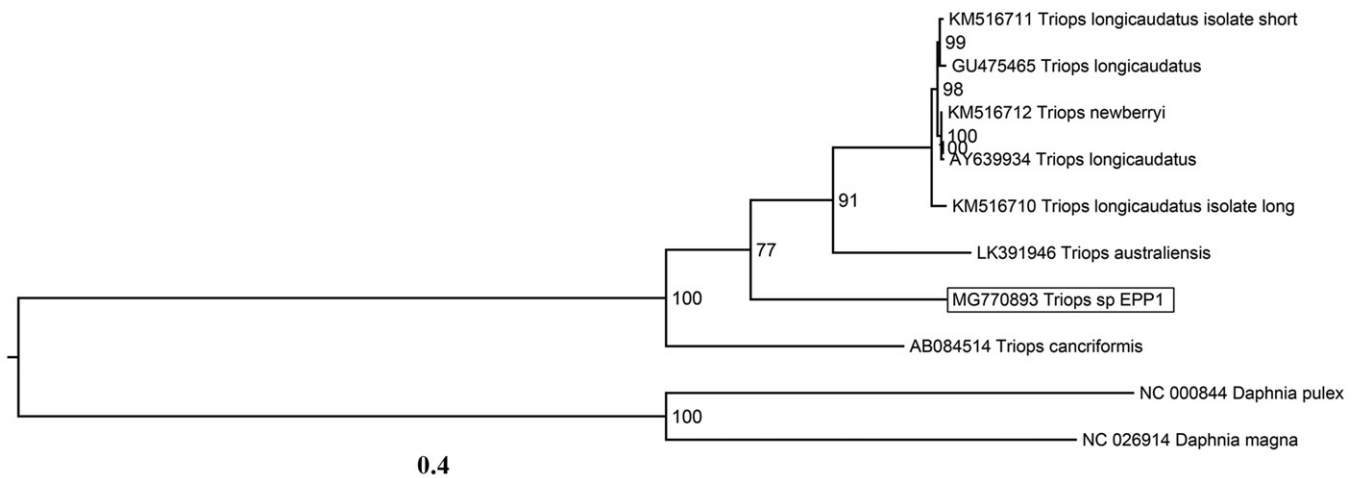
A whole specimen of *Triops* sp. was collected from an ephemeral pond located near Grahamstown, South Africa (–33.423817; 26.448509), and stored in a 1.5 mL tube containing SDS-lysis solution and proteinase-K. A voucher specimen has been lodged at the Kansas Biological Survey (DCR-1128). The DNA extraction was performed from the lysate as previously described (Sokolov 2000). The gDNA was sheared to

500 bp using an ultrasonicator (Covaris, Woburn, MA), processed with NEBUltra DNA library preparation kit (New England Biolabs, Ipswich, MA) and sequenced on a MiSeq sequencer (Illumina, San Diego, CA). Annotation of mitogenome used MITObim v1.8 and MITOS v1, respectively (Bernt et al. 2013; Hahn et al. 2013). Thirteen protein-coding genes were extracted from each mitogenome, partitioned by codon position and aligned using TranslatorX (Abascal et al. 2010). The 12S and 16S ribosomal rRNA genes were extracted and aligned using Muscle v3.8.31 (Edgar 2004). The resulting 41 partitions (13 protein-coding genes × 3 codon position + 2 rRNA genes) were trimmed with TriamAl v1.2 (‘-automated1 option’) (Capella-Gutiérrez et al. 2009). Model selection, partition merging, and maximum likelihood tree construction used IQTree v1.6.7 (Nguyen et al. 2015; Kalyaanamoorthy et al. 2017).

The mitogenome of *Triops* sp. EPP1 is 15,126 bp (30.95% GC content) with gene order that conforms to the primitive arrangement for Branchiopod crustaceans. A phylogenetic tree placed *Triops* sp. EPP1 within the *Triops* clade with moderate support for its basal position to all other *Triops* spp except *Triops cancriformis* (Bosc 1801) presumably due to an insufficient phylogenetic signal from uneven taxon sampling (Figure 1). COX1 sequence analysis indicates that *Triops* sp. EPP1 is the most closely related to a *T. granarius* specimen from India (Accession code: HF911376, BOLD database as of 16 October 2018) with a nucleotide similarity of 86.6%, suggesting that it may be a cryptic species of *T. granarius* (Korn and Hundsdoerfer 2006).

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**Figure 1.** Maximum likelihood tree depicting the evolutionary relationships of *Triops*. The tree was rooted with *Daphnia* spp. as the outgroup. Node labels indicate IQTree ultrafast bootstrap support values while branch lengths indicate number of substitutions per site.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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