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The complete mitochondrial genome of the viviparous freshwater snail *Tylomelania sarasinorum* (Caenogastropoda: Cerithioidea)

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ABSTRACT

Here, we present the first complete mitochondrial genome within the gastropod family Pachychilidae, using the viviparous freshwater snail *Tylomelania sarasinorum*. This species is a representative member of the lacustrine *Tylomelania* radiations of the Malili-Lakes-System (Sulawesi, Indonesia). The mitochondrial genome was 16,632 bp long and contained 13 protein-coding genes, 2 rRNA genes and 22 tRNA genes. A pronounced A + T bias was observed with an overall base composition of 29.5% A, 35.7% T, 18.3% G and 16.6% C. *Tylomelania sarasinorum* exhibited a novel mitochondrial gene arrangement, differing from all Caenogastropoda mitochondrial genomes published to date.

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Tylomelania (Caenogastropoda: Cerithioidea: Pachychilidae) is a genus of viviparous freshwater snails endemic to Sulawesi, Indonesia (von Rintelen et al. 2010). Renowned for their adaptive radiations in the ancient Malili Lakes System, *Tylomelania* represent a model system for freshwater invertebrate speciation (von Rintelen et al. 2010, 2014). While Caenogastropoda are the most speciose group of extant gastropods with ~85,000 species (Colgan et al. 2007), the phylogeny of gastropods in general, and Caenogastropoda in particular, is still a matter of debate (Colgan et al. 2007; Strong et al. 2011). Here, we present the mitochondrial genome of *Tylomelania sarasinorum* (Kruimel 1913), which constitutes the first complete mitochondrial genome within the family Pachychilidae and the third within the superfamily Cerithioidea (Zeng et al. 2014).

The specimen was collected at Loeha Island (Lake Towuti, South Sulawesi, 2.76075 S 121.5586 E) and is stored at the Museum für Naturkunde Berlin (accession number: ZMB Moll. 119994). DNA was extracted using CTAB extraction (Winnepenninckx et al. 1993) and shotgun sequenced (150-bp paired end) on an Illumia MiSeq® (Illumina, San Diego, CA) generating ~50 mio reads. The mitochondrial genome was reconstructed with MITObim (Hahn et al. 2013) using the COI sequence of the sister species *T. wallacei* (KJ933844) as seed reference and the following parameters: "-denovo", "-pair", "-proofread" and "-clean". The assembled mitogenome had a mean coverage of 495× and was manually inspected for repeats at the ends of the assembly to confirm circularity. Annotations were carried out with MITOchondrial genome annotation server (MITOS) (Bernt et al. 2012), followed by manual validation of the coding regions using the NCBI ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The annotated sequence file was submitted to NCBI (KU878411). The phylogenetic position of the new sequence is shown in Figure 1.

The complete mitochondrial genome was 16,632 bp in length, contained 13 protein-coding genes (PCGs), 2 rRNA genes and 22 tRNA genes. As described for other gastropods, in the mitochondrial genome of *T. sarasinorum* an A + T bias was evident with an overall base composition of 29.5% A, 35.7% T, 18.3% G and 16.6% C. The gene arrangement was most similar to the mitogenomes of the two cerithioideans *Semisulcospira libertina* (Gould 1859) and *Koreoleptoxis globus ovalis* (Burch & Jung 1987), differing only in the position of $tRNA^{Arg}$ and $tRNA^{Gln}$ (Cunha et al. 2009; Zeng et al. 2014; Osca et al. 2015). The light strand was clustered in the following order: $tRNA^{Cys}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Trp}$, $tRNA^{Glu}$, $tRNA^{Tyr}$, $tRNA^{Arg}$, $tRNA^{Gln}$, $tRNA^{Lys}$, *COX3*, $tRNA^{Met}$, *CYTB*, *ND6*, $tRNA^{Pro}$, *ND1*, $tRNA^{Leu2}$, $tRNA^{Leu1}$, *16S-rRNA*, $tRNA^{Val}$, $tRNA^{Gly}$, $tRNA^{Thr}$, *12S-rRNA*. The heavy strand genes clustered: $tRNA^{Ser1}$, *ND2*, $tRNA^{Asp}$, *ATP8*, *ATP6*, $tRNA^{Ile}$, *ND3*, *COX1*, *COX2*, $tRNA^{Ser2}$, *ND4L*, *ND4*, $tRNA^{His}$, *ND5*, $tRNA^{Phe}$. All PCGs had ATG as initiation codon. TAA was the most used termination codon with the exception of *COX3*, *CYTB*, *ND2*, *ATP8* and *ND3*, which used a TAG termination codon. The *12S* and *16S* genes had a length of 899 and 1382 bp, respectively. Overlaps were observed between *CYTB* and *ND6* (47 bp), *16S-rRNA* and $tRNA^{Val}$ (18 bp), and *16S-ND4L* and *ND4* (7 bp), $tRNA^{Leu1}$ and *16S-rRNA* (7 bp), *ND2* and $tRNA^{Asp}$ (2 bp) and between $tRNA^{Thr}$ and *12S-rRNA*.

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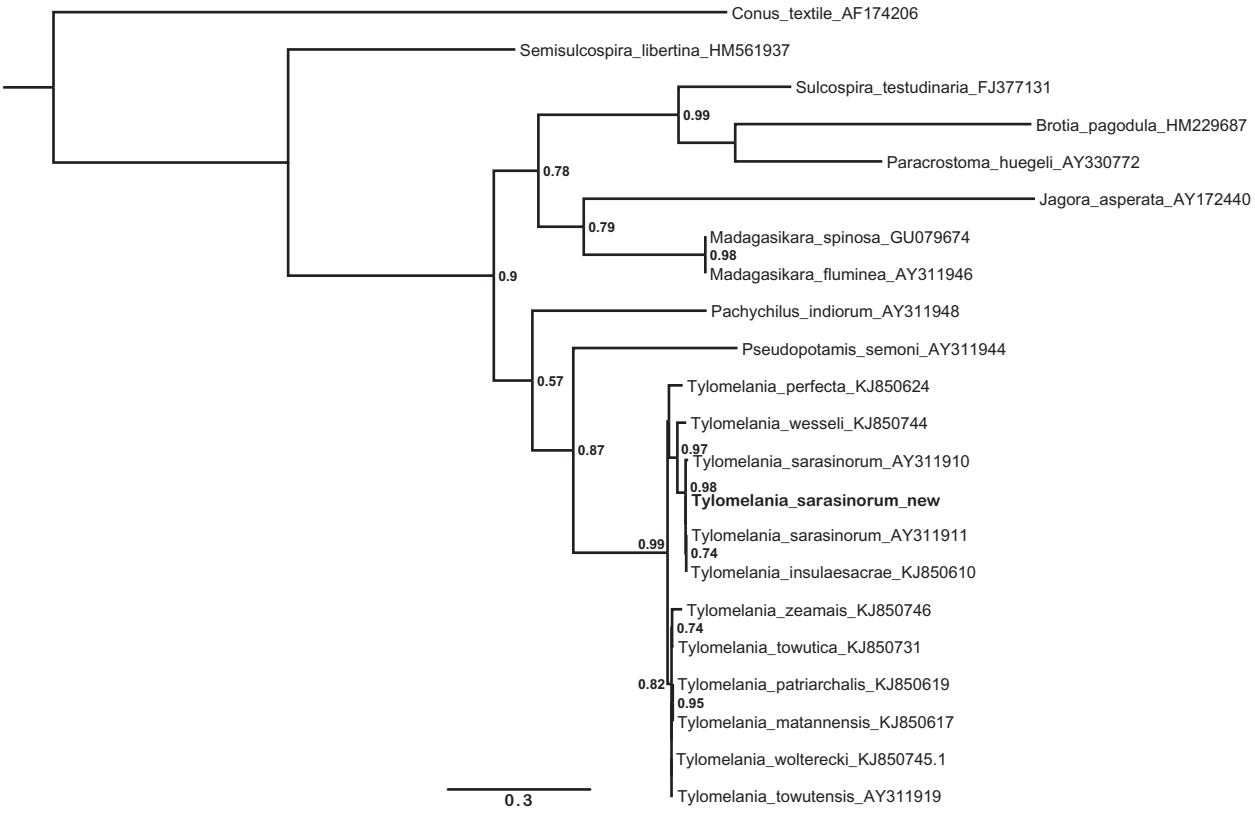

Figure 1. Maximum likelihood tree illustrating the position of the new *Tylomelania sarasinorum* 16s rRNA gene sequence within a subset of pachychilid species. Tree topology is largely in agreement with earlier work (Koehler & Glaubrecht 2010), but fails to resolve clades down to species level in the rapidly radiating genus Tylomelania which is in line with previous results (von Rintelen et al. 2004). Sequences were aligned using MAFFT 7.271 and highly divergent or poorly aligned regions were removed with Gblocks 0.91b (Castresana 2000) allowing for gap positions and smaller blocks. Trees were calculated using PhyML 3.1 (Guindon et al. 2010) with 12 rate categories, optimized equilibrium frequencies, GTR model of sequence evolution and combined heuristics (Nearest Neighbor Interchange and Subtree Pruning and Rerafting).

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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