**File S1.** Sample collection and library preparation of unpublished transcriptomes used in the study

***Mesobiotus philippinicus***

A single active *M. philippinicus* was obtained from a culture described in Mapalo et al. (2016) and was used for transcriptome sequencing following a modified protocol in Arakawa et al. (2016). The sample was first thoroughly washed on a sterile nylon mesh using Milli-Q water (Millipore), placed in a low-binding PCR tube with a minimal amount of water (<2 uL), and crushed using a pipette tip. The sample was then immediately lysed in TRIzol reagent (Life Technologies) and the RNA was extracted and purified using a standard DirectZol (Zymo Research) protocol. The quality of the extract was then checked using the TapeStation High Sensitivity RNA tape (Agilent). The cDNAs were synthesized and amplified with the SMART-Seq v.2 protocol using the SMARTer Ultra Low Input RNA Kit for Sequencing v.3 (Clontech) and prepared into an Illumina library using KAPA HyperPlus Kit (KAPA Biosystems). Then, the library was purified using AMPure XP purification method, quality-checked using TapeStation D1000 tape (Agilent), and the concentration was quantified using Qubit dsDNA BR assay (Thermo Scientific). A gel-based size selection of 300 bp was done using Nucleospin gel extraction kit (Clontech) and the library was sequenced using NextSeq500 High Output mode platform (Illumina) as 150 bp paired-end reads.

***Echiniscus testudo***

 Twenty *E. testudo* obtained from moss samples collected from the roof of a house in Nivå, Denmark (55°56’36.68” N, 12°29’55.85” E) was used in this study. They were all desiccated on Whatman 1 filters and stored at -80°C until use. Prior to RNA extraction, the animals were rehydrated and kept in mineral water for a minimum of 48 hours at 5°C to empty the gut content, minimize stress, and reduce contamination and acclimatized at room temperature (RT, approximately 20°C) for 1 hr. The samples were then divided into four set-ups: Active, Pre-tun, Desiccated, and Rehydrating. For the Active group (4 samples), the animals were kept in mineral water for 48 hours at 5° C and washed several times with molecular grade RNAse-free H2O prior to RNA extraction. For the Pre-tun group (5 samples), the animals were placed on a Whatman 1 filter submerged in molecular grade RNAse-free H2O. Then, the water was removed until only a film of water remains. The RNA extraction was done when the water reached a critical low-point and the tardigrades were observed to have stopped moving and started contracting into the tun formation. For the Desiccated group (7 samples), the animals underwent the Pre-tun step and all visible water was allowed to evaporate for approximately 40 minutes at ambient humidity (20-40% RH) so that the tardigrades can fully enter the tun formation (all legs and body contracted). The samples were then transferred to a desiccation chamber with silica gel beads for further desiccation of 48 hours at 0-10% RH at RT before RNA extraction. For the Rehydrating group (4 samples), the animals underwent the Desiccated step and was rehydrated by placing them in molecular grade RNAse-free H2O. The RNA extraction was done once the tardigrades exhibited muscle coordinated movement, which was approximately 5-10 minutes after rehydration.

 The total RNA of each sample was individually extracted using a modified protocol in Fernández et al. (2014). Using a needle, each individual sample encased in a 2 RNAse-free H2O water droplet was carefully transferred to an Eppendorf tube containing 500 μL TRIzol (Life Technologies). After vortexing, the tube was sequentially added with 5 μL glycogen and 50 μL of 1-bromo-3-chloropropane with 10 min incubation each at RT. The tube was then centrifuged at 16000 rpm for 15 mins at 4°C and the upper aqueous layer, which contained the total RNA, was recovered and with 500 μL isopropanol. After an overnight incubation at -20°C, the tube was centrifuged at 16000 rpm for 15 minutes at 4°C. The pellet was then washed by adding 1 mL 75% ethanol and centrifuged at 7600 rpm for 5 minutes at 4°C. After the washing step was done twice, the pellet was dissolved in RNA storage solution (Ambion). The mRNA was purified from the total RNA solution using Dynabeads mRNA Purification Kit (Invitrogen). In brief, the sample was incubated at 65°C for 5 minutes and added with 100 μL of magnetic beads. After 20 minutes incubation at RT on a rotator, the mRNA was eluted using 16 μL Tris-HCl incubated at 80°C for 2 mins. The quality of the mRNA extract was determined using the Bioanalyzer RNA Pico Assay Kit (Agilent). The cDNA libraries were created using the Apollo 324 platform using the PrepX mRNA kit (Wafergen) and amplified by PCR using the KAPA library amplification kit (KAPA Biosystems) at 18-20 PCR cycles. The libraries were then indexed and sent for sequencing on a HiSeq 2500 platform (Illumina) as 150 bp paired-end reads.

***Eperipatus* sp. and *Opisthopatus kwazululandi***

 Adult samples were obtained in the field, preserved in RNAlater, and flash frozen for storage. The RNA was extracted from 2-4 flash frozen trunk segments of an individual using the standard TRIzol protocol with 1μL glycogen as an RNA carrier. Then, mRNA was purified from the total RNA solution using Dynabeads mRNA purification kit and quantified using an RNA Pico Assay Kit. Paired-end cDNA libraries were constructed on an Apollo 324 platform using Wafergen kits and quantified using the Bioanalyzer HS DNA Assay Kit (Agilent). Single index from the PrepX mRNA library preparation kit from Illumina was added during the amplification of the cDNA libraries using 12-15 PCR cycles. Amplified libraries were then quantified using KAPA Library Quantification kits and qPCR and sent for sequencing on a HiSeq 2500 platform (Illumina) as 150 bp paired-end reads.

**References**

Arakawa K, Yoshida Y, Tomita M. 2016. Genome sequencing of a single tardigrade *Hypsibius dujardini* individual. Sci. Data. 3:1–7.

Fernández R, Laumer CE, Vahtera V, Libro S, Kaluziak S, Sharma PP, Pérez-Porro AR, Edgecombe GD, Giribet G. 2014. Evaluating topological conflict in centipede phylogeny using transcriptomic data sets. Mol. Biol. Evol. 31:1500–1513.

Mapalo MA, Stec D, Mirano-Bascos D, Michalczyk L. 2016. *Mesobiotus philippinicus* sp. nov., the first limnoterrestrial tardigrade from the Philippines. Zootaxa 4126:411-426.