Molecular Phylogenetics and Evolution 58 (2011) 553–558

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Short Communication

The mitochondrial genome of *Colossendeis megalonyx* supports a basal position of Colossendeidae within the Pycnogonida

Lars Dietz [a], Christoph Mayer [a,b], Claudia P. Arango [c], Florian Leese [a,*]

[a] Ruhr Universität Bochum, Evolutionsökologie und Biodiversität der Tiere, Universitätsstraße 150, D-44801 Bochum, Germany
[b] Forschungsmuseum Alexander Koenig, Adenauerallee 160, D-53113 Bonn, Germany
[c] Biodiversity Program, Queensland Museum, PO Box 3300, South Brisbane, Qld 4101, Australia

ARTICLE INFO

Article history:
Received 31 October 2010
Revised 26 December 2010
Accepted 27 December 2010
Available online 31 December 2010

Keywords:
Phylogeny
Arthropoda
Pycnogonida
tRNA
Mitogenomics
Pyrosequencing

ABSTRACT

We present the almost complete (16,007 bp) mitochondrial genome of a *Colossendeis megalonyx* specimen from the Southern Ocean and discuss gene order and tRNA structure in a comparative phylogenetic context. Our data suggest a basal position of the colossendeid lineage corroborating earlier phylogenetic studies but disagreeing with results of a recently published study that supported a highly derived sister-group relationship of Colossendeidae and Nymphonidae. Our results, together with BLAST searches and phylogenetic comparisons, indicate that the specimen presented as *Colossendeis* sp. in a series of recent studies had been misidentified. It has now been identified as a nymphonid species.

1. Introduction

Pycnogonida (sea spiders) are a group of aberrant, exclusively marine arthropods. Despite considerable research, their position in the arthropod tree of life is still controversially discussed (Dunlop and Arango, 2005; Edgecombe, 2010). The two main hypotheses suggest either a sister-group relationship of Pycnogonida to all other Euarthropoda ('Cormogonida hypothesis') or postulate Pycnogonida as the sister group to Euchelicerata. Evidence for both hypotheses is still found in most recent large-scale phylogenetic and phylogenomic studies (e.g. Meusemann et al., 2010; Regier et al., 2010). Within the Pycnogonida, recent studies have proposed group phylogenies based on nuclear 18S gene data (Nakamura et al., 2007) and on a combined morphological and multi-gene data set (Arango and Wheeler, 2007). Their inferred phylogenies agree in many aspects, however, with respect to the root of the Pycnogonida, their results differ. Nakamura et al. (2007) suggest ascorhynchids as the most basal taxon whereas Arango and Wheeler (2007) suggest a group of (Austrodecidae + Pycnogonidae + Colossendeidae) as the most basal Pycnogonida. Arabi et al. (2010) made additional analyses and suggest Austrodecidae, Pycnogonidae + *Rhynchothorax*, and Colossendeidae as the most basal groups. They interpreted the rooting within *Ascorhynchus* by Nakamura et al. (2007) as being artificially caused by inadequate outgroup choice (euchelicerates only). To further investigate pycnogonid relationships, evidence from mitochondrial gene order data can be a valuable tool.

Mitochondrial gene order data have been used extensively for phylogenetic inferences (e.g. Boore et al., 1998; Fahrein et al., 2007). However, mitochondrial gene order can be highly conserved at high taxonomic levels (e.g. in vertebrates) but it also can be highly variable between closely related species depending on the group of organisms in question (Gissi et al., 2008). Hence, the usefulness of mitochondrial genomes in phylogenetics needs to be regarded with caution, in particular when studying poorly known taxa.

Abbreviations: atp6, ATP synthase 6; atp8, ATP synthase 8; cox1, cytochrome oxidase 1; cox2, cytochrome oxidase 2; cox3, cytochrome oxidase 3; cytb, cytochrome b; nad1, NADH dehydrogenase 1; nad2, NADH dehydrogenase 2; nad3, NADH dehydrogenase 3; nad4, NADH dehydrogenase 4; nad4L, NADH dehydrogenase 4L; nad5, NADH dehydrogenase 5; nad6, NADH dehydrogenase 6; rrnL, large ribosomal subunit RNA (16S); rrnS, small ribosomal subunit RNA (12S); trnA, Alanine tRNA; trnC, Cysteine tRNA; trnD, Aspartate tRNA; trnE, Glutamate tRNA; trnF, Phenylalanine tRNA; trnG, Glycine tRNA; trnH, Histidine tRNA; trnI, Isoleucine tRNA; trnK, Lysine tRNA; trnL(CUN), Leucine tRNA, recognizing CUN codons; trnL(UUR), Leucine tRNA, recognizing UUR codons; trnM, Methionine tRNA; trnN, Asparagine tRNA; trnP, Proline tRNA; trnQ, Glutamine tRNA; trnR, Arginine tRNA; trnS(AGN), Serine tRNA, recognizing AGN codons; trnS(UCN), Serine tRNA, recognizing UCN codons; trnT, Threonine tRNA; trnV, Valine tRNA; trnW, Tryptophan tRNA; trnY, Tyrosine tRNA.

* Corresponding author. Fax: +49 0 234 3214114.
E-mail address: florian.leese@rub.de (F. Leese).

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doi:10.1016/j.ympev.2010.12.016

For pycnogonids, five complete and one partial pycnogonid mitochondrial genome sequences have been published so far. Hassanin et al. (2005) published a partial mitochondrial genome sequence of *Endeis spinosa* including the genes nad2, cox1, cox2, atp8, atp6, and cox3. This sequence shows the same gene order as the mitochondrial genomes of *Limulus polyphemus* and many other chelicerates, inferred to be the ancestral arthropod arrangement. Podsiadlowski and Braband (2006) presented the complete mitochondrial genome of *Nymphon gracile* which shows a highly derived gene order compared to the arthropod ground plan, with at least 10 translocations of tRNAs, a translocated control region, and an inversion of the region from nad2 to cox2. Park et al. (2007) sequenced the complete mitochondrial genome of the ammotheid *Achelia bituberculata*. Unlike that of *Nymphon*, it shows a gene order very similar to the arthropod ground pattern except for a translocation of trnQ. Just recently, Masta et al. (2010) published the complete or nearly complete mitochondrial genomes of the ammotheids *Ammothea hilgendorfi* and *Tanystylum orbiculare* and of *Colossendeis* sp. The ammotheids show the same gene order as that found in the ammotheid *Achelia* (Park et al., 2007), while *Colossendeis* shows a different arrangement of several tRNAs with apparent synapomorphies with *Nymphon* (Masta et al., 2010), contradicting proposed phylogenies for the group (Arango and Wheeler, 2007; Nakamura et al., 2007). In this study we present the almost complete (16,007 bp) mitochondrial genome of a Southern Ocean *Colossendeis megalonyx* specimen belonging to the clade E described by Krabbe et al. (2010). We analyze and discuss gene order and tRNA structure in a comparative phylogenetic context and show that the *Colossendeis* sp. that has been used in several recent studies (Regier et al., 2008, 2010; Masta et al., 2010) is apparently misidentified and not a colossendeid species.

2. Materials and methods

2.1. Study specimen, DNA extraction and sequencing

For this study a specimen of *C. megalonyx*, Clade E (Krabbe et al., 2010), collected from Bouvet Island during the ICEFISH 2004 expedition, was selected. DNA was extracted in 2010 using the Qiagen DNeasy Mini Kit and the tissue protocol according to the manufacturer's recommendations. For library preparation, 5 μg of genomic DNA of *C. megalonyx* was sonified and the 500 bp band gel excised. Adapters were ligated and the library analyzed on a GS-FLX Titanium (454). Library preparation and sequencing were carried out by Macrogen (Korea). Sanger sequencing with individually designed primers were performed on an ABI 3130xl automated sequencer.

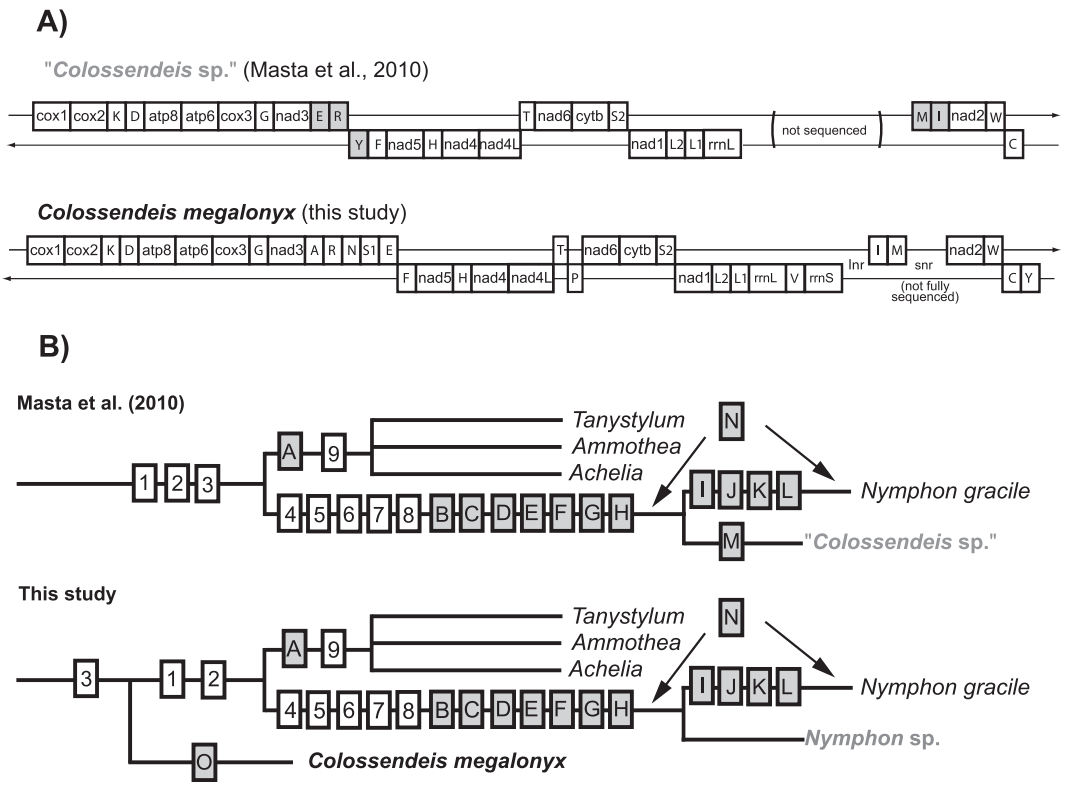

Fig. 1. A: Mitochondrial gene orders of *Colossendeis megalonyx* (this study) and "*Colossendeis* sp." (Masta et al. 2010). Genes marked in grey are those whose positions differ from the standard arthropod gene order in "*Colossendeis* sp.". L1=trnL(CUN), L2=trnL(UUR), S1=trnS(AGN), S2=trnS(UCN). Modified after Masta et al. (2010). B: Pycnogonid phylogeny with gene order and tRNA structural changes mapped on the tree according to Masta et al. 2010 (above) and this study (below). (A) trnQ translocated away from its position between trnI and trnM; (B) trnI translocated between trnM and nad2, on same alpha strand as original location; (C) trnY translocated next to trnF, on same beta strand as original location; (D) trnN translocated to near control region; (E) trnS(AGN) translocated to near control region; (F) trnA translocated to near control region; (G) either trnR or trnE translocated such that trnE is directly upstream of trnR on alpha strand; (H) trnP translocated to near control region; (I) translocation and inversion of trnK and trnD to near control region; (J) translocation of region coding for trnI-nad2-trnW-trnC-cox1-cox2 onto opposite strand, upstream of atp8; (K) trnM translocated to beta strand, between trnP and trnS(AGN); (L) control region translocated to between trnR and trnY; (M) translocation of trnI to between trnM and nad2, on same alpha strand (character M omitted in the phylogeny since it is redundant with character B); (N) trnV translocated away from location between rrnS and rrnL; (O) loss of trnQ. (1) Loss of the D-arm in trnA; (2) anticodon for trnK changes to UUU; (3) anticodon for trnS(AGN) changes to UCU; (4) variable loop in trnI decreases to 4 nt in *Nymphon* and "*Colossendeis* sp.", from 5 nt long in *C. megalonyx*, *Tanystylum* and *Achelia*; (5) loss of paired D-arm sequence in trnR; (6) mispaired acceptor stems in trnI; (7) mispaired acceptor stems in trnL(CUN); (8) mispaired anticodon stems of trnC and (9) reduced number of nucleotides in D-loop in trnA. The *Colossendeis megalonyx* sequence analyzed in this study is shown in bold, black letters, the "*Colossendeis* sp." of Masta et al. (2010) in bold, grey letters.