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SHORT PAPER

Complete Mitochondrial Genome of Calanoid Copepod *Bestiolina similis* (Sewell, 1914)

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Abstract

This study sequenced the mitochondrial genome of the copepod *Bestiolina similis*. DNA was extracted using a Qiagen Kit, and library preparation, PCR amplification, and sequencing were performed. The mitochondrial genome was 23,704 base pairs long, consisting of 13 protein-coding genes, 2 ribosomal RNA, and 22 transfer RNA genes. Its nucleotide composition is 43.42% adenine, 43.22% thymine, 6.48% guanine, and 6.88% cytosine. Phylogenetic analysis shows *B. similis* as a relict species, closely related to *Labidocera rotunda* and *Calanus simillimus*, with an independent evolutionary divergence from other copepods.

Introduction

Copepods, the most abundant metazoans found on Earth, represent a diverse group of organisms consisting of thousands of species distributed worldwide (Boxshall and Defaye, 2008). Among them, the order Calanoida stands out as a particularly rich and significant component of marine ecosystems, encompassing a large number of species that exhibit subtle variations in their morphological characteristics (Nawaz et al., 2023). Accurate identification of these species poses a challenge due to their cryptic nature, requiring careful examination and differentiation. To overcome this challenge, innovative approaches incorporating molecular markers have been developed, enabling more reliable and precise species identification (Blanco-Bercial et al., 2014).

Mitochondrial genes have proven to be highly variable and valuable tools in revealing taxonomically significant variation among copepod species. This variation enables clear discrimination between closely related species and facilitates the recognition of genetic divergence within conspecific groups, often associated with geographical isolation and cryptic speciation (Bucklin et al., 2003), Consequently, mitochondrial (mt) genes have gained significant attention in recent years for investigating the taxonomic position of copepods. Among these genes, mitochondrial cytochrome oxidase I (mtCoI) has emerged as a common marker for rapid species identification of copepods (Elías-Gutiérrez et al., 2018). However, while mtCoI is useful for species identification, it does not provide comprehensive insights into the phylogeny of copepod species, as it is known to be a fast-evolving gene (Lefébure et al., 2006). In contrast, recent studies utilizing complete

mitochondrial genome-based phylogenetic analyses have generated considerable interest in exploring the deep branching patterns of arthropods. Mitochondrial genome-based phylogenies have frequently revealed intriguing and sometimes striking topologies compared to traditional phylogenetic approaches (Nawaz et al., 2023b).

The family Paracalanidae comprises dominant calanoid copepods in tropical oceans, playing a crucial role in the grazing behavior of ocean ecosystems (Nawaz et al., 2023b). Among them, *Bestiolina similis* is a prominent calanoid species found abundant in some parts of coast of India (Hani and Jayalakshmi, 2023). It belongs to the genus *Bestolina* (Andronov 1991) within the Paracalanidae family, consisting of 11 species (World Register of Marine Species). *Bestiolina* is a new genus which was previously considered along with genus *Acrocalanus*. However, *Acrocalanus* species are characterized by their absence of a fifth leg in female (Kasturirangan, 1963). On the contrary, *Bestiolina* species have a small rudimentary fifth leg in female. In the past two decades, a significant number of *Bestiolina* species have been documented (Suárez-Morales and Almeyda-Artigas, 2016). However, their small size (>1mm), inadequate sampling techniques (using mesh size above 200 µm) and misidentification with copepodite stages of other paracalanid species makes them challenging to study (Dorado-Roncancio et al., 2019). Due to the following reasons, accurate taxonomic identification can be challenging, prompting the use of molecular approaches for taxonomy. Therefore, the objective of this study is to extract and sequence the complete mitochondrial genome of the marine copepod *B. similis* for the first time and deposit it in GenBank for further investigations. Currently, only a limited number of copepod mitochondrial genomes have been sequenced, with only a few belonging to the calanoid group. Hence, this study will construct a phylogenetic tree based on the extracted mitochondrial genome, incorporating the available data, to enhance our understanding of the evolutionary relationships among calanoid copepods. This research contributes to the expanding knowledge of copepod biodiversity and provides valuable insights into their taxonomic and phylogenetic relationships in marine ecosystems.

Materials and Methods

Copepods Collection and Identification

Zooplankton samples were collected from the Marina beach (13°03'00'' N, 80°16'56.64'' E, India) using a standard Bolton silk zooplankton net with a mesh size of 150µ five nautical miles away from the coast. The collected samples were preserved in 95% ethanol and transported to laboratory. Copepods were identified up to species level by carefully dissecting and studying the key characters (Boxshall and Halsey, 2004; Razoluls et al., 2024).

DNA Extraction and Quality Control

DNA from copepods was extracted using the Qiagen DNeasy Blood and Tissue Kit (Cat No.69506). The copepods were pelleted through centrifugation, and the supernatant was carefully removed, leaving behind the pellet, which was then air-dried. Subsequently, each microcentrifuge tube containing the pellet received ATL buffer and Proteinase K, followed by a brief vortexing and incubation at 56^{o}C for 2 hours. RNase A treatment (MP Biomedicals; Cat. No.210107683) was carried out at 65^{o}C for 20 minutes. The resulting lysate was thoroughly mixed with half the volume of absolute alcohol and loaded into a DNeasy mini spin column, placed within a 2 ml collection tube. The tubes were centrifuged at 8000 rpm for 1 minute, and the flow-through was discarded. The remaining column wash steps were conducted as per the manufacturer's instructions. Finally, DNA was eluted from the column using 10 mM Tris HCl, pH 8.0.

Library Preparation for Mitochondrial Genome

The DNA library preparation was carried out following the QIASeq FX DNA Library Preparation protocol (Cat#180475) in accordance with the manufacturer's instructions. The process involved enzymatic fragmentation, end-repair, and A-tailing of 1 ng to 10 ng of Qubit quantified DNA in a one-tube reaction using the FX Enzyme Mix provided in the QIASeq FX DNA kit. Subsequently, the end-repaired and adenylated fragments underwent adapter ligation, where an index-incorporated Illumina adapter was ligated to generate the sequencing library. The library was then subjected to 10 to 12 cycles of Indexing-PCR, including initial denaturation at 98˚C for 2 minutes, cycling (98˚C for 20 seconds, 60˚C for 30 seconds, 72˚C for 30 seconds), and final extension at 72˚C for 1 minute, to enrich the adapter-tagged fragments. Lastly, the amplified library was purified using Sera-MagTM Select beads (Cytiva, # 29343057), followed by a thorough quality control check of the library.

Illumina Sequencing and Data Analysis

Illumina-compatible sequencing library was quantified by Qubit fluorometer (Thermo Fisher Scientific, MA, USA) and its fragment size distribution was analyzed on Agilent 2200 TapeStation. The libraries were subsequently subjected to paired-end sequencing on an Illumina NovoSeq 6000 sequencer for 150 cycles, following the manufacturer's instructions (Illumina, San Diego, USA). The sequencing generated approximately 26.8 to 34.3 million Illumina short reads data. To ensure data quality, the raw reads were trimmed using Trimgalore-v0.4.01 to remove adapter sequences and low-quality reads. The processed high-quality reads were then utilized for assembly, gene prediction, and annotation using the MitoZ-v3.6 tool, a specialized

toolkit for animal mitochondrial genome assembly, annotation, and visualization (Meng et al., 2019).

Phylogenetic Tree Construction

The phylogenetic trees of mtDNA were constructed using the maximum likelihood (ML) method through MEGA-X software (Kumar et al., 1993). Existing mtDNA data from the NCBI database of relevant species were utilized for comparison and to produce the phylogenetic tree. Only data representing the genus level were considered for the analysis. Optimal trees were generated through heuristic searches with 1000 bootstraps (Felsenstein, 1985). The evolutionary history was inferred from the constructed trees using the Jukes-Cantor model (Grauer and Li, 2000). A bootstrap consensus tree was obtained from 1000 replicates, representing the evolutionary history of the analyzed taxa (Pattengale et al., 2009). Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates were collapsed. The initial tree(s) for the heuristic search were automatically obtained using Maximum Likelihood methods and BioNJ algorithms applied to a matrix of pairwise distances estimated through the Maximum Composite Likelihood (MCL) approach. The topology with the most favorable log likelihood value was selected. Species distances were compared at the phylogenetic levels of calanoid copepods to assess their evolutionary relationships.

Results

B. similis is a small calanoid copepod with a stout cylindrical body, with adult females having a size of around 0.80 mm in length. Its antennules (A1) in females have 21 segments, with extended terminal segments and no spinules' transverse rows on segments 1 to 7. There are also no spinules on the fifth pedigerous segment. The swimming legs (P1–P4) are biramous, with both exopod and endopod branches, and have a setal formula following a pattern typical of other Bestiolina species but with certain differences. The fifth pair of swimming legs (P5) in females is formed by a basal segment followed by four others, with the terminal segment having two unequal spines and the penultimate segment having a laterodistal spine. In males, P5 consists of an inflated basal segment preceded by four segments, with an analogous spine pattern. The urosome has four somites in females and five in males, with a distinct genital double-somite in females. The caudal rami are symmetrical, slightly elongated, and each carries five setae, a characteristic feature of calanoid copepods. Sexual dimorphism is observed, with males being slightly smaller than females and having asymmetrical fifth swimming legs, which are used for mating. These morphological characters are critical for the identification of *B. similis* and separating it from other copepods.

The complete mitochondrial genome sequence of *B. similis* found was to be 23,704 base pair in length, including 13 PCGs, 2 rRNA and 22 tRNA genes (Figure 1) (Accession No.-PRJNA1063006). The *B. similis* mitochondrial genome was confirmed based on compared with genbank database and shows 89.68% percentage of identity (E value-0.0). It comprises the two longest non-coding regions, from the beginning of the genome (0 bp to ∼6.5k bp) to the end (∼21.9k bp to 23.7k bp). Other non-coding regions were below 1000 bp in length. The complete mitochondrial genome's nucleotide composition is 43.42% Adenine, 43.22% Thymine, 6.48% Guanine and 6.88% Cytosine.

The mtDNA of *B. similis* reveals 13 protein-coding and 2 rRNA genes (Table 1) and NAD5, COX1, NAD4 and COB were observed to be the longer genes in the genome with 1678, 1537, 1300 and 1137 bp, respectively. NAD4L was observed to be the smallest gene with 292 bp. The mtDNA revealed 22 tRNAs coding genes each with ∼60 bp. There was ∼2k bp gap observed between the trnD(guc) and trnR(ucg) which COX2 and NAD4 occupied. Two gene coding for tRNA-Leu were observed-trnL(uaa) and trnL(uag) (Table 2).

The phylogenetic tree of copepods shows robust evolutionary affiliations among eight species, with high bootstrap values (100, 100, 92) validating them, representing high confidence in the inferred topology. The taxa were placed basal to derived, and *C. simillimus*, *L. rotunda*, and *B. similis* form a core clade (100% support), likely a recent common ancestor. *A. atopus* branches early in the analysis, followed by a clade with moderate support (92%), comprising *Pennella* sp., (a separate genus), *E. affinis, P. nana*, and the newly described *T. kingsejongensis*, representing shared ancestry among the derived taxa. The scale of genetic distance (0.20 substitutions per site) suggests moderate divergence, with *Pennella* as derived, perhaps due to unique evolutionary features (Figure 2).

This finding indicates a substantial disorientation within the order Calanoida when considering the families based on complete mtDNA. The phylogenetic tree constructed based on whole mitochondrial genome sequences revealed that *C. simillimus* and *L. rotunda* were grouped together, forming a closest cluster from *B. similis.* On the other hand, the positioning of *Eutemora* and *Pennala* is far from that of calanoid species, suggesting different evolutionary patterns within these calanoid species. While issues in long PCR amplification have traditionally made it difficult to extract full mitochondrial genomes, contemporary whole genome sequencing (WGS)-based methods like GetOrganelle and MitoZ have proven successful in reconstructing mitochondrial genomes without the need for long PCR. While *B. similis* was found to have <80% A-T content, which could affect some amplification approaches, other factors like DNA quality and structural complexities might also be responsible for the difficulties encountered.

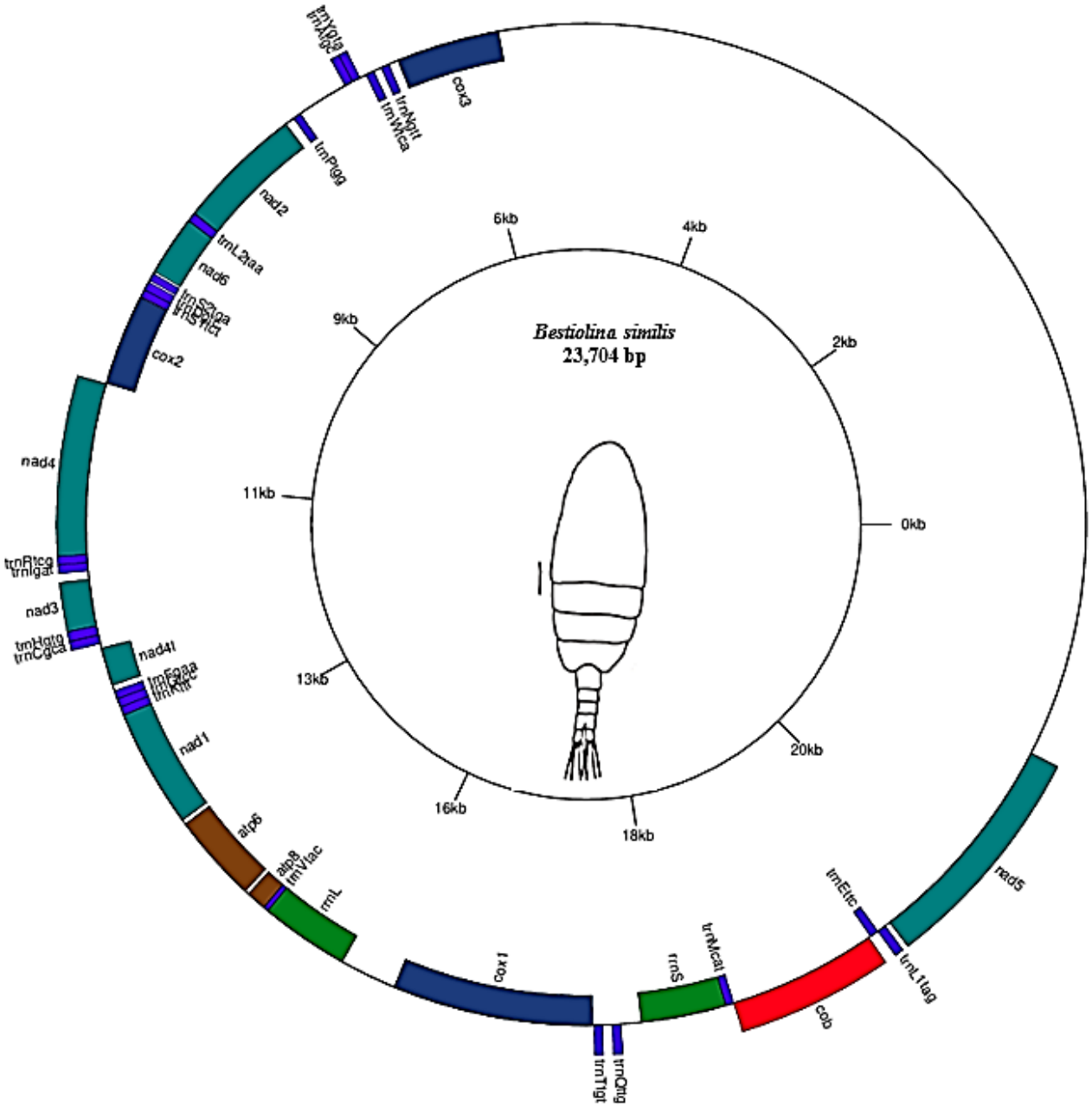

Figure 1. Complete mitochondrial genome of the calanoid copepod *B. similis*.

Table 1. Protein coding regions and rRNA of the mtDNA of *B. similis*

Gene Name	Start	End	Length(bp)
cox3	6590	7382	793
nad2	8356	9322	967
nad6	9394	9855	462
cox2	10078	10774	697
nad4	10779	12078	1300
nad3	12266	12620	355
nad4l	12793	13084	292
nad1	13326	14235	910
atp6	14270	14948	679
atp8	14980	15136	157
rrnL	15175	15856	682
cox1	16291	17827	1537
rrnS	18194	18846	653
cob	18909	20045	1137
nad5	20239	21916	1678

Table 2. tRNA genes of the mtDNA of *B. similis*

Gene Name	Start	End	Length(bp)
trnN(gtt)	7456	7520	65
trnW(tca)	7582	7646	65
trnY(gta)	7696	7759	64
trnA(tgc)	7760	7823	64
trnP(tgg)	8222	8283	62
trnL2(taa)	9325	9393	69
trnS2(tga)	9873	9930	58
trnD(gtc)	9946	10011	66
trnS1(tct)	10012	10070	59
trnR(tcg)	12079	12139	61
trnI(gat)	12140	12202	63
trnH(gtg)	12622	12689	68
trnC(gca)	12694	12757	64
trnF(gaa)	13129	13192	64
trnG(tcc)	13194	13260	67
trnK(ttt)	13261	13324	64
trnV(tac)	15136	15198	63
trnT(tgt)	17840	17900	61
trnQ(ttg)	17973	18041	69
trnM(cat)	18847	18908	62
trnE(ttc)	20061	20127	67
trnL1(tag)	20129	20196	68

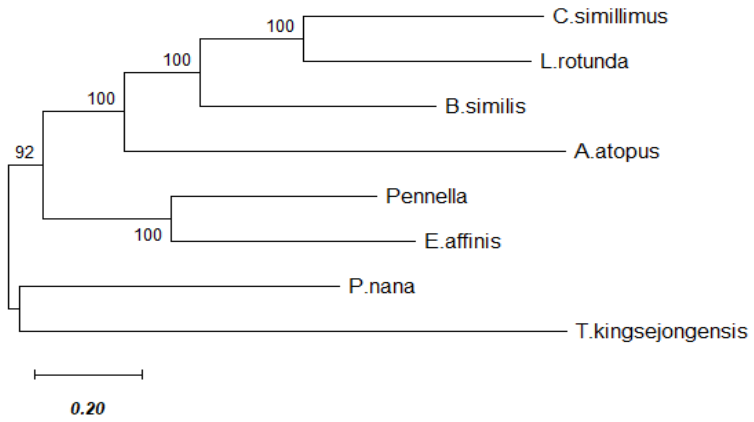

Figure 2. Phyologenetic tree based on ML method using complete mtDNA

Discussion

The genus *Bestiolina* (Andronov, 1991), previously known as *Bestiola* (Androv 1972) was distinguished from the genus *Acrocalanus* by the presence of 6 setae in P3 endopodite, which was 7 in the latter (Cornis and Blanco-Bercial, 2013). Since then, taxonomic and phylogenetic investigations on *Bestiolina* species have garnered considerable interest. The evolution of Paracalanidae species through both morphological and molecular datasets, revealing that *Bestiolina* species displayed a close relationship with *Acrocalanus* species in molecular datasets. However, their relationship differed significantly in morphological datasets (Cornis and Blanco-Bercial, 2013).

The size range of metazoan mitochondrial (mt) genomes varies from 14 to 48 kb (Crease, 1999) encompassing genes encoding at least 13 proteins, 22 transfer RNAs, and 2 ribosomal RNAs (rRNA). Notably, the mitogenome typically includes a substantial non-coding region that plays a role in transcription initiation and gene replication (Shadel and Clayton, 1997). In recent times, there has been an increasing trend in complete mitochondrial genome extraction for species identification and the study of phylogenetic and molecular evolution among closely related species (Li et al., 2022). The protein-coding genes (PCGs) of *B. similis* exhibit the same genes present in other calanoid mitochondrial genes, such as *C. hyperbolus* (Kim et al., 2013) and *E. affinis* (Choi et al., 2019). Unlike *Penella* and *Paracyclopina* species, *B. similis* does possess the ATP8 gene (Liu et al., 2022), indicating a genomic structure similar to other calanoid species. The vertebrate mitogenomes typically contain 2 rRNA genes, this feature is also common among most invertebrates (Boore, 1999). The presence of 2 rRNA genes was

observed in the present study. However, some cnidarins have been noted to exhibit reduced RNA genes in their genome (Lavrov, 2018), which may also apply to *B. similis*. However, given the limited availability of whole mitochondrial genome, it is currently not feasible to draw conclusive phylogenetic relationships between the species. Therefore, future studies are needed to further investigate the molecular evolution of calanoid species based on whole mitochondrial genome.

Ethical Statement

Not Applicable.

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Author Contribution

Conceptualization, K.S.K and M.G.K.; Methodology, K.S.K.; Software, K.S.K and M.G.K Validation, K.S.K and M.A.N.; Formal Analysis, K.S.K and M.A.N.; Investigation, K.S.K, P.M.P, S.V.M and M.A.N.; Resources, K.S.K and M.A.N.; Data Curation, K.S.K and M.G.K.; Writing – Original Draft Preparation, M.A.N. P.M.P and S.V.M; Writing – Review & Editing, K.S.K and M.A.N.; Visualization, K.S.K.; Supervision, K.S.K and M.A.N.; Project Administration, K.S.K; Funding Acquisition, K.S.K and M.A.N.

Conflict of Interest

The authors declare no conflict of interest.

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References

Blanco-Bercial, L., Cornils, A., Copley, N., & Bucklin, A. (2014). DNA Barcoding of Marine Copepods: Assessment of Analytical Approaches to Species Identification. *PLoS Currents*. https://doi.org/10.1371/currents.tol.cdf8b74-881f87e3b01d56b43791626d2

Boore, J. L. (1999). Animal mitochondrial genomes. *Nucleic Acids Research*, *27*(8), 1767–1780. https://doi.org/10.1093/nar/27.8.1767

Boxshall, G. A., & Defaye, D. (2007). Global diversity of copepods (Crustacea: Copepoda) in freshwater. *Hydrobiologia*, *595*(1), 195–207. https://doi.org/10.1007/s10750-007-9014-4

Boxshall, G., & Halsey, S. (2004). *An introduction to Copepod Diversity*. The Ray Society, London.

Bucklin, A., Frost, B. W., Bradford-Grieve, J., Allen, L. D., & Copley, N. J. (2003). Molecular systematic and phylogenetic assessment of 34 calanoid copepod species of the Calanidae and Clausocalanidae. *Marine Biology*, *142*(2), 333–343. https://doi.org/10.1007/s00227-002-0943-1

Choi, B., Han, J., Hwang, D., Souissi, S., Hagiwara, A., & Lee, J. (2019). Complete mitochondrial genome of the calanoid copepod *Eurytemora affinis* (Calanoida, Temoridae). *Mitochondrial DNA Part B*, *4*(2), 2731–2733. https://doi.org/10.1080/23802359.2019.1644558

Cornils, A., & Blanco-Bercial, L. (2013). Phylogeny of the Paracalanidae Giesbrecht, 1888 (Crustacea: Copepoda: Calanoida). *Molecular Phylogenetics and Evolution*, *69*(3), 861–872. https://doi.org/10.1016/j.ympev.2013.06.018

Crease, T. J. (1999). The complete sequence of the mitochondrial genome of *Daphnia pulex* (Cladocera: Crustacea). *Gene*, *233*(1–2), 89–99. https://doi.org/10.1016/s0378-1119(99)00151-1

Dorado-Roncancio, J., Gaviria, S., La Torre, L. B., & Ahrens, M. J. (2019). A new species of *Bestiolina* (Crustacea, Copepoda, Calanoida, Paracalanidae) from coastal waters of the Colombian Pacific, including a worldwide key for the identification of the species. *ZooKeys*, *846*, 1–18. https://doi.org/10.3897/zookeys.846.31497

Elías-Gutiérrez, M., Valdez-Moreno, M., Topan, J., Young, M. R., & Cohuo-Colli, J. A. (2018). Improved protocols to accelerate the assembly of DNA barcode reference libraries for freshwater zooplankton. *Ecology and Evolution*, *8*(5), 3002–3018. https://doi.org/10.1002/ece3.3742

Felsenstein, J. (1985). Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution*, *39*(4), 783. https://doi.org/10.2307/2408678

Graur, D., & Li, W. (1991). Fundamentals of molecular evolution. *Journal of Human Evolution*, *21*(3), 235. https://doi.org/10.1016/0047-2484(91)90069-8

Hani, P. M., & Jayalakshmi, K. J. (2023). Temporal variation in diversity, abundance and size class structure of planktonic copepods from a tropical estuary. *Aquatic Ecology*, *57*(1), 199–216. https://doi.org/10.1007/s10452-023-10003-3

Kasturirangan, L. R. (1963). *Key for the identification of the more common planktonic copepoda of Indian coastal waters* (Vol. 89). Council of Scientific and Industrial Research, New Delhi.

Kim, S., Lim, B., Min, G., & Choi, H. (2012). The complete mitochondrial genome of Arctic *Calanus hyperboreus* (Copepoda, Calanoida) reveals characteristic patterns in calanoid mitochondrial genome. *Gene*, *520*(1), 64–72. https://doi.org/10.1016/j.gene.2012.09.059

Kumar, S., Tamura, K., & Nei, M. (1991). *MEGA: Molecular Evolutionary Genetics Analysis*. Pennsylvania State University, University Park.

Lavrov, D. V. (2018). Mitochondrial genomes in invertebrate animals. In *Molecular Life Sciences*. Springer New York.

Lefébure, T., Douady, C., Gouy, M., & Gibert, J. (2006). Relationship between morphological taxonomy and molecular divergence within Crustacea: Proposal of a molecular threshold to help species delimitation. *Molecular Phylogenetics and Evolution*, *40*(2), 435–447. https://doi.org/10.1016/j.ympev.2006.03.014

Liu, B., Peng, Y., Zhang, K., Liu, Y., Li, J., Chen, J., Li, X., Jin, X., Zheng, S., Wang, Y., Zhang, S., Gong, L., Lü, Z., & Liu, L. (2022). Comparative analysis of the complete mitochondrial genomes of two species of Clupeiformes and the phylogenetic implications for Clupeiformes. *Journal of the Marine Biological Association of the United Kingdom*, *102*(6), 445–456. https://doi.org/10.1017/s0025315422000728

Liu, H., Fu, Z., Zhou, S., Hu, J., Yang, R., Yu, G., & Ma, Z. (2022). The Complete Mitochondrial Genome of *Pennella* sp. Parasitizing *Thunnus albacares*. *Frontiers in Cellular and Infection Microbiology*, *12*. https://doi.org/10.3389/fcimb.2022.945152

Meng, G., Li, Y., Yang, C., & Liu, S. (2019). MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization. *Nucleic Acids Research*, *47*(11), e63. https://doi.org/10.1093/nar/gkz173

Nawaz, M. A., Kandhasamy, S., & Gurunathan, B. (2023). Impact of Variation in Environmental Parameters on Abundance of Paracalanidae (Calanoida: Copepoda) from the Tropical Coast of India, Bay of Bengal. *Russian Journal of Marine Biology*, *49*(5), 391–400. https://doi.org/10.1134/s1063074023050073

Nawaz, M. A., Kandhasamy, S., Gurunathan, B., & Radha, V. (2023). Diversity rhythm in pontellid copepods (Pontellidae: Copepoda) from the Covelong coast pre- and post-COVID-19 lockdown, Bay of Bengal. *Turkish Journal of Zoology*, *47*(2), 71–80. https://doi.org/10.55730/1300-0179.3117

Pattengale, N. D., Alipour, M., Bininda-Emonds, O. R. P., Moret, B. M. E., & Stamatakis, A. (2009). Lecture notes in Computer Science. In *How many bootstrap replicates are necessary?* (pp. 184–200). Springer Berlin Heidelberg.

Shadel, G. S., & Clayton, D. A. (1997). Mitochondrial DNA maintenance in vertebrates. *Annual Review of Biochemistry*, *66*(1), 409–435. https://doi.org/10.1146/annurev.biochem.66.1.409

Suárez-Morales, E., & Almeyda-Artigas, R. J. (2016). A new species of Bestiolina (Copepoda: Calanoida: Paracalanidae) from the Northwestern Atlantic with comments on the distribution of the genus. *Revista Mexicana De Biodiversidad*, *87*(2), 301–310. https://doi.org/10.1016/j.rmb.2016.05.002