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Diversity of zooplankton in four perennial lakes of Coimbatore (India) and molecular characterization of *Asplanchna intermedia, Moina micrura, Mesocyclops edax* and *Cypris protubera* through mt-COI gene

P Saravana Bhavan, R Udayasuriyan, C Vadivalagan, R Kalpana, S Umamaheswari

Abstract
The distribution, diversity and population density of zooplankton species in four perennial lakes of Coimbatore city (Singanallur, Ukkadam, Sulur and Muthanan) were analyzed, and *Asplanchna intermedia* (Rotifera), *Moina micrura* (Cladocera), *Mesocyclops edax* (Copepoda) and *Cypris protubera* (Ostracoda) were discriminate by DNA bar coding. A total of 17 zooplankton species belonging to 13 genera with different population density were identified (Singanallur: 12 species and 1214 individuals L^{-1}; Ukkadam: 12 species and 995 individuals L^{-1}; Sulur: 12 species and 998 individuals L^{-1}; Muthanan: 10 species and 905 individuals L^{-1}). The genomic DNA was amplified with a set of universal primers (LCO1490 & HCO2198) for mt-COI gene, which revealed ~650 bp in each species, and the sequences showed 75-94% similarity with NCBI data base. The phylogenetic information revealed that these four zooplankton species are genetically distinct, conserved and less subjected to evolutionary forces. However, they are closely related and might have originated from a common ancestor.

Keywords: Zooplankton, Diversity, DNA barcoding, mt-COI gene, Divergence

1. Introduction
In water ecosystem, biological diversity measures are more useful, which harbor a large variety of phytoplankton, zooplankton, fishes etc., The diversity of a community can be described as the observed pattern of species abundance/ species richness [1]. Plankton are bioindicators for monitoring aquatic ecosystems and the integrity of water quality. Zooplankton assemblages are coupled to abiotic and biotic environmental factors (temperature, salinity, stratification, pollutants and food limitation, predation, competition) and therefore responding more rapidly to changes than do fishes, and are easier to identify than phytoplankton [2, 3]. In a freshwater ecosystem, since eutrophication influences both the composition and productivity of zooplanktons, studies on zooplankton population dynamics is important [4].

To study the distribution of zooplankton requires taxonomic identification traditionally performed by expert taxonomists employing optical techniques, which requires lot of time and great efforts. There are limitations in this method in some cases, such as those that appear while studying cryptic species (morphologically indistinguishable species), early developmental stages (eggs and larvae), parts of specimen bodies (e.g. one leg) or semi-digested samples (e.g. gut contents) [5]. Zooplankton identification often creates a bottle neck, species with different names and sibling species are universal, thereby increasing the difficulty in identification [6, 7]. While, genetic techniques have the potential of overcoming these limitations, and providing cheaper, faster and more accurate taxonomic identification [8].

The species level is recognized as the basic unit of biodiversity [9]. Nowadays, alpha taxonomy is still based mainly on morphology. Analyzing morphological differences is one of the possible methods to determine species between closely related populations [10]. With the advent of molecular technology for DNA sequencing, morphologically cryptic species have been increasingly revealed, and the use of DNA markers as a new tool to overcome morphological impediments [11]. The ideal DNA-based identification system would employ a single gene, and be suitable for any organism in the taxonomic hierarchy. Folmer *et al.,* [12] designed a universal primer for the mitochondrial cytochrome oxidase subunit I (mt-COI)

gene, which subsequently became a popular marker to study invertebrates. It has been suggested that the COI gene appears to be an appropriate molecular marker on several taxonomic scales, particularly at the species level [11-16]. Prior to phylogenetic analysis, DNA sequences are characterized by length and base composition, rate of nucleotide substitution, ratio of two specific instantaneous substitutions at which transitions and transversions occur [17].

This study dealt with morphological identification of few species of freshwater zooplankton groups belonging to Rotifera, Cladocera, Copepoda and Ostracoda, which are endemic to perennial lakes of Coimbatore city, Tamil Nadu, India, for assessing their diversity and population density. This study also aimed for molecular identification of one species of Rotifera (*Asplanchna intermedia*) and three crustacean zooplankton species (*Moina micrura*, *Mesocyclops edax* and *Cypris protubera* of Cladocera, Copepoda and Ostracoda respectively) by using DNA bar coding of mt-COI gene with analyses of sequence similarity, amino acids residues, sequence divergence, phylogenetic information such as synonymous and non-synonymous substitutions, transitional and transvertional substitutions, saturations, and phylogenetic tree topology.

2. Materials and Methods

Zooplankton were collected from four different perennial lakes in Coimbatore city, Tamil Nadu, India (Sulur Lake, Lat. 11º01'40" N and Long. 77º07'20" E; Ukkadam Lake, Lat. 10.99° N and Long. 76.96° E; Singanallur Lake, Lat. 10.98° N and Long 77.02° E; and Muthanan Lake, Lat. 11.03° N and Long. 76.89° E) for a period of three months once in fortnight from December-2014 to February-2015. For qualitative analysis of zooplankton, water samples were collected using Towing-Henson's standard plankton net (150 µm mesh) in zigzag fashion horizontally at a depth of 50 to 100 cm for about 10 minutes with a uniform speed of boat. For the quantitative analysis of zooplankton 100 liters of water was filtered through a plankton net made up of bolting silk (No: 10, mesh size: 150 µm) using a 10 liter capacity plastic container. Immediately after filtering out the water, the plankton biomasses were transferred to polyethylene specimen bottles (100 mL) filled with 5% of formalin (10 mL), the aqueous solution of formaldehyde.

Zooplankton sample was segregated/ assorted group-wise, which includes Rotifera, Cladocera, Copepoda and Ostracoda with the help of binocular stereo zoom dissection microscope. Individual species of plankton was mounted on microscopic slides with a drop of 20% glycerin after staining with eosin and rose Bengal. The identification of zooplankton was done by using the prescribed keys given in standard manuals, text books and monographs [18-26] with the help of compound microscope, and photomicrographs were taken using Inverted Biological Microscope (Model Number INVERSO 3000 (TC-100) CETI). Some of the important key characteristics of freshwater zooplankton are given here under. General elements that need to be assessed for all zooplankton groups are body shape and size, relative length of various appendages, including antennae, legs and setae, and presence and relative sizes of spines.

2.1. Rotifera

Rotifers are "wheel-bearer" refers to the crown of rotating cilia around the funnel-shaped mouth, which is used for locomotion and sweeping of food particles towards the mouth, and a specialized pharynx called the mastax, with its cuticular lining differentiated into trophy, a series of pieces that act as jaws.

2.1.1 Order/Family specific features

- Rotifers with paired generative organs (Monogononta).
- Rotifers with single generative organ, males present but mostly reduced (Monogononta).
- Marine forms: Corona not with two trochal discs, reduced, males fully developed (Seisonide).
- Freshwater forms: Corona with two trochal discs, latter rarely reduced in some forms; males not known (Bdelloidea).

2.1.2 Species specific features

- Inner margin tooth absent or rudimentary (*Asplanchna intermedia*). Body of *A. intermedia* is transparent with sacciform, greatly contractile, without lorica. Ciliary corona reduced to a thin course of cilia around the head, enclosing a large apical area; trunk with large body cavity (pseudocoel), stomach lying well away from body wall, intestine is absent; mastax is present over the trophy-incudate. Foot or any other extensions cuticle wanting. Retractile muscles are well developed [23].
- Lorica spherical (*Brachionus rubens*).
- Median anterior spines short (*Brachionus caudatus personatus*).
- Lorica vase-shaped; occipital spines small based; elevated lateral pectoral projections (*Brachionus rotundiformis*).
- Lorica thin, without plaques, anterior margin spines variable (*Brachionus calyciflorus*).

2.2. Cladocera

Cladocerans are a primary freshwater monophyletic micro-crustacean (water fleas) with compound eye, usually a carapace covering most of the body, except the head, and at least four pairs of trunk appendages which are in most cases broad lobed and fringed on the inner edges with bristles. No segmentation is visible on the carapace, but in many species the carapace forms a posterior spine. Sometimes there is also a spine on top of the head. The second antennae are very well developed. Their bodies are not divided into a separate thorax and abdomen. The tip of the trunk forms a "post-abdomen", which is bent towards the ventral trunk surface and is equipped with claws and spines for cleaning the carapace.

2.2.1 Order/ Family specific features

- Head with a protective head shield. Swimming antennae with less than ten natatory setae (Anomopoda).
- Head without a protective head shield. Swimming antennae with more than ten natatory setae (Ctenopoda).
- Antennules fused with rostrum (Bosminidae).
- Body not laterally compressed. Rostrum absent (Moinidae).

2.2.2 Species specific features

Antennule short and broad, length <10X width (*Moina micrura*). *M. micrura* is approximately 0.5 mm in length, relatively rounded in body shape, yet possesses a relatively large, distinct head [27]. The head is approximately ½ the length of the body and is curved or sloped ventrally. The first antennae are exposed (not covered by a beak), variable in length, flexible and are attached along the ventral surface of the head, rather than at the front. The tips of the antennae are blunt and exhibit short olfactory setae. The second antennae are large and used for swimming. The body is surrounded by a shell-like carapace which is open along the ventral surface and includes a notch-like cervical sinus (near the "nape") on the dorsal surface of the body. The dorsal surface also includes a

brood chamber to carry eggs. *M. micrura* lacks a rear shell spine, rostrum and ocellus, or eye spot, though does exhibit a single large, median compound eye. *M. micrura* does exhibit a post-abdominal claw with pecten of uniform length. Dorsal to the post abdomen are two pairs of relatively long abdominal setae [23].

- Small species as adult, (<0.5 mm); head with an acute rostrum; Valves distinctly reticulate, head small depressed and separated from body by a distinct ocular depression (*Ceriodaphnia cornuta*).
- Head small with a sloping dorsal side. Carapace with broad free flap. Swimming antennae weak and short (*Diaphanosoma sarsi*).

2.3. Copepoda

Copepods have short cylindrical bodies clearly divided into a number of segments. The head section is usually rounded and bears prominent, often very long antennae, which when held away from the body, serve to slow sinking rate. There are usually nine free trunk segments. The anterior segments bear the swimming appendages while the posterior segments taper, ending in a pair of caudal rami at the base of the abdomen. On the basis of major articulation of the body, Copepoda is divided into two groups, gymnoplea and podoplea. In gymnoplea (platycopida and calanoida), there are no appendages on the body segments posterior to the major articulation. In podoplea, there are reduced appendages on body segment posterior to the major articulation. Copepods are including three free living groups viz., calanoida, cyclopoida and harpacticoida.

2.3.1 Order/ Family specific features

- First antennae very short (<10 segment), do not reach past end of cephalothorax; Body cylindrical (Harpacticoida).
- First antennae up to 18 segments, may reach past the posterior end of cephalothorax; Body widest behind the head, tapers to urosome (Cyclopoida).
- First antennae long, >20 segments, extend to urosome or past end; Body torpedo like (Calanoida).

2.3.2 Species specific features

- Five caudal seta (inner margins of caudal ramus usually hairy) (*Mesocyclops edax*). *M. edax*, head is fused with the first one or two thoracic segments, while the remainder of the thorax has three to five segments, each with limbs. The first pair of thoracic appendages is modified to form maxillipeds, which assist in feeding. The abdomen is typically narrower than the thorax, and contains five segments without any appendages, except for some tail-like "rami" at the tip. Furcal rami bear prominent hairs on both the sides, inner margin of caudal ramus without hairs [26].
- Furcal rami without hairs on inner margin (*Mesocyclops leuckarti*).
- Furcal rami bear prominent hairs on both the sides, inner margin of caudal ramus without hairs (*Cyclops vernalis*).
- Antennule reaching the distal margin of prosomal somite 2. The pediger 5 with a longitudinal row of minute spinules on both of lateral sides (*Thermocyclops hyalinus*).
- Antennule overreaching caudal rami by last 3 or 4 segments and genital somite asymmetrical in females, left coxal spine stouter than right in leg 5 of female, right antennule with spine on each of segments 8th and 10-16 in male (*Heliodiaptomus viduus*).

2.4. Ostracoda

Ostracods are commonly called as 'seed shrimps' or 'mussel shrimps' and are very small, and the freshwater forms are usually smaller than a millimeter. Ostracods are equipped with a low Mg-calcite carapace attached by a dorsal hinge and a ligament. Members of Ostracoda are separated from other crustaceans by a laterally compressed body, undifferentiated head, seven or less limbs and a bivalve carapace with no growth lines.

2.4.1 Order/ Family specific features

- Carapace ovoid, inflated sub-triangular, oblong elongate, or compressed; no rostrum or incisura; valves overlap around free margin; 2nd antenna geniculate, pediform; with very small exopod with no more than two podomeres; much larger propulsive endopod with up to four podomeres; variable furca anterior to anus; no lateral eyes or Bellonci organ (Podocopa).
- Carapace elongate oblong to almost circular; with or without rostrum and incisura; valves usually not overlapping; 2nd antenna with large muscular protopod; long exopod with nine podomeres (rarely less) with long setae (often used for swimming); smaller (often much smaller) endopod with 1-3 podomeres, usually conspicuously dimorphic in adults; large lamellar furcae posterior to anus; with or without lateral eyes and Bellonci organ (Myodocopa).

2.4.2 Species Specific feature

- Valves with a prominent antero-ventral protuberance (*Cypris protubera*). In *C. protubera*, both paratype and holotype of carapace are present. Its furca is attached to the anterior end, maxillae are present with the zenker organ, and the zenker organ lies beneath the holotype carapace. Numerous antennae are present on the both ventral and dorsal region [19].
- Valves usually subvate; not tumid; unequal; left valve overlaps the right; left valve tuberculate. Furcal rami well developed, symmetrical and short (*Heterocypris dentatomarginatus*).
- Valve shape, variable greatest height in the middle or slightly behind the middle, valves moderately compressed and sub-equal. Penultimate segment of the second thoracic leg divided. Symmetrical furca, usually short, less than 1/2 the length of the valves (*Eucypris bispinosa*).
- Valve dorso-ventrally compressed (*Strandesia elongata*).

2.5. Enumeration of zooplankton

The sample (1 ml) was taken with a wide mouthed pipette and poured into the counting cell of the Sedgwick Rafter. After allowing for settle some time they were counted. At least 5 such counting was made for each lake. The species, sex and the developmental stage of the plankton was considered. The average values were taken. Total number of zooplankton present in 1 liter of water sample was calculated [28] using the formula ($N = n \times v / V$; Where, N = Total number of plankton per liter of water filtered, n = Average number of plankton in 1 mL of plankton sample, v = Volume of plankton concentrated (mL) and V = Volume of total water filtered in liter) and the zooplankton population is expressed in average (total number of individuals per litre).

2.6. Mass culture and molecular characterization of zooplankton

The collected zooplankton for qualitative analysis was also subjected to mass culture for 5 days in 10 litres of ground

water and they were fed with yeast. The water was maintained at pH, 7.4-7.6 and temperature, 26-28 ºC. From the mass culture of individual lakes, the dominant species was isolated, and 100 individual were subjected to monoculture in 1000 ml beaker for 7-14 days and fed with yeast. Invariably, the dominant species found in each group was subjected to molecular identification/ discrimination through mt-COI gene. Genomic DNA was isolated from the whole animal by using Qiagen Dneasy Blood and Tissue Kit (Germany). 1% Agarose Gel Electrophoresis (GENEI, Bangalore, India) was performed and the genomic DNA was detected in a Gel documentation system (Medicare, India). DNA amplification of mt-COI gene was carried out in Eppendorf Thermo Cycler with a universal set of primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTG -3'; GC%, 29.2) and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA- 3'; GC%, 34.6) [12, 16], and these primer sets were worked well with crustaceans, crabs and prawns [29 - 31]. Amplification was performed in a total volume of 50 µl containing 4 µl of DNA template, 20 p.mol of each primer, 400 µM of dNTP, and 0.4 µl of Taq DNA polymerase (Qiagen). The thermo cycler condition was as follows: 5 min at 95 °C for pre-running, 35 cycles of 60 s each at 95 °C for denaturation, 60 s at 57 °C for annealing, and 90 s at 72 °C for extension, followed by 5 min at 72 °C for a final extension. The amplified product was resolved with 2% AGE (GENEI, Bangalore, India). Sequencing was done by using ABI 3500 XL Genetic Analyzer with manufacturer's protocol of Chromous Biotech, Bangalore, India (outsourcing service was utilized).

The forward and reverse sequences were aligned pair wise by using CAP3. The sequence similarity available with NCBI database was identified by BLAST. The internal stop codon was removed by using BLAST. The reading frame shift was deducted by ORF finder. The trimmed sequence was authenticated with GenBank. The multiple sequence alignment was done by using T-Coffee and the aligned sequence was highlighted with multiple align show (MAS) as identical, similar and variable sites of amino acids. The nucleotide composition (AT and GC bias), nucleotide divergence (K2P model [32]) and some phylogenetic information were calculated by using MEGA v.6.01, and finally the phylogenetic tree (Maximum Likelihood model [33]) was reconstructed. The following phylogenetic information was also calculated: assessment of synonymous (Ks) and non-synonymous (Ka) substitutions for 3rd codon positions was calculated by Li93 method of DAMBE [34]; similarly, the inferred synonymous (dS) and inferred non-synonymous (dN) substitutions for 3rd codon positions was predicted by Muse-Gaut model of codon substitution [17]; the transitional (Ts) and transvertional (Tv) substitutions was determined by Felsenstein model of nucleotide substitution [35]; analysis of sequence saturation, index of substitutional saturation (Iss) and critical value of index of substitutional saturation (Iss.c) was done by Xia method using DAMBE [36, 37].

3. Result and Discussion

3.1. Diversity of zooplankton

In this study, in the Singanallur lake, 12 species (9 genera) of zooplankton were identified, of which 5 species belongs to Rotifera (*Asplanchna intermedia, Brachionus rubens*, *Brachionus calyciflorus*, *Brachionus rotundiformis*, and *Brachionus caudatus personatus*), 3 species belongs to Cladocera (*Moina micrura*, *Ceriodaphnia cornuta,* and *Diaphanosoma sarsi*), 3 species belongs to Copepoda (*Cyclops vernalis, Mesocyclops leuckarti*, and *Heliodiaptomus viduus*) and one species belongs to Ostracoda (*Cypris protubera*). Among these, the Rotifera, *Brachionus* species were found to be dominant, there were four *Brachionus* species (Table 1). In the mass culture with ground water under laboratory, the Rotifera species, *Asplanchna intermedia* was found to be dominant.

In the Ukkadam lake, 3 species of Rotifera (*Asplanchna intermedia*, *Brachionus rotundiformis,* and *Brachionus rubens*), 3 species of Cladocera (*Moina micrura, Ceriodaphnia cornuta,* and *Diaphanosoma sarsi*), 3 species of Copepoda (*Cyclops vernalis, Thermocyclops hyalinus,* and *Heliodiaptomus viduus*) and 3 species of Ostracoda (*Cypris protubera, Eucypris bispinosa,* and *Strandesia elongata*), totally 12 species of zooplankton were identified (Table 1). Each species belongs to different genus. In the mass culture with ground water under laboratory, the Ostracodan species, *Cypris protubera* was found to be dominant.

In the Sulur lake, 12 species of zooplankton (fall under 11 genera), of which 3 species of Rotifera (*Asplanchna intermedia*, *Brachionus rubens*, and *Brachionus caudatus personatus*), 3 species of Cladocera (*Moina micrura*, *Ceriodaphnia cornuta* and *Diaphanosoma sarsi*), 5 species of Copepoda (*Mesocyclops edax, Mesocyclops leuckarti*, *Thermocyclops hyalinus, Cyclops vernalis* and *Heliodiaptomus viduus*) and one species of Ostracoda (*Heterocypris dentatomarginatus*) were identified (Table 1). In mass culture, the Copepod species, *Mesocyclops edax* was found to be grown well.

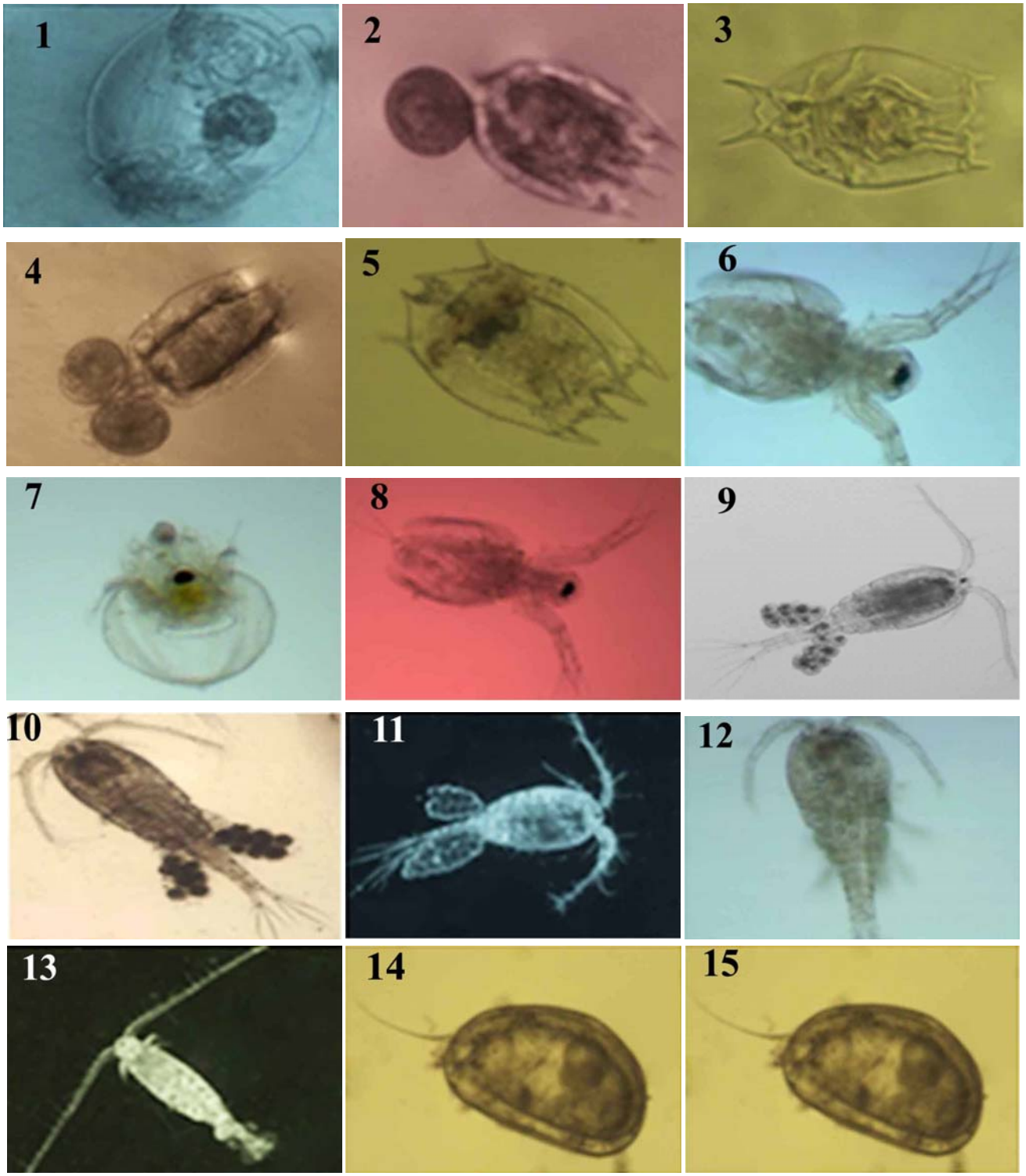
In the Muthanan Lake, 10 species of zooplankton (belongs to 9 genera) were identified, of which 3 species from Rotifera (*Asplanchna intermedia*, *Brachionus rubens,* and *Brachionus rotundiformis*), 3 species from Cladocera (*Moina micrura, Ceriodaphnia cornuta,* and *Diaphanosoma sarsi*) 3 species from Copepoda (*Cyclops vernalis, Mesocyclops edax* and *Heliodiaptomus viduus*), and one species from Ostracoda (*Cypris protubera*) (Table 1). In mass culture, the Copepod species, *Moina micrura* was found to be grown well.

Table 1: Distribution, diversity and population density of zooplanktons in different lakes of Coimbatore city

Zooplankton group	Zooplankton species	Lakes			
		Singanallur	Ukkadam	Slulur	Muthanan
Rotifera	*Asplanchna intermedia*	+++	++	++	++
	Brachionus rubens	++	+	+	+
	Brachionus caudatus personatus	++	-	+	-
	Brachionus rotundiformis	+	+	-	+
	Brachionus calyciflorus	+	-	-	-
Cladocera	*Moina micrura*	+	+	+	+++
	Ceriodaphnia cornuta	+	+	+	++
	Diaphanosoma sarsi	+	+	+	+
Copepoda	*Mesocyclops edax*	-	-	+++	+
	Mesocyclops leuckarti	+	-	+	-

	Cyclops vernalis	+	+	++	+
	Thermocyclops hyalinus	-	+	+	-
	Heliodiaptomus viduus	+	+	+	+
Ostracoda	*Cypris protubera*	++	+++	-	++
	Heterocypris dentatomarginatus	-	-	+	-
	Eucypris bispinosa	-	++	-	-
	Strandesia elongata	-	+	-	-
Species diversity		12	12	12	10
Population density (No. of individual L^{-1}). Mean ± SD of three individual observations.		1214±162	995±136	905±76	988±116

Note: +, Present; ++, moderately present; +++, luxuriantly present, -, absent.



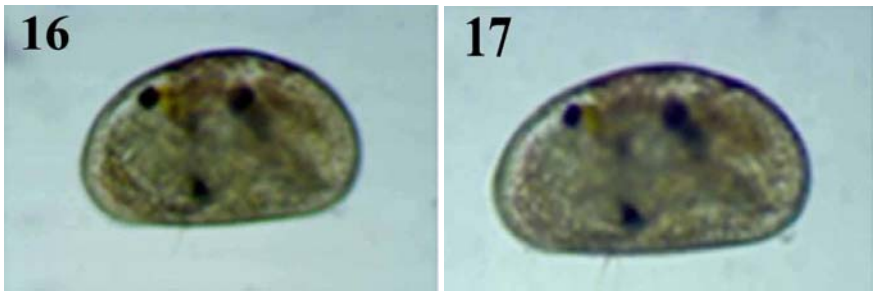


Plate 1: Species of zooplankton in different lakes (Singanallur Lake, Ukkadam Lake, Sulur Lake and Muthanan Lake) of Coimbatore city. Figs. 1-5, Rotifera: 1, *Asplanchna intermedia*; 2, *Brachionus rubens*; 3, *Brachionus caudatus personatus*; 4, *Brachionus rotundiformis*; 5, *Brachionus calyciflorus*; Figs. 6-8, Cladocera: 6, *Moina micrura*; 7, *Ceriodaphnia cornuta*; 8, *Diaphanosoma sarsi*; Figs. 9-13, Copepoda: 9, *Mesocyclops edax*; 10, *Mesocyclops leuckarti*; 11, *Cyclops vernalis*; 12, *Thermocyclops hyalinus*; 13, *Heliodiaptomus viduus*; Figs. 14-17, Ostracoda: 14, *Cypris protubera*; 15, *Heterocypris dentatomarginatus*; 16, *Eucypris bispinosa*; 17, *Strandesia elongata*

Therefore, in this study, totally 17 species of zooplankton (belongs to 13 genera) were identified: Rotifera, 5 species; Cladocera, 3 species; Copepoda, 5 species; Ostracoda, 4 species (Plate 1). Among these 17 species, only 7 species were present in all the four lakes, of which 2 species from Rotifera (*Asplanchna intermedia* and *Brachionus rubens*), 3 species from Cladocera (*Moina micrura*, *Ceriodaphnia cornuta* and *Diaphanosoma sarsi*) and 2 from Copepoda (*Cyclops vernalis* and *Heliodiaptomus viduus*). None of the Ostracodan species was found to be present in all the four lakes (Table 1). The average population density was maximum in the Singanallur Lake (1214 individuals L^{-1}), followed by the Ukkadam, Muthanan and Sulur Lakes (995, 988, and 905 individuals L^{-1} respectively).

Brachionus species are very common in temperate and tropical waters of alkaline in nature. Excess growth of rotifers in lakes indicates eutrophic conditions [38-41]. In this study, more Rotifera population was seen in the Singanallur Lake. Therefore, monitoring measures are warranted to minimize the water pollution of this lake. In this study, Cladocera population was higher in the Muthanan Lake. According to Balakrishna *et al.* [42] the most dominant Cladocerans inhabiting in the wetlands were *Moina* and *Ceriodaphnia* sp. It has also been reported that *Moina micrura* followed by *Moina brachiata* was observed in more numbers during summer [41]. The lake rich in organic matter supports higher number of Copepods. Thus, in this study, the recorded more Copepoda population suggests their preponderance in higher trophic state of water. It has been reported that micro-crustaceans mainly Copepods and Rotifers dominate in lentic habitats, while testate Amoebae and Rotifers were predominant in lotic habitats [43]. Generally, Ostracods are found in heavily polluted water and they serve as indicator species of climate and ecosystem changes [18, 44]. In this study, the recorded more Ostracoda population in the Ukkadam Lake suggests that this lake was being polluted. Therefore, adequate measures are needed to minimize the water pollution of this lake. The productivity of zooplankton in these lakes can be utilized for inland aquaculture production of fishes and prawns.

3.2. Molecular characteristics

The isolated genomic DNA showed greater than 10 kb nucleotides (Fig. 1) and the PCR amplified genomic DNA showed ~650 bp (Fig. 2).

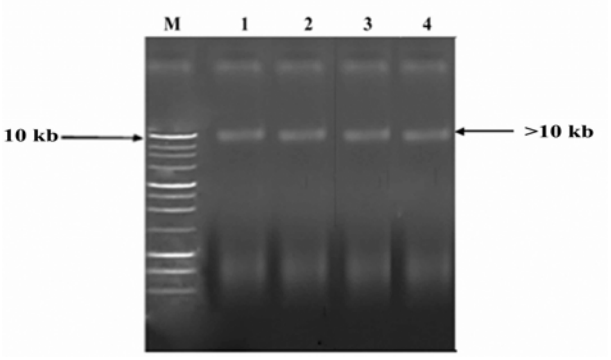


Fig 1: AGE (1%) of zooplankton genomic DNA
M, Marker (1 kb ladder);
1, *A. intermedia*; 2, *M. micrura*; 3, *M. edax*;
4, *C. protubera*

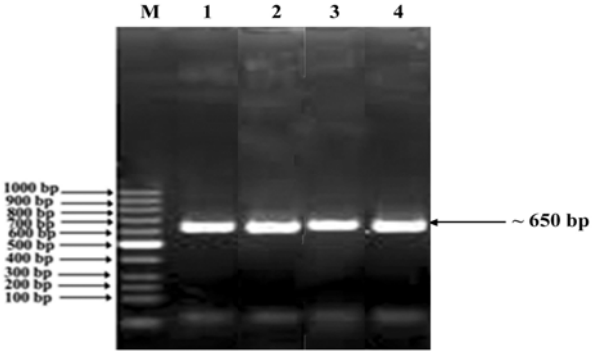


Fig 2: AGE (2%) of PCR amplified DNA product of zooplankton mt-COI gene
M, Marker (100 bp ladder);
1, *A. intermedia*; 2, *M. micrura*; 3, *M. edax*;
4, *C. protubera*

Actually the sequence analyses showed 646 bp, 686 bp, 651 bp and 657 bp for *A. intermedia*, *M. micrura*, *M. edax*, and *C. protubera* respectively. The BLAST similarity results for the sequences generated are given in Table 2. The similarity data available in the NCBI database were identified as 90% for *A. intermedia*, 94% for *M. micrura*, 86% for *M. edax* and 75% for *C. protubera*. The GenBank accession numbers of these sequences along with retrieved species are given in Table 3.

Table 2: BLAST similarity identification of COI gene sequences generated for subjected zooplankton species

Queried sequences	Identity (%)	Gap (%)	Matched Strand	Matched species	Reported from
Asplanchna intermedia	90	0	Plus	*Asplanchna silvestrii*	Mexico
Moina micrura	94	2	Minus	*Moina micrura*	Mexico
Mesocyclops edax	86	3	Plus	*Mesocyclops edax*	India
Cypris protubera	75	2	Plus	*Bennelongia timmsi*	Australia
Asplanchna intermedia (646 bp) TACTTTATATTTTATTTTCGGTATAGCTGGTTTTATTGGTCTAAGTATAAGTCTGCTTATTCGTTTAG AGCTAGGTGTTGTAGGACCTTATTTAGGTGATGAACATATTTACAATGTATTAATCACAGCTCATG CTTTTATTATGATTTTCTTCATAGTTATGCCTATTTCTATGGGTGGTTTTGGTAACTTAATTCCTTTA ATGTTAGGTGTTGCTGACATGGCTTTCCCTCGAATGAATAATCTTTCTTTTTTATTAGTTCCTTCTTT CATGTTCTTACTTCTTTCTTCTATTTTAGATGCAGGTGTTGGTACTGGTACAGTTTACCCTCCTTTAT CTGATTCTATGTATCATTCTGGTATTTCAGTAAACTTAGCTATTTTTAGTCTCCATCTAGCTGGTGTA TCTTCTATTTTAGGTAGAATCAACTTCTTAACAACTATTATCTGTTCTCGTACTACTAAATTAATCTC TTTAGATCGTTTACCTTTATTCTTAGCAGTTGCTGTTACAGCTGTTCTCCTTATTACTAGTCTTCCTG TTCTAGCTGGTGCTATTACTATGCTCTTAACAGATCGTAATTTTAATACGTCTTTCTTCGATCCGTCT GGTGGTGGTAATCCAGTATTATACCAACATTTATTC					
Moina micrura (686 bp) AGTTTTCCTCGGGTACTATGCTGTGTTTGTCTCACCTCCTGCAGGATCAAGAACGAAGTATTTAAAT TTCGATCTGTTAAAAGTATAGTAATAGCTCCTGCTAGAACAGGGAGGCTAAGTAAAAGAAGTAAT GCTGTAATTCCAACAGCTCAGACAAATAATGGAATTCGATCTAAAGTTATACCTTGAGTTCGTTTA TTAATAATTGTTGTAATAAAATTTACTGCTCCTAGAATTGATGAAATCCCTGCTAAATGTAATGAA AAAATTCTTAAATCAACTGAGGCTCCGGCGTGAGCAATTCCAGCAGATAAAGGAGGATAAACAGT TCATCCTGTTCCTGCTCCTCTTTCTACAGCTCCTCCTACTAATAATAAAGTTAAGGCAGGAGGAAGT ATTCAAAAACTTAAATTGTTAAGGCGAGGAAATGCTATATCAGGGGCTCCTAATATTAAAGGTACT TATCAATTTCCAAATCCTCCAATTAAGATGGGGTAACTATGAAGAAAATTATAATAAAAGCGTGA GCTGTAACGATACATTATAATTTGGTCATCACAATAAACTACCAGCTGGGCTAATCTACACGATAA TATACTTAGAGCAGTTCTACTATCCTGATCAATTCCAATATAAATATAGGGTTCATTATATATATAT GATAATGCCCGCGATAGCGTAAAATT					
Mesocyclops edax (651 bp) TAATTGGTACTGGACTAAGAGTTATTATTCGTCTAGAGCTAGGCCAACCTGGCTCTTTAATAGGGG ACGATCAAATTTACAATGTAGTAGTAACTGCCCATGCTTTTATTATAATTTTTTTTATGGTTATGCC TATTTTAATTGGTGGGTTTGGAAATTGATTAGTGCCATTAATATTAGGCTCTCCTGATATGGCTTTT CCTCGTATAAATAACATAAGATTTTGATTTTTAATGCCTGCTCTTTTTATACTTTTAACTAGATCCTT AGTAGAGAGAGGGGCCGGAACTGGATGAACAGTTTATCCTCCTTTAAGAAGAAATTTATCTCATTC TGGATCCTCAGTGGATTATGCTATTTTTTCTTTACATTTAGCTGGAATTTCTTCAATTTTAGGAGCA GTAAATTTTATTAGGACTTTAGGAAATATACGAACCTTAGGAATGTTTCTTGATCGTACTCCTTTAT TTGCTTGAGCTGTGTTAATTACTGCTATTTTTATTATTACTTTCTCTTACCTGTACTAGCAGGTGCGA TTACTATACTTTTTACAGATCGAAATTTAAATACTTCTTTTTATGATCCTAGAGGAGGAGGAGATCC TATTTATATCAACATTTATTTTGATTTTTTGGTCACCTGGGAAGTTTA					
Cypris protubera (657 bp) AGCTATGCTAGGCACAGGCGTCTTAGAGTAATTATTCGAGCTGAATTAGGGCAACCAGGCTCTCTT ATTGGCAATGATCAAATCTATAACACAATTGGCACAGCACATGCATTTATTATAATTTTCTTCATG GTTATACCTATTTTATCGGAGGCTTCGGCAATTGACTTGTTCCCCTAATATTAGGTGCCCCCGATAT GGCATTTCCGCGAATAGATAATATAAGAATTTGACTACTCCCCCCCTCACTATACCTACTAAGACT TGGATACTAACTGAAGGAGGGCTGGGACAGGTGAAAGGTTATCCCCCCTTATCCAGGAACCTCTCT CACTCAGGGACAAGAATACATTTTTCAATTTTTTCCTTACCTTTAACATGAGCAGGATCCATTTTAA GGGCCATTAACTTTATTACTACCATTGCTAATATACCAACTGCAAGAATATCCTCATATCGAATCC CCCTTATTCGATGGACTGTTGGGATCAACTGTCTTGCATTACTCACCTCCCCTCCTGGCCTAACTGG GGCAATCAACATACTTATAACCGATCCAAATCTTAATACCACCTTTTTTGACCCTGCAGGGGGAGG GAATCCCTATCTTATCCCACATCTCTTCTGAATTTTTGGCACCCGGGAAATTTAAATGGA					

Table 3: Details of GenBank accession numbers for the COI gene sequences generated for subjected zooplankton, and retrieved zooplankton species from the NCBI database

Zooplankton species	Country	Author	Accession number
Asplanchna intermedia	India	Paper Authors, 2015	KR106583
Asplanchna silvestrii	Mexico	Garcia-Morales and Elias-Gutierrez, 2013	JX216499
Asplanchna cf. sieboldi	Mexico	Garcia-Morales and Elias-Gutierrez, 2013	JX216495
Asplanchna sp.	Canada	Derry *et al*., 2003	AF499052
Moina micrura	India	Paper Authors, 2015	KR106584
Moina micrura	Mexico	Elias-Gutierrez *et al*., 2013	HQ336797
Moina cf. micrura	Mexico	Elias-Gutierrez *et al*., 2013	KC617710
Moina sp.	Mexico	Elias-Gutierrez *et al*., 2013	KC617696
Moina micrura	Mexico	Elias-Gutierrez *et al*., 2013	KC479042
Moina macrocopa	Mexico	Elias-Gutierrez *et al*., 2013	KC617129
Moina macrocopa	Mexico	Elias-Gutierrez *et al*., 2013	KC617134
Moina macrocopa	Mexico	Elias-Gutierrez *et al*., 2013	KC617132
Moina macrocopa	Mexico	Elias-Gutierrez *et al*., 2013	KC617131
Moina macrocopa	Mexico	Elias-Gutierrez *et al*., 2013	KC617408
Moina macrocopa	Mexico	Elias-Gutierrez *et al*., 2013	KC617133

Moina macrocopa	Mexico	Elias-Gutierrez *et al*., 2013	KC617129
Moina mongolica	China	Chen *et al*., 2014	KP027211
Moina rectirostris	China	Chen *et al*., 2014	KP027210
Mesocyclops edax	India	Paper Authors, 2015	KR106581
Mesocyclops sp.	Brazil	Young *et al*., 2013	KJ020570
Mesocyclops thermocyclopoides	Brazil	Young *et al*., 2013	KJ020572
Mesocyclops pehpeiensis	Brazil	Young *et al*., 2013	KJ020571
Mesocyclops edax	Mexico	Bojorquez *et al*., 2013	JQ284449
Mesocyclops leuckarti	Russia	Zagoskin *et al*.,2014	KF357729
Cypris protubera	India	Paper Authors, 2015	KR106580
Heterocypris incongruens	Turkey	Eldem *et al*.,2013	KF991575
Eucypris virens	Australia	Koenders *et al*.,2012	JN618114
Cypridopsis vidua	Turkey	Eldem *et al*.,2013	KF991565

The results of multiple sequence alignment with MAS for identification of identical, similar and variable sites of amino acids are shown in Plate 2. On the whole these four sequences together showed 174 identical amino acids residues, 59 similar amino acids residues and 531 variable amino acids sites. When the three crustacean zooplankton species (*M. micrura*, *M. edax* and *C. protubera*) alone was considered, they showed 211 identical amino acids residues, 61 similar amino acids residues and 451 variable amino acids sites (Table 4.) These data revealed more numbers of identical amino acids and similar amino acids, and less numbers of variable amino acids among the crustacean zooplankton when compared with Rotifera included group. It indicates discrimination of Rotifera from crustacean.

Table 4: Number of identical, similar and variable sites of amino acid residues in the COI gene sequences generated for different freshwater zooplankton species

Zooplankton species	Number identical amino acid residues	Number of similar amino acid residues	Number of variable amino acid sites
All zooplankton groups	174	59	531
Crustacean zooplankton groups	211	61	451

In this study, the base composition of the COI gene fragment varied among the species, AT biases were found to be higher in *A. intermedia*, *M. edax* and *C. protubera* than that of GC biases, whereas the GC bias was found to be higher in *M. micrura* (Table 5). The overall AT bias was nearly 60%, which indicates the lower abundance of nuclear copies of mt-DNA (NUMTs) genes known as pseudogenes, homologs or paralogs. Similarly, higher AT bias has been reported in a freshwater Ostracod, *Eucypris virens* (A+T (A = 27.4; T = 33.0); G+C (G = 17.3; C = 22.3) [45].

Table 5: Nucleotide composition of subjected zooplankton

Zooplankton species	AT bias (%)	GC bias (%)
Asplanchna intermedia	66.88	33.12
Moina micrura	43.73	56.27
Mesocyclops edax	66.07	33.93
Cypris protubera	58.00	42.00
Average	58.67	41.33

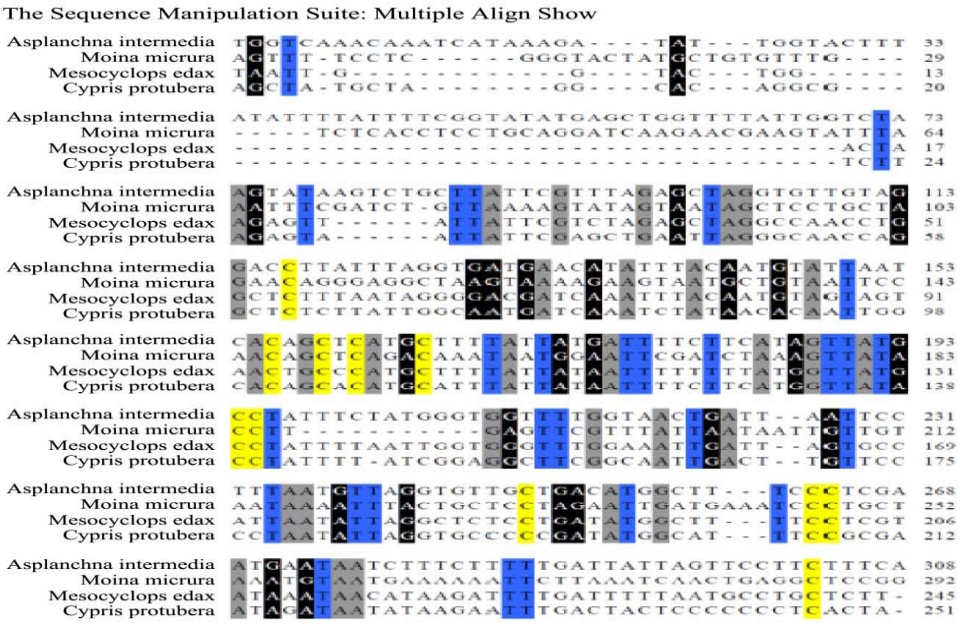


Fig 1: Multiple sequence alignment of COI gene sequences generated in all groups of zooplankton (Rotifera and crustacean groups, Cladocera, Copepoda and Ostracoda). An alignment is formatted by using multiple align show (MAS) with coloured background and a consensus setting of 100%. Identical residues are indicated by amino acid colour and similar residues are black in colour. Gaps and other residues are given in white background.

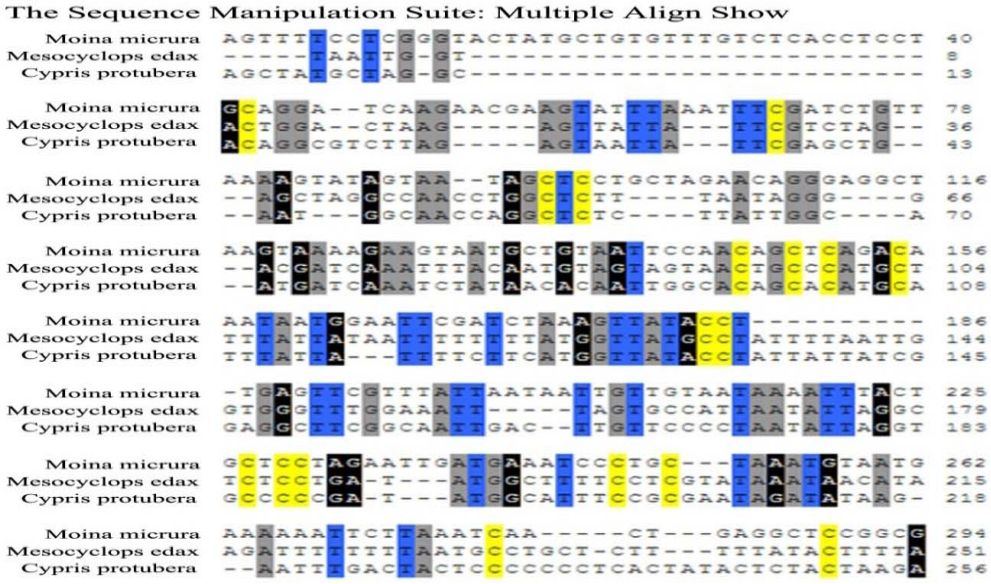


Fig 2: Multiple sequence alignment of COI gene sequences generated in crustacean zooplankton groups (Cladocera, Copepoda and Ostracoda). An alignment is formatted by using multiple align show (MAS) with coloured background and a consensus setting of 100%. Identical residues are indicated by amino acid colour and similar residues are black in colour. Gaps and other residues are given in white background.

Plate 2: Number of amino acid residues

3.3. Inter and intra species divergence

The inter species divergence rate calculated between different freshwater zooplankton species was ranged between 0.613 – 1.142 (*M. micrura* Vs. *M. edax*, and *M. micrura* Vs. *C. protubera* respectively). The divergence rate between crustacean zooplankton species was also ranged between 0.613 – 1.142. The intra species divergence was 0.768 between *M. micrura* of subjected and retrieved species of Mexico, and 1.016 between *M. edax* of subjected and retrieved species. No data is available with NCBI data base for other subjected species (Table 6; Plate 3).

Table 6: Inter and intra species divergence of zooplankton species

Divergence type	Between species	Divergence (%)
Inter species	*Asplanchna intermedia* vs. *Moina micrura*	0.715
	Asplanchna intermedia vs. *Mesocyclops edax*	0.708
	Asplanchna intermedia vs. *Cypris protubera*	0.810
	Moina micrura vs. *Mesocyclops edax*	0.613*
	Moina micrura vs. *Cypris protubera*	1.142*
	Mesocyclops edax vs. *Cypris protubera*	0.993*
Intra species	*Asplanchna intermedia*	Data not available
	Moina micrura vs. *Moina micrura* (Mexico)	0.841*
	Moina micrura vs. *Moina micrura* (Mexico)	0.862*
	Moina micrura vs. *Moina cf. micrura* (Mexico)	0.964*
	Moina micrura vs. *Moina* sp. (Mexico)	0.768*
	Mesocyclops edax vs. *Mesocyclops edax* (Mexico)	1.016*
	Cypris protubera	Data not available

Note: *Indicates the inter and intra species divergence between crustacean zooplankton

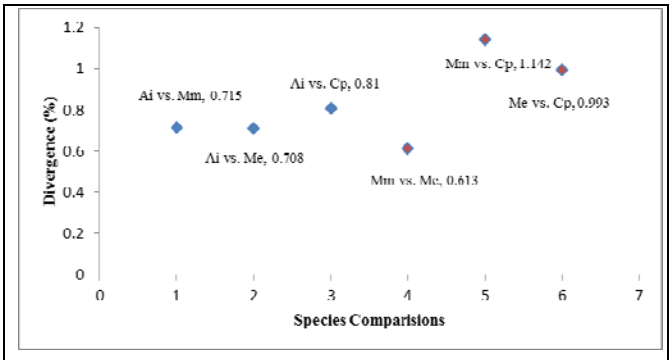


Fig 1: Inter species divergence between zooplankton groups. Blue color indicates inter species divergence between all groups of zooplankton (Rotifer and crustacean groups, Cladocera, Copepoda and Ostracoda). Red color indicates inter species divergence within groups of crustacean zooplankton (Cladocera, Copepoda and Ostracoda) alone. Ai, *A. intermedia*; Mm, *M. micrura*; Me, *M. edax*; Cp, *C. protubera.*

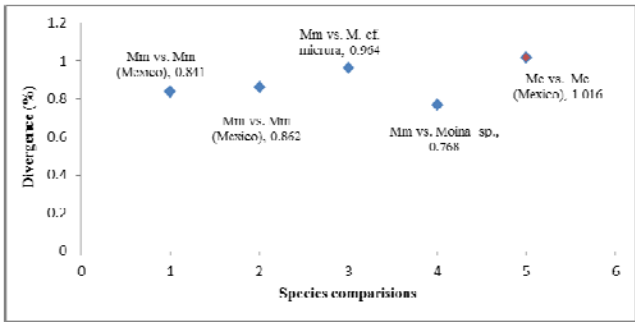


Fig 2: Intra species divergence between crustacean zooplankton groups alone. Red color indicates the divergence between *M. edax* of subjected species and retrieved species. Blue color indicates the divergence between *M. micrura* of subjected species and retrieved species. No. data available for *C. protubera*.

Plate 3: Nucleotide divergence

The genetic distances of the mitochondrial COI gene sequence between various animal taxa (mainly vertebrata and arthropoda) have been reported, and the general ranges for the intra and interspecies distances are 0.0001-0.05 and 0.04-0.21, respectively [46]. The most commonly identified divergence rates in crustaceans for mitochondrial COI genes are 1.4% and 2.6% per million years [47, 48]. A recent report says that a divergence rate of 0.3% between two Ostracoda species of *Physocypria biwaensis*, and suggested that they have been separated relatively recently [49]. Udayasuriyan *et al.,* [31] reported interspecies divergence of 0.500-2.697 between different freshwater prawn species. A very lower interspecies divergent rate of 0.09% has been reported between the freshwater crabs, *Spiralothelphusa hydrodroma* and *Barytelphusa jacquemontii* [29]. Similarly, the interspecies divergent rate of 0.497% has been reported between the mangrove crab, *Neosarmatium asiaticum* and the freshwater crabs, *S. hydrodroma* [29].

3.4. Phylogenetic information

The predicted phylogenetic information such as synonymous (Ks) and non-synonymous (Ka) substitutions, inferred synonymous (dS) and inferred non-synonymous (dN) substitutions, transitional (Ts) and transvertional (Tv) substitutions, and saturation, index of substitutional saturation (Iss) and critical value of index of substitutional saturation (Iss.c) are given in Table 7; Plates 4-6. When all the four groups of zooplankton were compared together, the following values in the phylogenetic information were observed. The Ks (occurrence of silent mutation, i.e. no change in the amino acid sequences of a polypeptide) was 0.592, and the Ka was 0.459 (occurrence of deleterious mutation), which indicates occurrence of more silent mutation and less deleterious mutation (Table 7; Fig. 1 of Plate 4). Similarly, the dS was 777.75 (94 sites) and dN was 312.84 (43 sites), which also indicates the fact that the silent mutation was greater than that of deleterious mutation. The dN-dS showed more negative signed (represents inferred synonymous) values than that of the positive signed (represents inferred non-synonymous) values (Table 7; Fig. 1 of Plate 5). The Ts (replacement of one nitrogenous base by another type of the same group of nucleotide) was 0.34 and the Tv (replacement of one type of nitrogenous base by another group) was 0.40. Therefore, there were occurrences of more transversional substitution and thus more phylogenetic information (Table 7; Fig. 1 of Plate 6).

Table 7: Overall phylogenetic information (average) of zooplankton sequences

Between zooplankton groups	Ks	Ka	dS	dN	Ts	Tv	Iss	Iss.c
Rotifera vs. Cladocera	0.358	0.555	252.36 (55)	182.27 (50)	0.38	0.25	0.732	0.658
Rotifera vs. Copepoda	0.344	0.425	219.39 (67)	154.53 (85)	0.33	0.21	0.711	0.703
Rotifera vs. Ostracoda	1.277	0.729	312.38 (86)	203.14 (62)	0.37	0.28	0.689	0.732
Cladocera vs. Copepoda	0.541	0.409	603.10 (111)	235.13 (56)	0.35	0.24	0.559	0.745
Cladocera vs. Ostracoda	1.203	0.672	384.71 (76)	137.00 (21)	0.35	0.27	0.640	0.660
Copepoda vs. Ostarcoda	1.076	0.674	241.27 (59)	235.37 (79)	0.40	0.29	0.692	0.703
All groups of zooplankton	0.592	0.459	777.75 (94)	312.84 (43)	0.34	0.40	0.670	0.700
Crustacean zooplankton	0.638	0.455	617.15 (86)	310.41 (57)	0.29	0.39	0.630	0.703

Ks, Synonymous substitution; **Ka**, Non-synonymous substitution; **dS**, Inferred synonymous substitutions; **dN**, Inferred non-synonymous substitutions; **Ts**, Transitional substitution; **Tv**, Transversional substitution; **Iss**, Index of substitutional saturation; **Iss.c**, Critical value of index of substitutional saturation. Values in parenthesis indicates No. of sites.

The difference between Iss.c and Iss (0.700-0.670=0.030) indicates the fact that there was no or little substitutional saturation, because Iss.c value is greater than Iss value, which means, there is some phylogenetic information. The one to one comparison between the four groups of zooplankton showed the maximum phylogenetic information through Iss.c value was observed between Cladocera and Copepoda (0.745), followed by between Rotifera and Ostracoda (0.732), Copepoda and Ostarcoda (703), and Cladocera and Ostracoda (660). The phylogenetic information recorded between Rotifera and Cladocera was less (Iss.c=0.658), and only least between Rotifera and Copepoda (Iss.c=0.703) (Table 7).

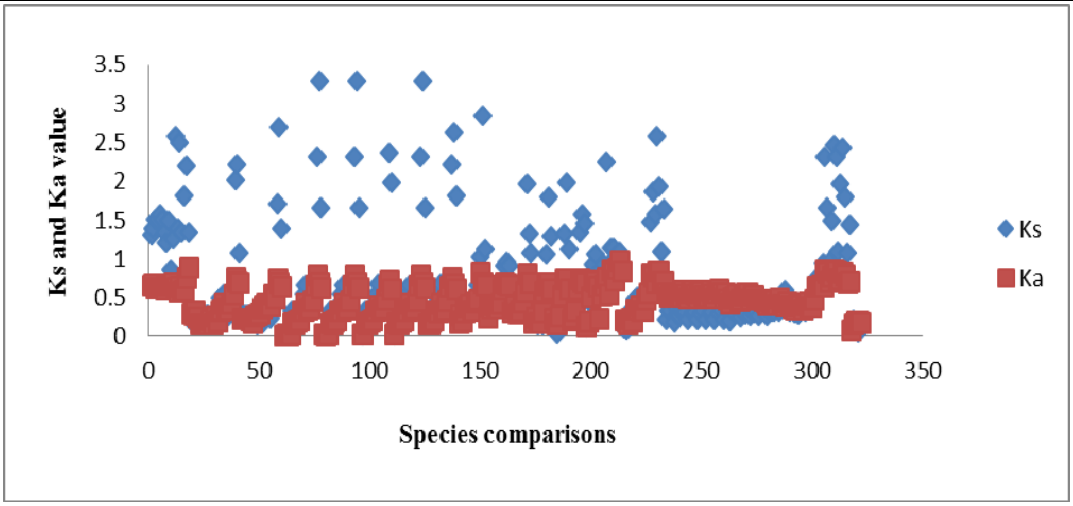


Fig 1: Number of synonymous (Ks) and non-synonymous (Ka) substitutions of nucleotides at 3rd codon position for COI gene sequences generated for all groups of zooplankton (Rotifera and crustacean groups, Cladocera, Copepoda and Ostracoda)

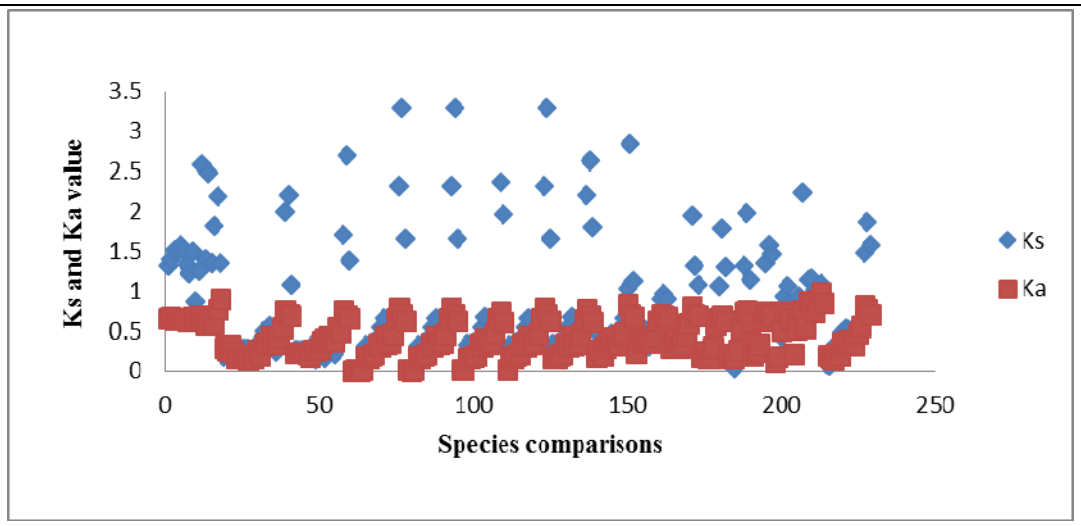


Fig 2: Number of synonymous (Ks) and non-synonymous (Ka) substitutions of nucleotides at 3rd codon position for COI gene sequences generated for crustacean zooplankton groups (Cladocera, Copepoda and Ostracoda)

Plate 4: Number of synonymous (Ks) and non-synonymous (Ka) substitutions

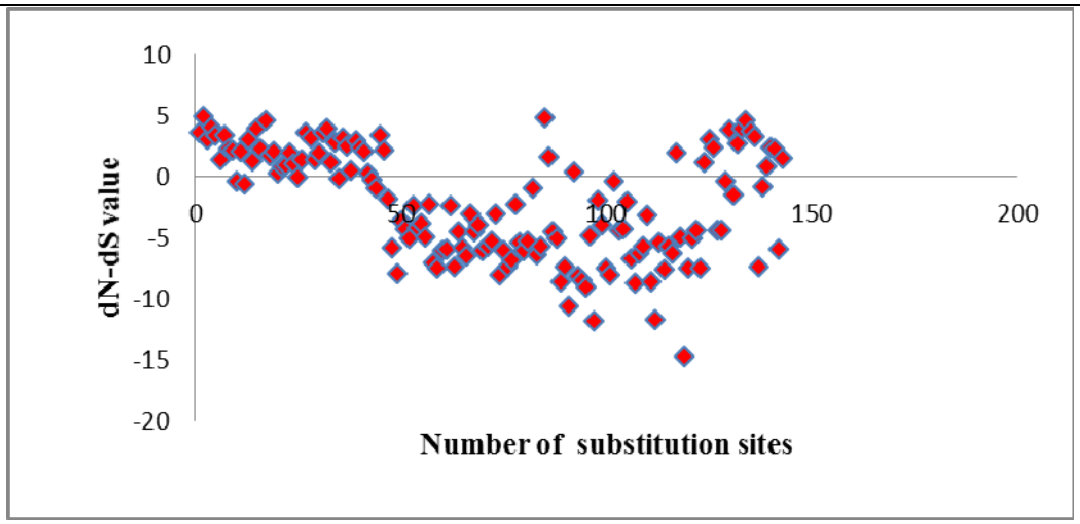


Fig 1: Number of inferred synonymous (dS) and inferred non-synonymous (dN) substitutions of nucleotides at 3rd codon position for COI gene sequences generated for all groups of zooplankton (Rotifera and crustacean groups, Cladocera, Copepoda and Ostracoda)

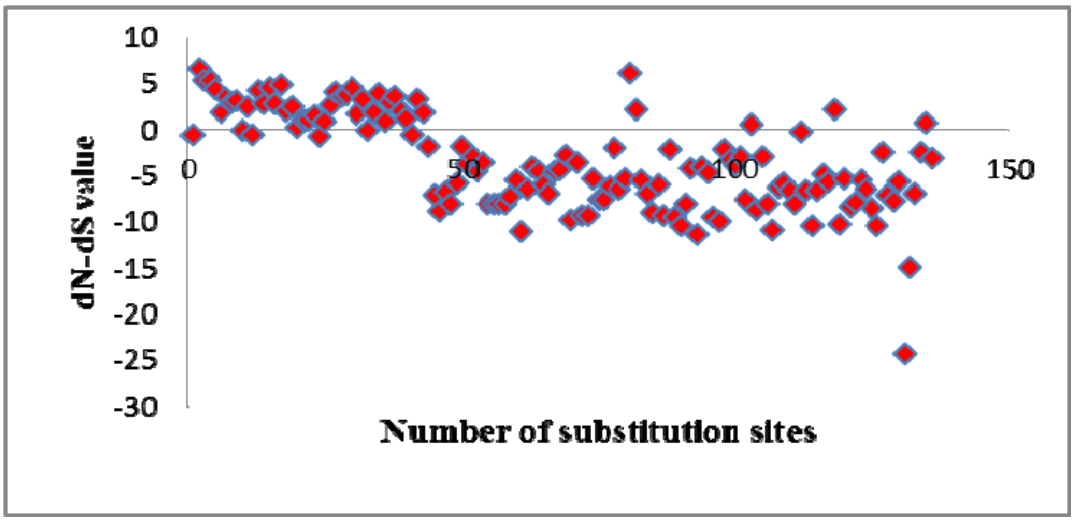


Fig 2: Number of inferred synonymous (dS) and inferred non-synonymous (dN) substitutions of nucleotides at 3rd codon position for COI gene sequences generated for crustacean zooplankton groups (Cladocera, Copepoda and Ostracoda)

Plate 5: Number of inferred synonymous (dS) and inferred non-synonymous (dN) substitutions

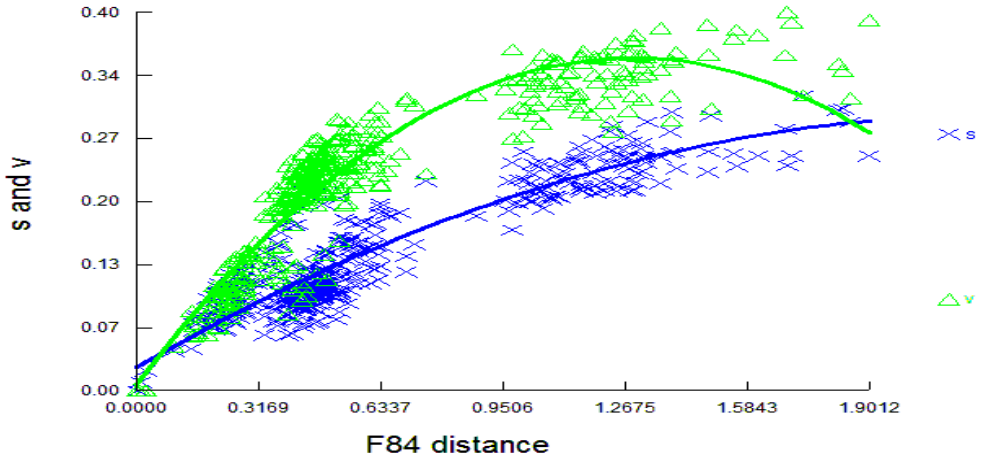


Fig 1: Scattergram shows transitional (Δ, blue) and tranversional (X, green) type substitutions occurred in COI gene sequences in all groups of zooplankton (Rotifera and crustacean groups, Cladocera, Copepoda and Ostracoda)

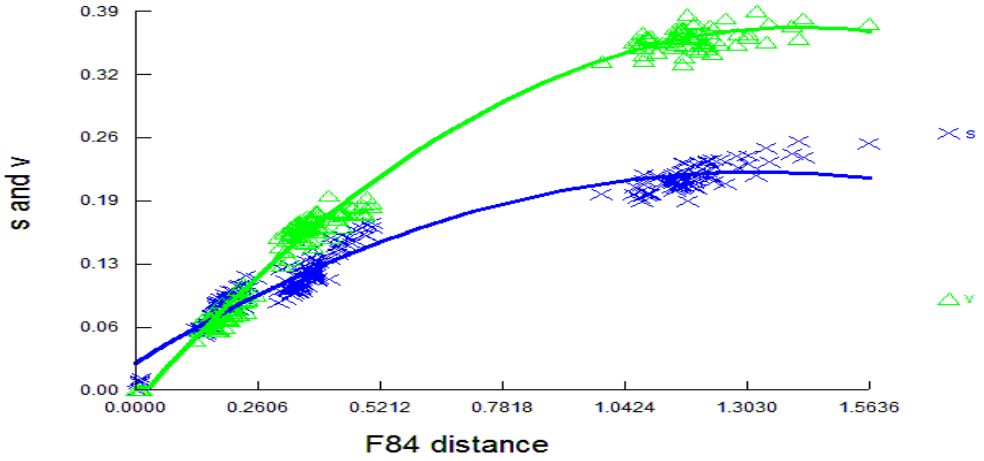


Fig 2: Scattergram shows transitional (Δ, blue) and tranversional (X, green) type substitutions occurred in COI gene sequences in crustacean groups of zooplankton (Cladocera, Copepoda and Ostracoda)

Plate 6: Number of transitional and transversional substitutions

In the phylogenetic tree, Rotifers including *A. intermedia* form a separate clade in between crustacean groups. The Ostracoda formed as another separate clade at the base, within which the subjected Cladoceran species *M. micrura* was aligned, but the subjected Ostracode species, *C. protubera* was formed as a separate clade in between Rotifera and Cladocera (Fig. 3). Actually, the subjected Cladoceran species, *M. micrura* formed as a sister taxan with Ostracoda (Fig. 3). The overall tree topology showed that there were two clusters, the first cluster consisted of Ostracoda and Cladocera (*M. micrura*) and the second cluster consisted of Copepoda (*M. edax*), Rotifera (*A. intermedia*), Ostracoda (*C. protubera*) and Cladocera. This suggests that the discrimination between Rotifera and crustacean zooplankton groups, Copepod, Ostracods and Cladocera was not wider (Fig. 3). Actually, in the phylogenetic tree the subjected species, *M. micrura* aligned at the base followed by *M. edax*, *A. intermedia* and *C. protubera*. Therefore, all the four subjected plankton species are closely related and might have originated from a common ancestor. However, they showed some discrimination.

Among the crustacean zooplankton, more silent mutation and less deleterious mutation have occurred (Ks=0.638 & Ka=0.455), thus, less changes occurred in the amino acid sequences between different crustacean zooplankton groups when compared with all four groups of zooplankton (Ka=0.459) (Table 7; Fig. 2 of Plate 4). Similarly, the dS was 617.15 (86 sites) and dN was 310.41 (57 sites), which also indicates the fact that the silent mutation was greater than that of deleterious mutation. The dN-dS showed more negative signed values (represents inferred synonymous) than that of the positive signed (represents inferred non-synonymous) values (Table 7; Fig. 2 of Plate 5). Similarly, less Ts and Tv have occurred (0.29 and 0.39 respectively) in crustacean group when compared with all groups of zooplankton (Ts=0.34; Tv=0.40)

(Table 7; Figs. 1 & 2 of Plate 6). However, the difference between Tv and Ts of crustacean zooplankton was wider (0.39-0.29=0.10) when compared with the difference of Tv and

Ts of all four groups of zooplankton (0.40-0.34=0.06), which indicates the fact that there

was more discrimination among crustacean zooplankton groups, and thus, Ostracoda formed

a separate cluster (except the subjected species, *C. protubera*). This was further

confirmed by the difference arrived between Iss.c and Iss values (0.703-0.630=0.073), which was greater than that of the difference arrived between Iss.c and Iss values of all groups of

zooplankton (0.030) (Table 7). Therefore, there was more phylogenetic information between crustacean groups than that of all zooplankton (Rotifera and crustacean) groups. Thus, here also the polyphyletic tree was resolved with two clusters the first cluster consisted of Ostracoda (except the subjected Ostracod, *C. protubera*, which has aligned between Copepoda and Cladocera as a separate clade) and the subjected Cladoceran species, *M. micrura*. The second cluster consisted of Copepod (*M. edax*), *C. protubera* (Ostracoda) and Cladocera (Fig. 4). Actually, the predicted phylogenetic information between the subjected crustacean zooplankton species, *M. micrura*, *M. edax* and *C. protubera* of Cladocera, Copepod and Ostracoda respectively are less discriminated, but when compared with retrieved species, the Ostracoda formed as separate cluster and showed some distance. All these evolutionary information of the present study are supported by literature [29 - 31, 50 - 52].

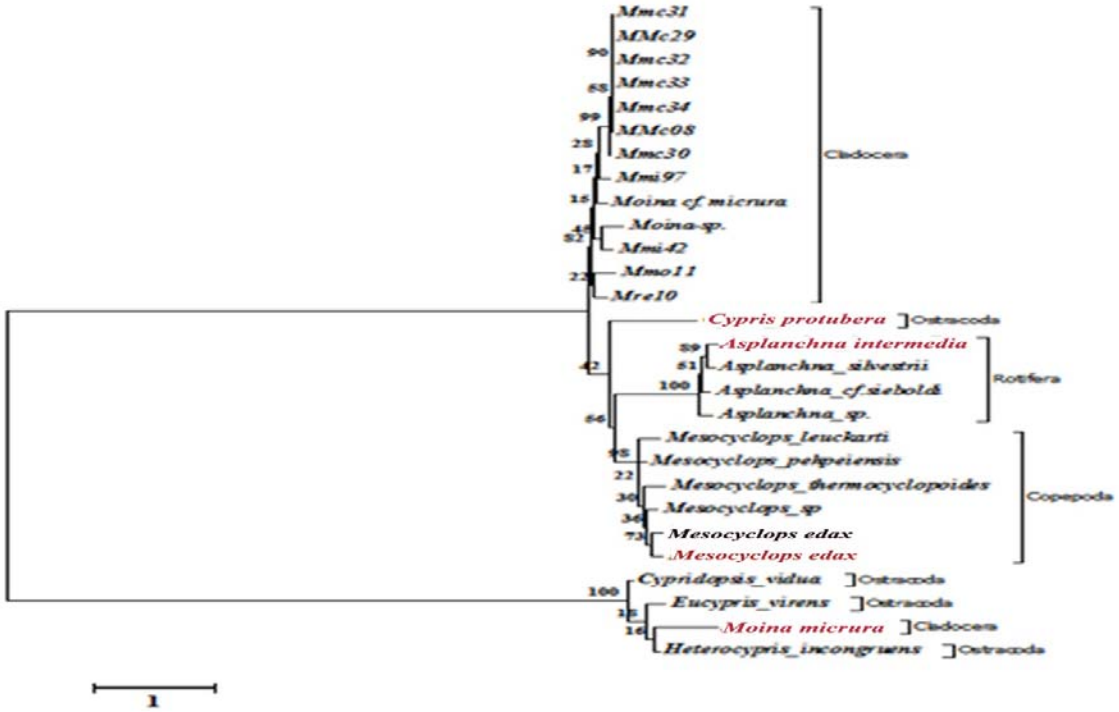


Fig 3: Phylogenetic significance of COI gene sequences generated in all groups of subjected zooplankton (Rotifera and crustacean groups, Cladocera, Copepoda and Ostracoda) and their retrieved species

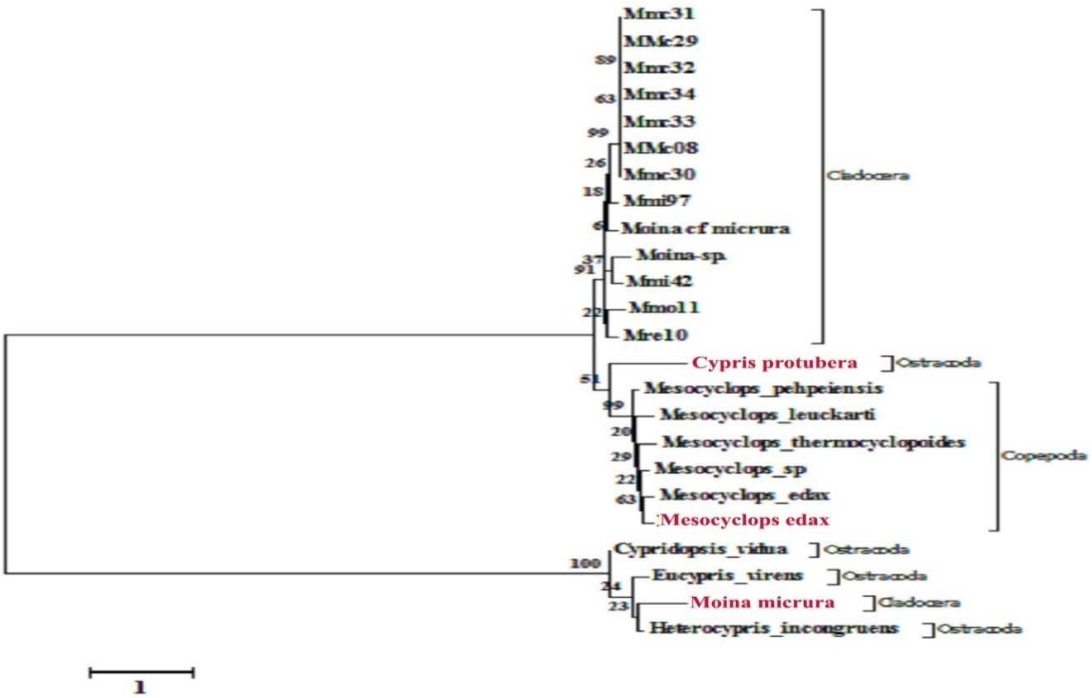


Fig 4: Phylogenetic significance of COI gene sequences generated in crustacean groups of subjected zooplankton (Cladocera, Copepoda and Ostracoda) and their retrieved species

4. Conclusion

This study concluded that, the subjected four zooplankton species showed some divergence and are aligned in separate clades with different bootstrap values. The Rotifers was aligned in between crustacean groups. The Cladoceran species, *M. micrura* was aligned within Ostracode group, and the Ostracode species, *C. protubera* was formed as a separate clade in between Copepods and Cladocerans. Therefore, they are genetically distinct, but closely related as per information available with mt-COI partial gene.

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