First record of a seed shrimp (Ostracoda: Podocopa) *Cypretta campechensis* (Cyprididae) in a perennial lake (Coimbatore, India): Its molecular identification

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**Abstract**

This study represents diversity of zooplankton species in a perennial freshwater lake located in Singanallur (Lat. 10.59° N and Long. 77.88° E) at Coimbatore, Tamil Nadu, India for a period of one year (June, 2017-May, 2018). Fifty three zooplankton species including roti-fish, cladocera, copepoda, ostracoda were observed. The first record of an ostracoda species *Cypretta campechensis* was observed during summer. This species was recognized according to its morphological characteristics. The molecular identification was done via analysis of the mitochondrial cytochrome c oxidase subunit I (mt-COI). The gene was amplified with universal primers (LCO1490 and HC02198), sequenced and authenticated with NCBI GenBank (MN641913). The BLAST showed 100% similarity with *C. campechensis* of Mexico (MF076727). The sequence showed more number of identical amino acid residues than that of variable amino acid sites. The noticed higher AT biases (62.4-63.0%) than GC biases (36.1-37.5%) indicated that lower abundance of nuclear copies of mt-DNA (NUMTs) genes. The divergence rate was very low between the subjected and retrieved species (0.000-0.290%). The endemism of *C. campechensis* can be used as a bio-indicator of water pollution status of this lake and may afford an opening to assess its importance in aquaculture production.

**Keywords:** Zooplankton, *Cypretta campechensis*, mt-COI gene, divergence, phylogeny

**1. Introduction**

Plankton are free-floating characteristic provide a crucial source of food to many small and large aquatic organisms, such as prawns, bivalves and fishes. Phytoplankton (earth's most critical organisms are microscopic in nature, such as diatoms and dinoflagellates as well as blue-green algae) possess photosynthetic capacity. Therefore, they are responsible for half of the atmospheric oxygen. Zooplankton diet consists of various phytoplankton species, which differ in their grazing resistance (e.g., size, shape, and toxins) and nutritional quality (Taipale et al., 2013; Taipale et al., 2016) [1,2]. Zooplankton species have different types of life histories influenced by seasonal variations of biotic factors, feeding ecology and predation pressure. The composition, abundance, and distribution of zooplankton species in any particular aquatic habitat usually provide information on the prevailing physical and chemical conditions in that habitat and hence, they are of great ecological importance (Abdul et al., 2016) [3].

The presence and abundance of zooplankton represents one of the ecological indicators of water quality. Zooplankton constitutes an important food sources for many omnivorous and carnivorous fishes and support the necessary amount of protein for their rapid larval growth (Bhavan et al., 2017) [4]. In any freshwater pond or lake, generally there are four zooplankton groups, namely Rotifers and crustacean zooplankton of Cladocera, Copepoda and Ostracoda. Among these, Ostracoda are of great interest as a model group in various ecological and evolutionary studies. This consists of lateral compressed bivalve carapace closing appendages and soft parts (Yousef, 2014) [5]. Ostracods can be found worldwide both in fresh and marine water bodies at different depths (Cohen et al., 2007) [6]. They are found in polluted water and serve as indicator species of climate and ecosystem changes (Martens et al., 2008) [7]. Globally, freshwater ostracods were documented and classified under the order Podocopida, which contains 15 families, 209 genera and 2103 species (Martens et al., 2008; Martens and Savatenalinton, 2011; Karanovic, 2012).
2012) [7-9]. They are playing a vital role in food chain and energy flow in the aquatic ecosystem (Li et al., 2018) [10]. The freshwater ostracods are the most important proxies in lacustrine environments, because of their high abundance and good preservation in sediments (Cohuo-Durán et al., 2013) [11]. There are at least 25,000 extant species, of which around 12,000 have been described (Cohen et al., 2007) [8]. Nevertheless, only few DNA barcoding data analyses have been conducted on these small crustaceans (Bhavan et al., 2016; 2017; Kalpana et al., 2018) [12-14]. The identification of cryptic species is in endemic taxon and biome and, therefore, these species have significant implications for evolutionary, biogeography and conservation studies (Bickford et al., 2007) [14]. Morphological identification of very similar species disregards certain evolutionary and ecological aspects because neither, does close morphological similarity come along with identical ecology nor with the same genetic background (Giere, 2009) [15]. Among various gene regions available for correct and quick discrimination of species, the mitochondrial cytochrome oxidase subunit-I (COI) gene region is unique, because its haplotypes are often used for studying the molecular ecology/taxonomy of animals (Mills et al., 2017) [16]. Actually, mt-COI gene has offered the most efficient and accurate barcoding method for species-level identification of animals including zooplankton regardless of their condition and size. There are often used for studying the energy flow in the aquatic ecosystem (Li et al., 2018) [10]. The freshwater ostracods are the most important proxies in lacustrine environments, because of their high abundance and good preservation in sediments (Cohuo-Durán et al., 2013) [11]. There are at least 25,000 extant species, of which around 12,000 have been described (Cohen et al., 2007) [8]. Nevertheless, only few DNA barcoding data analyses have been conducted on these small crustaceans (Bhavan et al., 2016; 2017; Kalpana et al., 2018) [12-14].

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Morphological and molecular taxonomic analysis is particularly useful and necessary to develop accurate tools for species identification, and thereby to ensure valid estimates of their diversity, distribution, and abundance in routine taxonomic analysis of zooplankton samples (Bhavan et al., 2016; Bhavan et al., 2015a; Kalpana et al., 2017) [12, 19, 20]. In this study, we have studied the distribution, morphology and molecular aspects of an ostracoda species, *Cypretta campechenesis* present in the Singanallur lake, Coimbatore, India.

2. Materials and methods

2.1 Description of the study area

The Singanallur (Lat. 10.59° N and Long. 77.88° E) Lake is located in Coimbatore city, Tamil Nadu, India, and is fed by canals derived from Noyyal River (Plate 1). The lake also receives water from Sanganur drain and sewage water. The water can be released through two sluice gates on the lake. In 2010, pipes were laid to connect the lake to Valankulam Lake (Coimbatore, Tamil Nadu, India) to drain the excess water during floods. Various birds including grebes, painted storks and purple moorhen can be spotted in this lake. Fishing is carried out by neighboring fishermen and enthusiasts.

![Image](satellite_view.png) a. Satellite image taken from Google maps

![Image](lake_view.png) b. The lake view

Plate 1: Images of the Singanallur Lake (Lat. 10.59° N and Long. 77.88° E), Coimbatore, Tamil Nadu, India.

2.2 Qualitative analysis of the zooplankton

The surface water sample was collected during the early morning hours (6.00 AM - 8.00 AM). For qualitative analysis of zooplankton, water samples were collected by Towing method using Henson’s standard plankton net (150 μm mesh) in zigzag fashion horizontally at a depth of 50 to 100 cm for about 10 min with a uniform speed of boat. The identification of zooplankton is made referring the standard manuals, text books and monographs (Sharma and Michael, 1987; Santhanam et al., 1989; Battish, 1992; Reddy, 1994; Shiel, 1995; Murugan et al., 1998; Altaff, 2005; Cohuo-Durán et al., 2013) [21-27, 11] with the help of a compound microscope. The photomicrographs were taken using Inverted Biological Microscope (Model Number INVERSO 3000 (TC-100) CETI) attached with a camera (Model IS 300). General elements that have been taken to assess all zooplankton groups were body shape and size, relative length of various appendages, including antennae, legs and setae, and presence and relative sizes of spines.

2.3 Mass culture of zooplankton

The collected zooplankton species were identified and segregated. The ostracoda species, *C. campechenensis* especially seen in summer was subjected to mass culture individually for 21 days and fed *ad libitum* with a mixture of phytoplankton culture, which contains *Spirulina* (*Arthrospira platensis*, *Spirulina* meneghiniana, *Labyrinthiformis* *magnusa*, *Oscillatoria brevis*). The zooplankton culture medium was maintained under the following conditions: temperature (°C), 28.40±2.10; pH, 7.17±0.46; salinity (ppt), 0.772±0.36;
dissolved oxygen, DO (mg/l$^{-1}$), 7.65±0.16; total dissolved solids, TDS (mg/l$^{-1}$), 1038±12.09; electrical conductivity, EC (μS/ cm), 2.113±0.12 and NH$_3$ (mg/l$^{-1}$), 0.024±0.004 with continued aeration. At the end of mass culture it was harvested and the number of individuals grown was counted by using counting chamber mounted on a microscope at a magnification of 10X and 40X.

2.4 Morphological characterization
The morphological characterization in C. campechensis was done based on anterior view, posterior view, dorsal margin, lateral view (triangular shape), maxillular palp, posterior seta, posterior claw, uropodal ramus and hemipenis. (Plate 2). Its SEM was also performed (Plate 3) using out sourcing service (Department of Nanoscience and Technology, Bharathiar University, Coimbatore, India).

| Taxonomic position |
|--------------------|
| Phylum | Arthropoda |
| Subphylum | Crustacea (Brunnich 1772) |
| Class | Ostracoda (Latreille 1802) |
| Order | Podocopa (Sars 1866) |
| Suborder | Podocopina (Sars 1866) |
| Family | Cyprididae (Baird 1845) |
| Subfamily | Cypretinae (Hartmann 1964) |
| Genus | Cypretta (Vavra 1895) |
| Species | campechensis (Colhu-Duran et al., 2013) |

2.5 Specific features of the class, 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

Plate 2: Morphology (400x) and taxonomic positions of first recorded zooplankton species, Cypretta campechensis from the Singanallur Lake, Coimbatore, Tamil Nadu, India.

Plate 3: SEM views of Cypretta campechensis. A: ventral view (1), dorsal view (2), hinge (3), anterior view (4), posterior view (5); B: right valve of external view (1), left valve of external view (2), dorsal margin strongly arched (3); C: hemipenis with two conspicuous terminal lobes (1); D: anterior marginal pore canals (1), antennula 1st and 2nd segment (2).
crustaceans by a laterally compressed body, undifferentiated head, and seven or less limbs and a bivalve carapace with no growth lines.

2.6 Specific features of the sub-class, podocopa
Carapace ovoid, inflated sub-triangular, oblong elongate, or compressed; no rostrum or incisura; valves overlap around free margin; 2nd antenna geniculate, pediform; 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.

2.7 Specific features of the order, podopocida
Carapace straight or concave in the oral region, valves strongly calcified, smooth or ornamented. Six or seven pairs of appendages, antenna biramous; exopodite often reduced to a single scale or seta, endopodite generally with four podomeres and with stout terminal chelate setae. Maxilla is with a branchial plate. Fifth limb with or without a branchial plate, either a maxilliped or a walking leg, or transformed (in males) into a claspers organ. Furca narrow, lamelliform bearing a few setae, or reduced, or absent (Sars, 1866) [30].

2.8 Specific features of the family, cyprididae
Bivalved carapace, jointed in dorsal region, encloses whole animal. Anterior margin of both valves are with a parallel row of radiating seta, or funnel-shaped radial pore canals (reduced seta), located perpendicular to the margin. Terminal segment elongated and cylindrical, developed claws and two stout setae. Surface ornamentation punctuate, reticulate or smooth. Right valve usually overlapping left one, although in some species the left valve overlaps the right one (Baird, 1845) [31].

2.9 Specific key characters of Cypretta campechensis (Cohuo-Duran et al., 2013) [11]
1. Lateral view sub triangular, anterior and posterior margins sub equally rounded, dorsal margin strongly arched, greatest height situated at mid-length …2
2. Terminal segment of maxillulal palp cylindrical and armed with two or three claws and two or three setae …3
3. Posterior seta less than half length of posterior claw, attachment of uropodal ramus long and narrow, distally bifurcated …4
4. Hemipenis with two conspicuous terminal lobes, small lobule between them, without spine-like process, internal canal double coiled…Cypretta campechensis.

2.10 Molecular analysis
The molecular analysis was performed using mt-COI gene. The genomic DNA was isolated from 10 individuals as a whole by using Qiagen Dneasy Blood and Tissue Kit (Germany). Agarose gel electrophoresis (AGE, 1%) 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 Applied Biosystem (ABI) Thermo Cycler (Veriti™ 96-Well Thermal Cycler) with universal primers of forward and reverse in nature, LCO1490 and HCO2198, respectively (Folmer et al., 1994) [34]. These primers set were worked well with crustaceans, crabs, zooplanktons and prawns (Udayasuriyam et al., 2017; Bhavan et al., 2016, 2017; Kalpana et al., 2018) [35, 12, 4, 13]. Amplification was performed in a total volume of 100 μl reaction mixture contain 1 μl of DNA template, 400 ng of each primer (Forward primer, 400 ng, 0.5 μl; Reverse primer, 400 ng, 0.5 μl), 4 μl each dNTPs (10mM), 10μl of 10X ChromTaq DNA polymerase Assay Buffer, 1μl of ChromTaq DNA Polymerase Enzyme (3U/μl) and milli q water (83μl). The thermo cycler condition was as follows: 5 min at 95°C for pre-running, 35 cycles of 30 s each at 95°C for denaturation, 45 s at 57°C for annealing,1 min at 72°C for extension and followed by 7 min at 72°C for a final extension. The amplified product was resolved with AGE (2%). Sequencing was performed with ABI 3500 XL Genetic Analyzer by using outsourcing service with a total volume of 20μl reaction mixture, which contains 3μl of template DNA, 3.2μM of primers (forward, 0.50 μl and reverse, 0.50 μl), 2μl of 5X big dye sequencing buffer and 4μl of 2.5X ready reaction premix and 10μl of DNase-RNase free water. The other conditions were as per company protocol (Chromous Biotech, Bengaluru, India). The forward and reverse sequences were aligned pair wise using CAP3. The sequence similarity available with NCBI database was identified and the internal stop codon was removed by BLAST. The reading frame shift was deduced by open reading frames (ORF) finder. The trimmed sequence was authenticated with GenBank. The multiple sequence alignment was done 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 biases), nucleotide divergence (Kimura two-parameter (K2P) substitution model; Kimura, 1980) [36] and some phylogenetic information were calculated by using MEGA v. 6.01. Assessment of synonymous (Ks) and non-synonymous (Ka) substitutions for 3rd codon positions was calculated by Li93 method using DAMBE (Xia, 2000) [37]. The transitional (Ts) and transversional (Tv) substitutions of nucleotides were determined (Felsenstein, 1981) [38]. Analysis of sequence saturation, index of substituitional saturation (Iss) and critical value of index of substituitional saturation (Iss.c) was done by Xia method using DAMBE (Xia, 2000; Xia et al., 2003) [37, 39]. Finally, the phylogenetic tree was reconstructed by Maximum Likelihood model (Tamura, 1992; Kumar et al., 2016) [40, 41].

3. Results
3.1 Identified zooplankton species
In this study, 53 zooplankton species were recorded under 13 families and 27 genera in the Sīnganallūr lake, which include 18 species of Rotifer, 10 species of Cladocera, 13 species of copepoda and 12 species of ostracoda (Table. 1). Among the rotifers, Brachionus calyciflorus, Brachionus caudatus personatus, Brachionus durgae, Brachionus quadridentatus, Brachionus leydigii, Brachionus rirsirumae, Brachionus urceolaris and Notholca laurentiae were present in all seasons; Brachionus rotundiformis and Brachionus diversicornis were absent in pre-monsoon season; Brachionus plicatilis and Brachionus ibericus absent in post-monsoon; Filinia longiseta was absent in pre-monsoon and summer; Asplanchna intermedia and Asplanchna brightwelli absent in monsoon season; Brachionus variabilis, Brachionus rubens and Brachionus nilsoni were absent in summer season. Among the cladocerans, Diaphanosaoma sarsi, Pseudochydorus globosus and Moina brachiata were present in all seasons; Moina macrocopa and Latonopsis australis
were absent in pre-monsoon; *Leydigia leydigia* was absent in pre-monsoon and post-monsoon; *Leydigia lousi* was absent in post-monsoon and summer season; *Ceriodaphnia cornutu* was absent in monsoon season; *Moina micrura* and *Macrothrix spinosa* were absent in summer season. Among the copepods, *Thermocyclops inversus*, *Thermocyclops crassus*, *Mesocyclops leuckarti* and *Macrocyctops albidus* were present in all seasons; *Acanthocyclops vernalis*, *Thermocyclops decipiens* and *Mesocyclops ogunnus* were absent in pre-monsoon; *Heliodiapomus viduos* was absent in post-monsoon; *Thermocyclops consimilis* was absent in monsoon season; *Cyclops strenuus*, *Eucyclops speratus*, *Mesocyclops edax* and *Mesocyclops pohpeiensis* were absent in summer season. Among the ostracods, *Cyprinotus nudus* was present in all seasons; *Cypris decaryi*, *Candona candida* and *Prionocypris glacialis* were absent in summer season; *Heterocypris denatamarginatus*, *Cypris protubera* and *Eucypris virens* were absent in monsoon and summer; *Heterocypris incongruens* was absent in post-monsoon; *Chriissia formosa* was absent in pre-monsoon; *Tanycypris pellucida* was absent in pre-monsoon and summer; *Eucypris bispinosa* was absent in post-monsoon and summer; the presence of *C. campchensis* only in summer season was the first record in this lake. The zooplankton abundance in this lake was as follows: Rotifera > Ostracoda > Copepoda > Cladocera.

Table 1: List of zooplankton species recorded in the Singanallur lake during the study period (June, 2017 – May, 2018)

| Group Phylum/Class/Order | Family | Genus | Species | Pre-Monsoon | Monsoon | Post-Monsoon | Summer |
|--------------------------|--------|-------|---------|-------------|---------|-------------|--------|
| Rotifera 18-species      |        |       |         |             |         |             |        |
| Brachionidae (Ehrenberg 1838) | Brachionus (Pallas 1776) | Brachionus rotundiformis (Tschugunoff 1921) | - | + | + | + |
|                          |        |       | Brachionus calyciflorus (Pallas 1776) | + | + | - | + |
|                          |        |       | Brachionus caudatus personatus (Ahlstrom 1940) | + | + | + | + |
|                          |        |       | Brachionus diversicornis (Daday 1883) | - | + | + | + |
|                          |        |       | Brachionus rubens (Ehrenberg 1838) | + | + | + | - |
|                          |        |       | Brachionus durgae (Dhanapathi 1974) | + | + | + | + |
|                          |        |       | Brachionus plicatilis (Muller 1786) | + | - | + | + |
|                          |        |       | Brachionus quadridentatus (Hermann 1783) | + | + | + | + |
|                          |        |       | Brachionus leydigii (Cohn 1862) | + | + | + | + |
|                          |        |       | Brachionus nelsoni (Ahlstrom 1940) | + | + | + | - |
|                          |        |       | Brachionus sriammonae (Segers, Ketethip and Sanamuang 2004) | + | + | + | + |
|                          |        |       | Brachionus urceolaris (Muller 1773) | + | + | + | + |
|                          |        |       | Brachionus ibericus (Ciros-Perez, Gomez and Serra 2007) | + | + | + | - |
|                          |        |       | Brachionus variabilis (Hempel 1896) | + | + | - | + |
|                          | Notholca (Gosse 1886) | Notholca laurentiae (Stemberger 1976) | + | + | + | + |
|                          | Trochosphaeridae (Harring 1913) | Filinia (Bory de St. Vincent 1824) | - | + | + | - |
|                          |        |       | Filinia longiseta (Ehrenberg 1834) | - | + | + | - |
|                          | Asplanchnidae (Eckstein 1883) | Asplanchna (Gosse 1850) | + | + | + | + |
|                          |        |       | Asplanchna intermedia (Hudson 1886) | + | - | + | + |
|                          |        |       | Asplanchna brightwelli (Gosse 1850) | + | - | + | + |
|                          |        |       | Ceriodaphnia (Dana 1853) | + | + | + | + |
|                          | Sididae (Baird 1850) | Diaphanasoma (Fischer 1850) | + | + | + | + |
|                          |        |       | Diaphanasoma sarsi (Richard 1895) | + | + | + | + |
|                          |        |       | Pseudocytherus (Fryer 1968) | + | + | + | + |
|                          | Chydoridae (Dybowski and Grochowski 1894) | Leydigia (Kurz 1875) | + | + | - | + |
|                          |        |       | Leydigia leydigia (Schodler 1863) | + | - | + | - |
|                          |        |       | Leydigia lousi (Elias-Gutierrez and Nieto 2003) | + | + | - | + |
|                          |        |       | Pseudocytherus globosus (Baird 1843) | + | + | + | + |
|                          | Daphnidae (Straus 1850) | Ceriodaphnia (Dana 1853) | + | + | + | + |
|                          |        |       | Ceriodaphnia cornuta (Sars 1853) | + | - | + | + |
|                          | Moinidae (Goulden 1968) | Moina (Baird 1850) | + | + | + | + |
|                          |        |       | Moina microura (Kurz 1874) | + | + | + | + |
|                          |        |       | Moina macroopa (Straus 1820) | + | + | + | + |
|                          |        |       | Moina brachiate (Jurine 1820) | + | + | + | + |
|                          | Sididae | Latonopsis (Sars 1888) | Latonopsis australis (Sars 1888) | + | + | + | + |
3.2 Mass cultured zooplankton

The ostracoda species, *C. campechensis* was subjected to mass culture for 21 days during the months of March-May, 2018 with mixed phytoplankton (Spirulina: *Spirulina meneghiniana*, *Arthrospira platensis*, *Arthrospira maxima* and *Labyrinthiformis*; Chlorophyceae: *Pediastrum duplex*, *Pediastrum tetrus*, *Spirogyra hyalina*, *Ulothrix zonata* and *Tabellaria fenestrata*; Cyanophyceae: * Aphanoocapsa pulchra*, *Chroococcus minutus*, *Oscillatoriasub brevis* and *Phormidium granulatum*) as feed, and found to be grown well. The details are given in Table 2.

Table 2: Number of individual (ind./L⁻¹) zooplankton species of *C. campechensis* grown under mass culture for 21-days with mixed phytoplankton as a feed during summer months

| Ostracoda Species | March, 2018 | April, 2018 | May, 2018 |
|------------------|-------------|-------------|-----------|
|                  | Introduced (ind./L⁻¹) | Harvested (ind./L⁻¹) | Introduced (ind./L⁻¹) | Harvested (ind./L⁻¹) | Introduced (ind./L⁻¹) | Harvested (ind./L⁻¹) |
| *Cypretta campechensis* | 11±4 | 40±5 | 7±1 | 36±2 | 13±4 | 41±6 |
3.3 Diagnosis of *C. campechensis*

Relatively big animal, the surface of the carapace is smooth and covered by short and sparsely located setae, except at the dorsal region. Both, anterior and posterior calcified inner lamella narrow. Funnel-shaped radial pore canals present at lower anterior margin on both valves. Anterior seta short and length of anterior claw, posterior claw thin and distally serrated and attachment of uropodal ramus long and narrow distally bifurcated. Hemipenis with two conspicuous terminal lobes, internal one anvil-like. A small lobe between them, without spine-like process, internal canal double coiled (Plates 2 and 3).

3.4 Genomic DNA and its amplification

The size of isolated genomic DNA from *C. campechensis* was >10 kb and its PCR amplified product was >540 bp (Plate 4 (a and b, respectively). Actually the size of the aligned sequence was 544 bp, which was authenticated with Gen Bank (MN641913) and the data of the specimen, photograph and their sequences were submitted in the BOLD database as well.

![Plate 4: DNA of zooplankton species. L, Ladder; Ccp, C. campechensis.](image)

The BLAST of *C. campechensis* sequence revealed 100% similarity with its matched sequence available in NCBI database (Table 3). The results of multiple sequence alignment with MAS for identification of identical, similar and variable sites of amino acids for *C. campechensis* with retrieved species showed 326 identical amino acids residues, 17 similar amino acids residues and 201 variable amino acids sites (Table 4; Plate 5). These data showed less numbers of variable amino acid sites and similar amino acid residues, and more number of identical amino acid residues. In this study, the base composition of the COI gene fragment showed 63.0% AT biases and 37.5% GC biases (Table 5).

| Queried sequences | Author, Country and Accession Number | I (%) | G (%) | Retrieved/Matched species | Author, Country and Accession Number |
|-------------------|---------------------------------------|-------|-------|---------------------------|---------------------------------------|
| *Cypretta campechensis* | Paper authors, India, MN641913 | 100 | 0 | *Cypretta campechensis* | Macario-Gonzalez et al., 2018 [42]; Mexico MF076727 |

*Table 3: BLAST identification of COI partial gene sequences of subjected and retrieved zooplankton species with their Gen Bank accession numbers*

| Comparison of zooplankton species | Number of identical amino acid residues | Number of similar amino acid residues | Number of variable amino acid sites |
|-----------------------------------|---------------------------------------|--------------------------------------|-------------------------------------|
| *Cypretta campechensis* with retrieved species | 326 | 17 | 201 |

*Table 4: Number of identical and similar amino acid residues, and number of variable amino acid sites of the COI gene partial sequences generated for subjected zooplankton species*

COI, Cytochrome C oxidase subunit I gene
Plate 5: Multiple sequence alignment of COI gene sequences generated for subjected zooplankton. 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.

Table 5: Nucleotide composition of COI gene partial sequences for subjected zooplankton species, *C. campechensis*

| Species Name                                      | Nucleotide % |       |       | AT | GC |
|--------------------------------------------------|--------------|-------|-------|----|----|
| *C. campechensis*, Paper Authors, MN641913       | 28.3         | 16.0  | 34.2  | 21.5 | 63.0 | 37.5 |
| *C. campechensis*, Mexico, MF076751              | 28.7         | 16.1  | 34.7  | 20.5 | 63.4 | 36.1 |
| *C. campechensis*, Mexico MF076727               | 28.3         | 16.0  | 34.2  | 21.5 | 62.5 | 37.5 |
| *C. campechensis*, Mexico MF076717               | 28.7         | 15.9  | 34.5  | 20.9 | 63.2 | 36.8 |
| *C. campechensis*, Mexico MF076716               | 28.4         | 16.2  | 35.5  | 19.9 | 63.9 | 36.1 |
| *C. maya*, Mexico MF076730                       | 26.1         | 15.8  | 36.5  | 21.6 | 62.6 | 37.4 |
| *C. maya*, Mexico MF076729                       | 26.1         | 15.8  | 36.5  | 21.6 | 62.6 | 37.4 |
| *C. maya*, Mexico MF076726                       | 28.3         | 16.0  | 34.6  | 21.1 | 62.9 | 37.1 |
| *C. maya*, Mexico MF076728                       | 26.1         | 15.8  | 36.5  | 21.6 | 62.6 | 37.4 |
| *C. maya*, Mexico MF076724                       | 26.5         | 15.8  | 36.2  | 21.5 | 62.7 | 37.3 |
| *C. maya*, Mexico MF076720                       | 26.7         | 15.6  | 35.7  | 22.1 | 62.4 | 37.2 |
| *C. maya*, Mexico MF076719                       | 26.7         | 15.6  | 35.7  | 22.1 | 62.4 | 37.7 |
| *C. elongata*, Mexico MF076723                    | 26.5         | 15.8  | 36.2  | 21.5 | 62.7 | 37.3 |
| *C. elongata*, Mexico MF076722                    | 26.5         | 15.8  | 36.2  | 21.5 | 62.7 | 37.3 |
| *C. elongata*, Mexico MF076721                    | 26.7         | 15.6  | 36.2  | 21.5 | 62.9 | 37.1 |
| Avg.                                             | 27.2         | 15.8  | 35.5  | 21.4 | 62.7 | 37.2 |

COI, Cytochrome C oxidase subunit I gene; A, Adenocine tri-phosphate; G, Guanocine tri-phosphate; T, Thymidine tri-phosphate; C, Cytidine tri-phosphate.

3.5 Inter species nucleotide divergence

Between subjected and retrieved inter species category, the mean divergent rate of *C. campechensis* showed a minimum value of 0.000 (*C. campechensis* Vs. *C. campechensis*, Mexico) and a maximum of 0.290 (*C. campechensis* Vs. *C. maya*, Mexico and, Vs. *Cypretta elongate*, Mexico) (Table 6).
3.6 Phylogenetic information

The subjected *C. campechensis* along with their respective retrieved species showed Ka value of 2.064 and Ks of 0.850, which indicated the possibility of occurrence of more deleterious mutation and less silent mutation. Similarly, the Tv value of 0.12 and the Ts of 0.09 were recorded, which indicated the fact that these sequences have more phylogenetic information (Table 7). However, saturation might have not occurred in this sequence, which was confirmed by the predicted higher Iss.c values of 0.741 than that of the Iss value of 0.252 (Table 7).

### Table 7: Phylogenetic information of subjected zooplankton species along with retrieved

| Zooplankton species | Phylogenetic information |
|---------------------|--------------------------|
|                     | Ks | Ka | Ks-Ka | Ts | Tv | Tvs | Iss | Iss,c | Iss,c-Iss |
| Cypretta campechensis and retrieved species | 0.850 | 2.064 | 1.214 | 0.09 | 0.12 | 0.03 | 0.252 | 0.741 | 0.489 |

Ks: Synonymous substitution; Ka: Non-synonymous substitution; Ts: Transitional substitution; Tv: Transversional substitution; Iss: Index of substitution saturation; Iss.c: Critical value of index of substitution saturation.

3.7 Phylogenetic tree topology

Plate 6 represent *Cypretta* phylogenetic tree topology, the subjected (*C. campechensis*) and retrieved (*C. campechensis*, *C. maya* and *C. elongata* from Mexico) species formed four clusters. The I-cluster was formed by three species of *C. campechensis* and one species of *C. maya*. The II-cluster was formed by three species of *C. maya*. The III-cluster was formed by two species of *C. maya*. The IV-cluster was formed by subjected *C. campechensis*, and retrieved (three species of *C. campechensis* and one species of *C. maya*).

### Plate 6: Phylogenetic tree topology of subjected zooplankton, *C. campechensis* along with retrieved species

4. Discussion

Planktonic organisms form the food for many aquatic animals including whales, fishes and prawns. The zooplankton serves as a main source of nutrition for freshwater and marine fishes and prawns larvae (Hamdy, 2019) (43). They supply necessary amount of protein, lipid, essential amino acids and fatty acids,
which provide immune stimulation, pigment enhancement, physiological regulations, and growth and quality larval production (Mayzaud et al., 2016; Manickam et al., 2017) [44, 45]. Zooplanktons are highly sensitive to detect any environmental disturbances through changes in species composition, abundance, and body size, distribution and diversification (Xiong et al., 2020; Eskinazi-Sant’Anna et al., 2020) [46, 47]. Zooplankton species composition in a particular water body is controlled by several ecological factors, including nutritional load and pollution status (Bhavan et al., 2015a) [19]. Different species of Rotifera, Cladocera, Copepoda and Ostracoda have been reported in heavily polluted water and t

The mass culture of zooplankton, such as Brachionus, Daphnia, Asplanchna intermedia, Ceriodaphnia, Eucyclops speratus, Mesocyclops edax, Moina, Cyclops, Lecanea, Keratella, Diaphanosoma and Diaptomus with different feeds, such as chlorella, Yeast, cow dung, mixed phytoplankton, condensed phytoplankton products, pulse bran water, poultry manure, snail faeces, chicken manure and fish waste diet have been reported (Bhavan et al., 2016; Kalpana et al., 2018; Ogello et al., 2019) [12, 13, 55]. In this study, the first reported zooplankton species C. campechensis has grown well in the laboratory with mixed phytoplankton diet.

The highest GC content was observed in North America using COI sequences. Similarly, Gilbert et al. (2005) [59] discriminated two cryptic species within the Daphnia obtusa complex in North America using COI sequences. Similarly, Gilbert et al. (2005) [59] described ten genetically distinct cryptic species of B. calicyflorus in eastern China. The higher AT biases have been reported in crustaceans and prawns (Bhavan et al., 2015b; Umamaheswari et al., 2016; Udayasuriyan et al., 2017) [60, 61, 35], and in freshwater zooplankton (Bhavan et al., 2016, 2017; Kalpana et al., 2018) [12, 13]. The higher A+T and lower G+C contents have also been reported by Wang et al. (2016) [62]. The highest GC content was reported in Parartemia longicaudata from saline lakes in Australia, while the lowest GC content was reported in the amphipod, Hyperia galba a parasite of jellyfish. Isopoda possessed the highest and lowest average GC content (Costa et al., 2018) [63]. Arisuryanti et al. (2020) [64] reported the highest percentage of nucleotide C and A+T, similar percentage of nucleotide A and G was observed in Parhippomyte uveae (red shrimp). The higher AT biases and lower GC biases have been reported in Phyllodiaptomus tunguensis (Zhang et al., 2020) [65]. The higher AT biases recorded in this study indicates the lower abundance of nuclear copies of mt-DNA (NUMTs) genes known as pseudogenes, homologs or paralogs in all the three species.

Generally, deep genetic divergence exists among allopatric populations of a single species. For example, five phylo groups of Daphnia ambigua (four in North America and one in South America) had been reported with >3% divergence (Hebert et al., 2003) [66]. In a study with six phylogroups of Sida crystalline with >5% divergence has also been reported by Cox and Hebert (2001) [67]. Zooplankton like rotifer, often have complex life cycles, high dispersal capacities and rapid local adaptations, which may facilitate inter specific gene flow and intra specific divergence (Gomez et al., 2002; Cristescu et al., 2012) [68, 69]. Barcode analysis enabled some forms and varieties of common species identified as separate
species; the cryptic species in \textit{Ascomorpha ovalis}, \textit{Lecane balla}, \textit{Lecane cornuta}, \textit{Lecane curvicornis}, \textit{Lecane crepida}, \textit{Lecane lunaris}, \textit{Lecane hastata}, \textit{Platyiuss quadricornis}, \textit{Keratella cochlearis}, \textit{B. calciflorus} and \textit{Testudinella patina}, as well as in some forms and varieties such as \textit{B. quadridentatus}, \textit{Mytilina ventralis}, \textit{Corbicula fluminea} and \textit{Leptodactliopium} \textit{(Elías-Gutierrez et al., 2008; García-Ia-Morales and Elías-Guti Errez, 2013)}\textsuperscript{72, 73}. The biogeographic range of \textit{Subeucalanus subtenius}, \textit{Subeucalanus mucronatus}, \textit{Subeucalanus subcrassus} and \textit{Parenalalanus langae} have been extended in the western Indian Ocean; the Indo-Pacific region consist of genetically divergent, allopatric populations of \textit{Subeucalanus pileatus}, \textit{Parenalalanus sewelli}, \textit{Rhincalanus rostrifrons}, \textit{Arietillus pavoninus}, \textit{Codium pulvinatum} and \textit{Boccardia proboscidea}; genetically divergent lineages of \textit{Subeucalanus crassus} and \textit{Rhincalanus nasutus} have been inadequately characterized (Sututoni et al., 2006; Zenetos and Galanidi, 2020)\textsuperscript{72, 73}. The sequence divergence has also been reported in \textit{Candacia}, \textit{Parenalalanus}, \textit{Rhincalanus} and \textit{Temora} \textit{(Pitz et al., 2020)}\textsuperscript{74}. In this study, the recorded lower inter-species divergence suggests that the first recorded species are closely related species with each other. The intra and inter specific genetic distances have considerably greater with larger proportions of closely related taxa \textit{(Funk et al., 2003; Moritz et al., 2004)}\textsuperscript{75, 76}. The smallest intra specific distances yield more consistent results \textit{(Meier et al., 2008)}\textsuperscript{77}. The identification success found to be declined when the overlap between intra- and inter specific distances increased \textit{(Ross et al., 2008; Virgilio et al., 2010)}\textsuperscript{78, 79}. Brando et al. \textit{(2010)}\textsuperscript{80} and Schön et al. \textit{(2010)}\textsuperscript{81} have found a very small (0.0–0.8 %) intra specific COI distances between western \textit{Australican Eucyris vires} and their closest European relatives. By using the distance-based approach, a species can be correctly identified when the mean distance to the most closely related species (nearest neighbor) is higher than the maximum intra specific distance \textit{(Aliabadian et al., 2009)}\textsuperscript{82}. The similarity between the sequences usually depends entirely on the similarity in nucleotide frequencies, which is based on level of substitutionsal saturation, which in turn decreases phylogenetic information \textit{(Xia and Lemey, 2009)}\textsuperscript{83}. The saturation of substitutions in sequences decreases phylogenetic information \textit{(Xia et al., 2003)}\textsuperscript{39}. When the sequences have experienced full substitutional saturation, the similarities between the sequences depend entirely on the similarity in nucleotide frequencies \textit{(Xia, 2000; Xia et al., 2003)}\textsuperscript{37, 39}, which often does not reflect on phylogenetic relationships. The phylogenetic information have previously been established by us for species of crabs, prawns, shrimps and plankton \textit{(Bhavan et al., 2016, 2017; Udayasuriyan et al., 2017; Dasilva et al., 2019)}\textsuperscript{12, 4, 35, 84}. The possibility for occurrence of more transversional substitutions have been reported when the sequences of \textit{Macrobrachium} and \textit{Cardinida} were compared \textit{(Udayasuriyan et al., 2017)}\textsuperscript{15}. In this study, the recorded more transversional substitution in comparison with transitional substitution suggests more phylogenetic information. Therefore, there are possibilities for evolutionary changes in these species in due course of time.

5. Conclusions

In the present work, the first recorded ostracoda species, \textit{C. campechensis} was largely found in the perennial standing freshwater of the Singanallur lake in summer season. This suggests that the water of this lake is being polluted during summer due to undiluted inflow of household let-outs and water evaporation. In other seasons, this effect may be diluted by rainwater inflow. Since this is the first report in India, we described by both morphological and molecular levels. The molecular analysis revealed that the species was distinct and showed significant variation. The mass culture of \textit{C. campechensis} would provide a feeding option in summer months for maintenance of sustainable aquaculture.

6. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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