www.genaqua.org
ISSN 2459-1831
Genetics of Aquatic Organisms 2: 13-22 (2018) DOI: 10.4194/2459-1831-v2_1_03

RESEARCH PAPER

Molecular Systematics of Freshwater Diaptomid Species of the Genus Neodiaptomus from Andaman Islands, India

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Received 10 April 2018
Accepted 29 July 2018

Abstract

Calanoid copepods belonging to the family Diaptomidae occur commonly and abundantly in different types of freshwater environment. Based on morphological taxonomic key characters 48 diaptomid species belonging to 13 genera were reported from India. Taxonomic discrimination of many species of these genera is difficult due to their high morphological similarities and minute differences in key characters. In the present study two species of the genus, Neodiaptomus, *N. meggiti* and *N. schmackeri* from Andaman Islands were examined based on morphological and molecular characters which showed low variation in morphology and differences in their distributions. The morphological taxonomy of Copepoda with genetic analysis has shown complementing values in understanding the genetic variation and phylogeny of the contemporary populations. In this study, a molecular phylogenetic analysis of *N. meggiti* and *N. schmackeri* is performed on the basis of mitochondrial Cytochrome c oxidase subunit I (COI) gene. The mtDNA COI sequence of *N. meggiti* and *N. schmackeri* is 650 bp long. The mtDNA COI 1 gene sequence data of *N. meggiti* exhibits a remarkable constancy throughout its distributional area ranging from North to south Andaman. Similarity of *N. meggiti* with other genera of the family varies from 67.6 to 75.5% while of *N. schmackeri* varies from 65.5 to 72.6% on the basis of COI gene sequences. While *N. meggiti* and *N. schmackeri* share a similarity of 92.3%, high COI gene sequence similarity among each other supports the morphological based data that they belong to the same genus. The phylogenetic relationship elucidated using three different tree-making algorithms, the neighbour joining, minimum evolution and maximum parsimony showed that five plankton specimens under study form two clusters and constituted a deeply rooted, evolutionarily distinct group, separated from the other related genera used in the analysis. The phylogenetic relationship of the two diaptomid copepod *N. meggiti* and *N. schmackeri* of Andaman is remarkably accurate when the Neighbor joining phylogenetic tree and as well as maximum parsimony phylogenetic tree are considered.

Keywords: Copepoda, diaptomid, molecular taxonomy, phylogeny, mtDNA COI.

Introduction

Calanoids are the dominant groups of the order Copepoda and they play vital role in energy transfer from primary producers to secondary consumers in aquatic ecosystem. Approximately 48 diaptomid species belonging to 13 genera were reported from Indian subcontinent against 59 genera andfig 470 species of freshwater diaptomid reported to occur around the globe (Dussart & Defaye, 2002). The most comprehensive study on the diaptomid has been reported by Reddy (1994), which provided detailed taxonomic keys to the genera Heliodiaptomus, Allodiaptomus, Neodiaptomus, Phyllodiaptomus, Eodiaptomus, Arctodiaptomus and Sinodiaptomus from India. He has also revised as many as twenty genera besides describing about fifty new species, establishing four new genera, two new families and reported about twenty new records from India (Reddy, 2013).

The molecular systematics and phylogenetic relationship of inland freshwater diaptomid copepods have till recently received little attention when compared with their marine counterparts (Bucklin, Guarnieri, Hill, Bentley, and Kaartvedt, 1999 and Bucklin, Frost, Bradford-Grieve, Allen, and Copley, 2003; Lee, 2000; Papadopoulos, Peijnenburg and Luttikhuizen, 2005; Bucklin & Frost, 2009; Minxiao, Song, Chaolun and Xin, 2011). Among the inland water calanoid taxa, the phylogeny and molecular systematics of Neritic diaptomids and Neotropical centropagids have been investigated using both nuclear (Thum, 2004; Marszalek, Dayanandan, and Maly, 2009) and mitochondrial markers (Adamowicz,

Menu-Marque, Hebert, and Purvis, 2007; Thum & Derry, 2008; Thum & Harrison, 2009; Marrrone, Brutto, & Arculeo, 2010, Marrone, Brutto, Hundsdoerfer, and Arculeo, 2013). In recent past the mitochondrial cytochrome oxidase I (mtCOI) gene sequences proved to be diagnostic molecular systematic characters for accurate identification and discrimination of the calanoid copepod species (Bucklin *et al*., 1999; Bucklin *et al*., 2003; Marrone *et al*., 2010). The sequences are also useful to reconstruct phylogenetic relationships among congeneric species, resolve large scale population genetic structure and taxonomically significant geographic variation, and may help to reveal cryptic species (Qinglong, Chatzinotas, Wang, and Boenigk, 2009). Further, sequencing and analysis of Mt COI gene sequence will allow us to clearly distinguish various species including sub species and cryptic species and permit to generate a DNA barcode specific to individual species.

Patterns of genetic isolation by distance in two sister species of freshwater copepods, *Eudiaptomus graciloides* and *Eudiaptomus gracilis*, using the polymorphism of microsatellite markers were demonstrated by Bohonak *et al*. (2006). Further, sequencing and analysis of mt COI sequence will allow us to clearly distinguish various species including sub species cryptic species and permit to generate a DNA bar code specific to individual species. Though there are a number of reports on the freshwater zooplankton community and diaptomid copepods from the main land India, there is no report from Andaman except on calanoid by Roy (1991) and Reddy (2000). In the present study, *Neodiaptomus meggiti* and *Neodiaptomus schmackeri* from different geographic locations of Andaman Islands were investigated to understand 1. The mitochondrial cytochrome oxidase I sequence, 2. Evaluate the phylogeny and genetic diversity of these genera from different geographical locations.

Materials and Methods

Zooplankton Sampling and Analysis

Zooplankton samples were collected from fourteen freshwater bodies covering the entire north, middle and south Andaman Islands by towing of zooplankton net (200 µm mesh size, 0.25 m diameter, and 0.5 m length) and the live zooplankton were transferred to one liter of filtered water. Live calanoids were separated from the zooplankton sample by filtering through a 300 µm mesh. The separated live calanoids were filtered on 100 µm nylon mesh and stored in 90% ethyl alcohol. *Neodiaptomus meggiti* and *N. schmackeri* were isolated and identified up to species (Rajendran, 1971 and 1973; Reddy, 1994).

DNA Extraction, PCR Amplification and Sequencing

Approximately 50 male calanoids were pooled and the genomic DNA was extracted with DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. Partial region of mitochondrial cytochrome oxidase I (mtCOI) gene, was amplified by polymerase chain reaction (PCR), using primers described by Folmer, Black, Hoeh, Lutz, and Vriejenhoek, (1994). The PCR amplification was carried out in a 50 µl reaction volume containing 30 - 50 ng of DNA template and 1X GT PCR master mix (TaKaRa). The condition for PCR included an initial denaturation at 94°C for 5 min followed by 35 cycles (denaturation at 94°C for 1 min, annealing at 45°C for 1 min, and extension at 72°C for 2 min) with a final extension at 72°C for 10 min. The amplified products were purified using Eppendorf Perfectprep gel clean up kit (Eppendorf, Germany). Sequencing reaction was carried out using BigDye terminator v3.1 cycle sequencing kit according to manufacturer's instructions (Applied Biosystems) and the sequences were determined by ABI-3500 genetic analyzer (Applied Biosystems Inc., Foster City, USA).

Phylogenetic Analysis

The forward and reverse mtCOI gene sequences were checked and assembled with Chromas Proversion1.5 software (www.technelysium.com.au/ChromasPro.html). The similarity of assembled sequences was determined using BLAST against GenBank database (www.ncbi.nlm.nih.gov). The mtCOI gene sequences of the closest homologs with highest scores were downloaded and the alignments were performed with CLUSTAL-X v 1.81 (Thompson, Gibson, Plewniak, Jeanmougi, & Higgins, 1997). The multiple sequence alignment was edited and flushed for gaps and missing data using DAMBE v 5.3.46 (Xia, 2013) and a degapped alignment of 561 nucleotides of mtCOI gene sequences was used to get an unambiguous sequence alignment. Evolutionary genetic distances of *N. meggiti* and *N. schmackeri* were computed according to the algorithm of Kimura two parameter (Kimura, 1980) using all codon positions. Tree topologies were inferred with the neighbour joining (Saitou and Nei, 1987), minimum evolution (Rzhetsky and Nei, 1992) and maximum parsimony (Fitch, 1971) methods, using MEGA v 5.05 (Tamura *et al*., 2011). The robustness of the topology of phylogenetic trees was evaluated by bootstrap analyses (Felsenstein, 1985) with 1000 replications. The DNA barcode was generated for individual species using barcode tools present in Barcode of Life Data System (http://www.boldsystems.org/index.php/databasesBold Base).

Results

Neodiaptomus meggiti collected from three locations namely Diglipur (DP-NA-2), Rangat (RG-MA-4) and Minne Bay (MB-SA-10), representing north, middle and south Andaman taken for molecular systematic study. While *N. schmackeri* were subjected for molecular systematic study from two locations namely Madupur (MP-NA-1) and Cattle gunj (CG-SA-2) due to their occurrence in these stations only. Extraction of DNA from the diaptomids found to be difficult due limited density of individual species and their smaller size (1.2 mm length). However, direct amplification from the organism can be done without extract (Bucklin *et al.*, 1999). A portion of COI gene was amplified using a set of standard primer described by Folmer *et al*., (1994). The initial amplification was feeble, hence gradient PCR (40-50 °C) was used and an optimum amplification was obtained at 48 °C. The size of amplified fragment of COI gene was estimated to be approximately of 700 base pairs (Figure 1).

The Mitochondrial Cytochrome Oxidase I gene sequences obtained in this study was deposited at the NCBI GenBank Database (www.ncbi.nlm.nih.gov/nucleotide). The accession numbers of *N. meggiti* haplotypes MB-SA-10, DP-NA-2 and RG-MA-4 are KF366714, KF366712 and

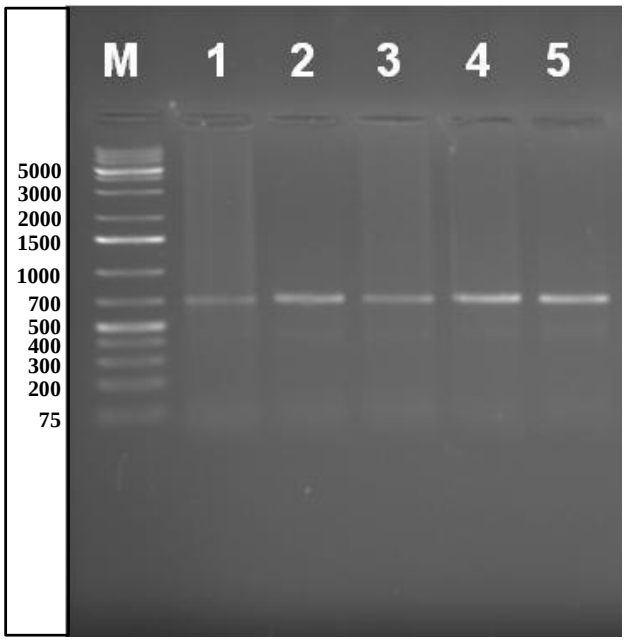

Figure 1. Agarose gel electrophoresis, of PCR Products of COI diaptomids 1. Madupur (MP-NA); 2. Cattle Gunji (CG- SA), 3. Diglipur (DP-NA); 4. Rangat (RG-MA); 5. Miniebay (MB-SA), M-Molecular marker; (100bp-10kb DNA ladder).

GTCTGGAATAGTAGGAACAGGCCTAAGAATAATTATCCGCTTGGAGCTGG
GGCAAGCTGGCAGACTCATTGGAGATGACCAAATTTATAATGTTGTAGTGACTGC
TCACGCTTTTATCATAATTTTTTTCATAGTAATACCAATTTTAATCGGAGGGTTCG
GAAACTGGTTGGTTCCTTTGATACTAGGGGCAGCTGATATAGCGTTTCCTCGTAT
AAATAATATAAGATTCTGGTTTCTAATTCCGGCATTAATCATACTTTTGACAAGA
TCCCTAGTTGAAAGGGGGGCAGGCACTGGTTGAACTGTGTATCCCCCGTTATCAA
GAAACATTGCGCACGCAGGAAGGTCGGTAGATTTTGCTATTTTTTCTCTCCATCT
GGCAGGAGTGAGATCAATTTTAGGGGCTGTAAATTTTATTAGCACCCTAGGGAAT
TTACGAGCTTTTGGTATAATTTTAGATCGTATGCCATTGTTTGCCTGAGCTGTGCT
GATTACTGCAGTCTTATTATTACTTTCATTGCCTGTGTTAGCTGGAGCTATTACCA
TGCTTTTAACGGACCGTAACCTCAACTCCAGATTTTACGATGCAGGTGGAGGGGG

Figure 2. KF366714 - *Neodiaptomus meggiti* (DP-NA-2 mtDNA COI subunit 1 gene, partialcds.

AGGAACAGGCCTAAGAATAATTATCCGCTTGGAGCTGGGGCAAGCTGGCA
GACTCATTGGAGATGACCAAATTTATAATGTTGTAGTGACTGCTCACGCTTTTAT
CATAATTTTTTTCATAGTAATACCAATTTTAATCGGAGGGTTCGGAAACTGGTTG
GTTCCTTTGATACTAGGGGCAGCTGATATAGCGTTTCCTCGTATAAATAATATAA
GATTCTGGTTTCTAATTCCGGCATTAATCATACTTTTGACAAGATCCCTAGTTGAA
AGGGGGGCAGGCACTGGTTGAACTGTGTATCCCCCGTTATCAAGAAACATTGCG
CACGCAGGAAGGTCGGTAGATTTTGCTATTTTTTCTCTCCATCTGGCAGGAGTGA
GATCAATTTTAGGGGCTGTAAATTTTATTAGCACCCTAGGGAATTTACGAGCTTT
TGGTATAATTTTAGATCGTATGCCATTGTTTGCCTGAGCTGTGCTGATTACTGCAG
TCTTATTATTACTTTCATTGCCTGTGTTAGCTGGAGCTATTACCATGCTTTTAACG
GACCGTAACCTCAACTCCAGATTTTACGATGCAGGTGGAGGGGGCGACCCC

Figure 3. KF366712 -*Neodiaptomus meggiti* (RG-MA-4) mtDNA COI subunit 1 gene, partial cds.

ATAGTAGGAACAGGCCTAAGAATAATTATCCGCTTGGAGCTGGGGCAAGC
TGGCAGACTCATTGGAGATGACCAAATTTATAATGTTGTAGTGACTGCTCACGCT
TTTATCATAATTTTTTTCATAGTAATACCAATTTTAATCGGAGGGTTCGGAAACTG
GTTGGTTCCTTTGATACTAGGGGCAGCTGATATAGCGTTTCCTCGTATAAATAAT
ATAAGATTCTGGTTTCTAATTCCGGCATTAATCATACTTTTGACAAGATCCCTAGT
TGAAAGGGGGGCAGGCACTGGTTGAACTGTGTATCCCCCGTTATCAAGAAACAT
TGCGCACGCAGGAAGGTCGGTAGATTTTGCTATTTTTTCTCTCCATCTGGCAGGA
GTGAGATCAATTTTAGGGGCTGTAAATTTTATTAGCACCCTAGGGAATTTACGAG
CTTTTGGTATAATTTTAGATCGTATGCCATTGTTTGCCTGAGCTGTGCTGATTACT
GCAGTCTTATTATTACTTTCATTGCCTGTGTTAGCTGGAGCTATTACCATGCTTTT
AACGGACCGTAACCTCAACTCCAGATTTTACGATGCAGGTGGAGGGGGCGACCC
CA

Figure 4. KF366713 -*Neodiaptomus meggiti* (MB-SA-10) mtDNA COI subunit 1 gene, partial cds.

KF366713 and *N. schmackeri* haplotypes MP-NA-1 and CG-SA-2 are KF366715 and KF366716, respectively. The DNA barcode was generated for individual species using (Bold Base) barcode tools (Figures 2 to 8).

Preliminary comparison of obtained COI gene sequences in the GenBank database indicated that all the plankton belong to the family Diaptomidae. Sequences of diaptomids MB-SA-10, DP-NA-2 and RG-MA-4; MP-NA-1 and CG-SA-2 are identical to each other respectively. COI gene sequence based similarity matrix shows that different genera of the family Diaptomidae share homologies of less than 80% with each other (Table 1). On the basis of morphological characteristics of plankton MB-SA-10, DP-NA-2 and RG-MA-4 belong to the same species *N. meggiti* while MP-NA-1 and CG-SA-2 belong to the species *N. schmackeri*. Similarity of *N. meggiti* with other genera of the family varies from 67.6 to 75.5% while of *N. schmackeri* varies from 65.5 to 72.6% on the basis of COI gene sequences (Table 1).

The results showed that *N. meggiti* and *N. schmackeri* share a similarity of 92.3%, high COI gene sequence similarity among each other supports the morphological based data that they belong to the same genus (Reddy, 1994).

The phylogenetic relationship elucidated using three different tree-making algorithms, the neighbour joining (Figure 9), minimum evolution (data not shown) and maximum parsimony (Figure 10) showed that five plankton specimens under study form two clusters and constituted a deeply rooted, evolutionarily distinct group, separated from the other related genera used in the analysis. The nodes grouping the five species has a high bootstrap value of around 100%, which indicates a strong stability of topology. High species divergence is present in the genus *Skistodiaptomus* in comparison to the genus

GGAACAGGCCTAAGAATAATTATCCGCTTGGAGCTGGGGCAAGCTGGCAGACTC
ATTGGAGATGACCAAATTTATAATGTTGTGGTGACTGCTCACGCTTTTATCATAA
TTTTTTTCATAGTAATACCAATTTTAATCGGAGGGTTCGGAAACTGGTTGGTCCCT
TTGATACTAGGGGCAGCTGATATAGCGTTTCCTCGTATAAATAATATAAGATTCT
GATTTCTAATTCCGGCATTAATTATACTTTTGACAAGATCCCTAGTTGAGAAGGG
GGCCGGCACAGGGTGAACTGTGTACCCCCCCTTTAAAAGAAACATTGCGCACGC
CGGGAGGTCCGGAGATTTTGCTATTTTTTCTCTCCATCTGGGAGGGGTGATATCA
TTTTTAGGGGCTGTAAATTTTATTACACCCTTAGGAAATTTACCAGCTTTTGGTAT
AATTTTAGAACCGATGCCCTTGTTTGCCTGAGCTGGGCTGAATACCGCCTTCTTAT
TATTACTTTCCTTGCCCGTGTTAGCTGGAGCTATTACCATGCTTTTAACCGAACGT
AACCTCAACTCCAGATTTTACGATGCAGGAGGAGGGGGCGACCCCATTCTCTA

Figure 5. KF366715 -*Neodiaptomus schmackeri* (MP-NA-1) mtDNA COI subunit 1 gene, partial cds.

ACAGGCCTAAGAATAATTATCCGCTTGGAGCTGGGGCAAGCTGGCAGACTCATT
GGAGATGACCAAATTTATAATGTTGTGGTGACTGCTCACGCTTTTATCATAATTTT
TTTCATAGTAATACCAATTTTAATCGGAGGGTTCGGAAACTGGTTGGTCCCTTTG
ATACTAGGGGCAGCTGATATAGCGTTTCCTCGTATAAATAATATAAGATTCTGAT
TTCTAATTCCGGCATTAATTATACTTTTGACAAGATCCCTAGTTGAGAAGGGGGC
CGGCACAGGGTGAACTGTGTACCCCCCCTTTAAAAGAAACATTGCGCACGCCGG
GAGGTCCGGAGATTTTGCTATTTTTTCTCTCCATCTGGGAGGGGTGATATCATTTT
TAGGGGCTGTAAATTTTATTACACCCTTAGGAAATTTACCAGCTTTTGGTATAATT
TTAGAACCGATGCCCTTGTTTGCCTGAGCTGGGCTGAATACCGCCTTCTTATTATT
ACTTTCCTTGCCCGTGTTAGCTGGAGCTATTACCATGCTTTTAACCGAACGTAACC
TCAACTCCAGATTTTACGATGCAGGAGGAGGGGGCGACCCCATTCTCT

Figure 6. KF366716 -*Neodiaptomus schmackeri* (CG-SA-2) mtDNA COI subunit 1 gene, partial cds.

Neodiaptomus, however all the species of each genus form a single monophyletic clade supported by high bootstrap values (Figure 9 and Figure 10).

Discussion

The genus *Neodiaptomus* Kiefer, 1932, is one of the most widely distributed and common diaptomid copepods in Southeast Asia. *Neodiaptomus* is represented by four valid species namely *N. schmackeri* (Poppe and Richard, 1892), *N. physalipus* (Kiefer, 1935), *N. lindbergi* (Brehm 1951), *N. intermedius* (Flobner, 1984) in Indian subcontinent. *Neodiaptomus meggiti* is recorded in Mayanmar and Malaysia, (Lai and Fernando 1978 and 1981) while never been reported in Indian subcontinent except of single report from Andaman (Reddy, 2000), whereas N. *schmackeri* is reported in Thailand (Sanoamuang & Athibai, 2002), mainland India (Reddy and Reddy, 1992) and adjoining countries to Andaman but not reported from Andaman.

Mitochondrial DNA sequence is considered to be the most powerful approach to resolve taxonomic uncertainties. The mtDNA COI sequence of *N. meggiti* and *N. schmackeri* is 650 bp long. Although phylogenies at the ordinal level are available for Copepoda there are no much published family-level phylogenies for the diaptomids (Huys and Boxshall, 1991). Since very few diaptomid species are represented in GenBank, it was essential to find homologous sequences using BLAST. The closest match (75.5% and 72.6% similarity) in GenBank for *N. meggiti* and *N. schmackeri* was the mtDNA COI sequence of *Mongolodiaptomus birulai*.

It is evident from the evolutionary similarity values and phylogenetic trees that five calanoid specimens under study belong to two different species of the same genus. The mtDNA COI 1gene sequence data of *N. meggiti* exhibits a remarkable constancy throughout its distributional area ranging from north

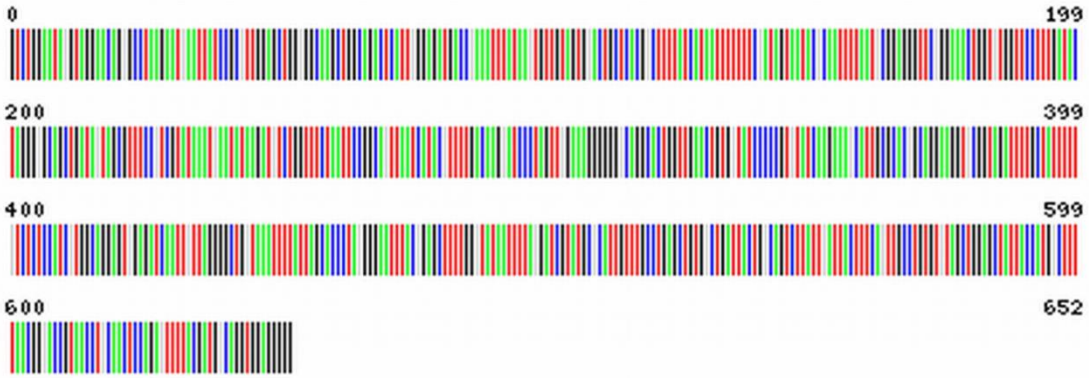

Figure 7. DNA Barcode *of Neodiaptomus schmackeri*) generated using mtDNA COI subunit 1 gene, partial cds.

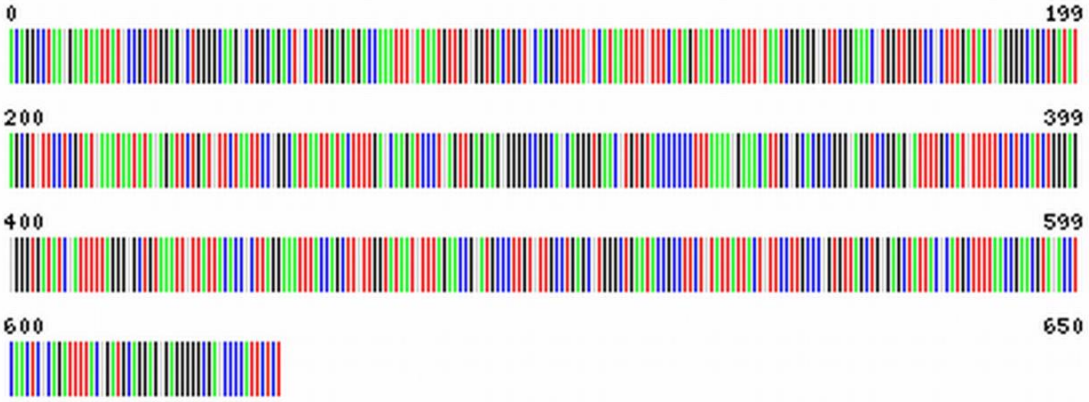

Figure 8. DNA Barcode *of Neodiaptomus schmackeri*generated using mtDNA COI subunit 1 gene, partial cds.

to south Andaman (intra-populations mitochondrial gene COI divergence is 0 %). According to Allendorf and Phelps (1981) the degree of genetic divergence among natural population will be affected by three basic evolutionary forces namely migration, genetic drift and natural selection. Even though the north, middle and south Andaman in isolated geographically by land and sea no genetic variation were recorded in the present study evinced the clear and constant migration of diaptomids between these locations. According to Burton (1987), if migration between populations is rendered by the presence of physical barriers, gene flow will be low and unique gene pool in local population could have resulted, such unique gene pool has not been recorded in the present study. The source of migration of mixing of gene pool is not clear. However, it suggested that gene migration could be through human, during the transportation and stocking of freshwater fish fingerling from south Andaman.

The phylogenetic relationship of the two diaptomid copepod *N. meggiti* and *N. schmackeri* of Andaman is remarkably accurate when the Neighbour joining phylogenetic tree and as well as maximum parsimony phylogenetic tree are considered. The generic relationships among the two species *N. meggiti* and *N. schmackeri* is unambiguously supported by the high COI gene sequence similarity of 92.3%. The observed inter population distances (maximum divergence of 7.7 %) between *N. schmackeri* and *N. meggiti* suggest that both the species are separate and share a different evolutionary lineage. The *N. schmackeri* is distributed throughout the Indo pacific region (Reddy and Reddy 1992; Sanoamuang & Athibai, 2002) whereas the distribution of *N. meggitti* is limited up to Andaman and Nicobar Islands and this species is not reported from mainland India. The occurrence of *N. meggitti* at Andaman Island might be due to its closeness of to Thailand and Malaysian land than the large oceanic barrier in between Andaman Island and mainland India.

The 18S DNA sequence resolves relationship among genera but not likely to resolve relationship within genera because of overall slow rate of molecular evolution of ribosomal gene as reflected by low bootstrap values of species relationship in North American diaptomid copepod species as reported by Thum (2004). Faster evolving mitochondrial DNA genes are generally preferred for resolving such relationships. However, DNA divergences within diaptomid genera are too high using mitochondrial cytochrome oxidase I DNA sequences for resolving phylogenetic relationships within genera and species (Thum, 2004).

The rare freshwater diaptomid *N. meggiti*s

Table 1. Estimates of evolutionary similarity between *CO*I gene sequences

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1														
2	79.0													
3	77.3	96.3												
4	77.2	72.9	72.7											
5	78.0	72.8	72.5	98.0										
6	75.2	72.5	73.2	76.3	76.7									
7	76.0	73.3	73.8	77.1	77.4	98.9								
8	71.9	67.6	68.1	74.6	73.6	75.5	75.8							
9	71.9	67.6	68.1	74.6	73.6	75.5	75.8	100						
10	71.9	67.6	68.1	74.6	73.6	75.5	75.8	100	100					
11	68.7	65.5	66.0	69.9	69.1	72.3	72.6	92.3	92.3	92.3				
12	68.7	65.5	66.0	69.9	69.1	72.3	72.6	92.3	92.3	92.3	100			
13	73.6	72.1	71.3	73.6	72.5	76.4	76.9	75.0	75.0	75.0	71.0	71.0		
14	73.9	71.8	71.0	73.8	72.8	76.6	77.1	75.5	75.5	75.5	71.5	71.5	99.5	

The evolutionary distance matrix generated in MEGA 5 program was used to determine percentage similarity values. The organisms are 1, *Skistodiaptomus carolinensis* (AY275437); 2, *S. oregonensis* VT801_106 (EU582593); 3, *S. oregonensis* MT001_2 (EU582589); 4, *Arctodiaptomus dorsalis* ZPLMX747 (EU770465); 5, *A. dorsalis* ZPLMX249 (EU770460); 6, *Mongolodiaptomus birulai* M_Hap_14 (AB593008); 7, *M. birulai* M_Hap_9 (AB593003); 8, *Neodiaptomus meggiti* RG-MA-4 (KF366713); 9, *N. meggiti* MB-SA-10 (KF366714); 10, *N. meggiti* DP-NA-2 (KF366712); 11, *N. schmackeri* MP-NA-1 (KF366715); 12, *N. schmackeri* CG-SA-2 (KF366716); 13, *Leptodiaptomus minutus* WI002_7 (EU825151); 14, *L. minutus* NS01_10 (EU825187).

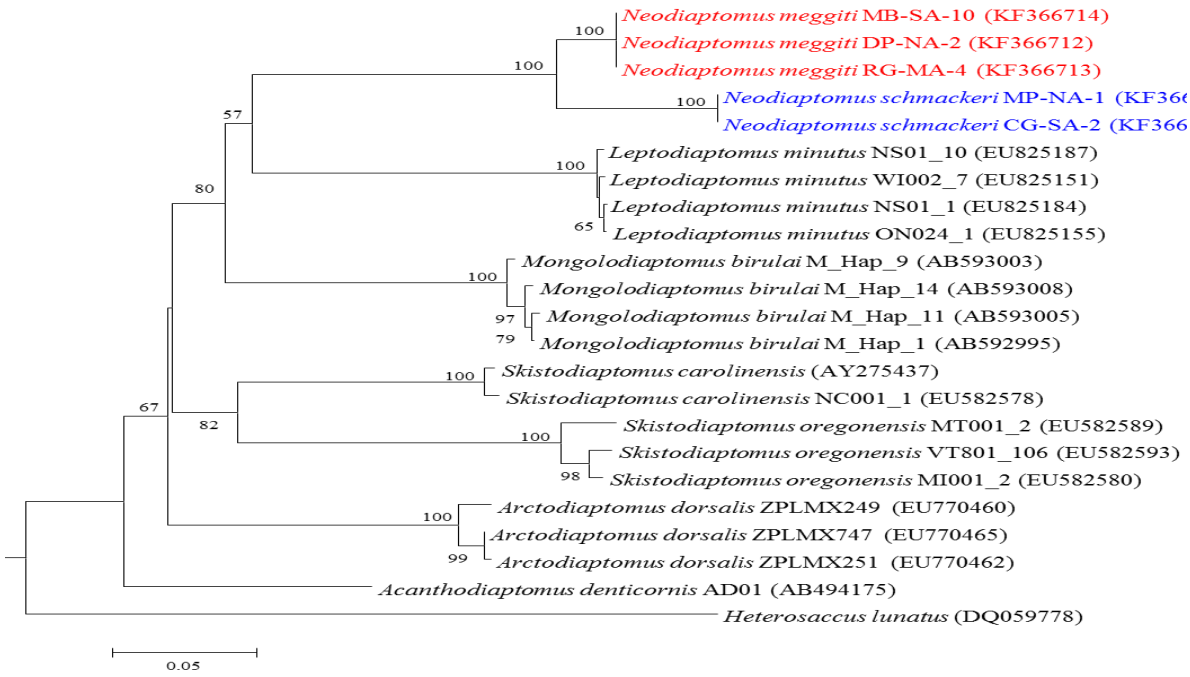

Figure 9. Neighbor-joining phylogenetic tree based on *CO*Igene sequences comparison, showing the phylogenetic position of *Neodiaptomus meggiti* and *Neodiaptomus schmackeri* haplotypes among related genera and species of the family *Diaptomidae*. *Heterosaccuslunatus* (DQ059778) was used as an out group. Bootstrap values greater than 50 % are shown at branch points. Bar 0.05 substitutions per nucleotide position.

species is so far known from Thailand, Sanoamuang and Athibai, (2002), and our report on the occurrence of *N. meggiti* throughout Andaman and Nicobar is noteworthy. Therefore, the distributional record of this species *N. meggiti* is being extended from Indonesian to Andaman and Nicobar Island of Indian subcontinent confirming the report of Reddy (2000).

The data obtained so far from the populations of *N. meggiti* and *N. schmackeri* indicates that there is very little or no variation occurring within populations and much more variation among other species. All of the above finding is based on the few individuals' samples. Phylogenetic studies of Sivakumar, Anandan, Muthupriya, Gopikrishna, & Altaff (2013) with reference to 18S rDNA of *Thermocyclops decipiens* also suggested no interpopulation variability in the 3' end fragment of the 18S rDNA molecule was observed in *T. decipiens*, *T. crassus*, *M. darwini*, *M.*

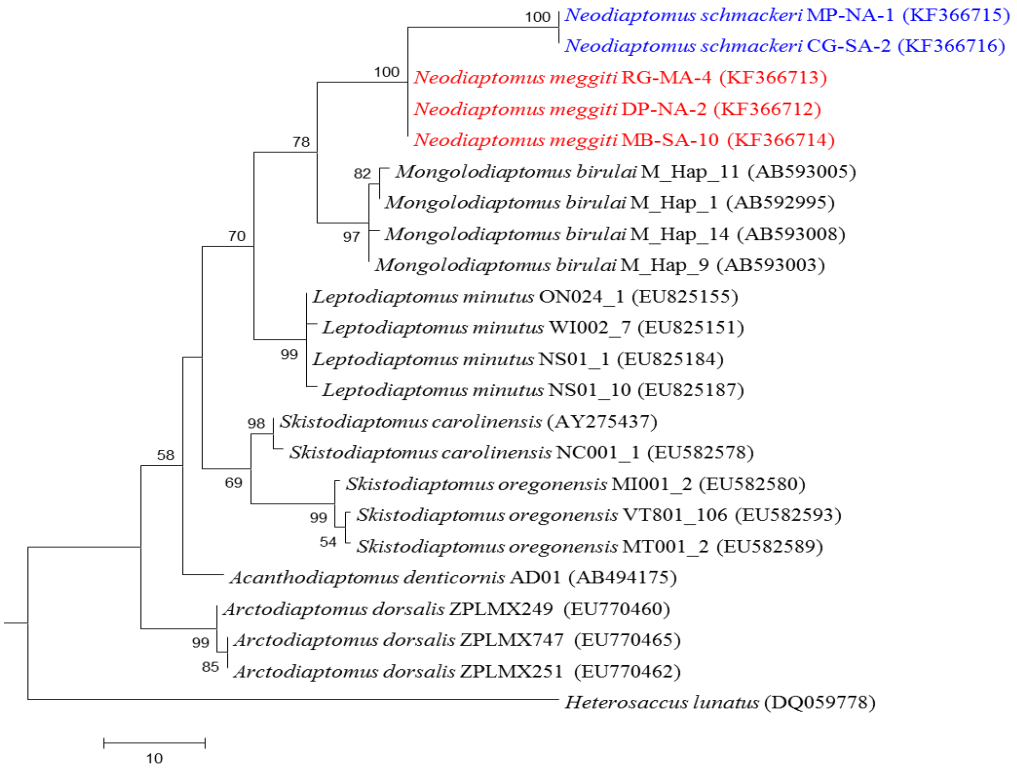

Figure 10. Maximum-parsimony phylogenetic tree based on *CO*I gene sequences comparison, showing the phylogenetic position of *Neodiaptomus meggiti*and *Neodiaptomus schmackeri* haplotypes among related genera and species of the family *Diaptomidae*. *Heterosaccuslunatus* (DQ059778) was used as an out group. Bootstrap values greater than 50 % are shown at branch points. Bar 10% nucleotide sequence divergence.

edax, *M. logngicetuscurvates*, *M. meridianus M. aspericornis*, *M. leuckarti*, *M. major*, *M. pehpeiensis* and *M. ogunnus*. Nevertheless, more than one aspects of the organism must be considered in order to make a reasonable conclusion about the mtCOI similarity and divergence among *N. meggiti*. In order to make a more accurate conclusion several alternative molecular techniques such as DNA sequences of nuclear introns, internal transcribed spacer 2 or large subunit ribosomal DNA (ITS2- 28S) may be combined to resolve diaptomid phylogenies at the species level as suggested by Hirai, Shimode, and Atsushi (2013).

Conclusion

The mtDNA COI sequence studies of *N. schmackeri* and *N. maggiti* suggested that these species are as distinct species of the genera *Neodiaptomus* and closest match to *Mongolodiaptomus birulai*. The distribution of *N. schmackeri* and *N. maggiti* in the main land and Andaman and Nicobar Islands of India showed variation. The former species is common in the main land while latter species is widely distributed in the Andaman and Nicobar Islands. Present study did not show genetic variation in the populations of *N. maggiti* from north, middle and south Andaman and Nicobar Islands that are separated by physical barriers.

Acknowledgements

The authors would like to thank reviewers for their valuable contribution to ameliorate the English language of this manuscript.

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