Phylogeography and genetic diversity of the copepod family Cyclopidae (Crustacea: Cyclopoida) from freshwater ecosystems of Southeast Nigeria

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Abstract

Background: Copepods are key components of aquatic ecosystems and can help regulate the global carbon cycle. Much attention has been paid to the species diversity of copepods worldwide, but the phylogeography and genetic diversity of copepods in Nigeria is unexplored.

Results: Using a mitochondrial cytochrome c oxidase subunit I marker, we performed phylogenetic and phylogeographic analyses for Cyclopidae copepods in Southeast Nigeria. A high species diversity of Cyclopidae in Nigeria: 5 species of Tropocyclops, 5 species of Mesocyclops and 2 species of Thermocyclops from Cyclopidae were identified in 15 populations. Moreover, we detected 18 unique haplotypes, which fell into two distinct clades. Pairwise genetic distances (uncorrected p-distances) among the species of Cyclopidae ranged from 0.05 to 0.257. Several species co-existed in the same lake, and some haplotypes were shared among different geographic populations, suggesting a dispersal of Cyclopidae in our sampling region. Finally, we found that the population genetic diversity for each species of Cyclopidae was low in Nigeria.

Conclusions: Our findings explored the species diversity and distribution of copepods within the family Cyclopidae for 15 Nigerian freshwater ecosystems: a high species diversity of Cyclopidae copepods was detected over a small geographic sampling range. Results from this study contribute to a better understanding of copepod diversity of Nigerian freshwater ecosystems.

Keywords: Cyclopidae, Species diversity, COI, Nigeria

Background

Copepods are one of the most taxonomically diverse groups of crustaceans, containing approximately 14,000 described species globally [1]. Copepods can be found in most kinds of aquatic habitats because of their remarkable evolutionary adaptability [1, 2]. They are key components in aquatic ecosystems, playing an important role in food webs [3, 4] and living as endo- or ectoparasites associated with aquatic animals [2, 5, 6]. Many previous studies have shown that copepods are sensitive to climate change [7, 8], because the range of copepods could track the rate of climate change [7]. Copepods can also help regulate the global carbon cycle [9, 10], and they can be used as indicators to natural and anthropogenic environmental stressors by tracing their responses to the elevation of atmospheric...
CO₂ levels [11]. Thus, much attention has been paid to the bio-diversity of copepods in aquatic ecosystems [12, 13].

Copepods are the intermediate hosts of the parasitic nematode Dracunculus medinensis, which causes a serious Guinea-worm disease in Nigeria and elsewhere [14]. Humans could become infected by drinking unfiltered water containing copepods which are infected with larvae of *D. medinensis*. Therefore, most studies on copepods from Nigeria have focused on their role in the dispersal of the pathogen [15, 16]. Only a few regional biogeographic studies have been conducted on copepods based on morphological species identification [17]. For example, based on the morphology, a previous study showed the occurrence of the genera *Mesocylops* Sars, 1914 and *Thermocyclops* Kiefer, 1927 in Nigerian freshwater ecosystems: six *Mesocylops* species and three *Thermocyclops* species were identified [18]. Moreover, it was believed that *M. aspericornis* was one of the most abundant species of *Mesocylops* in Nigerian waterbodies, and *T. decipiens* was the most abundant species of *Thermocyclops* from Nigeria [18]. However, the identification of different species of copepods solely based on morphology has technical limitations [19], as cryptic species are often detected. Therefore, more discerning methods such as DNA barcoding are needed to investigate copepod taxonomy, especially to recognize morphologically cryptic genetic lineages [20].

DNA barcoding has already been successfully applied to estimate the species/genetic diversity in many zooplankton taxa [21], as it can be used for rapid, accurate, reliable and remote identification of specimens of all metazoan [22]. A fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene has proved to be a useful marker for many biodiversity studies [23, 24], as COI has advantages of being effective for species identification from a wider range of metazoan phyla and possessing a phylogenetic signal which can be used over a wider range of taxonomic levels [22]. The COI marker has been successfully applied to species identification for cladocerans [25, 26]. For example, DNA barcoding was used to identify sibling cryptic species of the *Ceriodaphnia cornuta* species complex from Australia [27] and to examine 61 species of Cladocera, such as *Daphnia*, *Diaphanosoma*, *Ceriodaphnia*, *Moina* and *Alona*, from Mexico and Guatemala [28]. This approach has also been applied to copepods [29]. For instance, a study reported 800 new sequences of 63 marine copepod species by using a COI marker [30]. Elías-Gutiérrez et al. [28] examined 21 species of Copepoda from Mexico and Guatemala by applying a COI marker. Using COI is highly advantageous because it can also detect cryptic species, a phenomenon that is very common in copepod assemblages [31]. For example, three genetically divergent but morphologically similar forms of *Hemidiaptomus* (*Occidodiaptomus*) *ingens* were detected throughout the distribution range of this species complex [32]. Moreover, *Oithona similis* s.l. was found to be a complex of nine cryptic species instead of a single cosmopolitan species, according to a COI and a nuclear ribosomal 28S genetic marker [20]. Similarly, the nominal species “*Eudiaptomus hadzici*” in the Western Balkans consists of four cryptic species according to a mitochondrial (COI) and a nuclear (nH3) marker [33]. DNA barcoding often reveals differences between allopatric populations. In that situation, it is difficult to decide whether this indicates different genetic lineages or simply geographically intraspecific variation. For instance, several different genetic lineages of *Moina* which were allopatric in a phylogeny were assigned to a single species, because they had similar morphology [34].

The phylogeny of copepods had been widely studied using molecular data. Recently, a comprehensive study from Asia showed a high species diversity of copepods in South Korea [29]. In that study, 133 sequenced individuals represented 94 species belonging to six different orders [29]. Another study has shown that *Sinocalanus tenellus* consists of two very distinct clades in China, suggesting they are parts of a complex of cryptic species [35]. Moreover, Karanovic [36] detected a new species of *Schizopera* from Japan, which was the first member of its genus reported in Japanese freshwater ecosystems, and it had no close relatives from elsewhere in the world. Another study has revised the higher systematics of copepods and proposed the new taxa Canuelloida *ordo* nov., Smirnoviinidae *fam. nov.* and Cyclopicinidae *fam. nov.* [37]. Use of molecular data has not been restricted to species-level taxonomy [20], for example, the phylogeography of copepods has been also frequently investigated. They focused on the genealogical lineages of closely related species of copepods and their geographic distributions, by combining the information from phylogenetics, molecular genetics, population genetics, geology, paleontology, demography, ethnology and historical biogeography [38]. For instance, two species of copepods (i.e. *Neodiaptomus schmackeri* and *Mongolodiaptomus birulai*) occur in Chinese Taiwan: there was little gene flow among populations for both species [39]. Additionally, four populations of *Leptodiaptomus cf. sicilis* in Mexico were found to diverge into 3 distinct phenotypes, and their specialization was further supported by molecular data which showed persistence of a founder effect, limited gene flow, and a pattern of allopatric speciation [40].

There have been no studies on phylogeography and genetic diversity of copepods from Nigeria. In this study, we analyzed 15 copepod populations (out of 32 pools/lakes sampled) from Nigeria. By analyzing sequence variation in the COI gene, we aimed to explore the species diversity and distribution of copepods among these populations. Our expectation was to detect several members
of the Cyclopidae, as it is commonly observed worldwide [29, 37]. We also investigated the phylogeography of Cyclopidae in Nigeria.

Results
Species and COI genetic diversity
One to 9 specimens of Cyclopidae were sequenced per location, and a total of 88 Cyclopidae COI sequences were successfully obtained from 15 freshwater lakes around Southeast Nigeria, of which 18 unique haplotypes were detected (Tables 1 and 2). None of the COI sequences exhibited characteristics of nuclear pseudogenes (frame shifts or premature stop codons). Two independent species-delimitation methods (i.e. GMYC and bPTP) based on the COI Bayesian tree consistently identified 12 Cyclopidae species from Nigeria: 5 species of *Tropocyclops* (i.e. *T. decipiens*, *T. prasinus*, *T. prasinus shagamiensis* and *T. cf. mellanbyi*), 5 species of *Mesocyclops* (i.e. *M. cf. aspericornis*, *M. cf. dussarti*, *M. cf. ogunnus*, *M. cf. aequatorialis similis* and *M. cf. salinus*) and 2 species of *Thermocyclops* (i.e. *T. decipiens* and *T. cf. crassus*; Figs. 1 and 2). Species identified through molecular analyses fell into 2 distinct clades (i.e. clade I: *T. cf. confinis*, *T. cf. onabamiroi*, *T. cf. prasinus*, *T. cf. prasinus shagamiensis* and *T. cf. mellanbyi*; clade II: *M. cf. aspericornis*, *M. cf. dussarti*, *M. cf. ogunnus*, *M. cf. aequatorialis similis*, *M. cf. salinus*, *T. decipiens* and *T. cf. crassus*). Two *Thermocyclops* species were in the same clade as the *Mesocyclops* species. Pairwise genetic distances (uncorrected *p*-distances) based on COI sequence analysis ranged from 0.05 to 0.257 between species (Table 3). For each species, the population haplotype diversity (Hd) of COI ranged from 0 to 0.533, and the population nucleotide diversity (π) ranged from 0 to 6.86 × 10⁻³ (Table 2).

Geographic distribution of species
Based on the haplotype network, seven out of 12 species detected through analysis of the COI gene occurred at more than one locality in Nigeria (Fig. 1b). The most frequently occurring species in this study was *T. cf. prasinus*, which had 4 haplotypes and was distributed in 4 lakes, including A5G, AOR, O3M and U1H, and one of the 4 haplotypes was shared by 3 lakes (A5G, AOR, O3M). Such a pattern was also observed in species *M. cf. dussarti*, which had 2 haplotypes and one of them was shared by 3 lakes (N1O, N2O and UBS) (Fig. 1b). Different Cyclopidae species co-existed in the same lake. For example, three species (i.e. *T. cf. confinis*, *T. cf. prasinus* and *T. cf. crassus*) co-existed in Lake A5G (Fig. 1b). Similarly, *T. cf. onabamiroi*, *M. cf. aequatorialis similis* and *M. cf. salinus* co-existed in Lake UII (Fig. 1b). Moreover, five out of 18 haplotypes were shared by different lakes (Fig. 1b). The most abundant haplotype was CTH1, including 21 specimens shared by A1G, A2G and IHE. This was followed by CMS1, shared by N1O, N2O and UBS, and CTR1, shared by A5G, AOR and O3M (Fig. 1b). Four species (i.e. *T. cf. onabamiroi*, *T. cf. mellanbyi*, *M. cf. ogunnus* and *M. cf. aequatorialis similis*) expressed only one haplotype with a single individual (Fig. 1b).

| Lake (abbreviation) | Latitude | Longitude | Sampling time | Water surface temperature (°C) |
|---------------------|----------|-----------|---------------|------------------------------|
| Agu Ekwegbe Pool 1 (A1G) | 6.70 °N | 7.52 °E | August, 2018 | 30.3 |
| Agu Ekwegbe Pool 2 (A2G) | 6.71 °N | 7.51 °E | August, 2018 | 30.3 |
| Agu Ekwegbe Pool 5 (A5G) | 6.73 °N | 7.50 °E | August, 2018 | 30.7 |
| Adann Opona Rd. Pool 1 (AOR) | 6.75 °N | 7.02 °E | September, 2018 | 29.2 |
| Ihe (IHE) | 6.84 °N | 7.40 °E | August, 2018 | 29.7 |
| Nome 1 (N1O) | 6.80 °N | 7.41 °E | August, 2018 | 24.7 |
| Nome 2 (N2O) | 6.79 °N | 7.42 °E | August, 2018 | 23.9 |
| Nike Lake (NKL) | 6.51 °N | 7.51 °E | August, 2018 | 29.4 |
| Omasi Pool 1 (O1M) | 6.69 °N | 6.99 °E | September, 2018 | 30.6 |
| Omasi Pool 3 (O3M) | 6.70 °N | 6.98 °E | September, 2018 | 29.8 |
| Ogele Ube Lake Opi (OUL) | 6.76 °N | 7.49 °E | August, 2018 | 31.1 |
| Uhele Pool Opi (U1H) | 6.75 °N | 7.48 °E | August, 2018 | 29.6 |
| Ukwuado Pool 2 Opi (U2P) | 6.74 °N | 7.49 °E | August, 2018 | 30.3 |
| Ukwuado Bus Stop Opi Lake (UBS) | 6.75 °N | 7.49 °E | August, 2018 | 31.7 |
| Ushuiyi Isusu Ishandiagu (UII) | 6.82 °N | 7.58 °E | August, 2018 | 25.9 |
Discussion

Through analysis of COI sequence variation, we explored the species diversity and distribution of copepods within the family Cyclopidae for 15 Nigerian freshwater ecosystems, the first such study for West Africa. Our results suggested a high species diversity of Cyclopidae copepods over a small geographic sampling range.

High species diversity has already been reported in the copepods from Nigeria [18, 19]. Forty species of Cyclopidae copepods from Nigeria were described based on morphological characteristics in the 1990s [19]. Here, we did not detect any new species based on molecular data; all the 12 species identified in the present study were described in [19]. In agreement with a previous study based on morphology [18], we found that *T. decipiens* was the most abundant species of *Thermocyclops* from Nigeria. However, *M. aspericornis* was recorded as the most abundant species of *Mesocyclops* in Nigerian waterbodies [18], whereas we found that *M. cf. dussarti* is the most abundant species of the genus *Mesocyclops*. This inconsistency might be explained by the relatively small sampling region in our study in Southeast Nigeria.

Globally, high levels of species diversity of copepods have also been reported [29, 41]. For example, 53 *Cali- gus* species were present in Chinese Taiwan, and many more species remained to be discovered from this region [5]. Similarly, thirteen species of Copepoda, including three members of Calanoida (Diaptomidae) and ten members of Cyclopoida (Eucyclopinae and Cyclopinae), were recorded in Chiapas, Mexico [42]. Indeed, high species diversity, even in a relatively small area, has often been observed in copepods [41, 43, 44]. For example, a study identified 43 species that belonged to 11 genera of copepods in Sagami Bay [43]. Another study identified 48 species of copepods in Tolo Harbour, Hong Kong, and *Oithona rigida*, *O. simplex* and *Paracalanus crassirostris* were found to be the most abundant species [44].

Here, we detected 12 species with several species and

| Lake (abbreviation) | Mitochondrial gene (COI) | N₁ | N₂ | Haplotype | Hₜ | stdev of Hₜ | π | stdev of π | Species                        |
|---------------------|--------------------------|----|----|-----------|----|-------------|---|-----------|--------------------------------|
| Agu Ekwegbe Pool 1 (A1G) | 9 1 CTH1               | 0.00 | 0.00 | n.s       | n.s | n.s        | n.s | n.s       | *Thermocyclops decipiens*     |
| Agu Ekwegbe Pool 2 (A2G) | 6 1 CTH1               | 0.00 | 0.00 | n.s       | n.s | n.s        | n.s | n.s       | *Thermocyclops decipiens*     |
| Agu Ekwegbe Pool 5 (A5G) | 1 1 A5G1               | n.s | n.s | n.s       | n.s | n.s        | n.s | n.s       | *Tropocyclops cf. confinis*   |
|                      | 3 1 CTH2               | 0.00 | 0.00 | n.s       | n.s | n.s        | n.s | n.s       | *Thermocyclops cf. crassus*   |
|                      | 1 1 CTR1               | n.s | n.s | n.s       | n.s | n.s        | n.s | n.s       | *Tropocyclops cf. prasinus*   |
| Adanni Opar Rd. Pool 1 (AOR) | 7 1 CTR1             | 0.00 | 0.00 | n.s       | n.s | n.s        | n.s | n.s       | *Tropocyclops cf. prasinus*   |
| Ihe (IHE)          | 6 1 CTH1               | 0.00 | 0.00 | n.s       | n.s | n.s        | n.s | n.s       | *Thermocyclops decipiens*     |
| Nome 1 (N1O)       | 8 2 N1O1, CMS1         | 0.429 | 0.169 | 7.9 × 10⁻⁴ | 3.1 × 10⁻⁴ | Me *socyclops cf. dussarti* |
| Nome 2 (N2O)       | 7 1 CMS1               | 0.00 | 0.00 | n.s       | n.s | n.s        | n.s | n.s       | *Me *socyclops cf. dussarti* |
| Nike Lake (NKL)    | 1 1 NKL1               | n.s | n.s | n.s       | n.s | n.s        | n.s | n.s       | *Me *socyclops cf. ogunnus*   |
|                      | 1 1 NKL2               | n.s | n.s | n.s       | n.s | n.s        | n.s | n.s       | *Tropocyclops cf. mellanbys*  |
| Omasi Pool 1 (O1M) | 1 1 CTH2               | n.s | n.s | n.s       | n.s | n.s        | n.s | n.s       | *Thermocyclops cf. crassus*   |
| Omasi Pool 3 (O3M) | 6 2 CTR1, CMS1         | 0.533 | 0.172 | 9.8 × 10⁻⁴ | 3.2 × 10⁻⁴ | Me *socyclops cf. prasinus* |
| Ogele Ube Lake Opi (OUL) | 6 1 OUL1            | 0.00 | 0.00 | n.s       | n.s | n.s        | n.s | n.s       | *Me *socyclops cf. aspericornis* |
|                      | 1 1 OUL2               | n.s | n.s | n.s       | n.s | n.s        | n.s | n.s       | *Me *socyclops cf. salinus*   |
| Uhele Pool Opi (U1H) | 6 2 U1H1, U1H2       | 0.533 | 0.172 | 6.86 × 10⁻³ | 2.21 × 10⁻³ | Tropocyclops cf. prasinus |
|                      | 2 1 CTR2               | 0.00 | 0.00 | n.s       | n.s | n.s        | n.s | n.s       | Tropocyclops cf. prasinus shagamienis |
| Ukwuado Pool 2 Opi (U2P) | 1 1 CTR2            | n.s | n.s | n.s       | n.s | n.s        | n.s | n.s       | Tropocyclops cf. prasinus shagamienis |
|                      | 1 1 U2P1               | n.s | n.s | n.s       | n.s | n.s        | n.s | n.s       | *Tropocyclops cf. confinis*   |
| Ukwuado Bus Stop Opi Lake (UBS) | 8 1 CMS1         | 0.00 | 0.00 | n.s       | n.s | n.s        | n.s | n.s       | *Me *socyclops cf. dussarti* |
| Ushuwi Isusu Ihandiagu (UII) | 3 1 UI1              | n.s | n.s | n.s       | n.s | n.s        | n.s | n.s       | *Me *socyclops cf. aequatorialis similis* |
|                      | 3 1 UI2               | n.s | n.s | n.s       | n.s | n.s        | n.s | n.s       | *Me *socyclops cf. salinus*   |
|                      | 1 1 UI3               | n.s | n.s | n.s       | n.s | n.s        | n.s | n.s       | *Tropocyclops cf. onabamire*  |

*N₁* is the number of individuals used for COI sequencing, *N₂* is the number of haplotypes, *Hₜ* is haplotype diversity, stdev of *Hₜ* is standard deviation of haplotype diversity, *π* is nucleotide diversity, stdev of *π* is standard deviation of nucleotide diversity.
Fig. 1 (See legend on next page.)
species complexes across three genera in Nigeria; suggesting a high species diversity of Cyclopidae in South-East Nigeria.

In agreement with a previous study of Cyclopoida in Nigeria [18], our results showed that the same species could be found in geographically separate populations, which also suggests that there are not extensive and common cryptic species in these sampled lakes. *Thermocyclops decipiens* has also been detected in Antilles, Central America, Columbia, Venezuela, east of the Andes, Brazil [45] and Congo [46], indicating that this species has a wide distribution across continents. In contrast to several copepod species with high genetic divergence over short distances, e.g. *Tigriopus californicus* [47], our data showed genetic similarity of the *T. decipiens* populations from different continents. A similar phenomenon has been detected in some open-ocean copepods which have more obvious dispersal routes. For example, it was found that several mtCOI haplotypes of *Calanus pacificus* were distributed across multiple sampling location from the North Pacific Ocean [48]. By using restriction site-associated DNA sequencing, no significant genetic differentiation was found among *Centropages typicus* samples collected from different NW Atlantic regions with clear connectivity [49]. Zooplankton species often have vast ranges [34, 50]. For example, *Daphnia galeata* has been detected in both China and Europe with some haplotypes shared across large distances [51]. Birds are often regarded as the key vectors for the dispersal of resting eggs of aquatic zooplankton [52], across geographical barriers.

We found that different sibling species of Cyclopidae co-existed in the same Nigerian lake, a common finding in copepods [29]. For example, a study of the genus *Mesocyclops* conducted in Africa reported that *M. major* and *M. ogunnus* often co-existed in the same waterbody [53]. Similarly, another study from Nigeria reported that

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**Fig. 1** a. Geographic locations of sampling sites for Cyclopidae in Nigeria. b. Haplotype network of Cyclopidae from Nigeria, based on the COI gene (544 bp). Each circle represents a unique haplotype and its size reflects the number of individuals expressing that haplotype. Color codes denote geographic location of populations. Portion of circles indicate distribution of haplotypes among different populations. The number of marks on connecting lines indicates the number of mutations between haplotypes. For lake abbreviations see Table 1. The map was obtained from ArcGIS and edited in Adobe Illustrator.

**Fig. 2** Bayesian phylogenetic tree and species delimitation results for Cyclopidae from Nigeria, based on the COI gene (547 bp). The IDs for shared haplotypes are provided in Table 2; for origin of reference sequence IDs see Table S1. Only posterior probabilities > 0.70 are shown. Species delimitation according to the GMYC and bPTP methods are indicated. For the bPTP method, the statistical support (PP) for species membership is also shown. *Paracalanus parvus* was used as an outgroup.
Table 3 Uncorrected pairwise genetic distances (p-distances) among species of Cyclopidae copepods based on mitochondrial COI sequence analysis. The number in bracket denote the sample size of each species.

|                | Tropocyclops cf. confinis | T. cf. onabamiroi | T. cf. prasinus | T. cf. prasinus shagamiensis | T. cf. melinbyi | Mesocyclops cf. aspericornis | M. cf. dussarti | M. cf. ogunnus | M. cf. aequatorialis similis | M. cf. salinus | Thermocyclops decipiens | T. cf. cressus |
|----------------|---------------------------|-------------------|---------------|----------------------------|---------------|------------------------------|----------------|--------------|----------------------------|-------------|--------------------------|---------------|
| T. cf. confinis (2) |                           |                   |               |                            |               |                              |                |              |                            |             |                          |                |
| T. cf. onabamiroi (1) | 0.209                     |                   |               |                            |               |                              |                |              |                            |             |                          |                |
| T. cf. prasinus (20) | 0.224                     | 0.229             |               |                            |               |                              |                |              |                            |             |                          |                |
| T. cf. prasinus shagamiensis (3) | 0.243                   | 0.230             | 0.050         |                            |               |                              |                |              |                            |             |                          |                |
| T. cf. melinbyi (1) | 0.188                     | 0.210             | 0.193         | 0.199                      |               |                              |                |              |                            |             |                          |                |
| M. cf. aspericornis (6) | 0.211                    | 0.254             | 0.242         | 0.243                      | 0.219         |                              |                |              |                            |             |                          |                |
| M. cf. dussarti (23) | 0.205                     | 0.255             | 0.241         | 0.250                      | 0.205         | 0.161                        |                |              |                            |             |                          |                |
| M. cf. ogunnus (1)  | 0.209                     | 0.237             | 0.255         | 0.254                      | 0.202         | 0.173                        | 0.152          |              |                            |             |                          |                |
| M. cf. aequatorialis similis (1) | 0.195                   | 0.257             | 0.255         | 0.254                      | 0.204         | 0.143                        | 0.153          | 0.147        |                            |             |                          |                |
| M. cf. salinus (4)  | 0.210                     | 0.244             | 0.249         | 0.247                      | 0.220         | 0.161                        | 0.154          | 0.160        | 0.142                      |             |                          |                |
| T. decipiens (21)  | 0.199                     | 0.248             | 0.233         | 0.248                      | 0.206         | 0.165                        | 0.158          | 0.173        | 0.149                      | 0.170       |                          |                |
| T. cf. cressus (5)  | 0.201                     | 0.254             | 0.245         | 0.250                      | 0.184         | 0.176                        | 0.158          | 0.142        | 0.131                      | 0.153       | 0.125                    |                |
it was common for up to 3 Mesocyclops species co-
exists in a single locality [18]. Sympatry provides a pos-
sibility for interspecific hybridization, which is believed
to be a common phenomenon in zooplankton [54]. Hy-
bridization has often been observed in copepods. For
example, hybrids between Calanus glacialis and C. fin-
marchicus were detected along the Atlantic and Arctic
Canadian coast [55]. Another study also found that
hybridization occurred between a female Neocalanus cris-
tatus and a male N. plumchrus, and was then followed by
backcrossing to a N. plumchrus individual [56]. Our mo-
lecular data indicated paraphyly between Thermocyclops
and Mesocyclops, which might reflect introgression result-
ing from hybridization [57]. Paraphyly has also been ob-
served in other zooplankton, for example, in the Daphnia
pulex species complex [58] and Moina [34]. Future studies
and nuclear markers are needed to investigate gene intro-
gression among the copepod species from Nigeria.

Here, Cyclopidae in Nigeria showed a high species di-
versity, but for each species, the haplotype diversity and
nucleotide diversity were rather low. Consistently, low
haplotype and nucleotide diversities were observed in the
copepods Calanus finmarchicus [59], C. agulhensis and C.
sinicus [60]. Patterns of population genetic diversity could
be caused by different evolutionary forces, such as muta-
tion, migration, genetic drift and natural selection. How
these evolutionary forces affect the population genetic di-
versity depends on many factors, including species’ re-
ponse to environmental changes, and the past and pre-
sent sizes of the population [59]. Another explanation
might be that the structuring of a metapopulation to-
gether with founder effects resulted in low population
genetic diversity [61, 62]. However, the limited sampling
of each species among populations cannot be ruled out as
a cause for their low genetic diversity in this study.

Conclusions
In conclusion, our data revealed a high species diversity
of Cyclopidae in Southeast Nigeria: twelve species were
detected. Our geographical sampling scale in this study
was quite small, and therefore, further studies are called
for a comprehensive understanding of species distrib-
tion and genetic diversity of Cyclopidae in West Africa.

Methods
Sampling
Copepod specimens were collected from 15 freshwater
lakes around Southeast Nigeria (Fig. 1a and Table 1).
Samples were collected using a 125-μl plankton net
hauled vertically through the water column at three
different sites per location. Samples collected from
different sites in the same location were pooled to-
gether and preserved in 95% ethanol. All specimens
were identified morphologically according to the mor-
phological description of copepods in Nigeria [17–19,
63], which also worked as taxonomic keys in this paper.

DNA extraction, PCR amplification and sequencing
Copepods were processed for molecular analyses (Table 2).
The cephalosome portion of the prosome was ob-
tained from each individual copepod to avoid DNA con-
tamination from prey items in the gut, using a microscop-
ic tweezer and a sharp blade under the stereo-
scope. Total genomic DNA was extracted from the
head using H3 buffer with proteinase K (30 μL), contain-
ing 10 mM Tris-HCl, 0.05 M KCl, 0.005% Tween 20,
0.005% NP-40 and 10 mg/ml proteinase K (MERCK,
Germany). Samples were incubated overnight at 55 °C in
a water-bath with mild shaking. The proteinase K was ir-
reversibly denatured after a 12-min incubation at 95 °C.
The homogenate was centrifuged briefly and stored at
4 °C before use. A 680 base-pair fragment of the COI
gene was amplified using the consensus primer pair (for-
ward: 5’- GGT CAA CAA ATC ATA AAG ATA TTG
G-3’; reverse: 5’- TAA ACT TCA GGG TGA CCA
AAA AAT CA -3’ [64];). Polymerase chain reaction
(PCR) was carried out in a total volume of 20 μL, con-
sisting of 2 μL 10 X PCR buffer (10 mM Tris-HCl, pH
8.3, 5 mM MgCl2, 50 mM KCl), 2 μL 2.5 mM of each
dNTP, 1 μL 0.5 μM of each primer, 11.6 μL water, 2 units
of Taq DNA polymerase (SuperTherm DNA polymerase,
Taq HS from TAKARA BIO INC., California, USA) and
2 μL of genomic DNA. The PCR thermocycle protocol
was as follows: denaturation at 94 °C for 1 min, then 40
cycles of 1 min at 94 °C, 1.5 min at 40 °C and 1.5 min at
72 °C; followed by a final elongation at 72 °C for 6 min.
The success of amplification was checked using agarose
gel electrophoresis. Afterwards, the PCR products were
purified (High Pure PCR Product Purification Kit, Roche
Diagnostics) and sequenced in the forward direction on
an ABI PRISM 3730 DNA capillary sequencer by Invi-
trogen Trading Co., Ltd. (China). Chromatograms were
checked for ambiguous base calling and errors in base
calling were corrected using MEGA 6 [65], and the
Phred quality scores of the sequences were examined
with Chromas Lite Version 2.1 (Technelysium Pty. Ltd.,
South Brisbane, Australia). Sequences with double peaks
or noise were re-sequenced in the reverse direction, and
only chromatograms of high quality were applied to the
following genetic analyses. All newly obtained sequences
were submitted to GenBank under accession numbers
MN990471-MN990488.

Sequence alignment and genetic diversity
We identified unique haplotypes in DnaSP 5.10 [66].
MUSCLE [67] implemented in MEGA 6 was used to
align the sequences that were subsequently translated into amino acids to examine the presence of stop codons. Afterwards, all haplotypes were aligned, together with 8 reference sequences obtained from GenBank (Table S1), using the Clustal W algorithm [68] in MEGA 6. Then, all the sequences were timed to a uniform length of 544 bp in MEGA 6. For each species, the number of haplotypes (N2), haplotype diversity (Hd) and nucleotide diversity (n) per population (populations with sample size less than 3 were excluded) were calculated in DnaSP 5.10.

**Phylogenetic analyses**
The test of Xia et al. [69] implemented in DAMBE 5 [70] was used to inspect potential loss of phylogenetic signal resulting from substitution saturation at the COI locus. A phylogenetic tree was then constructed using the Bayesian method in BEAST 2 [71], with a tree sampled every 1000 generations among 10,000, a burn-in of 25%, and the final 10,000 trees summarized using TreeAnnotator. The best-fitting substitution model was GTR + G + I according to the corrected Akaike Information Criterion in jModeltest v. 2.1.7 [72]. We applied a strict molecular clock and set other tree prior to their default values. Tracer v1.6 [73] was applied to ensure that enough generations were computed. *Paracalanus parvus*, a member of the Calanoidea phylogenetically close to Cyclopoida, was used as an outgroup.

**Species identification and phylogeographic analyses**
To test the hypothesis that the Cyclopidae in Nigeria contains high biodiversity, two independent species delimitation methods were applied: the general mixed Yule coalescent model (GMYC) [74] and Poisson tree processes methods (PTP) [75]). The GMYC model is a likelihood-based method using an ultrametric tree to delimit species by fitting within- and between-species branching models to reconstruct gene trees. We performed the GMYC modeling using the “splits” package [76] in R 2.15 [77] and conducted the PTP calculations on the bPTP webserver (http://species.h-its.org/ptp/), with 100,000 Markov Chain Monte Carlo (MCMC) generations, thinning set to 100, burnin at 25% and a Bayesian search performed. The input phylogenetic tree was generated using BEAST 2 (see above). A network of COI haplotypes was then constructed to visualize genetic relationships among populations using Haploviewer [78]. The maximum likelihood tree inferred with MEGA 6 using the best model GTR + G + I (by jModeltest v. 2.1.7) was applied as input. Uncorrected pairwise genetic distances between species were calculated in MEGA 6 based on COI.

**Supplementary information**
Supplementary information accompanies this paper at https://doi.org/10.1186/s12862-020-01608-5.

**Additional file 1: Table S1.** List of reference COI sequences of Cyclopidae (from South Korea, Brazil and China) and the outgroup used in this study.

**Abbreviations**
COI: Mitochondrial cytochrome c oxidase subunit I gene; PCR: Polymerase chain reaction; bp: Base pairs; GMYC: The general mixed Yule coalescent model; PTP: Poisson tree processes methods

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**Authors’ contributions**
MY designed the study, CE, EO, CO, FA, CN, JO and PU collected samples, YN and JW carried out the molecular work. YN, WH and MY contributed to data analyses. MY wrote the manuscript with the help of YN. All authors read and approved the final version.

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**Availability of data and materials**
All the sequencing data are available via NCBI (under accession numbers MN990471-MN990488).

**Ethics approval and consent to participate**
Collection of zooplankton (copepods in this study) did not require specific permissions because these samples were obtained from unprotected lakes that are open for public activities. Our study did not involve the use or collection of endangered or protected species.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

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