RESEARCH

Morphologic and phylogenetic investigations revealed size-divergent clades in chelae morphotypes of freshwater prawn *Macrobrachium vollenhovenii* Herklots (Decapoda: Palaemonidae) in a lake and river system of Southwest Nigeria

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Abstract

Background The freshwater prawn *Macrobrachium vollenhovenii* is one of the largest *Macrobrachium* species, a biological agent against human schistosomiasis, and a cheap protein source in riverine communities in West Africa. However, its aquaculture development for sustainable utilization is challenged by cryptic identity amidst the presence of morphotypes of unknown size and genetic relatedness. This study aimed to investigate the maximum sizes and evolutionary links of chelae morphotypes in *M. vollenhovenii* for precise identification and utilization in a 3×2 randomized block experimental design. Ninety biggest encountered samples of *M. vollenhovenii* chelae morphotypes—those possessing equal left and right side chelae, longer left chelae, and shorter left chelae—were obtained from fisherfolks' catches at each of Asejire Lake and Ogun River during peak seasons (July–September) bimonthly field survey, representing EAAL, LLAL, SLAL—GAALs, and EAOR, LLOR, SLOR—GAORs. These were analyzed for differences ($p < 0.05$) in size-linked parameters—length (L (cm)), weight (W (g)), and condition factor (K). Specimens' 16S rRNA nucleotide sequences were utilized to infer phylogenetic linkages, single-nucleotide polymorphism (SNP), and amino acid translations alongside NCBI references (NCBIrefseq).

Results Weight (W) and condition factor (K), among GAALs, for SLAL and LLAL were similar; EAAL was significantly lowest; among GAORs, SLOR and LLOR were similar; and EAOR was significantly lowest. In GAALs, EAAL, LLAL, and SLAL had higher L, W, and K than counterpart GAORs. Sequences formed two polyphyletic groups: EAOR branch from EAAL, in which 100.0%EA rooted 75.0% NCBIrefseqs, forming a clade; and GAOR-SLOR and LLOR branch form GAOR-LLAL rooted SLAL, in which 100.0%LL and SL rooted KJ463387.1 (Badagry), forming another clade. SNP Locus 91 separated 100.0%GAOR from 100.0%GAAL and 100.0%NCBIrefseqs translating to valine; SNP Locus 171 separated 100.0%EA and its co-rooted NCBIrefseqs from 100.0%LL, SL, and their co-rooted NCBIrefseq, translating to glycine/glutamic acid change.

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Conclusion The equal left and right side chelae and the unequal left and right side chelae specimens are, respectively, small- and robust-sized, irrespective of habitat. They are divergent size-linked clades having protein translate differences, delineable at 16S rRNA SNP Locus 171; their size variant habitat strains are delineable at SNP Locus 91. These SNP markers will be useful for precision identification and selection of the size variant chelae morph strains for sustainable utilization.

Keywords Molecular taxonomy, Genotypes, 16S rRNA sequence, Freshwater prawn

Background

The prawn, *Macrobrachium* species belonging to the family Palaemonidae are decapod crustaceans of high economic importance worldwide and have been subjected to intense aquacultural practices, especially in Asia and America (Lawal-Are & Owolabi, 2012). *Macrobrachium vollenhovenii* is a freshwater prawn of biological and socioeconomic importance. It is an agent against human schistosomiasis as it feeds on a variety of mollusks that are intermediate hosts of schistosomes, responsible for schistosomiasis in humans (Sokolow et. al., 2013; Swartz et al., 2015; Savaya et al., 2018; Ndao et al., 2019). It serves nutritional purposes in riverine communities in West Africa and its *a*quaculture is developing in Africa, especially in the West African subregion. *Macrobrachium vollenhovenii* is a viable fishery in most West African countries (Konan et al., 2010; Nwosu & Wolfi, 2006), a part of the fauna of benthic and littoral zones of the intertropical zone of West Africa (Zabi & et Le Loeuff, 1992), and one of the largest species of *Macrobrachium* known (Marioghae, 1990).

However, there is a need for scientific clarification on the size and genetic structure of the species for precision in identification and sustainable utilization. Konan et al. (2010) analyzed morphometrics of the *Macrobrachium* species in West African rivers and reported that prawns could be of varied lengths of chelae. Williams (1984) reported that the species of the genus *Macrobrachium* have the second pair of walking legs chelae greatly lengthened, often equaling or exceeding body length. Meanwhile, variations in chelae and limb morphology could constitute taxonomic issues in some crustaceans. Subgenera in some crustaceans can be differentiated based on chelae morphology (Hobbs, 1969) and validated in molecular phylogenetic study (Breinholt et al., 2012). Divergence at 16S rRNA sequence recognized four new species and confirmed the specific and phylogenetic value of divergence in length of mesial spine on the basal antennal segment and length of walking legs in Decapod Raymunida species (Macpherson & Machordom, 2001).

Specimens of *M. vollenhovenii* having equal left and right side chelae and those having unequal length of the left and right side chelae obtained from a lake and a river system in Southwest Nigeria showed divergence in allozyme fingerprints (Oyebola et al., 2017), indicating that the chelae morphologic variants could constitute separate genetic entities. However, allozyme markers are less reliable in taxonomic studies and are increasingly been replaced by DNA-based markers for better efficiency in sustainable identification (Okumus & Ciftci, 2003). It is of interest to assess the 16S rRNA sequence of the observed chelae variants to establish the phylogenetic value of chelae morph divergence. Moreover, the phylogenetic/evolutionary relationship of the chelae morphotypes has not been established.

Interestingly, Jimoh et al., (2013) reported the cryptic genetic structure of *M. vollenhovenii* in Nigeria, after a phylogeography and systematic analysis of mitochondria gene sequences, including that of the 16S rRNA. This further indicates the need for a phylogenetic reconstruction of the species. The sampled *M. macrobrachium* by these authors could contain the chelae variants, which may be the product of evolutionary divergence. There is a need to evaluate the evolutionary link between the chelae subgroups and the earlier study to infer their relationship in this regard. A phylogenetic tree combining specimen nucleotides of the earlier and the current *M. vollenhovenii* samples with cognizance of the chelae subgroups would enhance understanding of their genetic/evolutionary link.

Further to this, sustainable molecular identification and management of the variants could be facilitated by the presence of single-nucleotide polymorphism (SNP) sites, as SNPs are capable of resolving outstanding questions in evolutionary ecology, conservation genetics, and wildlife management (Susan et al., 2013; Seeb et al., 2011; Van Tienderen et al., 2002; Luikart et al., 2003). The availability of sequence data could enable detection of single-nucleotide polymorphisms (SNP) across the variants.

The observed Macrobrachium vollenhovenii chelae variants were those possessing an equal length of chelae of left and right sides—EA; longer left side chelae—LL; and shorter left side chelae—SL. The availability of specimens of variants across locations provides an opportunity to evaluate intraspecific morphological variations and specific characters (Macpherson & Machordom, 2001). The encountered matured specimens of the variants seem to have varied sizes across the sampled habitats. Hence,

it is of interest to characterize these chelae variants by their maxima sizes in these habitats and to establish their phylogenetic relationships using divergence at a mitochondria sequence region. This study therefore aimed to investigate divergence in maximum encountered sizes of the chelae morphotypes of *M. vollenhovenii* obtained from its typical freshwater lake and river locations in Southwestern Nigeria, investigate phylogenetic/evolutionary relationship of the chelae variants using one of the earlier utilized markers, and investigate single-nucleotide polymorphisms (SNP) markers for their sustainable delineation.

Methods

Aim, design, and setting of the study

The study aims to investigate whether size-related parameters will not be significantly different across the encountered biggest size of the chelae morphs in the habitats, and whether the chelae morphs from the habitats will share the same descent and with similar sequences that are available on the gene repository, and whether there will not be any SNP at any locus in the genome of the chelae morphs of the habitats. The study utilized a 3×2 randomized block design, involving three chelae morphs of *M. vollenhovenii* as treatments and two freshwater habitats as the experimental blocks. The study setup included a peak season (July–September) bimonthly field survey conducted to obtain the largest sizes of the three chelae morphs of *M. vollenhovenii* encountered in fisherfolks' catch at the two freshwater habitats. This was followed by data collection and analysis for size and phylogenetic relationship.

Description of materials and processes

Study locations

The study was conducted at the Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria. Sequencing was carried out through Inqaba, International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. *Macrobrachium vollenhovenii* chelae morph variants were obtained from Asejire Lake (AL) and Ogun River (OR) in Southwestern Nigeria, West Africa, the locations being the habitat where the variants have been encountered and reported in a previous study (Oyebola et al., 2017). Asejire Lake is situated on coordinates 07° 21′N and 04° 07′E, at the borderline between Oyo and Osun States, Nigeria. It drains to Lagos Lagoon. Ogun River is on coordinates 06° 44″N and 03° 20′E, located in Oyo town, Oyo State, Nigeria. It runs through Ogun State to drain at Lagos Lagoon. Both locations are within the Ogun/Oshun River basin, the main water basin in Southwestern Nigeria. A hydrological map showing the sampled locations is presented in Fig. 1.

Experimental fish

Experimental fish are the freshwater crustacean *M. vollenhovenii* morphotypes that possessed equal left and right side chelae/arm (EA), longer left side chelae (LL), and shorter left side chelae (SL) which were obtained from each of Asejire Lake (AL) and Ogun River (OR), representing EAAL, SLAL, and LLAL (GAALs) and EAOR, SLOR, and LLOR (GAORs). *Macrobrachium* species live in freshwater and low-salinity waters not exceeding 10 parts per thousand, but their larval stages require brackish water conditions for their survival and optimum development (New & Singholka, 1985; Powell, 1982). Samples of the experimental fish were identified at the species level following Holthuis (1980) as utilized in Jimoh et al. (2013). They were then separated into chelae variants as described in Oyebola et al. (2017). Images of the experimental specimens are presented in Fig. 2.

Description of experimental processes

Determination of biggest sizes of the chelae morphotypes

The obtainable large sizes are important parameters in fisheries management (Moutopoulos & Stergiou, 2002) and aquaculture. Therefore, the largest sized samples of each of the morphs were targeted for sampling. Specimens were visually observed for relatively big sizes in the catch of encountered fisherfolk at the main landing sites of the water bodies, during the 2016 peak seasons' (July–September) bimonthly catch survey. The surveys were carried out during 08.00 and 10.00 and 16.00 and 18.00 h, coinciding with two hours each of the period of fishers return from fishing operation in respective morning and the evenings. An equal number of the visualized biggest specimens of each chelae variant category were collected from each location during each sampling period, transported to the laboratory inside ice crystal, and preserved inside a deep freezer at -20 °C. At the end of the final sampling, preserved specimens were thawed and utilized for data collection. The obtained 90 specimens per morph variant were utilized for data collection. Each specimen was measured in total length/total carapace length (L, cm) and wet weight (W, g). These parameters were measured to the nearest two decimal places using respective vernier caliper (Electronic Digital series DC 8001, USA) and electronic weighing scale (Ohaus explorerplus model EP 613). Fulton's condition factor (K) was derived from the length and weight data to infer relative robustness.
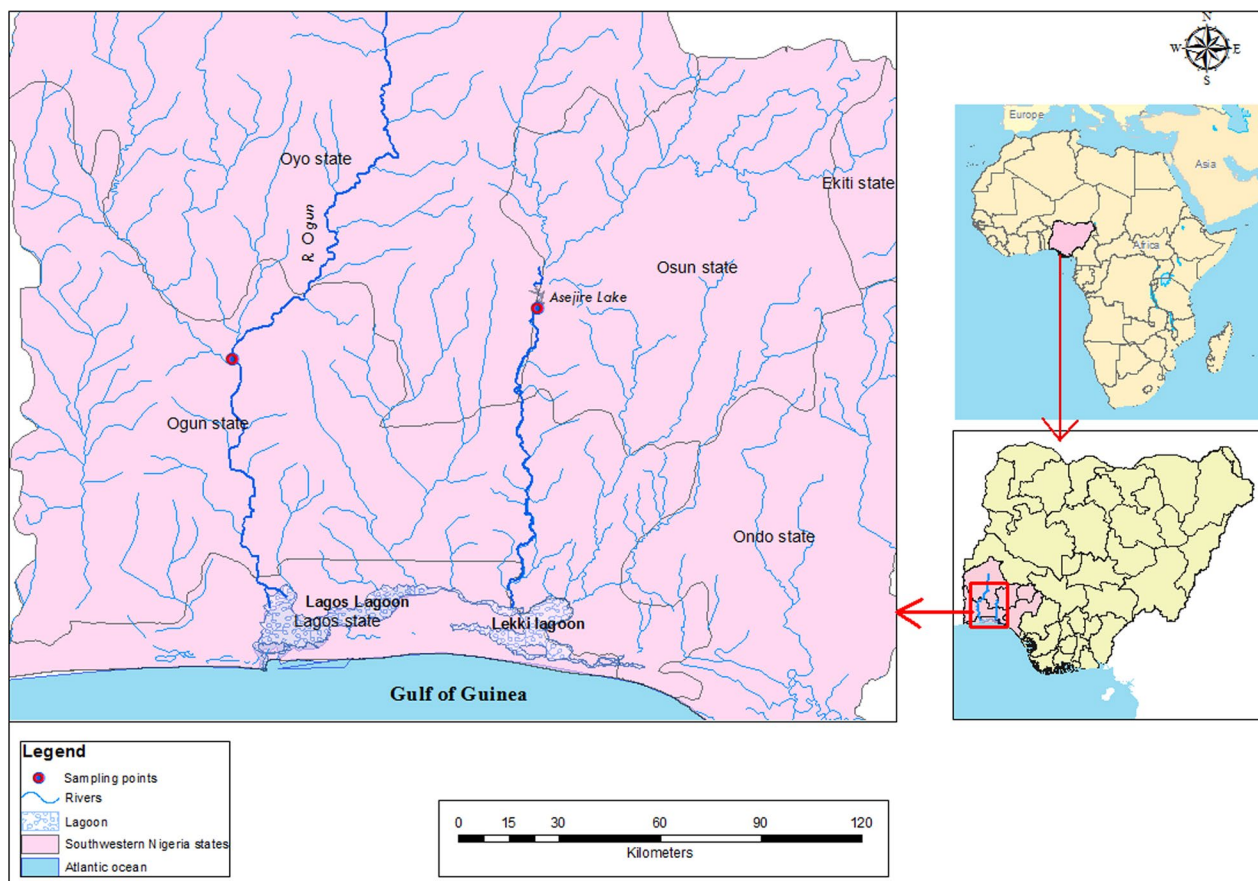
**Fig. 1** Hydrologic Map Showing the Sample Collection Sites (Asejire Lake and Ogun River), Southwest Nigeria

K was estimated following Htun-Han ([1978](#)): W divided by L^3, multiplied by 100 percent, where L = total length and W = wet weight.

Determination of phylogenetic/evolutionary relationships of the chelae morphotypes

Tissue collection, DNA extraction, and sequencing

Two grams of fleshy tissue was obtained from the dissected dorsal region of a randomly selected size-characterized specimen of each variant and extracted of DNA by homogenization and digestion with protease K at 37 °C overnight, followed by standard phenol/chloroform purification procedures (Sambrook et al., [1989](#)). DNA concentration was estimated on a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). The DNA specimens were amplified for the 16S rRNA gene region of the specimens through polymerase chain reaction (PCR). The 16S rRNA region was preferred for the study based on its capacity for phylogenetic mapping for crustaceans (Jose et al., [2016](#); Mantelatto et al., [2014](#)). The fragment was also utilized by Jimoh et al., ([2013](#)). The PCR mix contains 1.0 μl of 10 pmol forward 5′-CCGTGCGAA GGTAGCATAGTCAG-3′ and reverse primers 5′-AAC TCTCAAGGAAAATCACGCTG-3′ (Savaya et. al., [2014](#)), 10.0 μl of 5 × GoTaq colorless reaction buffer, 3.0 μl of 25 mM MgCl2, 1.0 μl of 10 mM dNTP, 0.3 μl of Taq DNA polymerase (Promega, USA), 8 μl of 10 ng/μl DNA template each, made up to 50 μl with sterile distilled water. Thermal cycling conditions include initial denaturation at 94 °C for 5 min and 94 °C for 1 min; 30 s at 35 cycles for final denaturation; annealing at 56 °C for 45 s and extension at 72 °C for 1 min; and final termination at 72 °C for 7 min carried out in GeneAmp 9700 PCR System Thermal cycler (Applied Biosystem Inc., USA). PCR products (4.0 μl) were mixed with 3.0 μl bromophenol blue loading dye, visualized on 1.5% agarose gel at 120 V for 1 h, and selected for direct sequencing. Sequences were determined on an ABI PRISM 310 Genetic Analyzer using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Inc, U.S.A.).
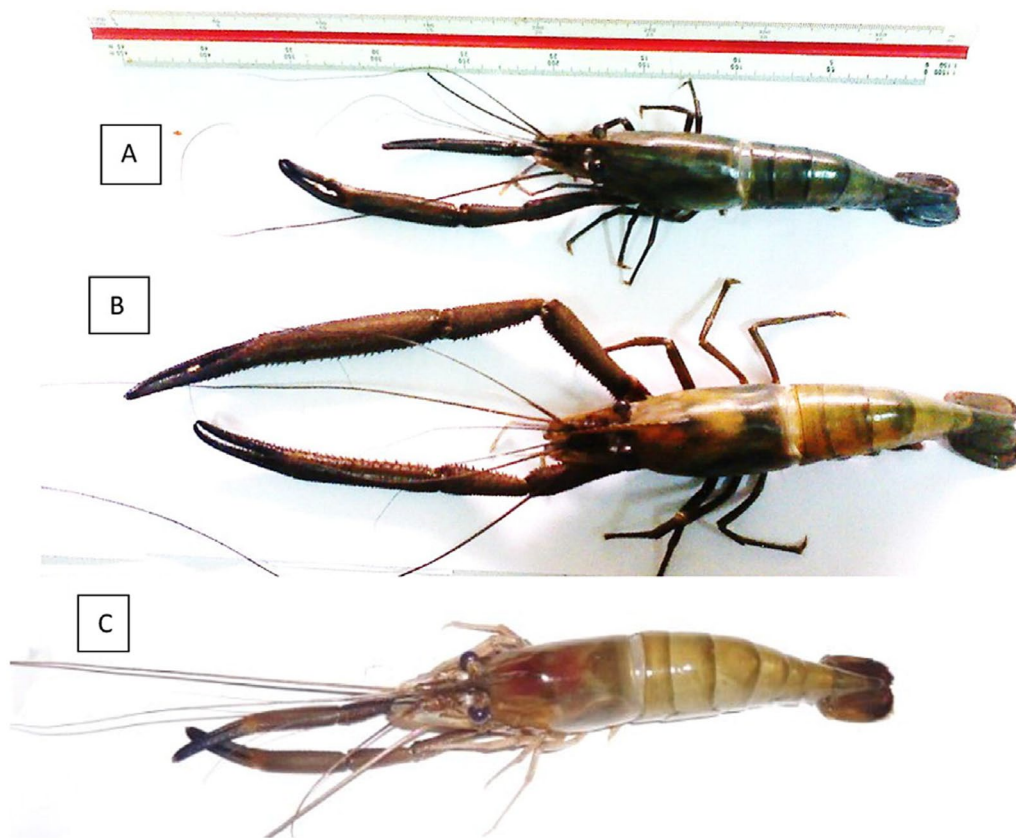
Fig. 2 Chelae Morphotypes of *M. vollenhovenii*. *A—long left (LL), B—short left (SL), C—equal arm (EA) *Source* Oyebola et al., 2017

Statistical analysis

Size data

Data on L, W, and K were presented using descriptive statistics and compared for differences ($p < 0.05$) across variants using ANOVA, carried out on the Paleodontological Statistics (PAST) computer program (Hammer et al., 2005).

Nucleotide similarity with NCBI specimens

Specimens' sequences were edited on BioEdit 7.0.5 (Hall, 2005) and identified by searching the NCBI GenBank database using the BLAST—Basic Local Alignment Search Tool—identification engine. Similarity matches of *M. vollenhovenii* samples with the corresponding NCBI reference specimens (NCBIrefseq) of *M. vollenhovenii* were recorded.

Construction of evolutionary tree and single-nucleotide polymorphism locus

The sequences of the chelae morph specimens, *M. vollenhovenii* NCBIrefseq, and an outlier *Macrobrachium rosenbergii* voucher on NCBI were included for the construction of the phyletic/evolutionary tree diagram.

Sequence data were subjected to a maximum likelihood estimate of the substitution matrix and characterized for the rate of transition and transversion substitutions. The Tajima's neutrality test to determine nucleotide diversity and the Tajimas D value was carried out; then, nucleotides were analyzed for phyletic rooting/clustering on a constructed evolutionary tree. The tree was inferred using the UPGMA—Unweighted Pair Group with the Arithmetic Mean method (Sneath & Sokal, 1973). Evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site (Tamura et al., 2004). The tree was drawn to scale at 1000 bootstraps and taxa that clustered together were shown next to the branches (Felsenstein, 1985). These analyses were carried out on MEGA 6 software (Tamura et al., 2013). Sequences of *M. vollenhovenii* specimens were aligned to detect single-nucleotide polymorphism (SNP) locus and translated to corresponding amino acid to detect associated amino acid changes using BioEdit 7.0.5 edition (Hall, 2005).

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Table 1 Length, weight, and condition factor of *M. vollenhovenii* Chelae Morphotypes at the studied locations

Variant	N	L(cm)	W(g)	K
EAAL	90	15.00 ± 0.79^a	52.06 ± 9.41^b	1.54 ± 0.18^b
EAOR	90	11.06 ± 0.92^b	19.27 ± 5.26^d	1.17 ± 0.46^d
SLAL	90	17.05 ± 1.31^a	101.66 ± 27.07^a	2.00 ± 0.25^a
SLOR	90	12.87 ± 2.02^b	38.79 ± 20.00^c	1.28 ± 0.28^c
LLAL	90	16.83 ± 1.41^a	99.25 ± 30.36^a	2.06 ± 0.43^a
LLOR	90	12.67 ± 1.96^b	38.80 ± 25.26^c	1.71 ± 0.38^c

Means with different superscripts along the same column are significantly different ($p < 0.05$)

L, body length; W, body weight; K, condition factor; EAAL samples having an equal length of chelae on both sides of the body (EA) sourced from a lake (AL); EAOR samples having an equal length of chelae on both sides of the body (EA) sourced from river (OR); SLAL samples having a shorter length of chelae on the left side (SL) sourced from lake (AL); SLOR samples having a shorter length of chelae on the left side (SL) sourced from river (OR); LLAL samples having a longer length of chelae on the left side (LL) sourced from lake (AL); LLOR samples having longer; length of chelae on the left side (LL) sourced from river (OR)

Table 2 16S rRNA Nucleotide Sequences Information of the Chelae Morphotypes of *M. vollenhovenii*

Variant	Accession ID	Base pairs (bp)	A:C:G:T
EAAL	MF683169.1	351	3:1:2:4
EAOR	MF683170.1	351	3: 1: 2: 4
LLAL	MF683171.1	351	3: 1: 2: 4
LLOR	MF683172.1	352	3: 1: 2: 4
SLAL	MF683173.1	352	3: 1: 2: 4
SLOR	MF683174.1	349	3: 1: 2: 4

A, C, G, and T indicates adenine, cytosine, guanine, and thymine, respectively

Results

Biggest sizes and condition factors of the chelae morphotypes

From Table 1, the sampled specimens of *M. vollenhovenii* indiscriminate of sexes had mean L range of 11.06 ± 0.92 cm (EAOR) to 17.05 ± 1.31 cm (SLAL), W ranged 19.27 ± 5.26 g (EAOR) to 101.66 ± 27.07 g (SLAL) while K ranged 1.17 ± 0.46 (EAOR) to 2.06 ± 0.43 (LLAL). In W and K, among GAALs, SLAL and LLAL were similar while EAAL was significantly lowest; among GAORs, SLOR and LLOR were similar while EAOR was significantly lowest. However, among GAALs, EAAL, SLAL, and LLAL had significantly higher L, W, and K than the respective GAORs—EAOR, SLOR, and LLOR.

Phylogenetic/evolutionary relationship, single-nucleotide polymorphism (SNP), and corresponding amino acid translates of the chelae morphotypes

From Table 2, a total of 2,106 nucleotides were generated and are accessible at NCBI. Nucleotide length ranged from 349 bp (SLOR) to 352 bp (LLOR and SLAL) at a 3:1:2:4 ratio of adenine:cytosine:guanine:thymine in 100.0% morphotype specimens. The NCBI blast result (Table 3) revealed EAAL was 100.0% similar with NCBIrefseqs JQ943724.1 (Calabar), JQ943722.1 (Badagry) and JQ943721.1 (Asejire-AL), SLAL and LLAL was 100.0% similar to KJ463387.1 (Badagry). EAOR, SLOR, and LLOR were 99.0% similar to 100.0% NCBIrefseqs. A 508-bp nucleotide length *Macrobrachium rosenbergii* voucher DQ004838.1 was included as an outlier in drawing the phylogenetic tree. The maximum likelihood estimate of the substitution matrix in the nucleotides' sequences that were utilized to draw the phylogenetic/evolutionary tree (Table 4) indicated 73.40 and 26.58 respective sums of transitional and transversion substitutions rates, the sum of which was approximated to 100 by the software. Tajima's neutrality test (Table 5) showed that the 11 sequences had 350 positions in the final dataset which had 49 segregated sites. The nucleotide segregation rate was 0.14, nucleotide diversity was 0.03 and the Tajima's D test result was −2.00. The evolutionary tree diagram (Fig. 3) showed two polyphyletic groups separated at 0.09 distances. On one group, 75.0% NCBIrefseqs rooted 100.0% EA specimens, in which the NCBIrefseqs JQ943724.1 (Calabar) and JQ943722.1 (Badagry) clustered, JQ943721.1 (Asejire, AL) rooted to the cluster, EAAL rooted to the JQ943721.1 (Asejire, AL) while EAOR branched from EAAL. On the other group, the remaining 25.0% NCBIrefseqs rooted 100.0% SL and LL specimens and branched from LLAL, which rooted to SLAL clustered KJ463387.1 (Badagry). In Fig. 4, two SNP loci were

Table 3 Similarity of nucleotides of the chelae morphotypes with the NCBI reference sequences (NCBIRefseq) of *M. vollenhovenii*

NCBIRefseq Accession (Location)	Similarity (%) with Chelae Variants					
	EAOR	EAAL	SLAL	LLAL	SLOR	LLOR
KJ463387.1 (Badagry)	99.0	99.0	100.0	100.0	99.0	99.0
JQ943724.1 (Calabar)	99.0	100.0	99.0	99.0	99.0	99.0
JQ943722.1 (Badagry)	99.0	100.0	99.0	99.0	99.0	99.0
JQ943721.1 (Asejire)	99.0	100.0	99.0	99.0	99.0	99.0

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Table 4 Maximum likelihood estimate of substitution matrix in nucleotides sequences utilized to draw the phylogenetic tree

	A	T/U	C	G
A	–	*5.08*	*1.31*	**13.87**
T/U	*3.84*	–	**8.63**	*3.06*
C	*3.84*	**33.5**	–	*3.06*
G	**17.4**	*5.08*	*1.31*	–

Each entry is the probability of substitution (r) from one base (row) to another base (column). Substitution patterns and rates were estimated under the model of Tamura and Nei (1993). Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics. Relative values of instantaneous r should be considered when evaluating them. For simplicity, the sum of r values is made equal to 100. The nucleotide frequencies are A = 28.91%, T/U = 38.21%, C = 9.84%, and G = 23.04%. For estimating ML values, a tree topology was automatically computed. The maximum log likelihood for this computation was -655.930. The analysis involved 11 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 350 positions in the final dataset. Evolutionary analyses were conducted in MEGA 6 (Tamura et al., 2013)

Table 5 Results from Tajima's Neutrality Test

m	S	ps	Θ	π	D
11	49	0.14	0.05	0.03	− 2

m = number of sequences, n = total number of sites, S = Number of segregating sites, ps = S/n, Θ = ps/a1, π = nucleotide diversity, and D is the Tajima test statistic. The analysis involved 11 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 350 positions in the final dataset. Evolutionary analyses were conducted in MEGA 6 (Tamura et al., 2013)

detected. At Locus 91, 100.0% GAOR (EAOR, SLOR, and LLOR) had 'G' nucleotide compliment, while 100.0% NCBIrefseqs and 100.0% GAAL (EAAL, SLAL, and LLAL) had 'A' nucleotide compliment. At Locus I71, 100.0% EA specimens (EAAL and EAOR), and the 75.0% NCBIrefseq JQ943724.1 (Calabar), JQ943722.1 (Badagry) and JQ943721.1 (Asejire, AL) had 'A' nucleotide compliment, while 100.0% SL and LL specimens

(SLAL, LLAL, SLOR, LLOR) and 25.0% NCBIrefseq KJ463387.1 (Badagry) had 'G compliment. Both G-A compliments at Locus 90 translated to valine, while G-A compliments at Locus 171 translated to glycine–glutamic acid change (Fig. 5).

Discussions

### Size and condition factor of the chelae morphotypes

The obtainable large sizes are important parameters in fisheries management (Moutopoulos & Stergiou, 2002). Encountered length and weight could implicate growth in crustaceans (Konan et al., 2014). *Macrobrachium vollenhovenii* is recognized as *a* target species for freshwater shrimp farming in West Africa based on its encountered big size (Konan et al., 2014). *Macrobrachium vollenhovenii* in streams and major basins of West Africa has a maximum total length of 182 mm and 159–190 mm as reported by respective Ikomi et al., (2005) and Konan et al. (2008). The observed mean length of 11.06 ± 0.92–17.05 ± 1.31 cm in the current study is equivalent to 110.6–170.5 mm, which closely compares to 182 mm and 159–190 mm maximum length of *M. vollenhovenii* reported by Ikomi et al., (2005) and Konan et al., (2008). This length value was supported by a weight range of 19.27 ± 5.26 g to 101.66 ± 27.07 g resulting in a condition factor of 1.17 ± 0.46 to 2.06 ± 0.43. Conventionally, the condition factor is a derivative of length and weight, symbolizing appearance, robustness, or well-being in fisheries. A condition factor above 1.0 is acceptable in fisheries (Jin et al., 2015) and has been reported in adult *M. vollenhovenii* (Uneke, 2014). The *K* values in the chelae variants were above 1.0, indicating that all the morphs were of acceptable robust conditions concerning their respective lengths and weights. However, the pattern of results ran contrary to the set null hypothesis of the study, as the size-related parameters were significantly different across the chelae morphs obtained within and across habitats. Specifically, in W and K, EAAL and EAOR were
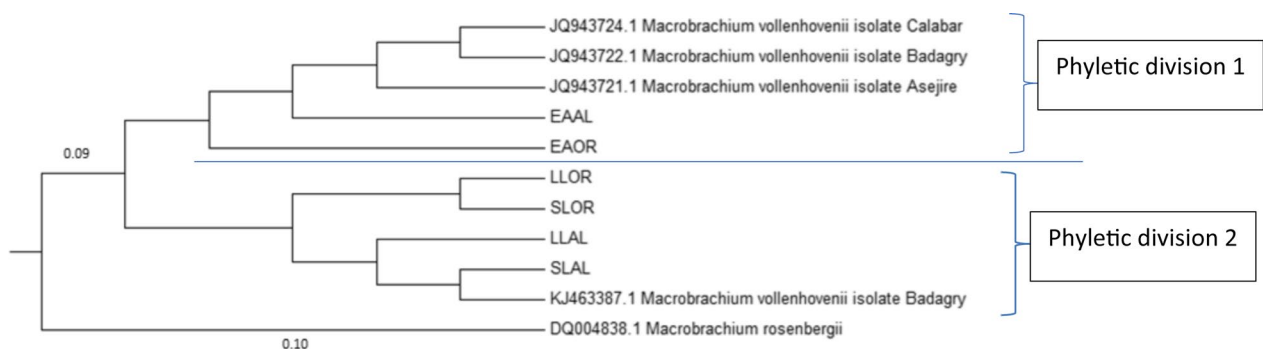
Fig. 3 Evolutionary Relationship of *M. vollenhovenii* Chelae Morphotypes and the NCBI References
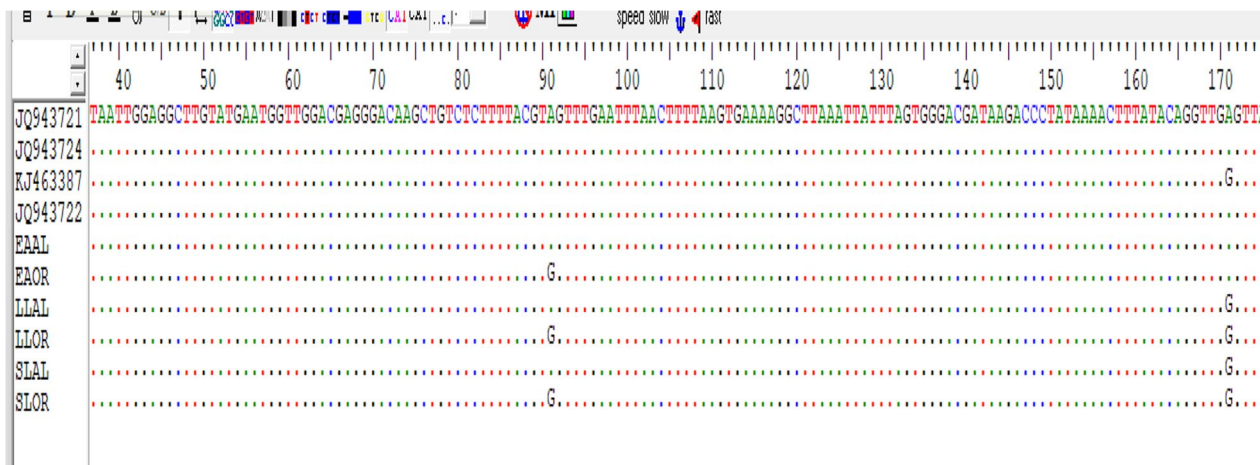
Fig. 4 Single-Nucleotide Polymorphism (SNP) Loci in *M. vollenhovenii* Chelae Morphotypes and the NCBI References. *G-A SNP occurred at Loci 91 and 171
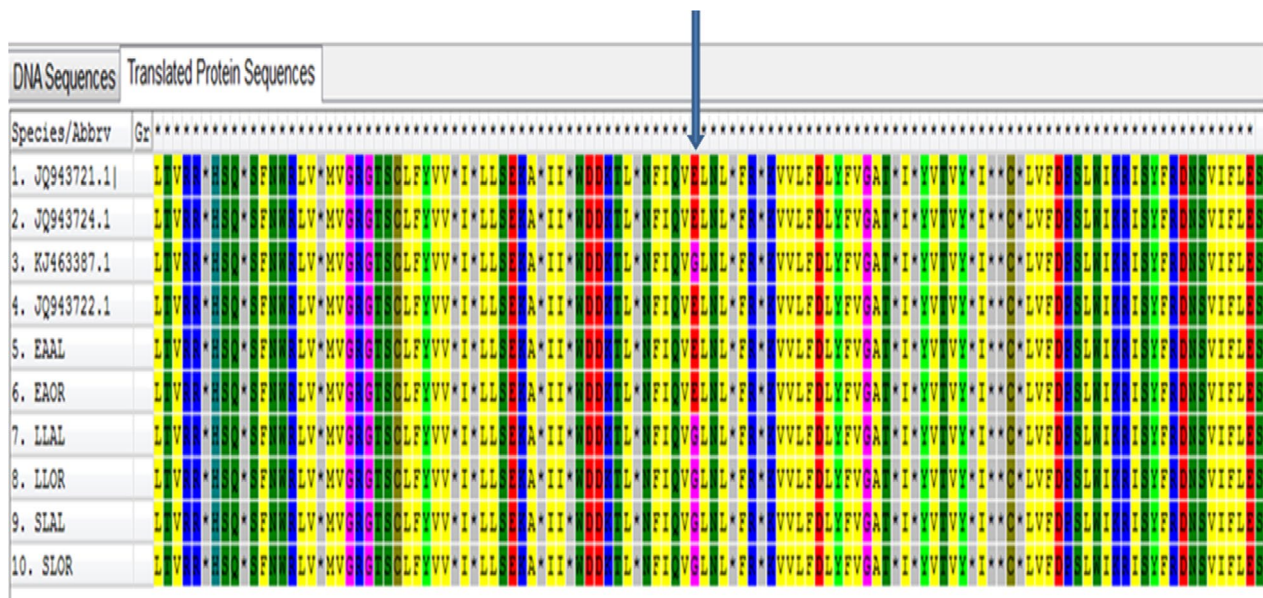
Fig. 5 Amino Acid Translation of *M. vollenhovenii* Chelae Morphotypes and the NCBI References Nucleotides. *Three nucleotides' codes for 1 amino acid on translation frame, G-A glycine–glutamic acid change occurred at Locus L171, E = glycine, G = glutamic acid at Locus L171, V = valine at Locus L91

significantly different and both were significantly lowest compared with respective SLAL and LLAL, which were similar, and from the SLOR and LLOR, which were similar. It is to be noted that their lengths were statistically similar. The similar but higher values of W and K in LL and SL in both habitats, while their respective EAs had the lowest values could indicate that the EAs were the relatively less weighted and least robust morphs. Meanwhile, the LL and SL were heavier and more robust morphs. Also, the GAALs—EAAL, SLAL, and LLAL—were higher than the respective GAORs—EAOR, SLOR, and LLOR—in all the assessed size-related parameters L, W, and K. This implies that the morphs diverged across the habitats in all the assessed size-related parameters, with respective AL morphs having higher values in all the parameters compared with the counterparts of the OR. The trend of results on size highlights that location/habitat and chelae morph types could influence the selection of big-sized *M. vollenhovenii* specimens.

Unequal chelae length is often linked to autotomy and re-autotomy within a group, which often leads to reduced biomass in crustaceans (Norman & Jones, 1991); loss of chelae leads to a reduction in molt increment due to energy diversion as a result of chelae regeneration load (Luppi, et al., 2004). In that case, the equal-armed EA would have superior weight and robustness, having had similar length with the unequal-armed LL and SL, but has not compensated for lost arms regeneration load. The observed result ran contrary to this phenomenon, indicating that other factor(s) may be responsible for the superior size (weight) and robustness in the SL and LL compared to the EA in the current study. The differences in sizes of the same morph type across the locations could be environmentally induced, possibly due to food availability and general habitat conditions. However, locations could only be presumed to have contributed in this regard, if the respective morphs are of the same genotype, having similar potentials for attained sizes across the locations. Genotypes may diverge in capacity to utilize food and general environmental resources within and across habitats resulting in varied encountered sizes. Such genetic divergence could occur across individuals, species, and populations. Moreover, phenotypic variation can stem from genetic differences between individuals from ontogenetic development (Schwander & Leimar, 2011).

Phylogenetic relationship, single-nucleotide polymorphism (SNP), and corresponding amino acid translates

Molecular data are a complementary approach to morphology, especially in discriminating cryptic or sibling species (Knowlton, 1993; Sarver et al., 1998), and in constructing phylogenetic relationships (Avise, 1994). The 16S ribosomal RNA region was widely utilized in drawing phylogenetic map for crustaceans (Jimoh et al., 2013; Jose et al., 2016; Mantelatto et al., 2014) and fisheries (Falade et al., 2016; Sultana et al., 2015). The relevance of this marker concerning crustaceans is further revealed in the current study. The sequenced region was polymorphic; having more of transitional than transversional substitutions, nucleotides' segregation rate was 0.14, nucleotide diversity 0.03, and the Tajima's D test resulted in -2.00. Conventionally, a positive Tajima's D value signifies low levels of polymorphisms while the negative value indicates the opposite. The obtained -2.00 Tajimas' D value in the current study indicates a high level of polymorphism. Moreover, the sequences had a similar structure of nucleotide ratio as reported in other *Macrobrachium* species (Rajkumar et al., 2017; Sharma et al., 2017; Yi et al., 2009). However, the evolutionary tree supported two divergent clades from a common root/ancestor.

Hence, the null hypothesis that the chelae morphs will share the same descent with each other and with similar sequences on gene repository is therefore rejected. The sequences formed two descend divisions/clades, each containing some chelae morphs and the NCBI references in divergent clusters and branches. This evolutionary pattern indicates two descends, having specimens of the morphs and the NCBI sequences in a spread of divergent clusters and branches. These represent polyphyletic groups, each having descendants. They are biological units, products of descent with modification.

Interestingly, most (75.0%) of the NCBIrefseqs rooted 100.0% EAs while EAOR branched from EAAL, indicating that while the EAAL shared descent with the majority of the encountered NCBI reference sequences, the EAOR is a recent divergent strain of the EAAL. This phyletic group specially had only the EA specimens possibly implying that the ancestor of the group and the co-rooted NCBI references would also be of the EA morphotype. Meanwhile, the EAOR branched from EAAL, indicating that EAOR is a strain that most likely recently diverged from EAAL. The EAAL and EAOR are two genotypes sharing the same recent ancestor, the EAOR being the most recent divergent genotype in the group. Going by the description of the sampled locations, the EAOR could be the river habitat strain, while the EAAL and JQ943721.1 (Asejire, AL) are the lake habitat strain. However, the phyletic group linked all the EAs irrespective of location and could therefore be insinuated as a lineage of EAs. The locations of the NCBI specimens JQ943724.1, JQ943722.1, and JQ943721.1, that is, Calabar, Badagry, and Asejire-AL, are coastal and inland waters of Southern Nigeria. These could indicate the possible areas of distribution of the EA genotype in the meantime.

Members of the second phyletic division would also have same descendant, with KJ463387.1 (Badagry) being the earliest in the group. This division had SLAL and LLAL rooted to KJ463387.1 (Badagry), the SLOR and LLOR clustered, taking root from LLAL. The tree pattern indicates that this phyletic division had the SLOR and LLOR and the SLAL and LLAL divergent genotypes. The division had all the SL and LL irrespective of location indicating that the ancestor of this division is of the SL or LL morphologic type. Meanwhile, the GAOR SL and LL and the GAAL SL and LL constitute divergent genotypes/strains originating from it, with the GAOR- SLOR and LLOR being the most recent divergent genotype. The reference (NCBIrefseqs) sequences of *M. vollenhovenii* and the studied chelae morphs shared two extractions from which genotypes EAAL, EAOR, SLOR, and LLOR, SLAL, and LLAL were linked. The EAOR, SLOR, and LLOR groups are the most recent divergent strains in the respective EA, SL, and LL extractions.

The phyletic divisions diverged at 0.09 (9.0%) agreeing with Maidin et al., (2017) who reported that *Macrobrachium* species can diverge at 0.6–10.1% distance. It is opined the lineages of the phyletic divisions in the current study are divergent of the *Macrobrachium* species, separated at this distance. Moreover, *M. rosenbergii* also outlies *M. vollenhovenii* specimens at a similar distance. Recent divergence origins occur at 0.6–2% genetic distance in crustaceans (Bhavan et al., 2016; Hebert et al., 2003; Umamaheswari et al., 2016); hence, separation of the morph lineages at 9.0% distance may not be considered recent. The reportage and analysis of the mixed chelae clades/genotypes as a genetic unit in previous studies could reflect ambiguous genetic structure, as this constitutes mixed genotypes. The phylogenetic pattern in the current study agreed with the observed size pattern, as it revealed divergence and similarity in the EAs compared with the SL cluster LL, and their habitat variants. It could be deduced that the highlighted genotypes would have differences in genetic potentials for attained sizes, but this has to be investigated using relevant size-/growth-related markers. Makombu et al., (2019) suggested a potential conflict between the morphological identification key and the genetic basis underlying speciation and species allocation for *Macrobrachium* species. In the meantime, even though additional works, including more specimens and species of related genera, are necessary, the results found in the present study illustrate a case of divergence at the mitochondria sequence region which recognized multiple clade/strains. The result agrees that the studied *M. vollenhovenii* in past studies would constitute a cryptic genetic structure. The study followed Mantelatto et al., (2014), who utilized molecular analysis to reveal possible cryptic species in commercial deep-sea crabs in the South Atlantic, with the possible genetic role of the chelae morphs in the case of cryptic *M. vollenhovenii* species in Southwestern Nigeria being highlighted in the current study.

Polymorphism in sequence data would avail the opportunity to investigate single-nucleotide polymorphism markers for sustainable discrimination of genetic variants. This assists in their sustainable management, utilization, and conservation. The SNP markers are valuable in investigating hybridization and introgression in captive or wild stocks of fisheries (Syaifudin et al., 2015). In the current study, SNP was discovered at Loci 91 and 171 of the 16S rRNA nucleotide. Hence, we reject the hypothesis that there will not be any SNP across the sequences. The SNP at Locus 91 separated OR specimens, from all NCBIrefseqs and all AL specimens. This implied that SNP at Locus 91 will discriminate between the AL and the OR chelae variants and their associated reference specimens. It also followed that each genotype at the two

locations is genetically different; they are possibly the divergent habitat strains. The SNP could represent the genetic site for the identification of the OR strains of the chelae variants. The OR specimens of the chelae variants represent habitat strains identifiable at Locus 91 in the sequenced partial genome region. However, the translated SNP did not reveal protein change, meaning that the SNP is underlined by functionally silent mutation. Silent mutations have occurred in barcode sequences of crustaceans such as *P. chinensis* (Hwang, 1996), *Portunus sanguinolentus, Charybdis natator, Portunus pelagicus, Portunus trituberculatus* and *Travancoriana napaea* (Umamaheswari et al., 2016). Silent mutation would not induce differences in amino acids of the divergent groups. The significant difference in size phenotypes of the chelae variants across OR and AL corresponded with nucleotide divergence but not with changes in protein/amino acids. However, the SNP would be useful for taxonomic purposes.

The SNP Locus I71 separated sequences containing the EA variants, EAOR and EAAL, and the NCBI sequences JQ943724.1 (Calabar), JQ943722.1 (Badagry), and JQ943721.1 (Asejire, AL) from the other sequences located on the divergent second phyletic division. This indicates that the SNP locus would discriminate the two main phyletic divisions, their ancestors and possibly their progenies. The SNP would discriminate samples of EA from either of SL/LL, irrespective of locations. The occurrence of glycine/glutamic acid change at the locus implies that the nucleotide variation is functional, having implications on changes in functional amino acids. The divergence in functional amino acid could highlight the genetic undertone of the significantly better quality of amino acid observed in tissues of EA compared to SL and LL chelae variants in previous studies (Oyebola et al., 2016). In the meantime, divergence in amino acid translates across EA and the SL/LL corresponds with divergent morphologically relative inferior biggest size and robustness of EA compared to the SL/LL genotype. The studied EA and SL/LL specimens are not only morphologic variants with respect to chelae but they are genotypes of divergent lineage, who have recently diverged habitat strains.

Conclusions
The studied equal left and right side chelae and the unequal left and right side chelae specimens are, respectively, small- and robust-sized, irrespective of habitat. The genotypes had two evolutionary links, being clades having protein differences, delineable at 16S rRNA sequence SNP Locus 171. The OR specimens are size variant habitat strains of the clades and are delineable at SNP Locus 91. The morphs have been

in existence prior to the current study; however, their sizes, genetic implications, and identity are revealed in the current study. The inability to separate *M. vollenhovenii* specimens into these genotypic subgroups could have informed the genetic ambiguity reported in the previous studies. Meanwhile, the identified markers in the current study will be useful for precision identification and selection of the size variant chelae morph strains for sustainable utilization. However, it is important to investigate the chelae morph strains for their spread in habitats and regions of the distribution of *M. vollenhovenii.*

Abbreviations

N	Number of sampled specimens
L (cm)	Total length measured in centimeters,
W (g)	Wet weight measured in gram
K	Condition factor
EA	Samples possessing equal left and right side chelae,
LL	Samples possessing longer left side chelae
SL	Samples possessing shorter left side chelae
AL	Asejire Lake
OR	Ogun River
EAAL	Samples having equal length of chelae on both sides of the body (EA) sourced from lake (AL)
EAOR	Samples having equal length of chelae on both sides of the body (EA) sourced from river (OR)
SLAL	Samples having shorter lengths of chelae on the left side (SL) sourced from lake (AL) SLOR Samples having shorter lengths of chelae on the left side (SL) sourced from river (OR)
LLAL	Samples having longer lengths of chelae on the left side (LL) sourced from lake (AL) LLOR Samples having longer; lengths of chelae on the left side (LL) sourced from river (OR)
GAAL	Asejire Lake samples comprising the EAAL, LLAL, and SLAL
GAOR	Ogun River samples comprising the EAOR, LLOR, and SLOR
RNA	Ribosomal nucleic acid
SNP	Single-nucleotide polymorphism
NCBIrefseq	Reference sequences in the GenBank (National Center for Biotechnology Information).
ANOVA	Analysis of Variance
PAST	Paleodontological Statistics Computer program
BLAST	The Basic Local Alignment Search Tool
MEGA	Molecular Evolutionary Genetics Analysis software
UPGMA	Unweighted Pair Group with Arithmetic Mean Statistical Method
A:C:G:T	Ratio of adenine:cytosine:guanine:thymine

Author contributions

Author OOO designed the study and prepared the draft of the manuscript, OOO and VA performed the molecular analysis, AO and OOO wrote the protocol and participated in fieldwork, and AO**,** OMC, and CFI did laboratory mensuration and managed the analyses. CO and OAA managed the pieces of literature and data interpretation. All authors read and approved the final manuscript.

Availability of data and materials

Macrobrachium vollenhovenii NCBI accessions Number MF683169.1–MF683174.1 datasets generated and analyzed during the current study are available in the [NCBI] repository, visible on page 2 of the https://www.ncbi.nlm.nih.gov/nuccore/?term**=**MF683169:MF683206[accn]. Other datasets used and/or analyzed during the current study are available with the author upon reasonable request.

Declarations

Ethics approval and consent to participate

The study was conducted with informal approval from the fishing community, which led to the fishers' readiness to release their stock for the study. Principles of animal use ethics were followed. Mortal samples meant for the market were collected from fisherfolk instead of making fresh catches. Sampling was specific on large sizes instead of all size grades and was not extended to a long period to reduce the number of sampled specimens. Meanwhile, specimens were not refined in any way.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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