Mitochondrial CO1 gene sequence variation and the taxonomic status of three *Macrobrachium* species in Nigeria

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Abstract. *Macrobrachium* species are ubiquitous being found all over the world except in Europe. They are the most speciose of the decapods showing very great variations in response to the prevailing environmental conditions. This has led to serious difficulties with their classification based on morphology. In Nigerian sympatric occurrences of *Macrobrachium vollenhovenii, Macrobrachium dux* and *Macrobrachium macrobrachion* have been reported in many water bodies. So far no record of a review of the morphological classification based on molecular markers have been reported. Sampling from four major water bodies namely Warri, Badagry, Calabar and Asejire Dam all in Southern Nigeria was done and mitochondria DNA marker CO1 genes were sequenced to determine the congruence of morphological and molecular classifications. A 556bp sequence was phylogenetically analyzed using the major methods. All the trees produced similar results. Instead the three species classification based on morphology, molecular phylogeny revealed consistently a two species grouping with *M. vollenhovenii* clearly mapping as a valid species. *M. dux* and *M. macrobrachion* were grouped together into one clade indicating a single species or at best morphotypes of a species. Genetic diversity within and between the species is low indicating a recent colonization process. The value of the bootstrap supporting the grouping was also low. Several primer combinations failed to amplify the CO1 gene of the Warri specimens. This is a quantitative measure of being distinct. The Nigerian *Macrobrachium* species maps closer to the Southern American species M*. brasiliense, M. carcinus* and *M. olfersi*. The Asian species *M. asperulum* and *M. rosenbergii* are the most distant from the Nigerian *Macrobrachium* species. Further studies to reveal deeper phylogenetic structuring using species specific primers may be needed to resolve fully the phylogenetic controversies. Determining the development patterns whether abbreviated or extended is also necessary. The sequencing of the complete mitogenome is also an important further research requirement.

Key Words: phylogeny, molecular systematics, population structure, barcoding, phylogeography, sympatry

Introduction. The Genus *Macrobrachium* Bate, 1888 is ubiquitous being found abundantly in all aquatic habitats of the world except Europe. In most parts of the world they form an important component of the shell fisheries sector as either capture or culture is done extensively. Currently over 200 species are described with more still being described as the procedure for species classification become more refined (Valencia & Campos 2007). The importance of the species vary with *Macrobrachium rosenbergii* being very important as a cultured species in Asia while the *Macrobrachium vollenhovenii, Macrobrachium dux and Macrobrachium macrobrachion* are important in the capture fisheries sector of the Nigerian economy (Nwosu & Wolfi 2006). These last three species are thought to occur in sympatry with no clear cut information about the mode of larva development as either being abbreviated or extended. Evidences of a dual life pattern exist in most of the members of the genus. Olivier et al (2012) refers to 'freshwaterization' due to various alterations in the ancestral marine planktonic larval development. Within *Macrobrachium*, the most speciose genus of the Palaemonidae, several authors, including Jalihal et al (1993) have described three forms of larval development, namely the abbreviated and the extended larval development and a third category called direct development, all being adaptations to various changes in the environment as the group evolved.

McDowall (1988, 1992) noted that amphidromy, a life cycle which requires migration between rivers and the sea because the juveniles and adults live and breed in fresh water but larval development in marine, is also an established phenomenon. This phenomenon points to evidences of marine descent of the group. Metric and meristic characters have formed the basis of their classification. Holthuis (1950, 1952), Bray (1976), Costa (1979), Choy (1984), Fincham (1987), Chace & Bruce (1993) are standard reference on the morphological classification of members of the genus. However the multiple factors of a need for expertise in taxonomy, plasticity of the major organs (the rostrum and second pereiopod), the influence of the environment which may affect growth especially where stocking density is high and there is seasonality in the salt content of the water body, all have effects of the pereiopod. Munasinghe (2010) noted that the genus has remained one of the most taxonomically challenging and difficult within the decapod group. These morphological characters have little phylogenetic value though they have remained very variable amongst species. As such classification of the group has been challenging. In Nigeria and most of Western part of Africa, the taxonomic challenge has been made difficult by the confusion created by the synonymous and inter-changeable use of names. *M. dux, M. macrobrachion* and *M. vollenhovenii* all occur in Nigeria. The basis of their morphological classification are as firstly described by both Lenz (1910) and Inyang (1981), for *M. dux* with males having rostrum equal of slightly shorter than antennal scale, dorsal edges being slightly convex over the eyes, few than 11 dorsal teeth of which 1 or 2 are on the carapace posterior to orbital margin. The second cheliped with carpus is shorter than the palm; palm much longer than fingers; fingers lacking fur-like covering and gaping with teeth on basal half of each finger.

For the *M. macrobrachion* the rostrum is equal or slightly longer than antennal scale, straight or with tip curved slightly or occasionally strong upwards; often an untoothed portion near the tip followed by 1 or 2 apical teeth post orbital. The second cheliped with carpus slightly longer than palm, palm much longer than fingers which are straight and uncovered with fur-like dense layer or short soft hairs; chela, carpus and merus uniformly dark coloured with row of visible spines along inner margin; ischium pale-coloured. The body is dark, with dorsal parts of last 3-4 abdominal semites which are light-coloured; side of carapace with dark line running from below eye towards base of the second cheliped.

For *M. vollenhovenii* the diagnostic features has rostrum equal or (more usually in adult) shorter than antennal scale; dorsal edge convex over eye; tip lacking prolonged toothless portion. The second chelipeds with carpus shorter than palm; movable finger with (in large adults) a single large tooth at mid-length of finger. Colour is generally pale without speckling or mottling; thin dark longitudinal lines on carapace and transverse ones on abdomen; also thin unbroken line along ventral margin of carapace. The third maxillipeds are bright yellow (white in small juveniles). Fingers of the 2nd cheliped dark blue, with yellow patch at articulation with palm; tips of fingers white in juveniles. The eggs are red or orange.

The above descriptions are however phenotypically plastic and are all affected by the prevailing environment leading to mis-classification.

No documented investigation has so far been conducted on the use of molecular markers for the classification of members of the group in Nigeria, especially *M. dux, M. macrobrachion* and *M. vollenhovenii* which are all thought to occur sympatrically. This research work investigates the congruence of morphological and molecular classification based on the CO1 gene segment. It also barcodes the species, thereby generating baseline molecular information about *Macrobrachium* species that occur in Nigeria.

Material and Method

Specimen collection. Samples were collected between January and June 2008 by the authors and identified by experts in decapod systematics based on morphological characteristics as described by Inyang (1981) for *M. dux*, Welcomme (1979) for *M. macrobrachion* and Powell (1979) for *M. vollenhovenii*. Further confirmation was done by two experts in decapod systematics. Samples were collected from the Badagry river,

location 1, Asejire dam, location 2, Warri river, location 3 and Calabar river, location 4. The samples from locations 1 and 2 were identified as *M. vollenhovenii*, while for locations 3 and 4 the samples were identified as admixture of *M. dux* and *M. macrobrachion*. The Figure 1 shows relative locations of sample collection.

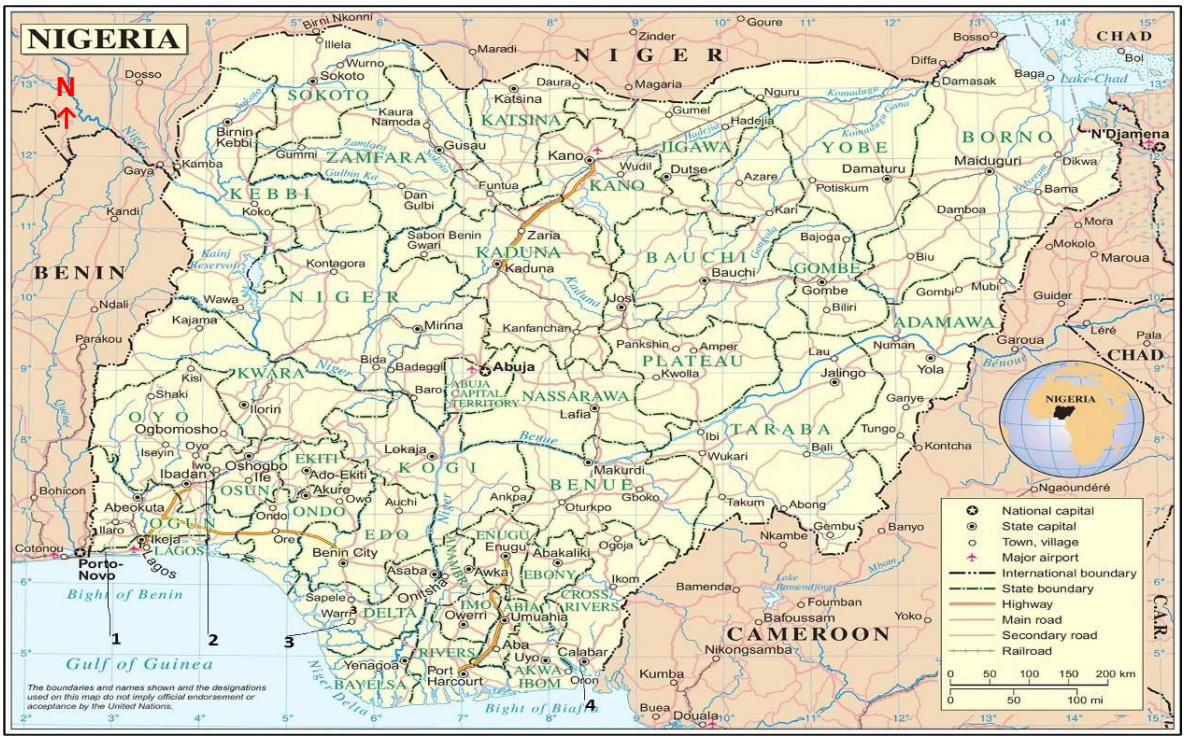

Figure 1. Map of Nigeria showing sample collection points: 1 – Badagry river; 2 – Asejire dam; 3 – Warri/Ogborogbo River; and 4 – Calabar River.

DNA extraction and amplification. Whole samples were taken to the lab preserved in 70% alcohol. A small piece of tissue was first washed for one hour in 1 mL GTE buffer (100 mM glycine, 10 mM Tris, 1 mM EDTA). DNA was extracted using commercial DNA extraction kit by Qiagen with the protocol recommended by the manufacturer. The extracted DNA was quantified using the nanodetector or running gel and highly concentrated samples were diluted using ddH_2O to achieve the optimum concentration for amplification in Polymerase Chain Reactions (PCR) (~50-300 ng μL^{-1}).

Polymerase Chain Reaction and sequencing. Polymerase Chain Reaction (PCR) was done according to Mullis et al (1986), using commercially available primers that is commonly used in decapod systematics for the CO1 gene segment was done. The primers used are LCO 1490 with sequence (-GGTCAACAAATCATAAAGAT ATTGG-) and HCO 2198 (-TAAACTTCAGGGTGACCAAAAAATCA-) (Folmer et al 1994). Amplification of targeted DNA was done in an Eppendorf Mastercycler EP gradient thermal cycler, using the following conditions: denaturation at 94°C for 3 minutes, 32 cylces of 30 seconds at 94°C, 40 seconds (annealing) at 50°C and 50 seconds at 72°C elongation, followed by extention at 72°C for 5 minutes and termination at 15°C for 5 minutes.

PCR master mixes for each primer were prepared using sterile 1.5 mL microfuge tubes. Each master mix of 25 µL had the following: 1. 17.25 µL millique water; 2. 2.5 µL Buffer; 3. 0.5 µL dNTP; 4. 1.0 µL Primer 1; 5. 1.0 µL Primer 2; 6. 0.25 µL Taq polymerase; 7. 2.5 µL template. Master mixes were vortexed gently to produce a homogenous solution. Successful amplicons/PCR products were then runout on a 2% agarose gel, impregnated with EtBr Agarose gel using Ultraviolet light and photographed. Successful aplicons were then purified and sent to the commercial laboratory - TechDragon for sequencing. Sequence files were viewed and edited in both directions using BioEdit 7.1.11 (Hall 1999). Sequences were read in both directions and consensus sequence subjected to exploratory data analysis using both DNAstar and MEGA

(Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Bootstrap value was set at 1000 replicates.

To increase the robustness of inferences from statistical analysis, and to account for known sensitivities in certain procedures, multiple analytical approaches were used. Each test has the ability to reveal different aspects of the data, and each has advantages and disadvantages. Utilizing multiple tests and comparing results allows the greater confidence in the interpretation of data.

Molecular data analysis. Base composition, estimates of evolutionary divergence, phylogeny based on the four major methodologies - maximum likelihood, neighbour joining, minimum evolution and the unweighted pair group method with arithmetic mean were used to determine phylogenetics relationship based on the partial sequences of the CO1 gene according to Page & Holmes (1998). Blast queries were also done to see how similar or dissimilar the sequences were to GenBank sequences of *Macrobrachium* species. Several indices of population structure like polymorphism between the sequences, singleton and parsimony informative sites, conserved DNA regions suitable for species specific primers, Fu and Li's tests, the Tajima's test, linkage disequilibrium and haplotypes was also determined. Based on the above, clades and population structure was determined. Specimens were also assigned to specific species. Three methods of phylogenetic inference were also employed in the determination of the relationship within and between species/locations. This is to further confirm the validity of the results obtained.

Results. A total of fifteen (15) sequences were obtained successfully from the forty (40) used in this experiment. No successful amplification was obtained even with the varying primer combinations and conditions for the Warri samples. The sequence alignment after editing yielded 556 sites for the three sites for phylogenetic analysis. The mean average composition was T = 27.38%, C = 27.06%, A = 27.82% and G = 17.70% for Badagry, T = 27.4%, C = 27.18%, A = 27.64% and G = 17.8% for Asejire, T = 27.46%, C = 27.24%, A = 27.72% and G = 17.56% for Calabar indicating that the CO1 mtDNA is Thiamine, Cytosine and Adenine rich in palaemonids. No successful CO1 amplification was obtained for Warri stock. The resulting sequences have been submitted to the GenBank (KC688272, KC688272.1, KC688273.1). There were also eighty seven variable sites (87), of which nineteen (19) were parsimoniously informative, eighty-two (82) were singletons with two variants. Table 1 shows the summary of sequence characteristics.

Table 1

Details of genetic parameters analysed for *Macrobrachium vollenhovenii, M. dux*/*M. macrobrachion* CO1 sequences from the three locations

Parameter	*Location*			
	Badagry (B)	*Asejire (A)*	*Calabar (C)*	*Warri (W)*
Number of samples (successful PRC reactions)	10(5)	10(5)	10(5)	10(0)
Alignment length (base pairs)	556	556	556	-
Average base composition (Thiamine)	27.38%	27.4%	27.46%	-
Average base composition (Cytosine)	27.06%	27.18%	27.24%	-
Average base composition (Adenine)	27.82%	27.64%	27.72%	-
Average base composition (Guanine)	17.7%	17.8%	17.56%	-
Total purine (Adenine + Guanine)	45.56%	45.44%	45.28%	-
Total pyrimidines (Cytosine + Thiamine)	54.44%	54.58%	54.90%	-
Number of polymorphic sites	27.38%	27.4%	27.46%	-
Singleton variable sites	87	87	87	-
Parsimony informative sites	19	19	19	-
Singleton variable sites (two variants)	82	82	82	-
Parsimony informative sites (two variants)	13	13	13	-
Singleton variable sites (three variants)	4	4	4	-
Parsimony informative sites (four variants)	6	6	6	-

Within location, the values range from 0.009 between M/A/4 and M/A/1 which is the lowest and 0.026 between M/A/5 and M/A/1. The least diversity is seen within the Asejire stock. It is noteworthy to indicate here clearly that Asejire is a dam created in the late seventies and that the stock found therein are introduced and completely isolated from all other sample site. The stock is also very young in terms of age and the possibilities of the exchange of genetic materials is absent unless there is deliberate re-introduction of new stock.

Equally low genetic divergence is also recorded in the Calabar stock. The waters are fresh and several miles in terms of contiguity from the Badagry waters. M/C/1 and M/C/2, M/C/3, M/C/4 have divergence of 0.005.

The element of being a different species is evident in the divergence between M/B/4 and M/B/1, M/B/2 and M/B/3 with values of 0.216, 0.220 and 0.229 respectively. This is also a quantitative measure of being different species. The levels of intra and inter specific divergence are shown in the Table 2.

The minimum and maximum pairwise genetic distances are also indicated in Table 3. Highest values are noted across species boundaries as seen between *M. vollenhovenii* and *M. dux/M. macrobrachion* samples from Badagry and both Asejire and Calabar.

Table 3

Indicating minimum and maximum pairwise genetic distances within and between locations

S/No	*Location*	*Taxon*	*Genetic distance (pairwise distance)*	*Overall mean distance*
1	Asejire	*Macrobrachium vollenhovenii*	0.009-0.026	0.017
2	Badagry	*M. vollenhovenii* *M. dux / M. macrobrachion*	0.007-0.229	0.222
3	Calabar	*M. vollenhovenii* *M. dux / M. macrobrachion*	0.007-0.229	0.222

Table 2

Measure of intra and inter genetic distances within and between species and locations. Note the coloured figures are within locations while black are between locations

	M/A/1	*M/A/2*	*M/A/3*	*M/A/4*	*M/A/5*	*M/B/1*	*M/B/2*	*M/B/3*	*M/B/4*	*M/B/5*	*M/C/1*	*M/C/2*	*M/C/3*	*M/C/4*	*M/C/5*
M/A/1															
M/A/2	0.020														
M/A/3	0.018	0.020													
M/A/4	0.009	0.015	0.016												
M/A/5	0.026	0.020	0.015	0.020											
M/B/1	0.026	0.024	0.018	0.024	0.018										
M/B/2	0.011	0.016	0.018	0.005	0.022	0.026									
M/B/3	0.026	0.020	0.018	0.020	0.015	0.015	0.022								
M/B/4	0.220	0.229	0.219	0.214	0.228	0.216	0.220	0.229							
M/B/5	0.011	0.016	0.018	0.002	0.022	0.026	0.007	0.022	0.211						
M/C/1	0.007	0.015	0.016	0.004	0.020	0.024	0.005	0.020	0.217	0.005					
M/C/2	0.011	0.016	0.018	0.002	0.022	0.026	0.007	0.022	0.217	0.004	0.005				
M/C/3	0.007	0.016	0.015	0.005	0.022	0.022	0.007	0.022	0.211	0.007	0.005	0.007			
M/C/4	0.011	0.016	0.018	0.005	0.018	0.026	0.007	0.022	0.220	0.007	0.005	0.007	0.007		
M/C/5	0.026	0.020	0.018	0.020	0.011	0.015	0.022	0.015	0.226	0.022	0.020	0.022	0.022	0.022	0.000

Key: M = *Macrobrachium*, A = location Asejire, 1 = sample 1; B = location Badagry; C = location Calabar. So M/A/1 is *Macrobrachium* from Asejire, sample 1 while M/C/5 is *Macrobrachium* from Calabar sample 5.

Phylogenetic analyses. To analyze the relationship between species and locations, phylograms were generated using three different methods (maximum likelihood, neighbor joining and minimum evolution) using the Kimura 2 parameter method with 1000 pseudo replications (Kimura 1980). They yielded very similar topologies (Figures 2-4) as such only two of the topologies were included. Each of these methods has its own strengths and weaknesses. The essence of several methods is to see whether there would be significant differences between them. The confidence levels were generally low but consistently the two clear clades were indicated for all the methods. One of the clades for all the topologies contains the *M. vollenhovenii* only indicating it as a valid species group while the other contained the *M. dux / M. macrobrachion* complex. All the topologies reveal two different clades with the M/B/4 being separated in all instances. The minimum evolution and neighbor joining topologies are essentially the same. For all the topologies, the overall were moderate, suggesting a loosely related intra and inter species relationship.

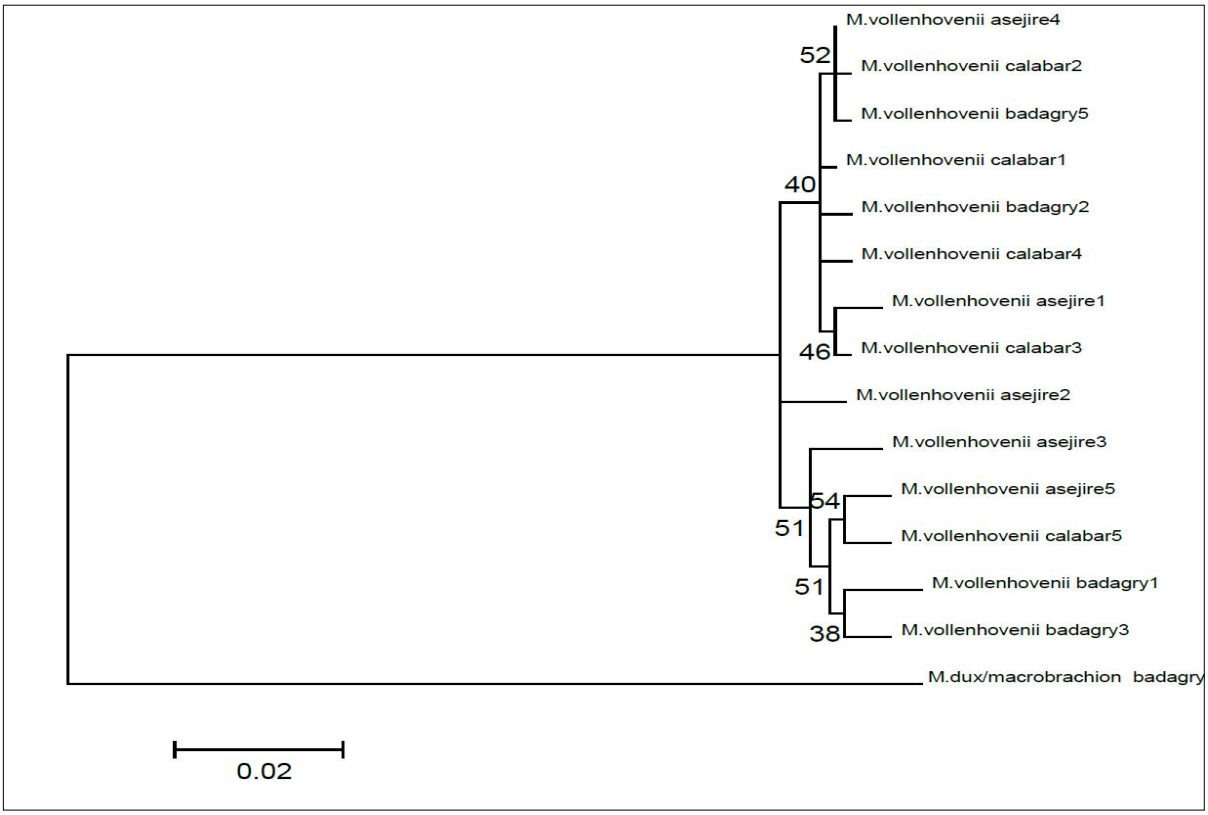

Figure 2. Phylogenetic tree using the Maximum Likelihood method.

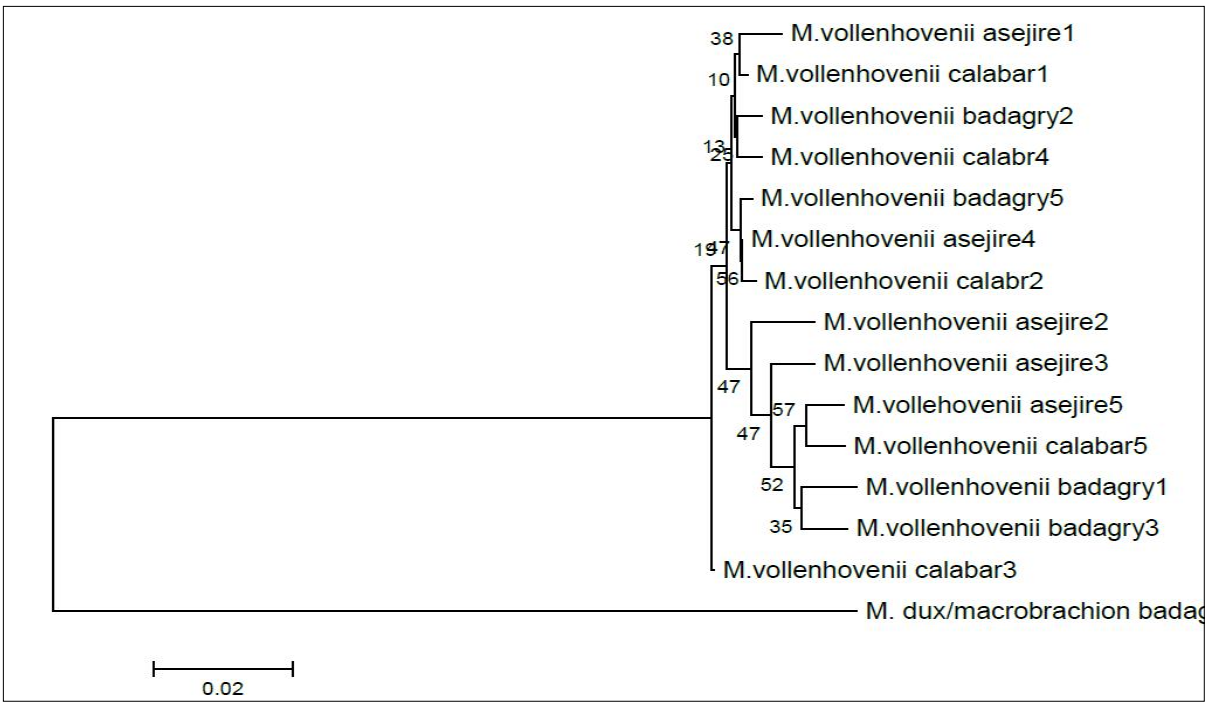

Figure 3. Phylogenetic relationship based on Minimum Evolution criteria.

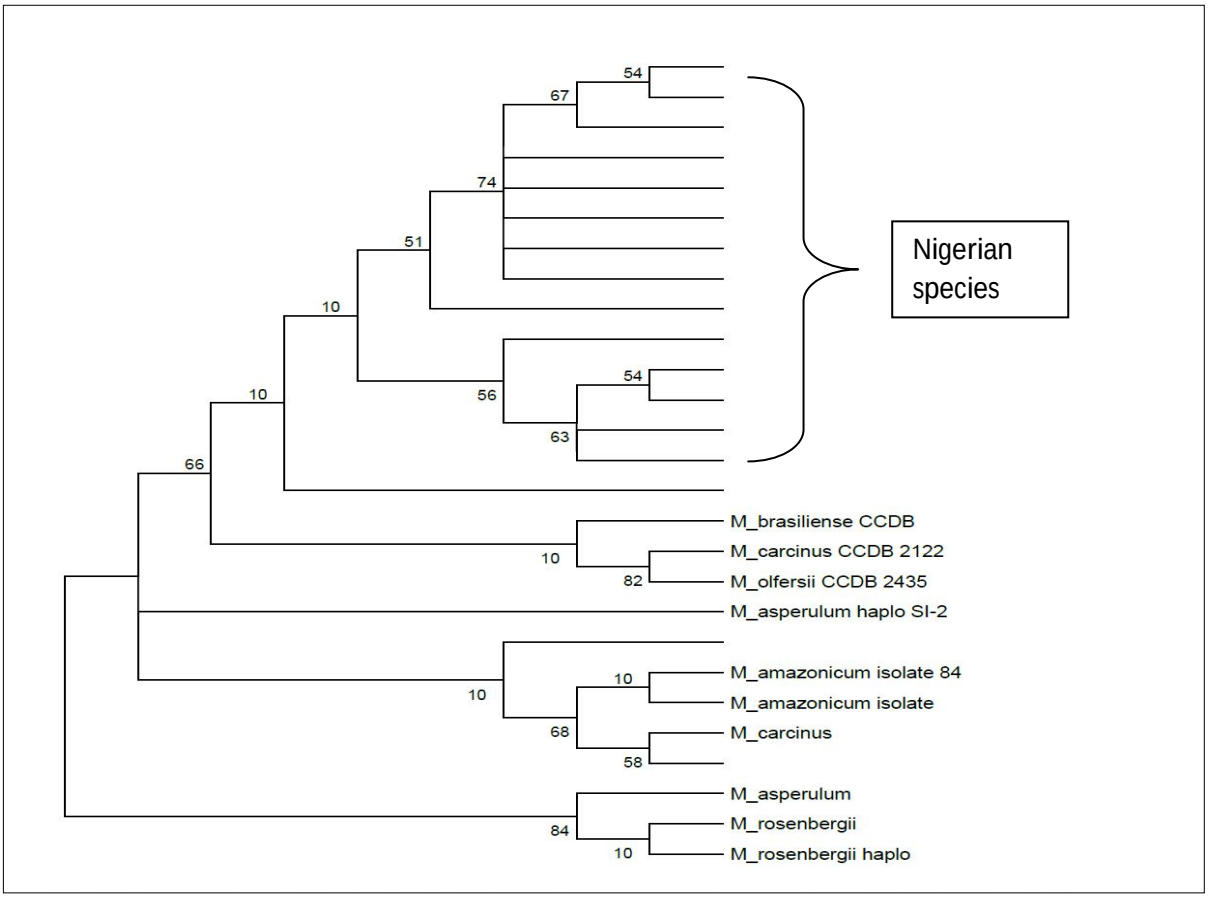

Figure 4. Indicating the phylogenetic position of the Nigerian *Macrobrachium* species in relations to the Asian, North and South Americans species based on the CO1 gene sequence.

Discussion. The result of this study clearly indicates that the morphological classification of the Nigerian species of *Macrobrachium* is questionable, since crustaceans are usually very speciose and exhibit various levels of morphological variation based on the ambient

environment which can be stocking density, availability of food, temperature, salinity, hybridization with other species, the nature of the water body especially when the water availability regime is unpredictable (Murphy & Austin 2005). *Macrobrachium* species are known to have shifted from the extended larvae development pattern (ELD) to the abbreviated larvae development pattern (ALD) in response to rivers volume being low. Across the distributional zone in Nigeria, the environmental conditions are diverse and this has been proven to lead to variation in phenotypes, reproductive periodicity and morphometric relationships (Bauer 2011). In the case of the Nigerian species, there are contradictions between morphometry and molecular systematics, as such a marriage of both methods is needed to resolve the ambiguities.

The inability of several combinations of CO1 primers that have been used successfully in resolving phenotypic relationships between and within species in Asia to successfully amplify segments of this gene in the Warri population is an indication of its being different from samples from other water bodies. In the resolution of issues of classification, several authors have relied on either morphometry, molecular of a combination of both methods. *M. rosenbergii* was thought to be one species group through-out Asia. Wowor & Ng (2007) using finite morphological features as described by Johnson (1973) which was subjected to multiple discriminant analyses (MDA) which revealed clearly morphometric disjunction into two taxas, indicating that they are actually two distinct species named *M. rosenbergii* and *M. dacqueti*. The primary variables separating both were the height of rostral base and number of teeth on ventral margin of the rostrum. The character was not affected by sex and age. The result of this study suggests that a natural grouping of samples across locations is observed and that *M. vollenhovenii* is a valid species. To be sure that the sequences obtained were not pseudo-genes, a blast search between one of the sequences obtained and the several GenBank submissions for *Macrobrachium* species from around the world was done. The sequence similarities were high for the Asian, American (north and south) species. One significant finding in the matching of these sequences is the absence of gaps. This is a further confirmation of the correctness of my findings and the unique nature of the CO1 gene which is established as having no insertions or deletions.

Further, the relationship between the Nigerian species and other established species was determined by mining the CO1 gene sequences available on the GenBank and determining the phylogenetic relationship. The North and South American group (*M. brasiliense, M. carcinus* and *M. olfersi*) are closest to the Nigerian group. The Asian group (*M. asperulum* and *M. rosenbergii*) are the farthest in terms of genetic divergence from the Nigerian stock.

The minimum evolution based on the CO1 gene is not different from the neighbour joining. The Nigerian group is shown as being distinct from the South and North American groups and the Asian groups. The Asian species are genetically farthest from the Nigerian species. The Nigerian *Macrobrachium* species are shown to be a distinct group.

Conclusions. After a thorough review of all the findings of this research, it is appropriate and right to conclude that *Macrobrachium vollenhovenii* is a valid species that is found in the tropics, with reported distribution as far as Senegal in the North Western fringes of West Africa. The controversies with *Macrobrachium dux* and *Macrobrachium macrobrachion* are age long and similar to past systematic difficulties reported in several other *Macrobrachium* species found both in Asia and the Americas. The resolution of these controversies will require more finite research methodologies that combine both recently developed morphological and molecular techniques. The use of nuclear genes in addition to mitochondrial genes may also be an option. The development of species specific primers are essential for the resolution of systematic controversies as this research essentially used general decapod primers that are available off the shelf. The determination of species and ecological boundaries based on the larval development patterns is also desirable. The gene order of the mitogenome is the resolution of this controversy can shed more light on the molecular systematics of the Nigerian *Macrobrachium* species.

References

Bauer R. T., 2011 Amphidromy and migrations of freshwater shrimps. I. Costs, benefits, evolutionary origins, and an unusual case of amphidromy. In: New frontiers in crustacean biology. Asakura A. (ed), Koninklijke Brill NV, Leiden, pp. 145-156.

Bray D. M., 1976 A review of two Western Australian shrimps of the genus *Palaemonetes, P. australis* Dakin 1915 and *P. atrinubes* Sp. Nov. (Decapoda, Palaemonidae). Rec West Aust Mus 4:65-84

Chace Jr. F. A., Bruce A. J., 1993 The Caridean shrimps (Crustacea: Decapoda) of the Albatross Philippine Expedition 1907-1910, Part 6: Superfamily Palaemonoidea. Smithsonian Contributions to Zoology 543:1-152

Choy S. C., 1984 On the freshwater Palaemonid prawns from the Fiji Islands (Decapoda, Caridea). Crustaceana 47(3):269-277.

Costa H. H., 1979 The Palaemonidae of the inland waters of Sri Lanka. Ceylon Journal of Science (Biological Sciences) 13:39-64.

Fincham A. A., 1987 A new species of *Macrobrachium* (Decapoda, Caridea, Palaemonidae) from Northern Territory, Australia and a key to Australian species of the genus. Zoologica Scripta 16:351-354.

Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R., 1994 DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3:294-299.

Hall T. A., 1999 BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-98.

Holthuis L. B., 1950 The Decapoda of the Siboga Expedition. Part X. The Palaemonidae collected by the Siboga and Snellius expeditions with remarks on other species. I. Subfamily Palaemoninae. Siboga Expeditie 39a9:1-268.

Holthuis L. B., 1952 The Decapoda of the Siboga Expedition. Part XI. The Palaemonidae collected by the Siboga and Snellius Expeditions with remarks on other species II. Subfamily Pontoniinae. Siboga Expeditie 39a10:1-253.

Inyang N. M., 1981 On the biology of *Macrobrachium felicinum* (Holthius) in the Lower Niger River of Southern Nigeria. Revista Zoologia Africana 98(2):440-449.

Jalihal D. R., Sankolli K. N., Shenoy S., 1993 Evolution of larval developmental patterns and the process of freshwaterization in the prawn genus *Macrobrachium* Bate, 1868 (Decapoda: Palaemonidae). Crustaceana 65:365-376.

Johnson D. S., 1973 Notes on some species of the genus *Macrobrachium* (Crustacea: Decapoda: Caridea: Palaemonidae). Journal of the Singapore National Academy of Science 3(3):273-291.

Kimura M., 1980 A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111-120.

McDowall R. M., 1988 Diadromy in fishes: migrations between freshwater and marine environments. Timber Press, Portland, OR, 308 pp.

McDowall R. M., 1992 Diadromy: origins and definitions of terminology. Copeia 1992(1):248-251.

Mullis K., Faloona F., Scharf S., Saiki R. K., Horn G., Erlich H., 1986 Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Symposia on Quantitative Biology 51(1):263-273.

Munasinghe D. H. N., 2010 Phylogenetic positions of some species of the genus *Macrobrachium* Bate, 1868 (Crustacea, Decapoda, Palaemonidae) in Sri Lanka. Journal of the National Science Foundation of Sri Lanka 38(3):193-199.

Murphy N. P., Austin C. M., 2005 Phylogenetic relationships of the globally distributed freshwater prawn genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidae): biogeography, taxonomy and the convergent evolution of abbreviated larval development. Zoologica Scripta 34(2):187-197.

Nwosu F. M., Wolfi M., 2006 Population dynamics of the giant African river prawn *Macrobrachium vollenhovenii* Herklots 1857 (Crustacea, Palaemonidae) in the Cross River estuary, Nigeria. West African Journal of Applied Ecology 9:1-14.

Olivier J. T., Conner S. L., Bauer R. T., 2012 Evidence of extended marine planktonic larval development in far-upstream populations of the river shrimp *Macrobrachium ohione* (Smith, 1874) from the Mississippi River. Journal of Crustacean Biology 32:899-905.
Page R. D. M., Holmes E. C., 1998 Molecular evolution: a phylogenetic approach. Blackwell Publishing Ltd., Oxford, UK.
Powell C. B., 1979 Three alpheid shrimps of a new genus from West African fresh and brackish waters: taxonomy and ecological zonations (Crustacea Decapoda Natantia). Revue de Zoologie Africaine 93(1):116-150.
Valencia D. M., Campos M. R., 2007 Freshwater prawns of the genus *Macrobrachium* Bate, 1868 (Crustacea: Decapoda: Palaemonidae) of Colombia. Zootaxa 1456:1-44.
Welcomme R. L., 1979 Fisheries ecology of floodplain rivers. Longman Press, London, 317 pp.
Wowor D., Ng P. K. L., 2007 The giant freshwater prawns of the *Macrobrachium rosenbergii* species group (Crustacea: Decapoda: Caridea: Palaemonidae). The Raffles Bulletin of Zoology 55:321-336.

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