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Genetics

THE GENUS MACROBRACHIUM (CRUSTACEA, CARIDEA, PALAEMONIDAE): A TAXONOMIC SYNOPSIS FROM THE CRADLE OF EVOLUTION IN AFRICA TO ASIA AND THE AMERICAS.

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ABSTRACT The genus Macrobrachium like all other decapod groups is very speciose. This make its systematic fairly cumbersome especially because the major morphological organ used in the classification is dependent on the ambient environment and also very plastic. The congruence therefore between classification based on morphology and molecular markers is an essential new process for deducing valid systematics of members of the group. The 16S rRNA mitochondrial gene is perhaps the most frequently used molecular marker for the systematics of the group. For this research work, we sequenced the three major Macrobrachium species found in Nigeria, namely the Macrobrachium vollenhovenii, Macrobrachium dux and Macrobrachium macrobrahion and used the obtained sequences to mine other sequences that were 95% similar to our sequences to deduce the phylogenetic affinities in fourty four species of Macrobrachium species found in four continents namely Africa, Asia, North and South America. There was conflict between morphological and molecular systematics in the Nigerian species as consistently the Macrobrachium dux and Macrobrachion macrobrachion were grouped together making them at best ecotypes of the same species. Very high affinities also existed between the Asian species as they grouped together consistently using three different methods of phylogenetic inference (maximum likelihood, minimum evolution and maximum parsimony). The African species also mapped closely with the north American species consistently. One very clear conclusion is that the genetic divergence between the grouping is small, evidence that the genus is just evolving. Further research need to include more African species as there is a dearth of sequences from African species in the Genebank.

KEYWORDS :

Members of the genus Macrobrachium are found in almost all clines with the distinguishing morphological characteristics which separate them from other palaemonids being a carapace with a projecting rostrum, telsons which are triangular that terminates in a single tip, mandibles with molar processes that are furnished with a triangular palp, a first pair of pereiopods chelate and slender with length as long as the carapace and a second pair of chelate which are often longer than the entire body in males amongst others. (Mossolini and Bueno, 2003, Holthuis, 1952, Bate, 1868). The presence of the hepatic and antennal spines and two pairs of spines on the dorsal surface of the telson is also a prominent feature. (Hedgpeth, 1949).

They are ubiquitous being found in diverse environments in the Americas, Africa and Asia as a whole. Being very speciose, they adapt well and are widely distributed contributing significantly to aquatic ecology and the food chain. Export of processed Prawns across geographical zones is a veritable income source for shrimping companies and countries. Being very speciose, the continued description of new and valid species strongly indicates there are still many species to be discovered. Macrobrachium's taxonomy has relied on a limited set of external morphological characters that are plastic, dependent on the environment and difficult to determine for the many stages of development. Description based on morphological characters alone has since become fairly difficult, hence an admixture of methods. The variability of key morphological characters is established. Hence distinguishing species across the several developmental stages is fraught with problems. Apart from this, no existing information about the developmental stages of the Nigerian species, whether they have abbreviated or extended larvae development pattern. A few recent studies based on morphometry concluded that there are two groups. Makombu. et.al., (2019) examined seven species that are commonly found in Cameroun and concluded closely that M. vollenhovenii and M.macrobrachion are conspecific while M.dux and M.sollaudii group together.

Delimitation based on molecular technique have therefore in recent times been used to resolve morphologically difficult taxonomies and also determine the phylogeny of species that are circumtropical. The use of the 16S and CO1 genes for the resolution and delimitation of species has rich literature with the Asian species where Macrobrachium has been very successful with speciation. Zheng et. al., (2019) resolved the structure of members of the genus Macrobrachium in the Zaomu Mountain Forest Park, Guangdong Province where Macrobrachium maculatum, M.inflatum, M.nipponsense and an undescribed new species M. laevis coexists in sympatry.

In Nigeria, there are several species that have been described. Majorly however, Macrobrachium vollenhovenii, Macrobrachium dux and Macrobrachium macrobrachion are the three most prominent species. With the very extensive aquatic drainage system that spans several hundreds of kilometers creating several niche types, the Nigerian populations have been expanding. Though Klaus (2013) notes in his research paper that the extant species of Macrobrachium show a world-wide distribution in the tropical belt, with a strongly decreasing diversity in the subtropical regions with only a few species in temperate zones, the "Centre of Origin Hypothesis" assumes that there is a correlation between extant diversity and the time available for diversification. Based on this biogeographical rule, we may therefore localize a tentative geographic center of origin within the tropical belt. This research work sets out to determine the phylogenic phylogeography of members of the genus Macrobrachium world-wide using the multilocus approach based on 16S rRNA which has been used several to determine in bits and pieces their phylogeny worldwide.

2. Materials and methods

2.1 Taxon samplings and Data Set

Fresh specimens for molecular analysis were obtained from field collections. Individuals were collected from fish landings at the various water bodies. Sampling for this study consisted of ten samples each from the four water bodies namely Badagry river 6.40785, 2.89162 (Location 1), Asejire dam 7.36347, 4.13384 (location 2), Warri river 5.51354,5.72986 (location 3) and Calabar river 4.93777, 8.28894 (location 4). For the sampling locations 1 and 2, identification based on morphology was done by an expert in the Fisheries and Aquatic Biology of the Lagos State University, Ojo in Lagos, Nigeria. They were identified as Macrobrachium vollenhovenii while for locations 3 and 4, identification based on morphology was done by Dr Francis Arimoro of the Ambrose Alli University, Ekpoma, Edo State Nigeria. Samples from 3 were identified as Macrobrachium dux while from location 4 were identified as Macrobrachium macrobrachion. My sampling covered also all the water bodies where the species are found in southern Nigeria. Identification in both cases followed criteria as specified by Powell, (1982) and Holthius (1980). With regards to taxon sampling, the two unique sequences obtained from my initial experiment were used as a basis for a GenBank search for all other Macrobrachium species that have 16S gene sequences deposited with

90% similarity to the sequences we obtained. The dataset included a total of two hundred and fifty similar sequences with ninety (90%) index of similarity. These sequences were initially checked for redundancies using DAMBE software. One hundred and fifty-seven (157) unique sequences were retained for further analysis. The dataset included 44 species, all already assigned to species and three other records identified at the genus level. These were analyzed and discussed in this paper.

2.2 DNA extraction and PCR amplification.

Whole samples were taken to the laboratory preserved in 75% alcohol. For DNA extractions, Qiagen DNA extraction kit was used according to the manufacturer's instructions. Total genomic DNA was isolated from the abdominal muscles tissue using the Qiagen kit for DNA extraction according to the manufacturer's instruction. Extracted DNA was quantified using the nano-detector or by running gel and highly concentrated samples were diluted using ddH2O to achieve the optimum concentration for amplification in polymerase chain reactions (~50 - 300 ng/μl).

Polymerase chain reaction (PCR) (Mullis et al.1986), using commercially available primers that are commonly used in decapod systematics. 16SAR (-CGCCTGTTTATCAAAACAT-) as the forward primer and 16SBR (-CCGGTCTGAACTCAGATCACGT-) (Palumbi 1996) as the backward primer. 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 cycles of 30 seconds at 94°C, 40 seconds (annealing) at 50°C and 50 seconds at 72°C elongation, followed by extension 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 25μl 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 run out on a 2% agarose gel, impregnated with Ethidium bromide (Etbr) Agarose gel as the intercalating agent using Ultraviolet light and photographed. Successful aplicons were then purified and sent to the commercial laboratory - TechDragon in Hong Kong for sequencing. Sequence files were viewed, edited and curated using JALVIEW software.

Phylogenetic analysis was based exclusively on the partial sequences of the 16S rDNA gene for the two major species found in Nigeria Macrobrachium vollenhovenii and Macrobrachium dux. To ascertain the genetic affinities of the Nigerian study population to other species of Macrobrachium found around the world, two hundred and fifty sequences were downloaded from the Genbank, of which 157 unique sequences were included in the analysis.

This was used in inferring the global relationship among species. With redundancies removed using DAMBE, One Hundred and Fifty-Seven sequences were retained. Multiple alignments were done and the ambiguous flanking regions were identified and removed with the program JALVIEW to remove tail ends and exploratory sequence analysis including the construction of phylogenetic relationship using MEGA 7.0 (Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods). Phylogenetic trees were constructed using neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods as implemented in MEGA 12. For the ML analysis, to determine the best substitution model to run the ML tree, the dataset was tested for goodness of fit on 24 models of evolution as implemented in MEGA. To assess the robustness of the NJ, MP and ML trees, bootstrapping (Felsenstein,1985) with 500 replicates was conducted.

2.3 Results

Mitochondrial partial 16S gene were either amplified (M. vollenhovenii and M. dux) or harvested from the GenBank for the remaining 42 species. The average sequence length was 462pbs. Accession numbers JQ943725.1, JQ943724.1, JQ943723.1, JQ943722.1, JQ943721.1, JQ943725.1, JQ943724.1, JQ943723.1, JQ943722.1 and JQ943721.1 are sequences from this project that have been verified and submitted to the GenBank. Sequence characterizations such as conserved sites (CS), variable sites (VS), and parsimony-informative sites (PIS), transition/transversion bias and ratio cum the best evolutionary model for the Macrobrachium 16S gene were checked using MEGA 11 (Kumar et al., 2011). There were 350 variable sites and 111 conserved sites. 312 of these sites were parsimoniously informative, 37 were singleton sites. The average nucleotide frequencies were T: 35.4, C: 11.7, A: 28.5 and G: 24.4. The sequences were found to be A+T rich (63.9%), with the estimated transition/ transversion bias (R) is 3,16. The ts/tv ratio is a parameter used in the estimation of phylogeny. Purvis and Bromham, 1997 noted that typically, it is intended to reflect that nucleotide substitutions are not all equally alike among the DNA sequences, so the tv/ts ratio is a very important aspect of modeling sequence evolution, expressing the relative probabilities of different types nucleotide changes, thus it is needed to correct measures of genetic distances. The patterns of molecular evolution can be ascertained by the ratio of ts/tv. Substitution patterns and rates were estimated under the Kimura (1980) 2-parameter model. These scores generally represent the relative ease with which one nucleotide or amino acid may mutate into or substitute for another, and they are used to measure similarity in sequence alignments. Table 1 below shows the maximum likelihood estimate of the substitution matrix. The rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics.

Table 1 Maximum Likelihood Estimate of Substitution Matrix.

	A	T/U	C	G
A	-	3.82	1.28	19.08
T/U	3.14	-	9.15	2.65
C	3.14	27.38	-	2.65
G	22.60	3.82	1.28	

Each entry shows the probability of substitution (r) from one base (row) to another base (column)[1]. For simplicity, the sum of r values is made equal to 100. Rates of different transitional substitutions are shown in bold and those of transversionsal substitutions are shown in italics. The nucleotide frequencies are 28.87% (A), 35.05% (T/U), 11.72% (C), and 24.37% (G). The transition/transversion rate ratios are $k1 = 4.445$ (purines) and $k2 = 5.179$ (pyrimidines). The overall transition/transversion bias is $R = 2.11$, where $R = [A*G*k1 + T*C*k2]/[(A+G)*(T+C)]$.

The Tajima's Neutrality Test was also performed. Table 2 below shows the results.

Table 2 Tajima's Neutrality Test

m	S	Ps	[illegible]	π	D
157	338	0.789600	0.138643	0.103091	-0.833983

Abbreviations: m = numbers of sequences, n = total number of sites, S = Number of segregating sites, Ps = ps /a1, π= nucleotide diversity and D is the Tajima test statistic.

The Tajima neutrality index D is sensitive to population fluctuation and significantly negative values (Tajima D = -0.800983) is observed for recent population expansion which is evident with the genus Macrobrachium (Freeland et al. 2011, Tajima 1989).

The evolutionary model for the 16S genes was also determined as the T92+G+1. It had the lowest Bayesian Information Criterion (BIC score). Phylogenetic trees were constructed using neighbor-joining(NJ), maximum parsimony (MP), and maximum likelihood (ML) methods as implemented in MEGA 7

Phylogenetic Relationships.

The phylogenetic relationship of Macrobrachium species based on the neighbor-joining (NJ) method is summarized in Figure 1. The first major clade consists of Macrobrachium americanum mainly and to a lesser extent M. tolmerum and M. carcinus. This is essentially a clade made up of species found in Mexico and Peru, Florida to the Southeastern coast of America, middle America, South America, the Caribbean, and North America. The largest known neotropical species of freshwater Prawns M. carcinus is also in this clade. The Nigerian species M. vollenhovenii also maps closely in this major clade. The mapping of the Nigerian species close to the American group is of note, especially because of the 93% support for the grouping. The waters of Africa and the Americas are not in any way contiguous. A major explanation for this may be that the species had migrated with the shipping routes a few hundred years ago, hence the similarity. The second major clade also has M. heterochirus, M. ohione, M.

occidentale, M. surinamicum, M. faustinum, M. olfersi, M. digueti, M. dentriculatum , M. crenulatum and M. meridionalis. It is a very diverse clade where ten of the species map. The species M. australiense forms the closest relative of this clade in the third major clade. Other species in the third major clade is the M. lar which all exclusively maps into this clade. The species M. lar is a unique population. There is a fourth clade consisting of M. lanchesteri and M. malcolmsonii. The fifth major clade has M. latidactylus, M. esculentum, M. lanatum, M. neglectum, M. dienbienphuense, M. rosenberghii, M. lanchesteri, M. trompii, M. hirsutimanus and to a large extent M. niphanae. The sixth major clade has majorly the M. asperulum and to a lesser extent M. anhuiense. The M. asperulum is a unique population too. The seventh major clade has the M. amazonicum, M. dux, M.latimanus and M. tenellum.

For the purposes of validation of outcomes in terms of the distribution of species, the maximum likelihood and the maximum parsimony of the dataset was also done. Apart from the location of the clades, the results obtained were essentially the same. The M.asperulum formed the basal clade of Macrobrachium species. They are a unique population, similar to what the neighbor-joining tree gave. The same with the M. lar, which mapped to a single clade, indicating the uniqueness of that population. The Nigerian species, M. vollenhovenii mapped net to next to the M.carcinus group, as seen with the NJ tree. Significantly, M. carcinus, M.americanum, and to a lesser extent M. latidactylus, M. esculentum, M. lanatum, M. hirsutimanus, and M. perspicax all map to the same clade. In all of the analysis (NJ, MP, ML) the Nigerian species (M. vollenhovenii) is sandwiched between the north, mid and the southern american species (M. americanum, M. carcinus, M. heterochirus, M. ohione, M. occidentale, M. fautinum, M.olfersi, M.digueti, M. denticulum, M. crenulatum, M. meridionalis).Macrobrachium lar which is a southern Asian species, it is certainly a unique species that has gone through several generations of evolution with stable genetic makeup. The M. lar group is mapped together in the NJ, ML, and MP phylogenetic tree. This also applies to the M. asperulum group. They are essentially southern Asia based and have gone through several generations of adaptations and evolution to become a unique population. The other Nigerian species the controversial M. dux, maps in all of the analysess closest to the American species of M. amazonicum and M. tenellum, the Eastern Pacific: Middle America and Costa Rica endemic species. The controversy with M. dux and M. macrobrachion subsists. Experts in decapod population genetics based on morphology believe that both species are different. But several molecular research outcome indicates that there are only two species collapsing M. dux and M. macrobrachion as being the same and M. vollehovenii as being the distinct species. The sequence divergence estimates amongst the Macrobrachium species range from 0.02% to 1.959% for the 16S gene. This also agrees with the findings of Chen, Tsai, and Tzeng (2009), who opined that the rapid radiation created numerous taxa within a short time period, hence a small genetic distance range within and between species resulting in severe convergence of the taxa. The latter is between M. asperulum and M. malcolmsonii, both south Asian species. The former being between M. lanchesteri (a south Asian species) and M. amazonicum (a south American species). Another major finding is that multiple samples from distant geographical populations were grouped into species-specific monophyletic groups with high bootstrap support. The Indo Pacific, Indian, Southeast Asia, and northern Australia group of M. lanchesteri, M. rosenbergii, M. trumpii and M.niphanae is also well defined by all (ML, NJ and MP) methods of analysis. The monophyly of the Macrobrachium species clades could also not be confirmed using the 16S data because the outspecies group the Chyphiops caementarius nested in one of the polyphelytic clades made up of M. occidentale, M.ohione and M. heterochirus in the NJ and MP analysis. This is consistent with the findings of Pilegii and Mantelatto (2010). Both authors advocate that given the strong relationship between Cryphiops and Macrobrachium , the phylogenetic position of Cryphiops remains questionable. The genetic distance estimated among species indicates low genetic variability in line with great morphological conservatism. With reference to previous research works aimed at resolving the phylogeny and origin of Macrobrachium species Murphy and Austin (2005), this work has included more species. One major outcome of this is that the phylogeny of Macrobrachium species using the 16S rRNA reveals poorly resolved relationships that lack internal structure with short internal branch lengths in most cases and longer tips among species of Macrobrachium. This outcome is probably due to the weakness as it gets to saturation or by a lack of power of the data to resolve relationships amongst the many taxas (Albertson et al. 1999). Several authors have reported the difficulty of the genus because of phenotypic plasticity of the taxonomically important traits namely the rostrum and the 2nd pereiopod (Holthius,1950) for reasons of both changing very much and gradually during growth. This has led to morphologically similar species being often quite genetically distinct and morphologically dissimilar species being genetically the same as with the Macrobrachium macrobrachion and Macrobrachium dux, which are species found in Nigeria and have been classified as being morphologically distinct but several research findings say the opposite (Sokefun, 2017, Shih, 2009 unpublished). Members of the genus despite being very speciose, they are relatively conserved in general appearance and taxonomic mistakes are commonplace. In this research, we didn't find an admixture in species groups that are from different locations. Another apparent finding is that molecular variation was not wide indicating the occurrence of gene flow. The molecular data here unravels the specific positions of the North and South American groups, the Asian group and the affinity between the African species and the North American species. I recommend amongst others for further research the inclusion of more African species so that more robust phylogenies can be deduced.

REFERENCES.

1. Albertson, R.C, Markert, J.A, Danley, P.D, Kocher, T.D. (1999). Phylogeny of a rapidly evolving clade: the cichlid fishes of Lake Malawi, East Africa. Proc Natl Acad Sci USA, 96: 5107-5110.
2. Bate S. (1868). On a new genus with four new species of freshwater shrimps. Proceedings of the Zoological Society of London 363-368.
3. Felsenstein J. (1985). Confidence Limits on Phylogenies: An Approach Using the Bootstrap. Evolution. Jul;39 (4):783-791.
4. Freeland, J.R., Kirk H, Peterson. S.D (2011). Molecular Ecology, 2nd ed. Wiley-Blackwell, 449 pp.
5. Hedgpeth. J.W (1949). The North American species of Macrobrachium (river shrimp). Sci. 1:28-38.
6. Holthuis L. B. (1952). A general revision of the Palaemonidae (Crustacea Decapoda Natantia) of the Americas II. Occasional Paper Allan Hancock Foundation 12 1 – 332.
7. Holthuis, L.B. (1980). FAO Species Catalogue. Vol. 1 Shrimps and prawns of the world. An annotated catalog of species of interest to fisheries. FAO Fish. Synop. 125(1):271p.
8. Kimura M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111-120.
9. Klaus. A (2013). Neotropical Macrobrachium (Caridea: Palaemonidae): On the Biology, Origin, and Radiation of Freshwater-Invading Shrimp, Journal of Crustacean Biology, Volume 33, Issue 2, 1 March. Pages 151–183.
10. Kumar S., Stecher G. and Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870-1874.
11. Makombu, V. F., Stomeo, P.M., Oben, E., Tilly, O. O Stephen… -, (2019) Morphological and molecular characterization of freshwater prawn of genus Macrobrachium in the coastal area of Cameroon. Ecology and Evolution. John Wiley and Sons., Ltd
12. Mossolini. E, and Bueno S. L. (2003). Relative growth of the second pereiopod in Macrobrachium olfersi (Wiegmann, 1836) (Decapoda, Palaemonidae). Crustaceana. 76 363- 376.
13. Mullis. K, Faloona. F, Scharf. S, Saiki. R., Horn. G., Erlich. H. (1986). Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harb Symp Quant Biol. 51 Pt 1:263-73. doi: 10.1101/sqb.1986.051.01.032.
14. Palumbi, S.R. (1996). Nucleic acids II: The polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK (eds) Molecular systematics. Sinauer Associates, Inc, pp. 205–247.
15. Pileggi. L.G.A and Mantelatto. F.L (2010). Molecular phylogeny of the freshwater prawn genus Macrobrachium (Decapoda, Palaemonidae), with emphasis on the relationships among selected American species.
16. Powell, C. B. (1982). Fresh and brackish water shrimps of economic importance in the Niger Delta. Proc. 2nd. Ann. Conf. Fish. Soc. Nigeria, 254 – 285.
17. Purvis, A and Bromham. L. (1997). Estimating the transition/transversion ratio from independent pairwise comparisons with an assumed phylogeny. J. Mol.Evol.44: 112-119.
18. Tajima. F. (1989). Genetics, 123:585-595.
19. Tajima. F. (1989). The effect of change in population size on DNA polymorphism. Genetics 123: 597–601.
20. Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101:11030-11035.
21. Tamura. K, Stecher. G and Kumar. S (2021). MEGA 11: Molecular Evolutionary Genetics Analysis version 11. Molecular Biology and Evolution (https://doi.org/10.1093/molbev/msab120).
22. Zheng, X. Z., Chen, W. J., & Guo, Z. L. (2019). The genus Macrobrachium (Crustacea, Caridea, Palaemonidae) with the description of a new species from the Zaomu Mountain Forest Park, Guangdong Province, China. ZooKeys, 866, 65–83. doi:10.3897/zookeys.866.32708.