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Mismatch between molecular (mtDNA) and morphological classification of *Macrobrachium* prawns from Southern Nigeria: Cryptic freshwater species and brackish water morphotypes

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ABSTRACT

With a wide distribution across brackish and freshwater habitats in West African coastal regions, the giant prawns, *Macrobrachium vollenhovenii* and *Macrobrachium macrobrachion*, are potential candidates for aquaculture in the region. Here, we present the first molecular investigation of the phylogeography and systematics of these prawns. Morphological analyses unambiguously classed individuals into two clusters corresponding with the recognized species. However, phylogenies based on 3 mitochondrial DNA regions (CO1, 16S rRNA,12S rRNA) consistently recovered two highly divergent clades. One clade comprised all individuals from two geographically distant upstream (freshwater) populations of *M. vollenhovenii*, the other all individuals from brackish water sites, comprised of both morphospecies. Within mtDNA clades, there was no apparent genetic differentiation between morphospecies or geographic location, which is most consistent with gene flow through human-mediated translocation. Our results indicate a cryptic *Macrobrachium* species which appears to be adapted to freshwater conditions and therefore highly suitable for freshwater aquaculture. Further investigations are required to determine whether the existence of two apparent morphospecies in brackish water results from intraspecific polymorphism, recent speciation or extensive hybridization.

1. Introduction

Freshwater prawns are important for commercial fisheries and aquaculture, with an estimated annual global production of around 300,000 t in 2001 (New, 2003), reportedly all belonging to *Macrobrachium*, the largest genus in the family Palaemonidae (New, 2002), with about 200 species so far identified (Jayachandran, 2001). Most species require brackish water during the initial stages of their life-cycle, and so are found in water that is directly or indirectly connected to the sea (New, 2003). However, populations of a few species, including *Macrobrachium nipponense* (Kutty, 2005), *Macrobrachium australiense* (Cook et al., 2002) and *Macrobrachium vollenhovenii* (Anetekhai, 1986; Marioghae, 1990) are believed to complete their entire life cycle in freshwater.

Macrobrachium are distributed worldwide in tropical and subtropical regions (New and Singholka, 1985) and occur throughout West Africa (Etim and Sankare, 1998), with four species reported from Nigeria (Bello-Olusoji et al., 2004): *M. vollenhovenii* — the African river prawn, *Macrobrachium macrobrachion* — the brackish water prawn, *Macrobrachium felicinum* — the Niger river prawn and *Macrobrachium dux* — the Congo river prawn. Despite their common names, all species are known to occur in brackish waters. These species can be reliably distinguished on the basis of their morphologies (Marioghae, 1982, 1987, 1990; Meye and Arimoro, 2005; Powell, 1982). Only the larger *M. vollenhovenii* and *M. macrobrachion* are considered to be of economic importance.

Although both *M. vollenhovenii* and *M. macrobrachion* are potentially suitable for aquaculture, nothing is known of the distribution of genetic variability within and among natural populations, or of the phylogenetic relationships among species. Such questions are of interest for aquaculture because genetically isolated populations might also differ in quantitative traits (e.g. growth rate, size of maturity), or they might be adapted to different environments.

Both species occur in a number of drainage systems with limited potential for migration between them. Such geographical isolation is typical for freshwater organisms often leading to pronounced genetic

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population structure (Hänfling and Brandl, 1998; Liu et al., 2011; Sharma and Hughes, 2009; Ward et al., 1994). Furthermore, *M. vollenhovenii* occupies a wide range of habitats ranging from coastal brackish water to upstream riverine environments, providing the potential for local adaptations.

Genetic studies using mitochondrial DNA (mtDNA) have proven useful in addressing such questions (Avise, 1998, 2000; Murphy and Austin, 2005) and have been employed in a number of studies of crustaceans (e.g., de Bruyn et al., 2004a,b; Mather and de Bruyn, 2003; Murphy and Austin, 2004a,b; Pileggi and Mantelatto, 2010; Trontelj et al., 2004).

This study aims to investigate whether genetically distinct populations of *M. vollenhovenii* and *M. macrobrachion* exist in Nigeria in order to provide information for the selection of aquaculture stocks and insights into the evolution of the genus *Macrobrachium*. We employed mtDNA sequence analyses of the CO1 and 16 s and 12S rRNA regions and morphological analysis to examine relationships between a number of freshwater and brackish water populations from different river systems in southern Nigeria.

2. Materials and methods

2.1. Sample collection and transportation

Using baited non-return valve traps, samples of *M. vollenhovenii* and *M. macrobrachion* were collected from five sites in the southern region of Nigeria (Fig. 1 and Table 1): Asejire Lake and Ebonyi River are freshwater habitats from different river catchments with no freshwater connections between them; the remaining three sites are brackish water habitats, with two being from the same catchment. *M. vollenhovenii* was collected from all sites, but *M. macrobrachion* was found only at the three brackish water sites (Table 1). Fresh samples were preserved, at the point of collection, in 98% ethanol, transported to the laboratory and then stored at −20 °C.

Table 1
Details of sampling localities and sample sizes.

Sample site	Site code	River catchment	Ecology	Lat.	Long.	Sample Size (M/F)	
						MV	MM
Asejire Lake	A	Oyo	Freshwater	7° 22′	4° 08′	10/20	–
Badagry Creek	B	Lagos	Brackish	6° 26′	2° 43′	09/21	11/19
River Cross	C	Cross River	Brackish	4° 54′	8° 10′	10/20	10/20
River Ebonyi	E	Ebonyi	Freshwater	5° 58′	7° 59′	02/28	–
River Cross	I	Akwa-Ibom	Brackish	5° 13′	8° 01′	11/19	12/18

M = Male; F = Female; MV = *Macrobrachium vollenhovenii*; MM = *M. macrobrachion*.

2.2. Molecular methods

Three regions of the mitochondrial genome were investigated. The 12S rRNA region has provided resolution of deep divergences as well as among closely-related taxa (Goebel et al., 1999). The 16S rRNA region has been described as one of the most conserved mtDNA sequences (Schubart et al., 2000), useful for elucidating phylogenetic relationships in decapod crustaceans, including a broad range of *Macrobrachium* species (Munasinghe et al., 2003; Murphy and Austin, 2004b; Murphy et al., 2004; Nguyen and Austin, 2005; Pileggi and Mantelatto, 2010). Cytochrome oxidase c subunit 1 (CO1) is a highly variable region, widely used for phylogeographic and phylogenetic studies of more recent events (Liu et al., 2011; Sharma and Hughes, 2009). In the first instance the 12S region of 5–8 individuals per population was sequenced. Subsequently, a subset of individuals representing the major lineages and all populations was sequenced for 16S and CO1 to attempt finer resolution, and to test whether the results were influenced by PCR artifacts or amplification of pseudogenes (Table 2).

Tissue samples were obtained from either the distal segment of the 2nd pair of walking legs (pereiopods) or the tail muscle. Total DNA was extracted using the cetyl-trimethyl ammonium bromide (CTAB) extraction protocol (Doyle and Doyle, 1985). A fragment of

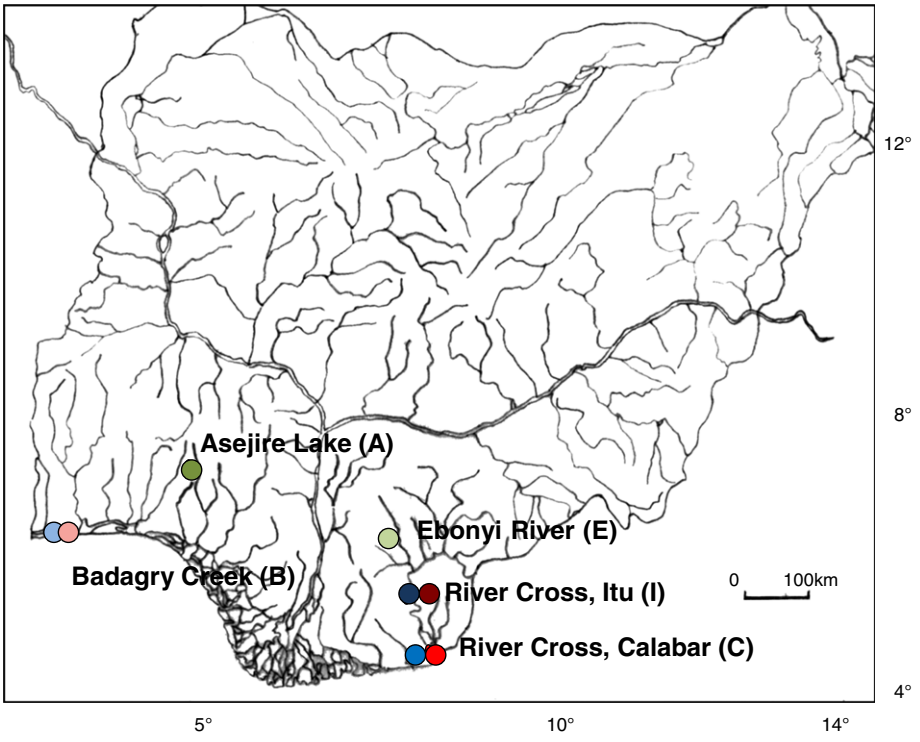

Fig. 1. Map of Nigeria showing sampling sites. Populations are color-coded according to the legend in Fig. 3.