

## Linking genetic lineages of giant freshwater prawn *Macrobrachium rosenbergii* to their global populations

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### Abstract

Globally, giant freshwater prawn, *Macrobrachium rosenbergii* is considered as a commercially important aquaculture species. Genetically, this species is differentiated into Western, Central and Eastern populations. Molecular studies reported population difference in *M. rosenbergii* inhabiting Indian waters and suggested possibilities for the existence of a separate genetic stock in Vembanad Lake (Kerala, S. India). This study was carried out to address this quest regarding the lineages of *M. rosenbergii* inhabiting this lake. Since the lake is divided into 'downstream' and 'upstream' parts, *M. rosenbergii* samples were collected from both these regions and *COI* sequences were developed. Sequence alignment revealed base substitutions for certain samples, indicating possibilities of population difference. AMOVA analysis also provided significant *Fst* value (0.874; *p*-value = 0.00000 +/- 0.00000) confirming population difference in individuals inhabiting the lake. Genetic lineages of these populations towards the global *M. rosenbergii* populations were investigated using developed and acquired *COI* sequences of this species. Phylogenetic tree based on neighbour joining (NJ) analysis and population network revealed presence of western and central populations in Vembanad Lake. This study recommends identification of natural stocks of *M. rosenbergii* inhabiting different geographical realms through genetic approaches.

**Keywords** *Macrobrachium rosenbergii*. Vembanad Lake. *COI*. Population analysis. Genetic lineages.

## Introduction

Giant freshwater prawn *Macrobrachium rosenbergii* (De Man 1879) represents the largest known palaemonid in the world (Munasinghe and Thushari 2010). It is considered as one of the commercially important aquaculture species with global acceptance (Banu and Christianus 2016; Jose et al. 2019). However, for several decades, *M. rosenbergii* was facing considerable disputes related to nomenclature. Lindenfelser (1984) conducted certain systematic studies on the basis of molecular (allozyme) markers and differentiated *M. rosenbergii* populations inhabiting east and west of the prominent biogeographic barrier, Huxley's Line. Single taxon status was also challenged by Hedgecock et al. (1979), who categorized *M. rosenbergii* as three discrete populations (two population groups inhabiting east and west of Huxley's line and a third group inhabiting northern Australia) on the basis of certain morphological differences. At first, individuals distributed along the eastern side of Huxley's Line were accounted as *M. rosenbergii rosenbergii* (De Man 1879) while those inhabiting the western region of Huxley's Line were considered as *M. rosenbergii dacqueti* (Sunier 1925). Monotypic status of *M. rosenbergii* remained intact till the renaming of eastern and western 'forms' as *M. spinipes* Schenkel (1902) (previously *M. wallacei*) and *M. rosenbergii* respectively (Wowor and Ng 2007; Ng and Wowor 2011). Later, phylogeography and molecular data were coupled to confirm the eastern group as *M. spinipes* (wild populations inhabiting Philippines, West Irian and South Papua New Guinea (PNG), Northern Australia) and the western group as *M. rosenbergii* (wild populations inhabiting the coastal rivers of Pakistan and northwestern India, tropical and subtropical regions of southeast (SE) Asia and Vietnam) (De Bruyn et al. 2004a 2004b; Hurwood et al. 2014; Rajkumar et al. 2016).

*Macrobrachium rosenbergii*, the western group, emerged as the most economically important freshwater prawn with aquaculture potential within its natural distribution and beyond (Munasinghe and Thushari 2010; Hasnita et al. 2014). It was also introduced in Africa, America, Asia, Europe and Oceania (New et al. 2010). In Asia, *M. rosenbergii* is used for tropical and subtropical aquaculture since 1980s. In India, *M. rosenbergii* is a potential species for inland freshwater prawn farming with its production reported upto 42,870 tons in 2005 followed by a drastic decline in 2010-11 (Rajkumar et al. 2016; Mohanty et al. 2016; Suresh et al. 2015). However, natural diversities within the wild stocks of *M. rosenbergii* were unknown and populations across its full natural range were treated systematically as a single taxon. Intraspecific variations within the wild stocks of this taxon were addressed using subsequent molecular studies. Hurwood et al. (2014) examined molecular diversity of *M. rosenbergii* wild populations across their complete range of

distribution. They performed phylogeographic analyses for identifying diversity in wild stocks of the western forms (Wowor and Ng 2007) for which samples were collected from different stations including northwestern (Gujarat), southwestern (Kerala) and northeastern (Orissa) parts of India. Hurwood et al. (2014) suggested population events in *M. rosenbergii* inhabiting Vembanad Lake since its stock was represented by individuals inhabiting five confluent rivers draining into the lake. However, a study carried out using mitochondrial *ATPase 6/8* gene revealed lack of population difference in *M. rosenbergii* inhabiting Vembanad Lake (Rajkumar et al. 2016).

*M. rosenbergii* inhabiting Vembanad Lake and its confluent rivers has been supporting a lucrative fishery with high potential for export in the form of whole frozen and frozen headless prawns, as well as domestic trade. In the early 1960s, annual average landings of *M. rosenbergii* were recorded nearly to 400 tonnes which dwindled to mere 38 tonnes in the 1980s (Raman 1967; Kurup et al. 1992). This drastic decline in the stock of this species has been attributed to the impact of human interventions in the ecosystem (Kurup and Harikrishnan 2000; Jayachandran and Indira 2010). During the 1990s, the stock showed some improvement with landings accounted upto 129 tonnes followed by a decline (Harikrishnan and Kurup 2001). Recently, Hasnita et al. (2014) recommended the need for assessment of intra and inter population genetic variations in *M. rosenbergii* which will lead to the identification of commercially important traits with higher growth rate, survival instincts and disease resistance which are necessary for high quality broodstock development. In addition, genetic manipulation of stocks owing to the improvement and sustainability of the farming industry has also been suggested. Suburamanian et al. (2015), performed a genetic level approach towards the stock improvement of *M. rosenbergii* inhabiting Vembanad Lake for improving aquaculture production. Bala et al. (2017) also examined the genetic variability in wild and hatchery populations of *M. rosenbergii* using randomly amplified polymorphic DNA markers.

Hurwood et al. (2014) delineated three distinct haplotype sets (i.e., Eastern, Central and Western) in *M. rosenbergii* populations using *COI* gene. They also indicated the possibilities of population events in Vembanad Lake. Since the lake is differentiated into ‘downstream’ and ‘upstream’ regions (Harikrishnan 1997), this study was carried out to investigate the occurrence of population events in *M. rosenbergii* inhabiting both these regions of Vembanad Lake and to find out their lineages towards global populations. For achieving this goal, homologous *COI* regions from *M. rosenbergii* individuals inhabiting Vemaband Lake were sequenced and analyzed.

## Materials and methods

### Sample collection

A total of 101 *Macrobrachium rosenbergii* individuals were collected from fourteen stations (Fig. 1) of Vembanad Lake.

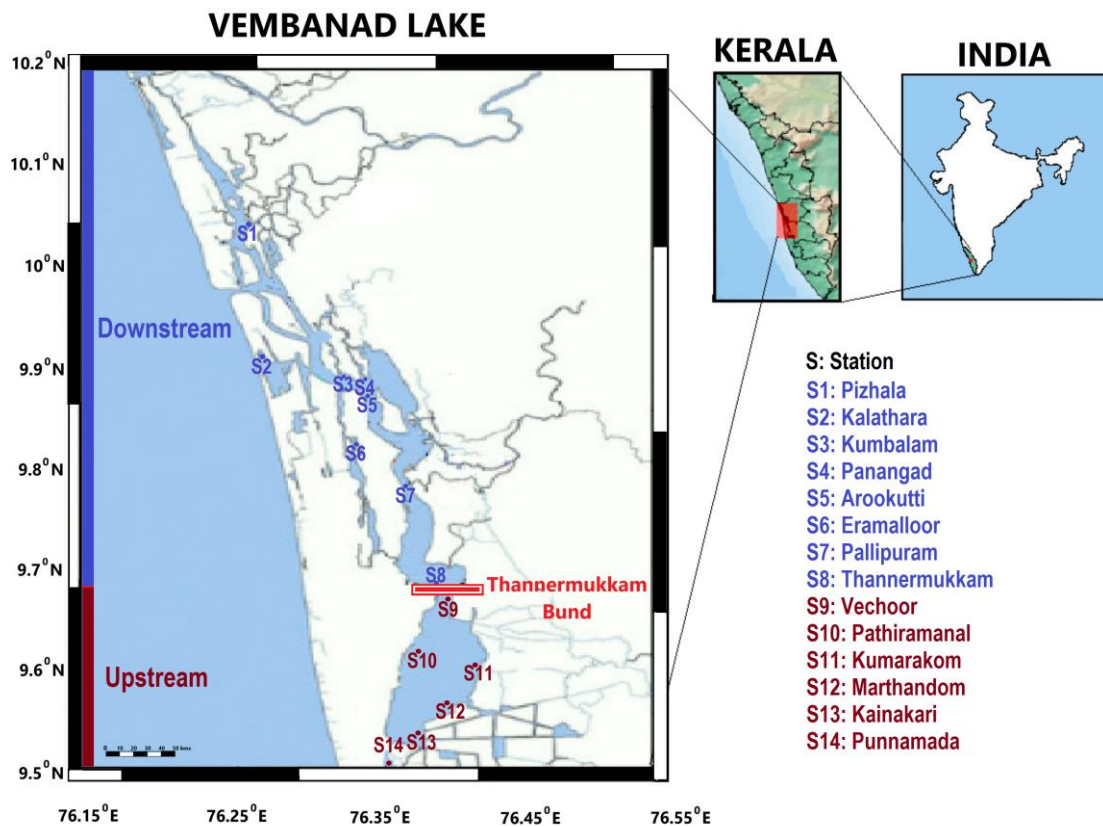


Fig. 1 Sampling locations of Vembanad Lake

Stations 1 to 8 represent locations extending from Cochin to Thanneermukkam ("downstream part" of the lake). 'Upstream' part (region lying between Thanneermukkam and Alappuzha) of the lake is represented by stations 9 to 14 (Harikrishnan 1997). Details regarding sampling locations and collected individuals are given in Table 1.

**Table 1.** Details regarding regions of Vembanad lake, sampling locations, samples collected and *COI* sequences generated for *Macrobrachium rosenbergii* including their accession numbers.

Region of the lake	Station (S)	Sampling station name (Location data)	Individuals collected	<i>COI</i> sequenced	Accession number(s)
Downstream	S1	Pizhala (Lat. 10°05'N; Long. 76°24'E)	5	3	KR871628 MK782915-16
	S2	Kalathara (Lat. 9°92'N; Long. 76°25'E)	5	3	KR871629 MK782919-20
	S3	Kumbalam (Lat. 9°89'N; Long. 76°32'E)	6	3	KR871630 MK782925 MK782930
	S4	Panangad (Lat. 9°87'N; Long. 76°34'E)	6	3	KR871631 MK782927 KR081247
	S5	Arookutti (Lat. 9°86'N; Long. 76°32'E)	6	3	KR081245 MK782921-22
	S6	Eramalloor (Lat. 9°82'N; Long. 76°32'E)	6	3	KR081246 MK782917 MK782923
	S7	Pallipuram (Lat. 9°77'N; Long. 76°37'E)	7	3	MK782924 MK782926 MK782928
	S8	Thannermukkam (Lat. 9°68'N; Long. 76°39'E)	10	5	KP756689 MK782929 KX019980-82
Upstream	S9	Vechoor (Lat. 9°66'N; Long. 76°40'E)	6	3	MK782914 KX019983-84
	S10	Pathiramanal (Lat. 9°62'N; Long. 76°38'E)	6	3	MK782932 KX019985-86
	S11	Kumarakom (Lat. 9°61'N; Long. 76°43'E)	12	6	KP756692 MK782931 KX019987 MK782935-36 MK782938
	S12	Marthandom (Lat. 9°55'N; Long. 76°40'E)	8	4	MK782933 KX019988 MK782939-40
	S13	Kainakari (Lat. 9°53'N; Long. 76°38'E)	8	4	MK782918 MK782941-43
	S14	Punnamada (Lat. 9°51'N; Long. 76°34'E)	10	5	MK782934 MK782937 MK782944-46

## Genotyping

Genomic DNA was isolated using DNeasy Blood and Tissue Kit (Qiagen) following spin column protocol for purification of total DNA from pleopods of collected specimens. Quality and quantity of DNA extracts were analyzed using UV spectrophotometer (Nanodrop 2000). Ratio of absorbance between 260 nm and 280 nm (Optical density (OD) at 260/280 ratio) was measured to analyze the quality of DNA isolates. Value(s) between 1.7 and 2.0 were selected (data not provided) which indicated good quality.

Partial mitochondrial *COI* gene sequences were amplified from genomic DNA using Sigma Aldrich ReadyMix™ Taq PCR Reaction Mix with MgCl<sub>2</sub> in Corbett gradient thermal cycler. *COI* region was amplified using LCO1490-HCO2198 crustacean primer (Folmer et al. 1994) and thermal profile according to Costa et al. (2007). The temperature profile consisted of an initial denaturation for 4 min at 95 ° C; followed by 10 cycles of denaturation at 95 ° C for 40 s, 40 ° C (annealing temperature) for 40s, extension at 72 ° C for 40 s; 25 cycles of 95 ° C for 40 s, 55 ° C annealing temperature for 40 s, 72 ° C for 40 s; and a final extension step at 72 ° C for 8 min. Amplicons exhibiting intense bands after agarose gel electrophoresis (1.2%) were selected and outsourced for purification and sequencing.

## Nucleotide sequence analyses

Sequences were manually edited and compiled with BioEdit 7.0.9 (Hall 1999) and aligned with Clustal X (Thompson et al. 1997). Primary screening of generated *COI* sequences was done by comparing with Basic Local Alignment Search Tool (BLASTn) annotations of National Centre for Biotechnology Information (NCBI) to assure homology and speciation (Deepak and Harikrishnan 2016). Different genetic parameters were characterized using DnaSP 5.10 (Librado and Rozas 2009) and Arlequin 3.1 (Excoffier et al. 2005). The hierarchical AMOVA (analysis of molecular variance) was performed to assess the genetic differentiation within *COI* sequences (Steinert et al. 2012). Details pertaining to the *COI* sequences submitted in NCBI are given in Table 1. Molecular analyses were performed using 1000 bootstraps for obtaining phylogenetic tree and pair wise sequence distance data based on Kimura 2-Parameter model using MEGA 5 (Tamura et al. 2011). Population network was generated using PopART (Population Analysis with Reticulate Trees) based on Median joining network (Bandelt et al. 1999; Leigh and Bryant 2015).

## Results

Out of 101 morphologically characterized samples of *M. rosenbergii*, fifty-one samples were selected (approx. 1:2 ratio) and *COI* sequences were generated from them using the mentioned primer pair and protocols. Sequences showing identity within 97% for BLASTn search were selected for molecular analyses (Shen et al. 2016). Sequence length for *COI* ranged from 636 to 657 base pairs. Among the fifty-one aligned sequences, considerable base substitution pattern was observed, revealing possibilities for population level divergence within *M. rosenbergii* individuals. Final alignment resulted in *COI* sequences with 636 base pairs (excluding sites with missing data).

Even though selected samples exhibited similarity in morphology and morphometry, they were differentiated based on molecular data based on *COI* gene. *COI* sequences were differentiated as population 1 (KR871628-31; KR081245-47; KP756689; KP756692; MK782914-MK782934) and population 2 (KX019980-988; MK782935-46) based on base pair substitution pattern. Genetic parameters were analyzed for confirming population level divergence persisting among them and results are detailed in Table 2 and 3.

**Table 2.** Details of genetic parameters inferred from *COI* sequences of *Macrobrachium rosenbergii* populations inhabiting Vembanad Lake

Taxon	<i>Macrobrachium rosenbergii</i>	
	Population 1	Population 2
Number of Sequences	30	21
Alignment length (excluding gaps)	636	636
Number of monomorphic sites	630	634
Number of polymorphic sites	6	2
Number of haplotypes	6	2
Haplotype diversity ( <i>Hd</i> )	0.586 +/- 0.098	0.467 +/- 0.072
Nucleotide diversity ( $\pi$ )	0.0089 +/- 0.0049	0.0015 +/- 0.0012
Base frequency (%)	A	26.87
	C	25.88
	G	19.47
	T	27.78
		26.52
		25.89
		19.39
		28.20

Within the first population, 630 monomorphic sites and 6 polymorphic sites accounted for 6 haplotypes. Haplotype 1 included three individuals from station 3 (KR871630, MK782925 and MK782930) and one representative from station 4 (KR081247), while haplotype 2 was represented by single individuals each from stations 9 (MK782914) and 10 (MK782932). Within the 3<sup>rd</sup> haplotype, two individuals (KR871631 and MK782927) inhabiting station 4 were present. Individuals from station 1 (KR871628; MK782915-16), station 2 (KR871629; MK782919-20), station 5 (KR081245; MK782921-22), station 6 (MK782923), station 7 (MK782924, MK782926, MK782928), station 8 (KP756689; MK782929), station 11 (KP756692; MK782931), station 12 (MK782933), station 14 (MK782934), constituted the 4<sup>th</sup> haplotype. Fifth haplotype contained individuals from station 6 (KR081246, MK782917) and a single individual from station 13 (MK782918) formed the sixth haplotype within first population. On the contrary, population 2 contained only two polymorphic sites in addition to the 634 monomorphic sites, accounting for two haplotypes (7<sup>th</sup> and 8<sup>th</sup>) only. Haplotype 7 contained 14 individuals viz. two individuals each from station 8 (KX019980-81) and station 9 (KX019983-84), one representative from station 10 (KX019986), three inhabitants of station 11 (KX019987, MK782935-36), two individuals from station 13 (MK782942-43) and four individuals from station 14 (MK782937, MK782944-46). Haplotype 8 contained seven individuals, one from station 8 (KX019982), one each from station 10 (KX019985) and station 11 (MK782938), three from station 12 (KX019988, MK782939-40) and a single individual from station 13 (MK782941). Diversity indices ('*Hd*' and ' $\pi$ ') and base frequencies also showed considerable divergence, supporting the presence of two populations.

#### **AMOVA analysis for obtaining 'Fixation index (*Fst*)' with respect to the population difference within *M. rosenbergii***

Analysis of molecular variance (AMOVA) was carried out to prove the presence of population difference in *M. rosenbergii*. Generated results revealed higher percentage of variation among populations (87.41%) and lower percentage of variation within populations (12.59%). Fixation index (*Fst*) value was 0.874 (*p*-value = 0.00), high enough to support the presence of population difference between population 1 and population 2 of *M. rosenbergii*. Details regarding the AMOVA analysis are given in Table 3.

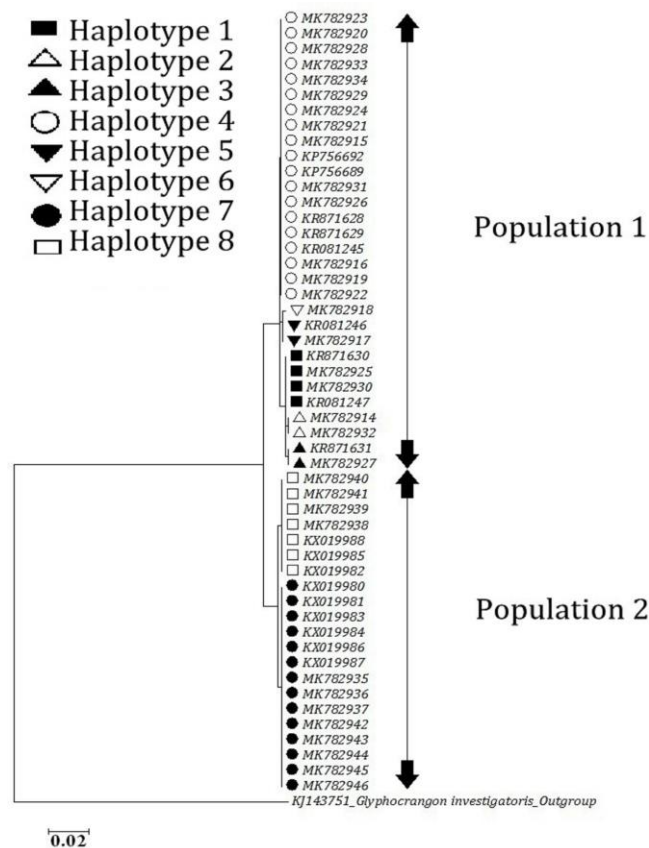


**Table 3.** AMOVA analysis performed for *COI* sequences of *Macrobrachium rosenbergii* populations

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	1	332.143	13.36594 Va	87.41
Within populations	49	94.367	1.92585 Vb	12.59
Total	50	426.510	15.29179	

Fixation Index (*Fst*): 0.87406 (*p*-value = 0.00000 +/- 0.00000)

**Phylogenetic tree (NJ), haplotype network and pairwise genetic distance for *M. rosenbergii* populations**



**Fig. 2** Haplotypes present in two populations of *Macrobrachium rosenbergii* identified from Vembanad Lake

Phylogenetic tree supported previous findings regarding the presence of two different populations of *M. rosenbergii* in Vembanad Lake. Cladistic array represented eight haplotypes

within two populations having two independent lineages (Fig. 2). Identified populations were compared with global *M. rosenbergii* populations using additional *COI* sequences acquired from NCBI (Table 4).

**Table 4.** *COI* sequences acquired from NCBI for population analysis

Taxon	Accession numbers of acquired <i>COI</i> sequences	No. of sequences	References
<i>Macrobrachium rosenbergii</i>	KF499322-KF499355	34	Hurwood et al 2014
	AY554293-AY554327	35	De Bruyn et al 2005
	AY614545-AY614587	43	De Bruyn and Mather 2007
	DQ060194-DQ060208	15	
<i>Glyphocrangon investigatoris</i> (Outgroup)	KJ143751	1	Deepak et al 2014

The relationship between *M. rosenbergii* inhabiting Vembanad Lake and other countries across the globe were investigated through haplotype network based on Median joining analysis (Fig. 3 (a, b, c)).

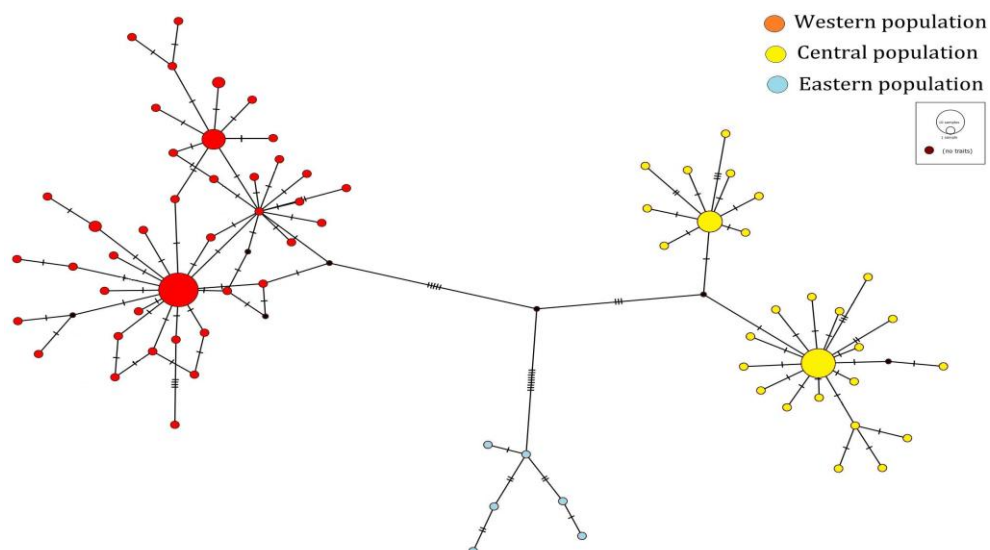


Fig. 3 Haplotype network of global populations of *Macrobrachium rosenbergii*

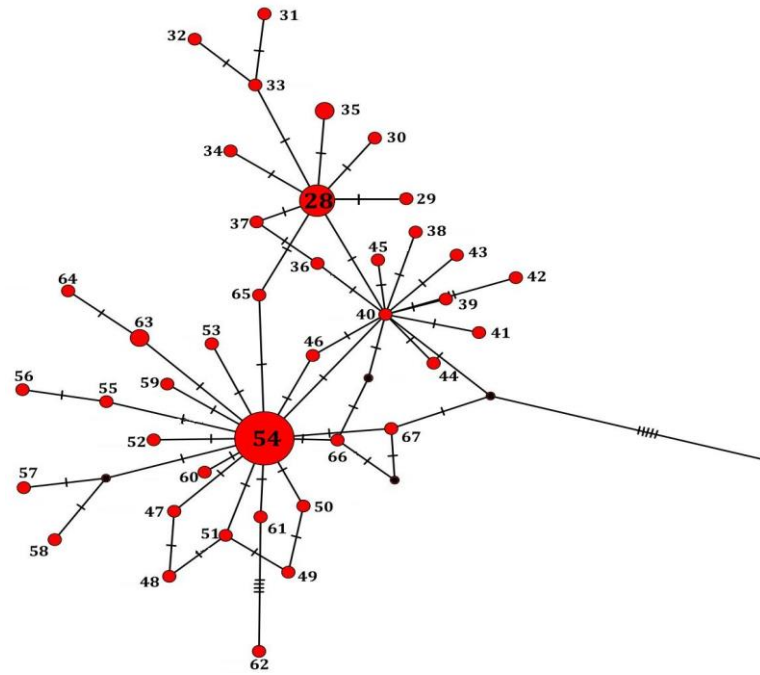


Fig. 3a Haplotype network of Western population of *Macrobrachium rosenbergii* (detailed)

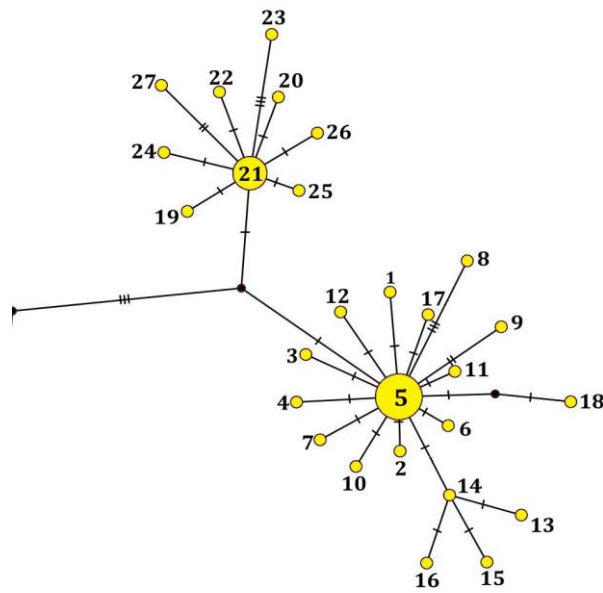


Fig. 3b Haplotype network of Central population of *Macrobrachium rosenbergii* (detailed)

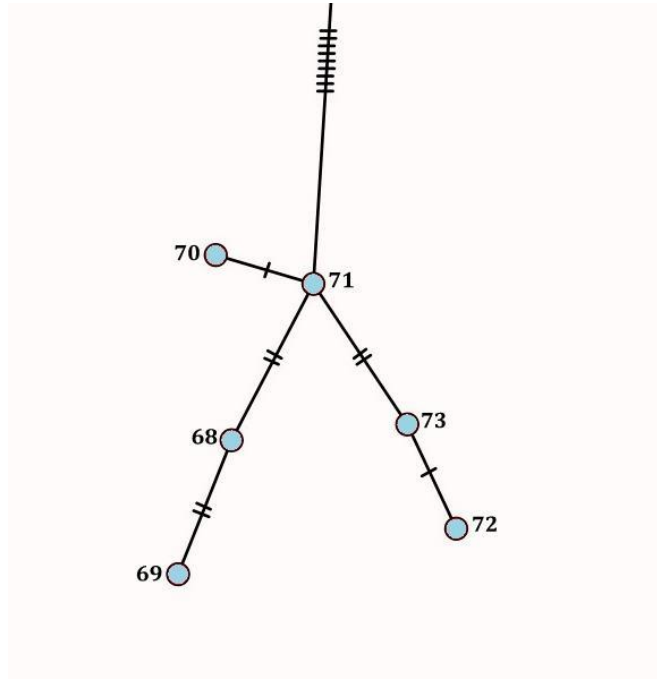


Fig. 3c Haplotype network of Eastern population of *Macrobrachium rosenbergii* (detailed)

Globally, about 73 haplotypes were accounted within the western, central and eastern populations of *M. rosenbergii* (Online Resource). Phylogenetic tree (Fig. 4) based on Neighbour Joining (NJ) analyses using Kimura 2 Parameter model and 1000 bootstrap replications showed array of these haplotypes as three distinct clades in which representatives of population 1 within the lake showed genetic lineage towards the western clade while representatives of population 2 arrayed along with the representatives of central clade. However, both of these populations lacked affinity towards Eastern clade.

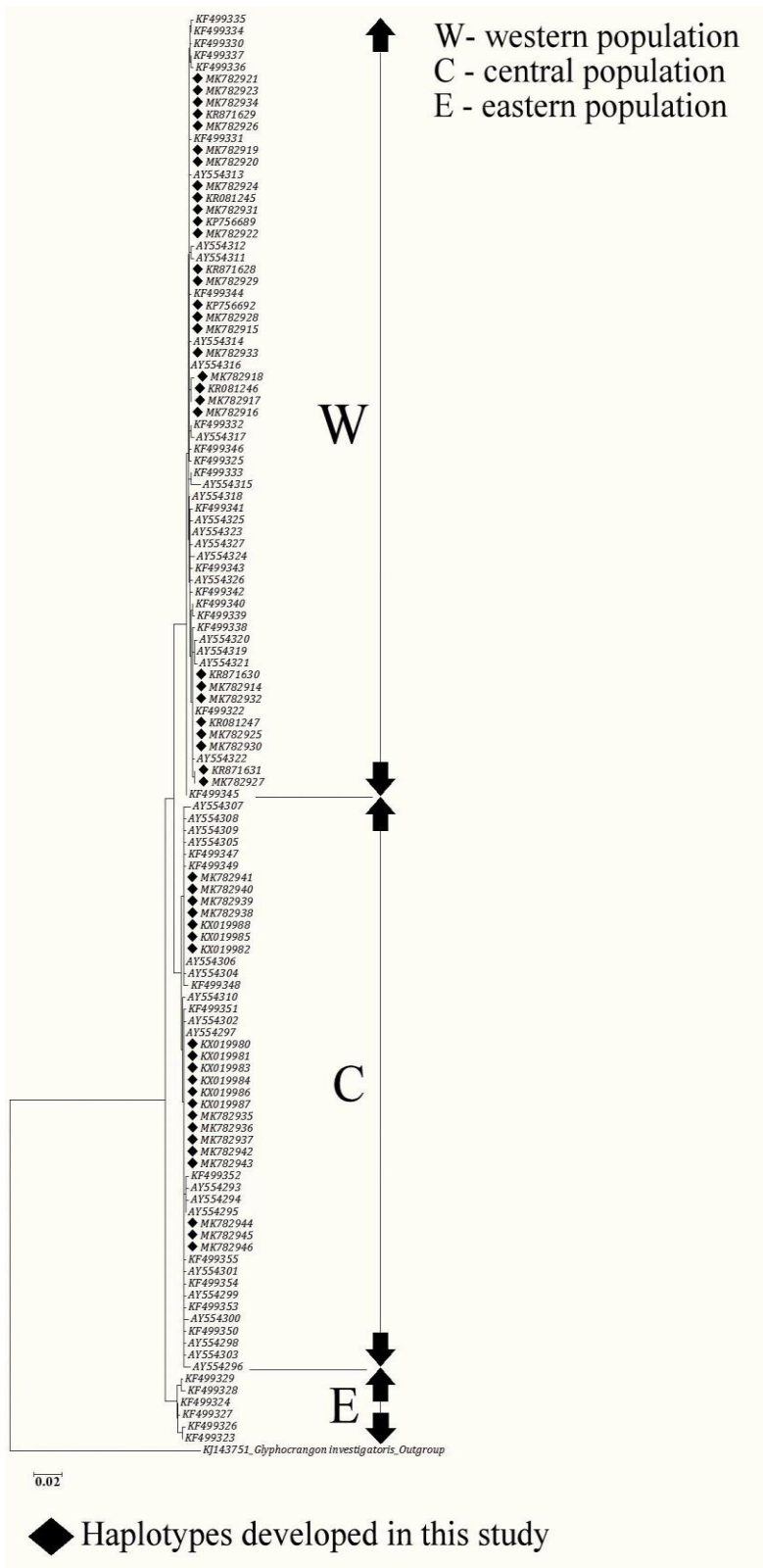


Fig. 4 Phylogenetic tree based on Neighbour Joining analysis for global populations of *Macrobrachium rosenbergii*

Genetic distance between Population 1 and 2 were calculated using pairwise distance (Table 5) which reached up to 2.10%, sufficient enough to confirm the presence of population difference. However, genetic distance was low within each population.

**Table 5.** Genetic distance data obtained from *COI* sequences of *Macrobrachium rosenbergii* populations

Serial Number	Population	Pairwise genetic distance (in %)
1	Within population 1	0.20 to 0.80
2	Within population 2	0.0 to 0.30
3	Among populations 1 and 2	1.80 to 2.10

## Discussion

Molecular markers are used for the assessment of genetic structure and population variability in aquaculture species (Bala et al. 2017; Elsheikh et al. 2015; Liu and Cordes 2004; Mohanty et al. 2016). Previously, fishes were mainly selected for molecular studies and hence, a paucity in such type of studies over other related aquaculture species including *M. rosenbergii* was reported (Mohanty et al. 2016; Sui et al. 2018). Later, Khan et al. (2014) studied the genetic diversity of *M. rosenbergii* inhabiting Bangladesh Rivers (Paira, Naaf and Pashur) using microsatellite DNA and identified moderate genetic variability among its populations inhabiting these rivers which was higher than that in the Indian populations of *M. rosenbergii*. Recently, problems regarding *M. rosenbergii* production in Bangladesh was also studied by Bala et al. (2017) through genetic characterization using randomly amplified polymorphic DNA and their results proposed loss of genetic variations as the reason for lowered productivity. Rajkumar et al. (2016) revealed population structuring in natural stocks of *M. rosenbergii* inhabiting Indian waters using *ATPase 6/8* region and proposed lack of population diversity among individuals from adjacent rivers like Mahi, Narmada, and Tapti; Vembanad Lake and Bharatpuzha; Hoogly and Roopnarayan. An extensive differentiation of global *M. rosenbergii* populations as Australian, western, eastern and central groups were also provided (Hurwood et al. 2014; De Bruyn et al. 2005; De Bruyn and Mather 2007). Hurwood et al. (2014) also pointed out the possibilities for combination of individuals belonging to different gene

pools at certain sites and proposed the chance of population differences in Vembanad Lake (Kerala, India), due to the amalgamation of individuals from five river systems draining into the Lake. A recent study by Mohanty et al. (2016) using microsatellite markers, also reported population difference in *M. rosenbergii* from Kerala. However, no attempt has so far been reported to ascertain the population difference among stocks of *M. rosenbergii* inhabiting Vembanad Lake.

Present study investigated the presence of population difference in *M. rosenbergii* using *COI* sequences (since mitochondrial *COI* gene delineates the underlying genetic divergence regarding population difference) and compared them with the global population groups reported by Hurwood et al. (2014). Populations identified from Vembanad Lake could be grouped under the western (population 1) and central (population 2) clades only. However, when combined with haplotypes belonging to global populations, haplotypes of central population of Vembanad lake i.e., population 2 was accounted first followed by the rest of the haplotypes of the lake within population 1 (please refer Online Resource). Within population 1, individuals contained within haplotype 1 (please refer Hap 28 in Online Resource) corroborated with haplotype 36 (KF499322), reported by Hurwood et al. (2014) which was an amalgamation of individuals from South western India (Locality: Kerala); Northeastern India (Locality: Orissa), Northwestern India (Locality: Gujarat), Sri Lanka (Bandattara river), Bangladesh (Meghna River) and Southern Thailand\_West Coast (Kraburi River). Even though Hurwood et al. (2014) reported certain samples from Kerala within this group, their sampling station was different (10°52'N 76°01'E) from ours (refer Table 1). An unexpected genetic congruence was observed between the individual accounted under fourth haplotype within population 1 (please refer Fig. 2 and Hap 54 in Online Resource) and haplotype 24 (AY554316; *M. rosenbergii* inhabiting Mekong River, Vietnam; Geographical location 10°02'N 105°50'E), reported by De Bruyn et al. (2005). Haplotypes 1, 2, 3,4, 5 and 6 (corresponding to Hap 28, 29, 35, 54, 63 and 64 in Online Resource) were accounted within the first population (i.e., western population inhabiting Vembanad lake), while haplotypes 7 and 8 (corresponding to Hap 5 and 21 in Online Resource) were accounted with in second population (i.e., central population inhabiting the lake). Haplotype 4 was found distributed uniformly in entire lake as it was sourced from polyhaline and mesohaline parts of the lake. On the contrary, haplotype3 and 5 (Hap 35 and 63 in Online Resource) was unique in that they could be sourced only from mesohaline regions of the lake (stations 4 and 6), in the downstream part of the lake. Haplotypes 2 and 6 (Hap 29 and 64) belonging to western population were also unique in that they could be sourced only from oligohaline part of Vembanad lake (stations 9, 10 and 13). Similarly, the haplotypes representing central population (7 and 8) could be sourced only from oligohaline upstream part of the lake, south of Thanneermukkam salinity barrier. These haplotypes

revealed no genetic overlaps between them and may be representatives of native stocks of *M. rosenbergii*. However, haplotype 7 (refer Hap 5 in Online Resource) corroborated with haplotype (AY554297; Fifth haplotype according to De Bruyn et al. 2005) from Raimangal River (South West Bangladesh; Geographical location 22°00'N 89°20'E). Haplotype 8 (please refer Hap 21 in Online Resource) exhibited genetic congruence with fourteenth haplotype (AY554306) from Meghna River (South East Bangladesh; Geographical location 22°29'N 91°25'E as reported by De Bruyn et al. 2005). Results obtained from the present study revealed possibilities for population dispersion since haplotypes of Vembanad Lake exhibited genetic congruency with haplotypes inhabiting diverse geographical regions (Western and Central clade).

NJ tree also reflected the findings of haplotype network i.e., inhabitation of *M. rosenbergii* individuals belonging to Western and Central group in Vembanad Lake. In short, the occurrence of two major populations of *M. rosenbergii* (representing Western and Central clades) in Vembanad Lake was confirmed by molecular analyses. Among the identified haplotypes of the lake, hap 7 and 8 were accounted under second (central) population while the majority (hap 1 to 6) contained within the first (western) population. It could be noted that individuals belonging to population 2 was concentrated more towards the upstream region. An exception was observed for individuals sampled from station 8 (Thanneermukkam, which is considered as a border of downstream and upstream region of the lake), where almost equal proportion of individuals representing population 1 and 2 was present. Moreover, individuals representing population 1 seems to be dispersed along the entire lake. Presence of localized population events may result in subpopulations or populations differing from the present. Even though the identified populations did not represent such events, the division of Vembanad Lake as downstream and upstream regions (Harikrishnan 1997) might have resulted in this population difference. It has been reported that the operation of Thanneermukkam salinity barrier resulted in retaining estuarine conditions in downstream part of Vembanad lake (Cochin to Thanneermukkam) and freshwater habitat in the upstream part (Thanneermukkam to Alleppey) (Kurup et al. 1992).

Rivers are important reservoirs of *M. rosenbergii* and riverine populations could be screened for collecting mother prawns for broodstock development in hatcheries. Hurwood et al. (2014) provided a significant categorization to global *M. rosenbergii* populations as four distinct groups and this could be utilized as a reference for identifying populations across the globe. Present study proposes the first approach towards the identification and comparison of populations of *M. rosenbergii* (inhabiting Vembanad Lake) and global populations. A major advantage of this approach



is the characterization of appropriate brooders from Vembanad Lake with desired traits. Vembanad Lake has the potential to provide good quality brood stock for aquaculture industry. The seeds produced from these brood stocks are considered to be more disease resistant with better growth and yield. These qualities make them potential candidates for foreign exchange (US\$ 300 per 100 numbers) (Nair and Salin 2012). Swamy (2003) highlighted the need for quality brood stock based on wild populations for satisfying the annual requirement of freshwater prawn seeds in India. Natural broodstock of *M. rosenbergii* available in Vembanad Lake are transported seasonally to neighboring states like Tamil Nadu and Andhra Pradesh for catering to the requirements of hatcheries (Balamurugan et al. 2005).

Amalgamation of individuals belonging to different gene pools from different locations at certain sites leads to genetic diversities as a result of demographic or genomic effects (Khan et al. 2014; Hurwood et al. 2014). Significant variability in *M. rosenbergii* populations due to high water salinity, local adaptations and existence of geographic barriers was also reported (Bala et al. 2017; Hasnita et al. 2014). However, populations of Vembanad Lake were less influenced by such effects since they were representatives of broad populations rather than localized. Since the populations identified in this study corroborated with the larger population groups inhabiting the natural distribution zones of *M. rosenbergii*, its sustainability could be assured, as the genetic diversity of larger populations are stable when compared to smaller populations (Khan et al. 2014). Improvement and sustainability of the farming industry could be achieved by identifying and manipulating the genetics of the local stocks. In addition, protection of weaker populations and subpopulations may be done through the estimation of their distribution and genetic structure as well as regulation of harvest based on their genetic properties (Utter 1991). Genetic characterization is also important for evaluating the evolutionary potential and sustainability of populations (Koljonen et al. 2002; Reed and Frankham 2003; Sui et al. 2018). Higher genetic variations within populations are utilized for improving production traits like growth rate, viability, disease and stress resistance as well as fecundity. Hence, conservation of genetic variation is important for efficient fisheries management programs (Halim et al. 2021; Khan et al. 2014; Mohanty et al. 2016; Sui et al. 2018).

The knowledge of the genetic variability in Indian populations could be potentially used in the future research to select desired populations prior to genetic improvement programme since a narrow genetic base could support present and future studies in improving desirable phenotypic traits. We suggest a more comprehensive approach towards the populations of *M. rosenbergii* for understanding the nature of geographical distribution of this species that could be applied for

conservation of wild genetic resources. Aquaculture interest of *M. rosenbergii* across the globe demands the necessity for identification of population difference since geographically diverse wild populations are screened for broodstock development in culturing programs (New et al. 2010; Rajkumar et al. 2016; Hasnita et al. 2014; Khan et al. 2014; Bala et al. 2017).

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### **Data availability statements**

The data underlying this article are available in the article and in its online resource.

### **Disclosure statement**

The authors declare responsibility for the entire contents of this paper and have no conflicts of interest.

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	Haplotype	No. of sequences	Accession number(s)
CENTRAL (POPULATION 2)	Hap 1	1	[KF499355]
	Hap 2	1	[KF499354]
	Hap 3	1	[KF499353]
	Hap 4	1	[KF499350]
	Hap 5 (H7)	15	[AY554297, KX019980*, KX019981*, KX019983*, KX019984*, KX019986*, KX019987*, MK782935*, MK782936*, MK782937*, MK782942*, MK782943*, MK782944*, MK782945*, MK782946*]
	Hap 6	1	[AY554298]
	Hap 7	1	[AY554299]
	Hap 8	1	[AY554296]
	Hap 9	1	[AY554300]
	Hap 10	1	[AY554301]
	Hap 11	1	[AY554303]
	Hap 12	1	[AY554302]
	Hap 13	1	[KF499352]
	Hap 14	1	[AY554295]
	Hap 15	1	[AY554293]
	Hap 16	1	[AY554294]
	Hap 17	1	[KF499351]
	Hap 18	1	[AY554310]
	Hap 19	1	[KF499349]
	Hap 20	1	[KF499347]
	Hap 21 (H8)	8	[AY554306, KX019982*, KX019985*, KX019988* MK782938*, MK782939*, MK782940*, MK782941*]
	Hap 22	1	[AY554305]
	Hap 23	1	[AY554307]
	Hap 24	1	[AY554309]
	Hap 25	1	[AY554308]
	Hap 26	1	[AY554304]
	Hap 27	1	[KF499348]
WESTERN	Hap 28 (H1)	5	[KR871630*, KF499322, KR081247*, MK782925*, MK782930*]
	Hap 29 (H2)	2	MK782914*, MK782932*
	Hap 30	1	[AY554322]
	Hap 31	1	[AY554321]
	Hap 32	1	[AY554320]
	Hap 33	1	[AY554319]
	Hap 34	1	[KF499338]
	Hap 35 (H3)	2	[KR871631*, MK782927*]
	Hap 36	1	[KF499340]

EASTERN	Hap 37	1	[KF499339]
	Hap 38	1	[KF499343]
	Hap 39	1	[KF499341]
	Hap 40	1	[AY554323]
	Hap 41	1	[AY554327]
	Hap 42	1	[AY554324]
	Hap 43	1	[AY554326]
	Hap 44	1	[AY554325]
	Hap 45	1	[KF499342]
	Hap 46	1	[AY554318]
	Hap 47	1	[KF499337]
	Hap 48	1	[KF499336]
	Hap 49	1	[KF499335]
	Hap 50	1	[KF499334]
	Hap 51	1	[KF499330]
	Hap 52	1	[KF499344]
	Hap 53	1	[KF499331]
	Hap 54 <b>(H4)</b>	20	[KR871628*, KR871629*, AY554316, KR081245*, MK782924*, MK782926*, MK782931*, MK782929*, MK782934*, MK782933*, MK782928*, MK782923*, MK782922*, MK782921*, MK782920*, KP756689*, KP756692*, MK782915*, MK782916*, MK782919*]
	Hap 55	1	[KF499332]
	Hap 56	1	[AY554317]
	Hap 57	1	[AY554312]
	Hap 58	1	[AY554311]
	Hap 59	1	[AY554313]
	Hap 60	1	[AY554314]
	Hap 61	1	[KF499333]
	Hap 62	1	[AY554315]
	Hap 63 <b>(H5)</b>	2	[KR081246*, MK782917*]
	Hap 64 <b>(H6)</b>	1	[MK782918*]
	Hap 65	1	[KF499325]
	Hap 66	1	[KF499346]
	Hap 67	1	[KF499345]
Hap 68	1	[KF499329]	
Hap 69	1	[KF499328]	
Hap 70	1	[KF499327]	
Hap 71	1	[KF499324]	
Hap 72	1	[KF499326]	
Hap 73	1	[KF499323]	

(\*Represent haplotypes developed in this study)



**Supplementary material 1. Haplotype data table for all the incorporated individuals.  
Haplotype numbers of *M. rosenbergii* populations developed for this study is given  
inside brackets in bold.**