PHYLOGENETIC RELATIONSHIPS IN SOME SPECIES OF THE GENUS *MACROBRACHIUM* BASED ON NUCLEOTIDE SEQUENCES OF THE MITOCHONDRIAL GENE CYTOCHROME OXIDASE I

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ABSTRACT

A phylogeny for four species of the freshwater prawn genus *Macrobrachium* (*M. carcinus, M. olfersii, M. acanthurus* and *M. rosenbergii*) based on the partial nucleotide sequence of the mitochondrial cytochrome c oxidase subunit I gene is presented. The results are consistent with previous studies based on morphological data.

I. INTRODUCTION

The genus *Macrobrachium* comprises a group of approximately 250 species distributed in the tropics worldwide, they are very common in freshwater rivers and one species, *Macrobrachium rosenbergii* (De Man) is cultivated worldwide. The alpha taxonomy of the genus as well as that of the whole subfamily Palaemoninae has remained without major changes after the monographic studies of Holthuis (1950, 1951, 1952), who combined several subgenera within the single genus *Macrobrachium*. Regarding the phylogenetic relationships within the genus, some authors have recognized several species groups, but no formal action has been taken to reorganize the genus so that it reflects phylogenetic relationships. Pereira (1997), based on previous work (Pereira 1989) showed a cladogram of the

subfamily Palaemoninae in which the most obvious conclusion was that the subfamily shows different degrees of paraphyly at the subfamily and generic levels (Fig. 1). However, further corroboration is necessary in order to improve this phylogenetic hypothesis towards a more stable model. Molecular systematics may provide further evidence independent of previous morphological analysis that would be of great help in order to improve our understanding of phylogenetic relationships among the group.

The objective of the present study is to determine the partial nucleotide sequence of the mitochondrial cytochrome c oxidase subunit I gene (COI) of representative species of *Macrobrachium* to begin a genetic database of palaemonid shrimps and to establish phylogenetic hypothesis to compare it with previously published phylogenies based on morphological characters.

II. MATERIALS AND METHODS

Four species of freshwater shrimps in the family Palaemonidae (*Macrobrachium carcinus* (L), *M. olfersii* (Wiegmann), *M. acanthurus* (Wiegmann) and *M. rosenbergii* (De Man)) and one species in the family Atyidae (*Potimirim potimirim* (Müller)) were selected. Additionally, five taxa whose sequences were obtained from

GenBank were included (*Litopenaeus vannamei* (Boone), *Artemia franciscana* (L), *Daphnia pulex* (Leidig), *Anopheles gambie* (Giles) and *Drosophila yakuba*).

The laboratory methods used in this study followed Hillis et al. (1996). DNA was extracted from muscle tissue and visualized in agarose gels. mtDNA fragments were amplified ·by the polymerase chain reaction (PCR) using tested primers for COI (Table 1)

Table 1. *Sequence information for oligonucleotide primers (Simon et al. 1994, Folmer et al.1994).*

A2963	5' AGGTAGTTCTTCATTATAIGAATGTTC 3'
A2771	5' GGATAA/GTCAGAA/GTAACGTCGA/TGG/TGGTATA/C 3'
S1718	5' GGAGGATTTGGAAATTGATTAGTTCC 3'
S1991	5' GTAATTAATATACGACCTAAAGG 3'
LCO1490	5' GGTCAACAAATCATAAAGATATTG 3'
HCO2198	5' TAAACTTCAGGGTGACCAAAAAATCA 3'

with the following thermal cycle protocol (40 cycles): 30 sec at 94 °C (DNA denaturing); 30 sec at 50 °C (primer annealing); 60 sec at 72 °C (primer extension). The product of this reaction was purified after being run on an agarose gel (QIAquick columns, Qiagen, Valencia, CA, USA) and visualized in agarose gel. Sequencing was carried out using DNA automatic sequencing machine (ABI PRISM 370A). The sequences were edited, translated to amino acids of the COI, aligned and translated back to DNA sequences. DNA sequences for related species were obtained fron GenBank. Phylograms were generated using a distance method (Saitou and Nei 1987) and maximum parsimony (MP). Several outgroups were used simultaneously in order to use the overall outgroup parsimony (Madison et al. 1984, Pereira 1997). Phylogenetic trees were generated with the computer program PAUP (Swofford 1998), using the Branch and Bound algorithm excluding 3rd position.

Figure 1. *Partial view of the morphological based cladogram after Pereira (1997). (*)refers to species of* **Macrobrachium** *used in the analysis*
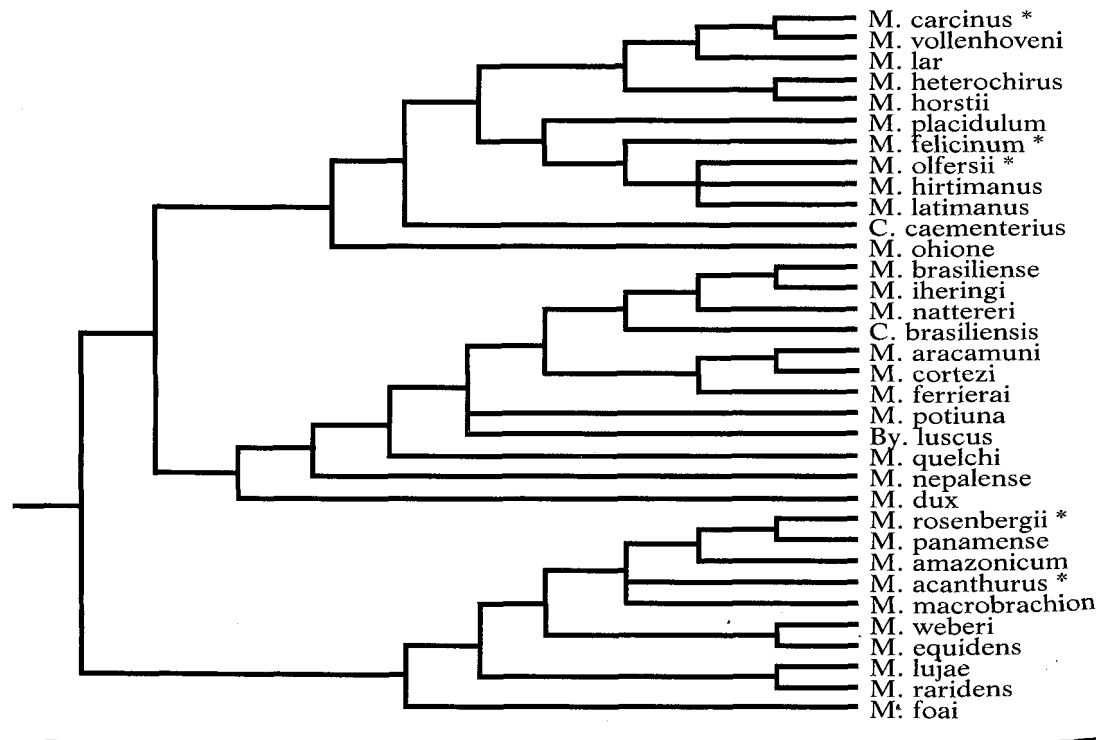

M. carcinus *
M. vollenhoveni
M. lar
M. heterochirus
M. horstii
M. placidulum
M. felicinum *
M. olfersii *
M. hirtimanus
M. latimanus
C. caementerius
M. ohione
M. brasiliense
M. iheringi
M. nattereri
C. brasiliensis
M. aracamuni
M. cortezi
M. ferrierai
M. potiuna
By. luscus
M. quelchi
M. nepalense
M. dux
M. rosenbergii *
M. panamense
M. amazonicum
M. acanthurus *
M. macrobrachion
M. weberi
M. equidens
M. lujae
M. raridens
M. foai

Table 2. *Sequence and alignm*

Potimirim potimirim

?????????????????????????
ATGATCAAATTTACAACGTAWT
GACTGGTCCCACTAATGCTAG(
TTACTATCCAGAGGAATAGTAG,
TCTGTCGACCTTGGTATTTTT
AGAACAGGAATACTAATAGACC(
GGAGCTATTACTATACTTTCAA(

Macrobrachium acanthurus

CTCATCTTGATCTTCGGAKCT(
GAGAAATTATCAGATCTACAACC
ACTGACTACTGCCCCTAATACT,
TCCTTTTATCGAGAGGTATGGT
ATCAGTAGACCTCGGGATTTT(
GTCACCGGGCATAACCATGGA(
CCGGAGCAATTACCATGTTACT.

Macrobrachium olfersii

CTATCTTGGATCTTCGGAGCT
TGGGAATGACCAAATCTACAAC
TAATTGACTAGTCCCTCTAATA(
CTTCTTCTATCCAGAGGAATG(
CCTCAGTTGACCTCGGTATCT
GATCTCCTGGAATAACTATAGA(
TGGAGCTATCACTATGGTTATA,

Macrobrachium carcinus

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TGATCAAATCTACAACGTTATT(
GCTAGTCCCCCTTATACTAGG/
ACTATCTAGAGGAATAGTGGA/
CAGTAGACCTTGGAATCTTTT(
TCACCAGGAATAACCATAGACC
GGAGCCATCACTATATTATTGA,

Macrobrachium rosenbergii

CCCATCTTGGACYTCGGAGCC
TCGGAAATGACCAAATCTACAA(
AATTGACTAGTACCCCTAATATT
TCTTCTCTCCAGAGGAATAGT/
TCGGTAGATCTAGGTATTTTTT
CCCCAGGAATAACTATAGATCG,
AGCCATTACCATACTCTTAACT(

Table 2. *Sequence and alignment of 651 nucleotide for the Cytochrome C Oxidase Subunit I for five species used in the analysis.*

Potimirim potimirim

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??????????????????????????????GAATAGTAGGAACTGCCCTAAGTCTCTTAATTCGAGCTGAACTAGGTCAGCCAGGCAGACTAATTGGGA
ATGATCAAATTTACAACGTAWTTGTTACAGCCCACGCCTTCGTTATAATCTTCTTCATAGTTATACCGATTATAATTGGTGGATTCGGCAACT
GACTGGTCCCACTAATGCTAGGAGCCCCAGATATGGCTTTTCCCCGGATAAACAACATAAGATTCTGACTTTTACCACCCTCCCTAACTCTT
TTACTATCCAGAGGAATAGTAGAAAGGGGGCGTGGGCACAGGATGAACTGTGTACCCACCCCTTGCTAGAGGAATTGCTCATGCCGGGGCT
TCTGTCGACCTTGGTATTTTTTCACTCCACTTAGCAGGAGTGTCTTCTATCCTAGGGGCCGCTAACTTCATATCTACAGTAATCAATATACGA
AGAACAGGAATACTAATAGACCGGATACCCCTCTTTGTGTGATCTGTCTTTATTACTGCCATTTTATTACTTCTCTCTCTCCCGGTACTAGCC
GGAGCTATTACTATACTTTCAACCGACCGTAATTTAAATACCTCATTCTTTGACCCCGCAGGAAGTGGTGACC?????????????????????
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Macrobrachium acanthurus

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CTCATCTTGATCTTCGGAKCTCGAGCTGGCATATTAAGCACATCTCTTTAAGACTTAATCCGGGCTGAACTAGGGCAGCCAGGCAGACTAAT
GAGAAATTATCAGATCTACAACGTCATTGTCACTGCCCACGCATTCGTGATAATCTTCTTCATGGTTATACCCATCATAATTGGAGGATTCGGTA
ACTGACTACTGCCCCTAATACTAGGTGCCCCCGACATGGCCTTCCCCCGAATAAACAACATGAGATTCTGACTTTTACCCCCATCACTCACAC
TCCTTTTATCGAGAGGTATGGTAGAAAGCGGGGTAGGCACGGGGTGAACTGTATACCCCCCACTAGCAGCCGGGACTGCACATGCAGGAGC
ATCAGTAGACCTCGGGATTTTTCTCATTACACCTCGCAGGTGTTTCTTCCATCCTAGGAGCGGTCAATTTTTATTACCACAGTAATTAACATGCG
GTCACCGGGCATAACCATGGACCGACTCCCCCTATTTGTATGGGCGGTATTCCTGACAGCCATTCTTCTTTTTATTTCCCTTCCTGTATTAG
CCGGAGCAATTACCATGTTACTAACAGACCGAAACCTAAATACTTCCTTCTTTGACCCTGTTGGGGGAGGAGACCCA??????????????????
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Macrobrachium olfersii

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CTATCTTGGATCTTCGGAGCTTGAGCAGGTATAGTCGGCACATCCCTAAGACTCTTAATTCGAGCTGAATTAGGTCAACCCGGGAGACWGAT
TGGGAATGACCCAAATCTACAACGTCATWGTCACCGSWCACGCTTTCGTAATAATTTTCTTCATGGTAATGCCTATCATAATTGGAGGATTCGG
TAATTGACTAGTCCCTCTAATACTAGGAGCACCTGATATGGCCTTCCCCCGAATGAATAACATAAGATTCTGACTCCTGCCCCCCTCTCTAAAT
CTTCTTCTATCCAGAGGAATGGTAGAAAGAGGTGTAGGAACCGGGTGAACCGTGTATCCCCCCCTAGCTGCAGGAACCGCCCACGCCGGAG
CCTCAGTTGACCTCGGTATCTTTTCCCTCCACTTAGCCGGAGTCTCATCAATCCTAGGAGCCGTAAATTTCATTACAACTGTAGTTAACATGC
GATCTCCTGGAATAACTATAGACCGACTACCCCTATTCGTTTGAGCGGTATTATTAACCGCCATTATTGTCGTTCTTTCCCTCCCAGTATTAGC
TGGAGCTATCACTATGGTTATAACTGACCGAAACCTAAATACATCCTTCTTTGACCCTGCCGGGGGGTGGAGACCCAATTTTAT???????????
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Macrobrachium carcinus

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??????????????????????????????????????GCACATCTCTGANACTCTTAATTCGTGCTGAGTTAGGACAACCGGGCAGACTAATCGGAAA
TGATCAAATCTACAACGTTATTGTCACAGCTCACGCGTTCGTAATAATTTTCTTCATGGTAATGCCAATTATAATTGGAGGCTTTGGATATTG
GCTAGTCCCCCTTATACTAGGAGCCCCAGACATGGCCTTCCCGCGAATAAATAATATAAGATTCTGGCTCTTACCTCCCTCTCTAACTCTCCT
ACTATCTAGAGGAATAGTGGAAAGAGGGGTGGGGACAGGATGAACTGTGTACCCCCCTCTAGCAGCAGGAACTGCTCACGCGGGACCTG
CAGTAGACCTTGGAATCTTTTCCCTTCACCTTGCCGGTGTCTCATCTATCCTGGGTGCCGTCAATTTCATCACCACTGTAATTAACATGCGA
TCACCAGGAATAACCATAGACCGGCTACCCCTATTAGTGTGAGTCGTNTTGTTAACAGCAATTTTATTTCTATTATCCCTGCCCGTGTTAGCG
GGAGCCATCACTATATTATTGACAGACCGAAACTCAAATACTGANNNTTTYGACCCAGCAGGNGGAGGCGACCTCAAT??????????????????
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Macrobrachium rosenbergii

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CCCATCTTGGACYTCGGAGCGTGAGCAGGCATGGTAGGTACGTCACTAAGACTCTTAATTCGAGCAGAATTAGGGCAGCCGGGCAGACTGA
TCGGAAATGACCCAAATCTACAACGTAATTGTCACTGCCCACGCATTCGTAATAATTTTTTTCATGGTTATACCGATCATAATTGGTGGTTTCGGT
AATTGACTAGTACCCCTAATATTAGGGGGCCCCAGACATAGCATTCCCACGCATAAACAACATAAGATTCTGACTCCTACCCCCCATCTCTAACACT
TCTTCTCTCCAGAGGAATAGTAGAAAGAGGGGTTGGCACAGGATGAACTGTTTATCCACCACTAGCGGCCGGTACCGCCCACGCCGGGGCA
TCGGTAGATCTAGGTATTTTTTCCCTCCACCTAGCAGGAGTTTCTTCAATCTTAGGGGCTGTCAACTTTATTACCACAGTGATTAACATACGAG
CCCCAGGAATAACTATAGATCGACTGCCCCTATTCGTATGAGCCGTATTTCTAACAGCCATCCTGCTTCTTCTCTCATTACCAGTTTTAGCCGG
AGCCATTACCATACTCTTAACTGATCGAAACCTAAATACATCCTTTTTCGACCCAGCGGGAGGAGGGGACCCTATTCTCTACCGACA????
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L = 869
IC ≈ 0.612
IC excluding uninformative
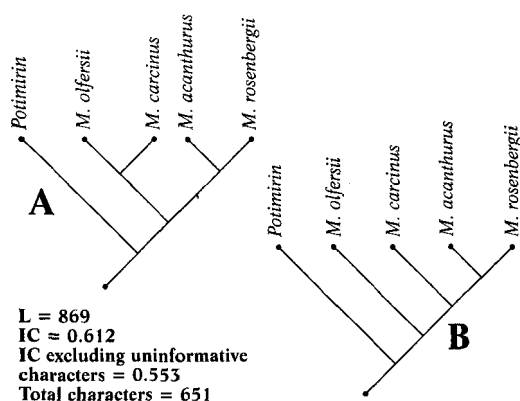characters = 0.553
Total characters = 651

Figure 2. *A: distance based tree (Neighbor-Joining). B: maximum parsimony tree.*

III. RESULTS

We sequenced 651 DNA nucleotide bases at the 5' end of the COI mitochondrial gene (Table 2), for four species of freshwater shrimps in the family Palaemonidae and one species in the Family Atyidae. The results of the distance and phylogenetic analyses are shown using neighbor-joining (Fig. 2A) and maximum parsimony (Fig. 2B). Maximum parsimony resulted in a single tree of 191 steps and a consistency index (CI) of 0.6. The distance based (DB) tree (Fig. 2A) agrees more closely with morphological-based hypotheses. Regarding outgroups, insects cluster together and outside of crustaceans, showing the progressive splitting of *Artemia* and Cladocera, both within the Class Branchiopoda and considered a primitive group; then follow the Decapoda, with *Litopenaeus* appearing first, traditionally considered a primitive genus. Next the representatives of the two caridean families, first *Potimirim potimirim* from the family Atyidae and then all the members of the Palaemonidae, *M. olfersii* and *M. carcinus* in a single cluster and *M. acanthurus* and *M. rosenbergii* in another.

The MP tree (Fig. 2B) does not provide resolution at higher levels, however results within caridean species are almost identical using both methods. When compared with previous phylogenetic trees based on morphological data (Fig. 1), the results are fully consistent. They agree in that Atyidae is the sister group of the Palaemonidae, *M. carcinus* and *M. olfersii* are more closely related to each other as well as the pair *M. acanthurus-M. rosenbergii*. This result agrees with

Pereira (1997), however more species should be included in order to have a more robust hypothesis. We hope that this work will motivate further research on molecular systematics of this important group.

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The descrip
of *Macrobrachium*
rodriguezi, Pereira, 19
The origin and signif
in *Macrobrachium* is

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The shrimp
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far from estuaries. E
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17 species of *Macrob*
occur in South Amer
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1979), *M. jelskii*
(Magalhães, 1989),