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ANTÔNIO VITOR CAMPÊLO RIBEIRO

ANÁLISE DA VIABILIDADE DO GENE RDNA 28S PARA A DELIMITAÇÃO DE ESPÉCIES DO GÊNERO *Macrobrachium*

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Trabalho de Conclusão de Curso apresentado ao curso de graduação em Biotecnologia para obtenção do grau de Bacharel em Biotecnologia; Universidade Federal do Oeste do Pará, Instituto de Biodiversidade e Florestas.

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Conceito:

Data de aprovação: _____/_____/_____

Termo de Aprovação:

_____
Prof. Dr. Gabriel Iketani Coelho – Orientador
Universidade Federal do Oeste do Pará – UFOPA (ICED)

_____
Prof. Dr. Cléo Rodrigo Bressan – 1° Examinador
Universidade Federal do Oeste do Pará – UFOPA (IBEF)

_____
Profa. Msc. Luciana Pimental da Silva – 2° Examinador
Universidade Federal do Oeste do Pará – UFOPA (ICTA)

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RESUMO

Os crustáceos são um grupo importante ecologicamente e economicamente. A ordem dos Decápodes destaca-se por sua diversidade, mas principalmente por sua relevância no mercado, pois, dentro desse grupo estão os indivíduos mais consumidos globalmente. Entretanto, ainda há uma carência de técnicas capazes de fazer análises completas visto que atualmente a identificação é feita principalmente de forma morfológica abrindo margem para dúvidas e classificações errôneas. Uma das possíveis alternativas para sanar esse problema, prover dados seguros e gerar informações confiáveis é aliar diversas abordagens morfológicas, morfométricos, ambientais e moleculares. Com isso, a Biologia molecular começou a explorar o uso de marcadores moleculares como uma possibilidade, porém essa abordagem também enfrenta suas limitações, uma delas é a presença de heteroplasmia e pseudogenes nas sequências de nucleotídeos. Esses fatores podem levar a uma amplificação errônea das bases e consequentemente dados poucos seguros. Uma alternativa para sanar esse problema é fazer uso de regiões mais completas, possuindo porções conservadas e variadas, com isso, começou-se a fazer uso do gene 28S para identificação molecular desse grupo. Este trabalho visou analisar a viabilidade do gene 28S na delimitação molecular de *Macrobrachium* através de análises filogenéticas, cálculos de distância intra e interespecífica e por fim, análise do ABGD. Para isso foram obtidas e triadas 321 sequências do *Genbank* e submetidas as análises acima. O resultado da Árvore e do ABGD mostraram-se positivos, pois o agrupamento foi satisfatório reunindo todas as espécies próximas, os dados provenientes das distâncias nos confirmarem que houve a formação do *barcode gap* necessário para distinção das espécies dentro do gênero. Portanto, foi comprovada a eficiência desse marcador para discriminar espécies, uma vez que as análises se mostraram congruentes com a árvore gênica e com as distâncias inter e intraespecíficas, evidenciando a presença de um barcode que pode ser usado como ferramenta complementar alternativa de identificação para as espécies as quais há dificuldade de separação usando apenas dados morfológicos

Palavras-chave: Crustáceos, 28S e barcode gap

ABSTRACT

Crustaceans are an important ecologically and economically important group. The order of the Decapods stands out for its diversity, but mainly for its relevance in the market, because within this group are the individuals most consumed globally. However, there is still a lack of techniques capable of making complete analyzes, since the identification is mainly done in a morphological way, making room for errors and erroneous classifications. One of the possible alternatives to solve this problem, provide reliable data and generate reliable information is to combine several morphological, morphometric, environmental and molecular approaches. With this, molecular biology has begun to explore the use of molecular markers as a possibility, but this approach also faces its limitations, one of which is the presence of heteroplasmy and pseudogenes in the nucleotide sequences. These factors can lead to an erroneous amplification of the bases and consequently few insurance data. An alternative to cure this problem is to make use of more complete regions, having conserved and varied portions, with that, the use of the 28S gene for molecular identification of this group was started. This work aimed to analyze the viability of the 28S gene in the molecular delimitation of Macrobrachium through phylogenetic analyzes, intra and interspecific distance calculations and, finally, ABGD analysis. For this 321 Genbank sequences were obtained and sorted out and subjected to the above analyzes. The results of the Tree and ABGD were positive, since the grouping was satisfactory, bringing together all the species close to each other. The data from the distances confirmed that the barcode gap was necessary to distinguish species within the genus. Therefore, the efficiency of this marker to discriminate species, since the analyzes were shown to be congruent with the gene tree and with the intra and intraspecific distances, evidencing the presence of a barcode can be used as a complementary tool as an alternative identification the species that are difficult to separate using only morphological data.

Keywods: Crustaceans, 28S and barcode gap

SUMÁRIO

1. Introdução

Os crustáceos pertencem ao filo dos artrópodes, onde encontram-se espécies comestíveis bem conhecidas como os camarões, as lagostas, os caranguejos e os lagostins (Da Silva et al.,2016). Este filo engloba, aproximadamente, 52 mil espécies descritas e, segundo a classificação atualizada de Martin e Davis (2001), contém seis classes, nomeadamente: Branchiopoda, Remipedia, Cephalocarida, Maxillopoda, Ostracoda e a Malacostraca. A ordem Decapoda pertence à classe Malacostraca, se destaca, uma vez que esta reúne cerca de 18 mil espécies e agrupa os indivíduos comestíveis, sendo o maior e mais diferenciado grupo de espécies de crustáceos (HICKMAN et al., 2013; MOLTSCHANIWSKYJ, 2005).

Os camarões, foco deste trabalho, estão distribuídos em duas subordens: Dendrobranchiata e a Pleocyemata, esta, comporta 31 famílias, sendo a família Palaemonidae a segunda maior em número de espécies, distribuídas principalmente nas regiões Oriental e Neotropical do globo (DE GRAVE et al., 2008). A família Palaemonidae compreende quatro subfamílias: Typhlocaridinae, Pontoniinae, Euryhynchinae e Palaemoninae. A primeira ocorre em águas subterrâneas e cavernícolas, da região mediterrânea, e as demais subfamílias estão amplamente distribuídas em corpos fluviais por todo o globo terrestre. Os Pontoniinae são exclusivamente marinhos, normalmente encontrados em corais e são predominantemente comensais. Os Euryrhynchinae são de água doce e ocorrem principalmente em igarapés da região amazônica. Da subfamília Palaemoninae, são conhecidas, até a presente data, sete gêneros, os quais são importantes destacar *Palaemon* e *Macrobrachium*. (BOND-BUCKUP et al.,1994; SANTOS et al., 2015).

Palaemoninae inclui camarões que habitam todos os tipos de ambientes aquáticos, desde o ambiente marinho até as águas estritamente dulcícolas (FERREIRA et al., 2010). A diversidade de ambientes ocupados se repercute na sua ampla distribuição geográfica, o que faz deste grupo um táxon muito representativo e importante, não só ecologicamente, mas também economicamente (MELO, 2003).

Fundamentado em seu valor intrínseco como patrimônio biológico, o camarão deve ser conservado pela importância ecológica compondo a base da cadeia alimentar de

outros seres, além de servirem como vetores intermediários para ciclos de vida de outros indivíduos, bem como seu valor antropogênico, ao sustentar todo um modo de vida (ALHO, 2008).

Economicamente, os camarões são um alimento muito apreciado em todo o mundo e a sua produção global (captura e aquicultura) representa mais de 50% da produção total de crustáceos. A importância crescente da carcinicultura no Brasil tem estimulado o estudo dos camarões-de-água-doce em todos os estados do país. Nos estados da região sul (Paraná, Santa Catarina e Rio Grande do Sul), o desenvolvimento da carcinicultura vem exigindo informações mais precisas sobre as espécies que ocorrem nos diversos ambientes aquáticos continentais da região, porém enfrenta a dificuldade na obtenção precisa de dados, visto que a grande maioria dos trabalhos utiliza apenas dados morfológicos (FILHO e RONÇANI, 2010).

A identificação de crustáceos apenas tendo como base a morfologia enfrenta diversos desafios, um exemplo claro disto, é o ocorre com o gênero *Macrobrachium*, tal condição deve-se ao fato da variação dos caracteres desse gênero como comprimento total, da carapaça, do quelípodo e do própodo quelar. Portanto, entre as alternativas para a resolução deste problema estão o aumento dos caracteres que são avaliados ou a junção de mais técnicas e abordagens que possam prover dados mais completos, aliando informações moleculares com dados filogenéticos e morfológicos. (PILEGGI e MANTELATTO, 2010, PILEGGI et al., 2013 e PILEGGI et al.,2014)

Nos últimos anos, com a aplicação de abordagens moleculares, associadas ao avanço da disponibilidade de dados para os camarões palaemonídeos, evidenciaram-se as limitações das classificações de base morfológica, contribuindo para o processo de delimitação de espécies e estudo das relações filogenéticas para o grupo (PILEGGI e MANTELATTO, 2010; VERGAMINI et al., 2011; ROSSI e MANTELLATO, 2013). Por exemplo, análises filogenéticas com base em dados moleculares realizadas para o gênero *Macrobrachium* evidenciam os reflexos das limitações das classificações morfológicas, além de contribuírem para o processo de identificação e discriminação de organismos morfologicamente similares (LIU et al., 2007, PILEGGI e MANTELATTO, 2010).

O gene mitocondrial Citocromo Oxidase C subunidade I (COI) foi proposto por Hebert et al. (2003) como um marcador para identificação a nível de espécie para diversos organismos. Desde então, vários trabalhos têm confirmado sua eficácia nos mais diferentes animais como mamíferos (NGA et al., 2016), insetos (RAUPACH et al., 2010), aves e peixes (Ward, 2009), surtindo efeito inclusive para a conservação de espécies (Francis et al., 2010). Para crustáceos, Da Silva et al. (2011) e Radulovici et al. (2009) são exemplos de trabalhos robustos e eficientes utilizando a COI.

Entretanto, há presença de heteroplasmia e/ou pseudogene para COI em alguns crustáceos (SONG et al., 2008; BUHAY, 2009). Heteroplasmia é a ocorrência de duas variedades de DNA mitocondrial dentro do citoplasma de uma única célula (KMIEC et al., 2006) o que pode ser causado, por exemplo, por herança de DNA mitocondrial paterno (WHITE et al., 2008). Já os Pseudogenes ou NUMTs, são, segundo Lopez et al. (1994), cópias de genes mitocondriais incorporadas no genoma nuclear através de transposição, e por isso, perderam a capacidade de expressão. Em ambos os casos, o pressuposto da ortologia é violado (sequencias parálogas são inadvertidamente tratadas como ortólogas) levando a inferências incorretas (FUNK e OMLAND, 2003) necessitando, assim, de uma análise detalhada para não serem incorporadas, ou ao menos consideradas em análises moleculares, principalmente em trabalhos de filogenia e taxonomia (WHITE et al., 2008; CALVIGNAC et al., 2011). Para o gênero *Macrobrachium*, já foram apontados indícios de heteroplasmia mitocondrial e/ou NUMTs em sequências de COI nas espécies *M. ferreirai* (ROBE et al., 2012), *M. jelskii* (MORAES, 2017), *M. brasiliense* (Ávila, 2017), já para *M. amazonicum*, tais ocorrências foram confirmadas a partir de diferentes métodos de extração de DNA e clonagem gênica por Iketani (2012). Dessa forma, tais ocorrências apontam que a COI deve, no mínimo, ser utilizada com extrema cautela.

A presença de NUMTs e heteroplasmia em *Macrobrachium* também apontam para necessidade de marcadores alternativos. Assim, uma possibilidade é o marcador rDNA 28S (DNA ribossomal 28S) que segundo Castelin (2013) tem potencial e aplicabilidade para este grupo. O rDNA 28S consiste em uma mistura de regiões conservadas e variáveis organizadas *in tandem* com centenas de cópias por genoma (HASSOUNA; MITHOT; BACHELLERIE, 1984). Chen (2009) faz uma comparação entre mtDNA 16S (DNA mitocondrial) e rDNA (DNA ribossomal) 28S e Wowor (2009)

traz uma abordagem multiloco de gerar inferências mais completas indo de encontro com a abordagem tradicional encontrada na literatura que é a uniloco.

É importante frisar que Chen et al. (2009), ao comparar as topologias obtidas a partir dos marcadores mDNA 16S e rDNA 28S para espécies de *Macrobrachium* provindas de Taiwan, expõe resultados que indicam que as análises das árvores filogenéticas derivadas do 28S tiveram uma resolução consideravelmente mais alta do que a do 16S, principalmente devido ao alto índice de transições e transversões das sequências do marcador mitocondrial, além das fracas divergências observadas. Por outro lado, a sobreposição da topologia de ambos os genes se mostraram convergentes para alguns táxons.

Diante desse cenário, surge a necessidade do desenvolvimento de ferramentas genéticas que complementem os dados apontados pela morfologia e que embasam a taxonomia. Logo, é essencial revisar a utilização da COI como marcador de identificação molecular para crustáceos bem como avaliar a viabilidade do uso do 28S como marcador molecular. Dessa forma, o objetivo desse trabalho é testar a aplicabilidade do 28S para identificação de camarões da família Palaemonidae, abrangendo espécies do gênero *Macrobrachium*.

2. Metodologia

2.1 Construção do banco de Dados

Todas as sequências foram obtidas através do *Genbank* (plataforma online de anotações de sequências de nucleotídeos) usando o acesso *Macrobrachium* + 28S. Após essa busca, foi realizada a triagem preliminar dos dados, a priori, dividindo por sequências provindas de artigos publicados e excluindo aquelas provindas de artigos não publicados. Conseguinte, todas as sequências foram triadas e analisadas pelos seguintes fatores: localidade e espécie.

O segundo passo da triagem envolveu uma organização mais específica das sequências, com isso, dentro do fator local foram realizadas duas subdivisões, uma macro, considerando apenas os países e uma micro, considerando territórios menores dentro do mesmo país. Para o fator Espécie, foi realizada a quantificação do número de espécies de *Macrobrachium* encontradas e posteriormente quantificado as demais espécies, devido à baixa representatividade numérica das demais sequências, criou-se um grupo denominado de "outros". Por fim, para o fator artigo de origem, apenas foi realizada uma quantificação de sequências por artigo.

2.2 Banco de Dados

Todas sequências obtidas para *Macrobrachium* foram alinhadas e, visualmente, otimizadas (editadas quando necessário) no programa CodonCode Aligner v7.0.1 (CodonCode Corporation), o alinhamento foi feito na plataforma online MAFFT v. 7 (Disponível em <https://mafft.cbrc.jp/alignment/server/>) (Castresana, 2000; Talavera & Castresana, 2007).

2.3 Análises Filogenéticas e Delimitação de Espécies

A árvore filogenética foi construída através do método de agrupamento de vizinhos (NJ), baseada em distância genética simples (p), a significância dos agrupamentos foi suportada através da análise de *bootstrap* e, a partir de 1000 pseudoréplicas, foram estimadas as distâncias genéticas (distância p) entre e dentro de

cada espécie, ambas as análises foram realizadas no programa MEGA v10 (Kumar et al., 2018).

O método Automatic Barcode Gap Discovery (ABGD) (Disponível em <http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html>) baseia-se na identificação das distâncias genéticas entre diferentes espécies, a partir da detecção do chamado "*barcode gap*", estima-se então a significância do gap gerado e, posteriormente, o programa particiona os dados (Puillandre et al., 2012), o resultado gerado pelo algoritmo deve indicar uma maior divergência interespecífica, e menor intraespecífica (Da Silva, 2017). O ABGD foi executado usando distâncias simples (p) e o valor utilizado para a largura relativa do gap foi de X=1,5, todos os demais parâmetros foram mantidos como padrão.

3. Resultados e Discussão

3.1 Banco de Dados

Foram obtidas 321 sequências usando o acesso *Macrobrachium* + 28S. O resultado da triagem utilizando o fator Artigo resultou em 83 sequências provenientes de artigos não publicados e 238 de trabalhos publicados, distribuídas em 13 artigos, sendo que, 100 destas sequências estão concentradas em apenas um artigo de Wowor et *al*., (2008), seguido por Castelin et al., (2013 e 2018) com 58 e 49 sequências consecutivamente. (Tabela 1).

Tabela 1: Quantificação de espécies do gênero *Macrobrachium* para o gene 28S relacionando o artigo de origem com número de sequências depositadas, considerando apenas trabalhos publicados.

NOME	QUANTIDADE DE SEQUÊNCIAS
BRACKEN et al., 2010	1
CHEN et al., 2009	1
Direct Submission	1
KOU et al., 2013	2
VON RINTELEN et al., 2012	2
PORTER et al., 2005	2
LU et al., 2009	3
CUTMORE et al., 2013	4
AZNAR-CORMANO et al., 2015	6
FUKE et al.,2018	9
CASTELIN et al., 2018	49
CASTELIN et al ., 2013	58
WOWOR et al., 2008	100
TOTAL	**238**

Foram identificadas 64 espécies, destas, 58 pertencentes ao gênero *Macrobrachium* e 6 aos gêneros *Palaemon*, *Expopalaemon* e *Pseudophyllodistomum*. As espécies com maior representatividade, que detém 125 das 238 das sequências publicadas, são: *Macrobrachium rosenbergii* (7), *Macrobrachium australe* (56) e *Macrobrachium lar* (65).

No fator Local, foram obtidas sequências de 12 países, principalmente da região asiática. Destacando-se Indonésia com 26 sequências, China com 22, Japão com 17 e Malásia com 16, representando 81 das 119 sequências com locais informados. Por fim,

não foi possível determinar o local de origem de 119 das sequências, pois não havia informações suficientes para esta identificação no *GenBank* e/ou no artigo (Tabela 2).

Tabela 2: Lista de locais relacionado a quantidade de sequências dos artigos publicados para o gênero *Macrobrachium* com o gene 28S.

LOCAL	QUANTIDADE DE SEQUÊNCIAS
Laos	2
Camboja	3
Tailândia	4
Austrália	4
Vietnã	4
Singapura	8
Taiwan	11
Malásia	16
China	22
Indonésia	26
Japão	17
Índia	2
Local não informado	119
TOTAL	**238**

Após construção do banco de dados e do alinhamento das sequências, formaram-se cinco grupos. O grupo D1-D2 é composto por 251 sequências, seguido das duas partições do grupo D4, com 31 e 21 espécies em cada. Os outros dois grupos foram pouco representativos e juntos correspondem a apenas oito sequências majoritariamente das espécies *Macrobrachium rosenbergii* e *Macrobrachium potuina,* infelizmente, não foi possível identificar a qual região de 28S essas sequências pertencem (Tabela 3).

É importante frisar que Sonnberg (2007) cita e explora a importância da região D1-D2 devido a variabilidade de 28S, bem como permite a aplicação do *barconding gap* para *Metazoa.* Além disso, ainda aponta essa região como potencial para substituir a região comumente usada que é a D3-D4, pois infere que os resultados obtidos na região D1-D2 possuem uma melhor resolução oferecendo assim inferências mais completas. Além disso, ainda reforça que uso dessa região é importante para colocar as sequências no contexto filogenético correto.

Tabela 3: Resultado dos grupos formados pós alinhamentos baseado em sua região de origem

GRUPO	NÚMERO DE SEQUÊNCIAS	PRINCIPAIS ESPÉCIES
D1D2	251	*Macrobrachium lar*
D4.1	31	*Macrobrachium placidulum*
D4.2	21	*Macrobrachium rosenbergii*
***Contig* 4**	8	*Macrobrachium rosenbergii*
***Contig* 8**	2	*Macrobrachium rosenbergii*

3.2 Análises Filogenéticas e Delimitação de Espécies

3.2.1 Árvore filogenética

A árvore gerada para o primeiro grupo do D4 agrupou corretamente a maioria das espécies, sendo que houve a formação do clado composto por *M. sp e M. clymene* que pode indicar uma maior proximidade entre essas espécies. Com isso, podemos ter como hipótese que esta sequência não identificada a nível de espécie (*sp.*) possa ser um *M. clymene*. Porém, para a confirmação, seria necessário a realização de outras análises moleculares, pois essa espécie é originaria da Malásia e é comumente associada a *M. leucodactylus* de acordo com Worror (2009) (Figura 1).

O único resultado da árvore que difere do ABGD refere-se ao clado de *M. placidulum* com a inserção de *M. tolmerum,* isto pode ser explicado pelo fato das partições do ABGD constituírem uma análise inicial, portanto *M. tolmerum* destingiu-se em um grupo somente baseado no algoritmo usando pelo programa (Figura 1).

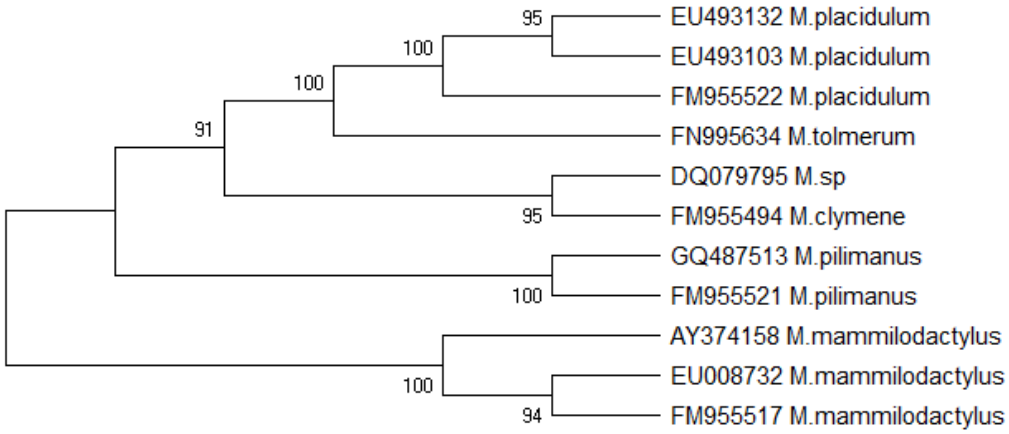

Figura 1. Árvore filogenética de agrupamento de vizinhos (NJ) para a região rDNA 28S, baseada na distância *p*, para espécies pertencentes ao gênero *Macrobrachium* região D4.1. Os números em cada ramificação indicam os valores de *bootstrap*.

O grupo D4.2, apesar de se dividir em outros dois grandes grupos, não possui apoio estatístico significativo, visto que clados formados estão apoiados em nós de respectivamente 8 e 41, ou seja, pouco significativo (Figura 2). A análise dessa árvore entra em concordância com o resultado obtido no ABDG, o qual indicou a impossibilidade de distinção de grupos dentro da partição, apesar de ter agrupado corretamente todas as espécies.

Para o grupo D1-D2 o resultado da árvore foi majoritariamente preciso e com apoio estatístico, variando de 86 a 99 nos nós principais, mostrando a eficácia do 28S para a diferenciação de espécies desse gênero (Figura 3). Sonnenberg *et al.*, (2007) ressalta o potencial desse marcador para identificação molecular pois possui partes conservadas e variáveis. Este autor ainda ressalta que um marcador eficiente é aquele que consegue diferenciar até as espécies com maior proximidade genética. Hirai *et al.*, (2013) apresentam em seu trabalho a eficiência do 28S para diferenciação molecular a nível de espécie, enfatizam a alta qualidade do produto da PCR e ainda exibem que em comparação ao resultado da COI, o 28S apresenta resultados mais coerentes.

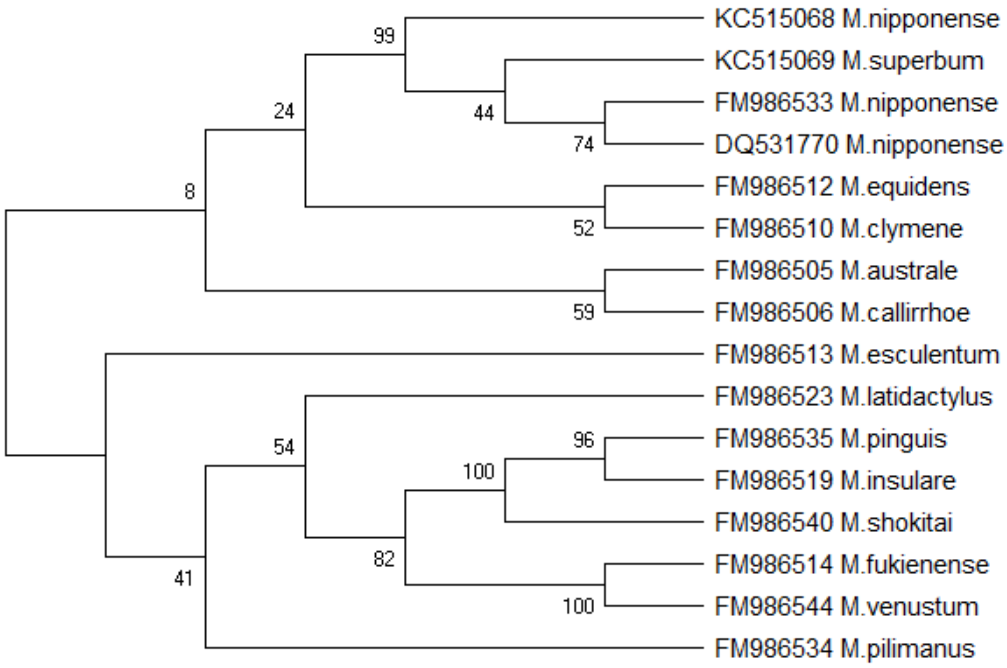

Figura 2. Árvore filogenética de agrupamento de vizinhos (NJ) para a região rDNA 28S, baseada na distância *p*, para espécies pertencentes ao gênero *Macrobrachium* região D4.2. Os números em cada ramificação indicam os valores de *bootstrap*.

Obteve-se um clado formado por três sequências de *M. rosenbergii* e uma de *M. dacqueti*, esse agrupamento já possui explicações claras na literatura. Wowor e Ng (2001) sugeriram baseado em dados morfológicos e morfométricos que havia necessidade de elevação a nível de espécie dessas duas subespécies à época. Wowor (2007 e 2008) relata o agrupamento de *M. dacqueti* com *M. rosenberg~~u~~ii* e sugere que esse agrupamento, na verdade, se deve ao fato de uma divergência na nomenclatura envolvendo essas espécies, a qual ela elucida nesses trabalhos.

A única espécie que agrupou de forma inesperada foi a *M. asperulum*, pois teve suas três sequências dispersas em clados diferentes com suporte estatístico variando de 73 a 98. Liu et al., (2011) fornece uma hipótese para essa diferenciação, baseada no surgimento de uma barreira física que causou isolamento geográfico temporário. Os padrões de distribuição da fauna de água doce nesta ilha certamente foram moldados por eventos geológicos e oscilações climáticas (Yang e Tzeng,1986).

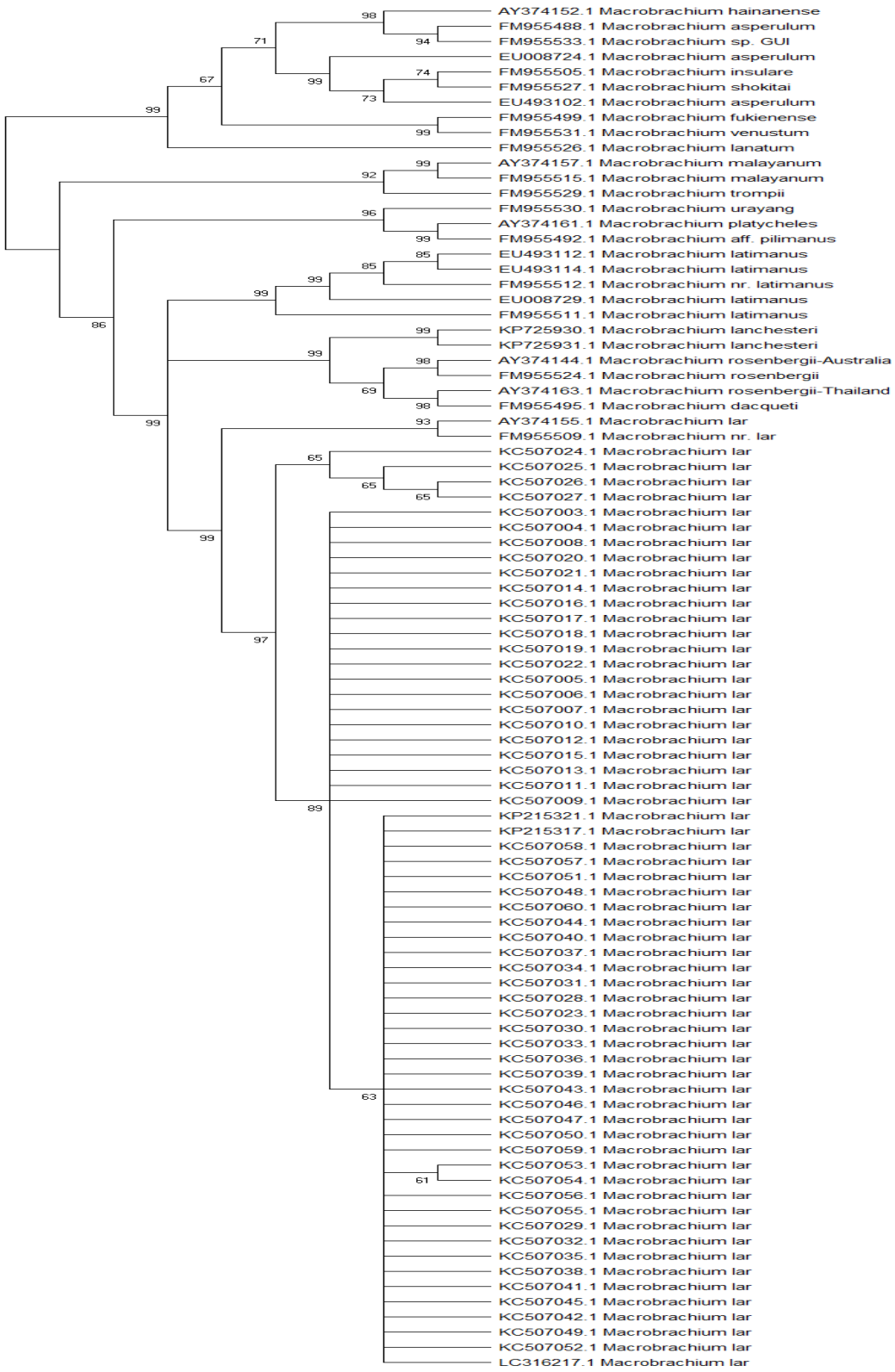

Figura 3. Árvore filogenética de agrupamento de vizinhos (NJ) para a região rDNA 28S, baseada na distância *p*, para espécies pertencentes ao gênero *Macrobrachium* região D1-D2. Os números em cada ramificação indicam os valores de *bootstrap*.

3.2.2 Distâncias

Os valores obtidos para as distâncias intra e interespecíficas (Tabela 4) corroboram com os dados previamente apresentados. Por exemplo, para distância máxima do grupo D1-D2 tivemos um outlier com 6,25%. Analisando essa espécie, podemos supor através dos dados já apresentados sobre o *M. asperulum* que essa alta divergência dentro da espécie pode ser relacionada a separação da espécie pela barreira geográfica, consequentemente dando um valor tão alto e fora do padrão. Por isso, ele foi desconsiderado e o segundo valor foi tido como principal. No mais, os valores mínimos e máximos para distância intraespecífica convergem com a literatura. De acordo com, Aznar-Cormano (2015) não foi encontrada variação intraespecífica para 28S. Comparativamente, dos 6 resultados obtidos neste trabalho 2 foram 0%, 2 n/c (não calculado) e apenas para o grupo D1-D2 e para D4-1 conseguiu-se detectar variação dentro da espécie, fato esse que pode ser explica~~n~~do pela diversidade de locais de origem das sequências, mesmo que lidem da mesma espécie (Tabela 4).

Na literatura ainda não é possível encontrar um valor padrão para 28S porém, Hebert e Hogg (2013) e Hebert et al., (2003) propõem 2% de distância para sequências similares. Figueiredo (2015) obteve para o gene da COI uma variação intra de 0 a 2,5% e inter de 4,9% a 23,6%. Já para 16S apresentou uma distância interespecifica nula e intraespecifica de 0 a 3%. No presente estudo, é possível que a distância intraespecífica possa estar subestimada visto que existem muitas espécies com apenas uma sequência como representante. A distância interespecífica obtida está dentro do padrão do grupo tendo como outlier apenas a distância máxima do grupo D4-1 que obteve 36%. É importante frisar que o valor obtido ao comparar *M. lar* e *M. lepidactyloides* foi inesperado visto que não se espera que espécies distintas tenham 0% de distância interespecífica. Ao analisar o banco de dados e a literatura, é possível inferir que essas sequências, possam ter sido identificadas e depositadas erroneamente, porém Sonnenberg (2007) discute que a depender do método usado para eliminação de bases ambíguas o resultado pode variar. (Tabela 4).

Tabela 4: Distâncias intra e interespecífica com valores mínimos e máximos, para as regiões DI-D2, D4.1 e D4.2 do gênero Macrobrachium para o gene 28S.

Nível Taxonômico	**Distância Mínima**			**Distância Máxima**		
----------	D1-D2	D4-1	D4-2	D1-D2	D4-1	D4-2
Intraespecífica	0,0%	0,0%	NC	0,88 (6,25%*)	0,2%	NC
Interespecífica	1,0%	2,0%	1,0% (0,0%**)	25%	36%	23%

*Valor obtido ao comparar diferentes sequencias de *M. asperulum*
**Valor obtido ao comparar *M. lar* e *M. lepidactyloides*

3.2.3 ABGD

D1D2

Foram obtidas 11 partições, sendo nove inciais e duas recursivas, com variação da distância *p* entre 0,1 e 0,001, com a formação de 26 a 2 grupos. A partição mais coerente é o inicial 3, pois agrupa mais as espécies semelhantes e possui menos divergências, corroborando com a árvore filogenética. Além disso, a análise do histograma demonstra a formação clara de um *gap* entre 0,02 e 0,06 demonstrando a clara diferenciação dos indivíduos (Figura 4 e 5).

Smithet *et. al.*, 2005 e De Salle 2006, reforçam que o resultado depende da diferenciação do *gap*, isso pode ser indicativo de uma nova espécie e de que a tecnologia do *barcoding gap* é uma ótima ferramenta para análises iniciais. Puillandre et al., (2012) enfatiza que a análises das partições não deve ser tida como uma diferenciação final e sim como uma hipótese inicial. Além disso, reforça a necessidade de analisar o *output* do ABDG fazendo uso de dados complementares e pré-existentes, como espécies previamente definidas, outros estudos de códigos de barras que identificaram um valor de divergência anterior para a mesma taxa ou um grupo estreitamente relacionado, ou quaisquer outros dados disponíveis para as espécies estudadas (Figura 4 e 5).

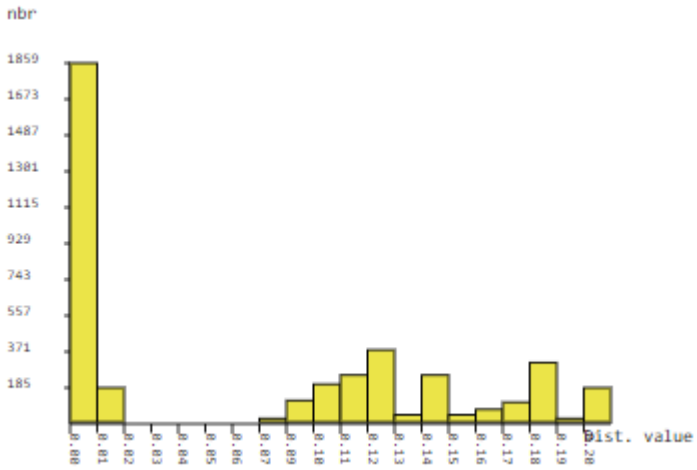

Figura 4. Gráfico resumindo os resultados do ABGD para o marcador nuclear rDNA 28S região D1-D2 mostrando a distribuição de distâncias genéticas e de "*barcode gaps*" para espécies do gênero *Macrobrachium*

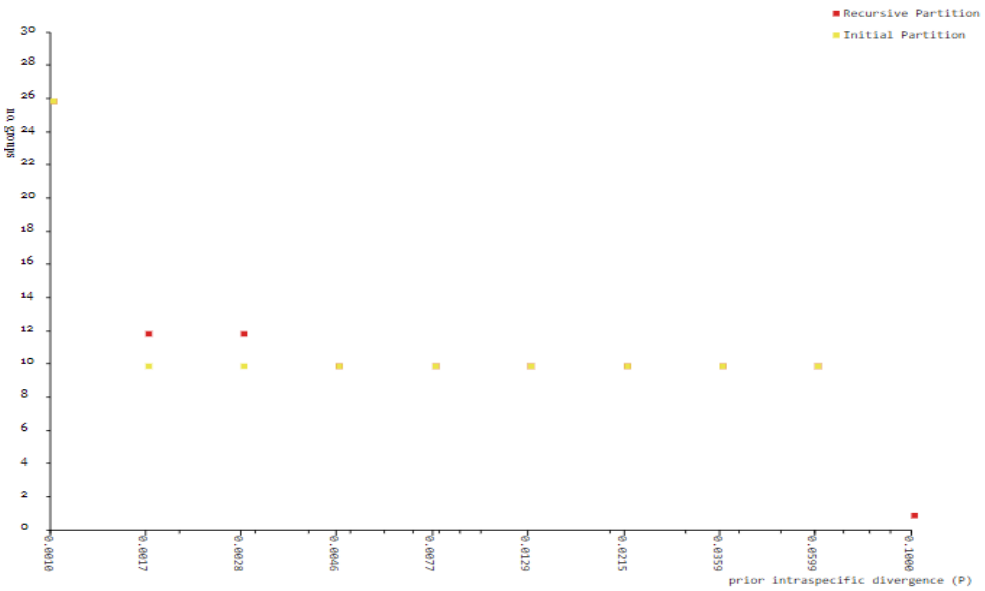

Figura 5. Gráfico resumindo os resultados do ABGD para o marcador nuclear rDNA 28S região D1-D2, registrando o número de grupos para as partições primárias e recursivas geradas pelo ABGD como função do limite de divergência intraespecífica das espécies

D4.1

Foram obtidas dez partições, sendo nove inciais e uma recursiva, com variação da distância *p* entre 0,1 e 0,001, com a formação de 1 a 5 grupos. A partição mais coerente é o inicial 1. A única diferença entre a árvore e esse resultado está no agrupamento do *M.*

tolmerum, visto que no ABDG foi tido como grupo único enquanto que a árvore o agrupou com *M. placidulum.* A análise do histograma demonstra a formação clara de um *gap* entre 0,03 a 0,07 deixando clara a diferenciação dos indivíduos (Figura 6 e 7).

Nesses casos D1D2 e D4.1, pode-se observar a maior consistência das partições iniciais, que corresponderam aos agrupamentos observados na árvore de agrupamento de vizinhos. Segundo Yang *et al.* (2016), os resultados do ABGD podem ser influenciados pelo valor empregado para a largura relativa do *gap*, nesse sentido o valor de X=1,5 superestimou o número de grupos observados nas partições iniciais (Figura 6 e 7).

De acordo com Puillandre *et al*., (2012) na presença de um conjunto de dados de referência com espécies previamente caracterizadas, as espécies de um organismo podem ser automaticamente identificadas usando sua sequência de código de barras, por isso torna-se tão relevante o aparecimento desses *gaps*.

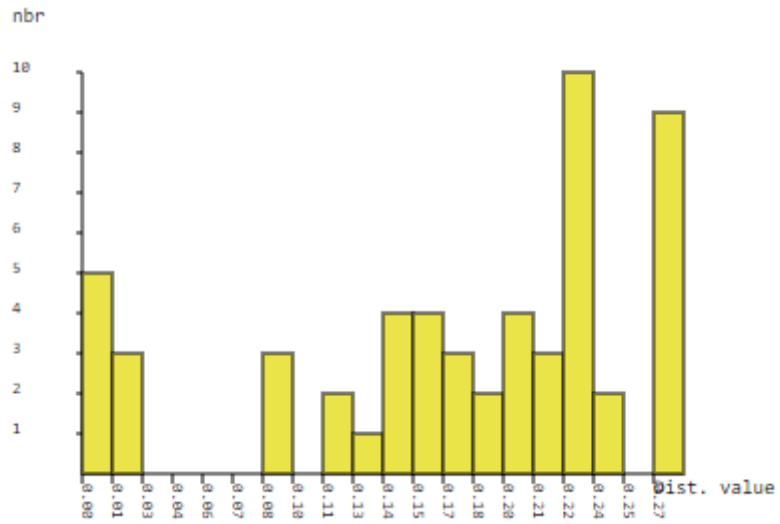

Figura 6. Gráfico resumindo os resultados do ABGD para o marcador nuclear rDNA 28S região D4.1 mostrando a distribuição de distâncias genéticas e de "*barcode gaps*" para espécies do gênero *Macrobrachium*

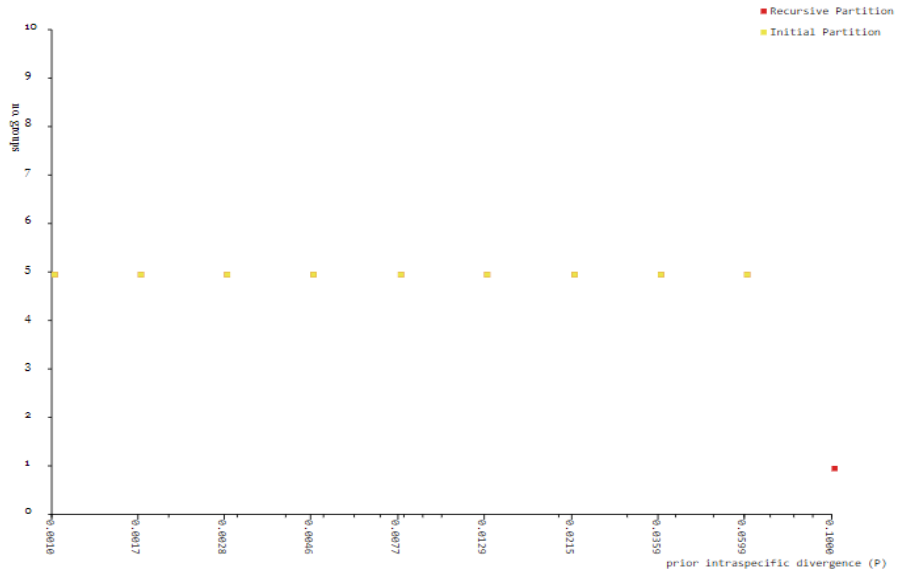

Figura 7. Gráfico resumindo os resultados do ABGD para o marcador nuclear rDNA 28S região D4.2, registrando o número de grupos para as partições primárias e recursivas geradas pelo ABGD como função do limite de divergência intraespecífica das espécies

D4.2

Não houve a formação de grupos no ABGD, o que sugere que as espécies são tão próximas que não foi possível a separação em grupos e por isso não gerou o *output* com as partições. A árvore demonstra algo similar, visto que a separação baixa valores de *bootstrap*.

4. Conclusão

Os resultados suportaram a consistência do status taxonômico do gênero analisados topologia observada correspondeu, de forma geral, com as análises do ABGD e, com as distâncias genéticas calculadas, com poucas divergências como discutido anteriormente. Comparando o padrão da distribuição da divergência genética entre e dentro das espécies, ficou evidente a presença de um *barcode gap*. Mesmo o 28S apresentando baixa variabilidade no caso de algumas espécies, os resultados obtidos nesse trabalho comprovam sua aplicabilidade em substituição ao COI, mas, é importante estabelecer que a busca de marcadores nucleares mais variáveis é de extrema importância para auxiliar no melhor entendimento do comportamento taxonômico da subfamília

Palaemoninae. Portanto, foi suportada a eficiência desse marcador para discriminar espécies servindo como uma ferramenta complementar de identificação para as espécies.

5. Referências

ALHO, Cleber José Rodrigues. Biodiversity of the Pantanal: response to seasonal flooding regime and to environmental degradation. Brazilian Journal of Biology, v. 68, n. 4, p. 957-966, 2008.

Ávila FF. (2017). Identificação molecular de camarões de água doce (Decapoda, Palaemonidae) do baixo amazonas (Dissertação de Mestrado, Brasil).

AZNAR-CORMANO, L. et al. An improved taxonomic sampling is a necessary but not sufficient condition for resolving inter-families relationships in Caridean decapods. Genetica, v. 143, n. 2, p. 195-205, 2015.

Bond-Buckup, G., and L. Buckup. 1994. A família Aeglidae (Crustacea, Decapoda, Anomura). Arquivos de Zoologia 32: 159-347.

Buhay JE. (2009). "COI-like" sequences are becoming problematic in molecular systematic and DNA barcoding studies. Journal of Crustacean Biology, 29(1), 96-110.

Calvignac S, Konecny L, Malard F, Douady CJ. (2011). Preventing the pollution of mitochondrial datasets with nuclear mitochondrial paralogs (numts). Mitochondrion, 11(2), 246-254.

Castelin, M., Feutry, P., Hautecoeur, M., Marquet, G., Wowor, D., Zimmermann, G., Keith, P., 2013. New insight on population genetic connectivity of widespread amphidromous prawn *Macrobrachium lar* (Fabricius, 1798) (Crustacea: Decapoda: Palaemonidae). Mar. Biol. 160, 1395–1406. https://doi.org/10.1007/s00227-013-2191-y.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Nat. Nat. Proc. R. Soc. Lond. B Bioinforma. Nucleic Acids Res. Trends Biol. Sci. Maddison, Phylogeny Character Evol. Mol. Biol. Evol 390, 31–32.

Chen, R.T., Tsai, C.F., Tzeng, W.N., 2009. 16S and 28S rDNA Sequences in Phylogenetic Analyses of Freshwater Prawns (*Macrobrachium* Bate, 1868) from Taiwan. J. Crustac. Biol. 29, 400–412. https://doi.org/10.1651/08-3069.1.

Da Silva JM, Creer S, Dos Santos A, Costa AC, Cunha MR, Costa FO, Carvalho GR. (2011). Systematic and evolutionary insights derived from mtDNA COI barcode diversity in the Decapoda (Crustacea: Malacostraca). PLoS one, 6(5), e19449.

DA SILVA, Catarina Ribeiro. Desenvolvimento de métodos moleculares para a diferenciação de espécies de crustáceos em alimentos. 2016.

Da Silva, D.J.F., 2017. Delimitação molecular de espécies de pargos vermelhos (Lutjanidae – Perciformes) do atlântico. Dissertação de Mestrado, 55.

DE FIGUEIREDO, Carla Kühl. Variação morfológica e análise da distribuição do caranguejo *Pyromaia tuberculata* Lockington, 1877 (Crustacea: Decapoda). 2015. Tese de Doutorado. Universidade de São Paulo.

De Grave S, Cai Y, Anker A. (2008). Global diversity of shrimps (Crustacea: Decapoda: Caridea) in freshwater, 595:287–293.

De Salle R, Egan MG, Siddall M. (2005). The unholy trinity: taxonomy, species delimitation and DNA barcoding. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 360 (1462), 1905-1916.

Ferreira, R.S., Vieira, R.R.R., D'Incao, F., 2010. The marine and estuarine shrimps of the Palaemoninae (Crustacea: Decapoda: Caridea) from Brazil. Zootaxa 24, 1–24.

Francis CM, Borisenko AV, Ivanova NV, Eger JL, Lim BK, Guillén-Servent A, Hebert PDN. (2010). The role of DNA barcodes in understanding and conservation of mammal diversity in southeast asia. PLoS One, 5(9).

FUNK, Daniel J.; OMLAND, Kevin E. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annual Review of Ecology, Evolution, and Systematics, v. 34, n. 1, p. 397-423, 2003.

Hassouna, N., Michot, B., Bachellerie J.P., 1984. Nucleic Acids Research. Nucleic Acids Res. 36, ii–ii. https://doi.org/10.1093/nar/gkn907.

Hickman, C. P., Roberts, L. S., Keen, S. L., Eisenhour, D. J., Larson, A., & l'Anson, H. (2011). Crustaceans. In Integrated Principles of Zoology 15th ed., (pp. 421-443). New York: McGraw-Hill.

HICKMAN, C.L.; ROBERTS, L.S.; LARSON, A. Princípios integrados de Zoologia. Rio De Janeiro: Editora Guanabara Koogan S.A., 2013.

HOGG, Ian D.; HEBERT, Paul DN. Biological identification of springtails (Hexapoda: Collembola) from the Canadian Arctic, using mitochondrial DNA barcodes. canadian Journal of Zoology, v. 82, n. 5, p. 749-754, 2004.,

Iketani G. (2012). Desenvolvimento e Aplicação de Marcadores Moleculares Mitocondriais em Camarões de Água Doce do Gênero *Macrobrachium* (Decapoda: Palaemonidae) (Tese de Doutorado, Brasil).

Kmiec B, Woloszynska M, Janska H. (2006). Heteroplasmy as a common state of mitochondrial genetic information in plants and animals. Current genetics, 50(3), 149-159.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol. Biol. Evol. 35, 1547–1549. https://doi.org/10.1093/molbev/msy096.

Liu, M., Cai, Y., Tzeng, C., 2007. Molecular Systematics of the Freshwater Prawn Genus. Zool. Stud. 46, 272–289.

Lopez JV, Yuhki N, Masuda R, Modi W, O'Brien SJ. (1994). Numt, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. Journal of Molecular Evolution, 39(2), 174-190.

Melo, G., A., S., 2003. Manual de Identificação dos Crustacea Decapoda de Água Doce do Brasil. Edições Loyola, São Paulo.

Moltschaniwskyj, N. A. (2005). Edible Shellfish. In Handbook of Food Science, Technology, and Engineering - 4 Volume Set: Boca Raton: CRC Press.

Moraes SASN. (2017). Variabilidade fenotípica e genotípica do camarão *Macrobrachium jelskii* (Miers, 1877) das bacias do noreste brasileiro: uma abordagem de sistemática integrativa. (Dissertação de Mestrado, Brasil).

Nga T, Anh T, Huyen D, Son N, Thong V, Sang N, Thanh H. (2016). Assessment of Genetic Relationship of some Horseshoe Bats (Chiroptera: Rhinolophidae) in Vietnam Using Cytochrome oxydase Subunit I (COI) Gene Sequence. VNU Journal Of Science: Natural Sciences And Technology, 32 (1S).

Pileggi, L.G., Magalhães, C., Bond-Buckup, G., Mantelatto, F.L., 2013. New records and extension of the known distribution of some freshwater shrimps in Brazil. Rev. Mex. Biodivers. 84, 563–574. https://doi.org/10.7550/rmb.30504

Pileggi, L.G., Mantelatto, F.L., 2010. (Decapoda, Palaemonidae), with emphasis on the relationships among selected American species. Invertebr. Syst. 24, 194. https://doi.org/10.1071/IS09043

Pileggi, L.G., Rossi, N., Wehrtmann, I.S., Mantelatto, F.L., 2014. Molecular perspective on the American transisthmian species of *Macrobrachium* (Caridea, Palaemonidae). Zookeys 131, 109–131. https://doi.org/10.3897/zookeys.457.6818

Puillandre, N., Modica, M. V., Zhang, Y., Sirovich, L., Boisselier, M.C., Cruaud, C., Holford, M., Samadi, S., 2012. Large-scale species delimitation method for hyperdiverse groups. Mol. Ecol. 21, 2671–2691. https://doi.org/10.1111/j.1365-294X.2012.05559.x

Radulovici AE, Sainte-Marie B, Dufresne F. (2009). DNA barcoding of marine crustaceans from the Estuary and Gulf of St Lawrence: a regional-scale approach. Molecular Ecology Resources, 9, 181-187.

Raupach MJ, Astrin JJ, Hannig K, Peters MK, Stoeckle MY, Wägele JW. (2010). Molecular species identification of Central European ground beetles (Coleoptera: Carabidae) using nuclear rDNA expansion segments and DNA barcodes. Frontiers in zoology, 7 (1), 26.

Robe LJ, Machado S, Bartholomei-Santos ML. (2012). The DNA barcoding and the caveats with respect to its application to some species of Palaemonidae (Crustacea, Decapoda). Zoological science, 29(10), 714-724. Ruiz

RONÇANI, Liara Darabas; GOULARTI FILHO, Alcides. A trajetória recente da economia catarinense (2000-2010): na contramão do crescimento?. Acta Scientiarum. Human and Social Sciences, v. 37, n. 1, p. 53-64, 2015.

Rossi, N., Mantelatto, F.L., 2013. Molecular Analysis of the Freshwater Prawn *Macrobrachium olfersii* (Decapoda, Palaemonidae) Supports the Existence of a Single Species throughout Its Distribution. PLoS One 8, 1–13. https://doi.org/10.1371/journal.pone.0054698.

SANTOS, Sandro et al. Three New Species of Aeglidae (Aegla) from Paraná State, Brazil. Journal of Crustacean Biology, v. 35, n. 6, p. 839-849, 2015.

Song H, Moulton MJ, Hiatt KD, Whiting MF. (2013). Uncovering historical signature of mitochondrial DNA hidden in the nuclear genome: the biogeography of Schistocerca revisited. Cladistics, 29(6), 643-662.

Sonnenberg, R., Nolte, A., Tautz, D., 2007. An evaluation of LSU rDNA D1-D2 sequences for their use in species identification. Front. Zool. 4, 1–12. https://doi.org/10.1186/1742-9994-4-6.

Talavera, G., Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. 56, 564–577. https://doi.org/10.1080/10635150701472164

Vergamini, F.G., Pileggi, L.G., Mantelatto, F.L., 2011. Genetic variability of the Amazon River prawn *Macrobrachium amazonicum* (Decapoda, Caridea, Palaemonidae). Contrib. to Zool. 80, 67–83.

Ward RD. (2009). DNA barcode divergence among species and genera of birds and fishes. Molecular ecology resources, 9 (4), 1077-1085

White DJ, Wolff JN, Pierson M, Gemmell NJ. (2008). Revealing the hidden complexities of mtDNAe inheritance. Molecular Ecology, 17(23), 4925-4942. Williams

Wowor, D., Muthu, V., Meier, R., Balke, M., Cai, Y., Ng, P.K.L., 2009. Evolution of life history traits in Asian freshwater prawns of the genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidae) based on multilocus molecular phylogenetic analysis. Mol. Phylogenet. Evol. 52, 340–350. https://doi.org/10.1016/j.ympev.2009.01.002

WOWOR, D.; NG, P. K. L. Identity of the Giant Prawn, *Macrobrachium rosenbergii* (De Man, 1879)(Crustacea: Decapoda: Caridea: Palaemonidae). In: Proceedings of the Fifth International Crustacean Congress, Melbourne, Australia. 2001. p. 9-13.

YANG, D. C. H.; TZENG, S. W. Simplification and linearization of manipulator dynamics by the design of inertia distribution. The International journal of robotics research, v. 5, n. 3, p. 120-128, 1986.

HEBERT, Paul DN et al. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences, v. 270, n. 1512, p. 313-321, 2003.

8. Anexos

Anexo 1

Normas da revista

BIOCHEMICAL SYSTEMATICS AND ECOLOGY

DESCRIPTION

Biochemical Systematics and Ecology is devoted to the publication of original papers and reviews, both submitted and invited, in two subject areas: I) the application of **biochemistry** to problems relating to **systematic biology** of organisms (**biochemical systematics**); II) the role of biochemistry in interactions between organisms or between an organism and its environment (**biochemical ecology**).

In the Biochemical Systematics subject area, comparative studies of the distribution of (secondary) metabolites within a wider taxon (e.g. genus or family) are welcome. Comparative studies, encompassing multiple accessions of each of the taxa within their distribution are particularly encouraged. Welcome are also studies combining classical chemosystematic studies (such as comparative HPLC-MS or GC-MS investigations) with (macro-) molecular phylogenetic studies. Studies that involve the comparative use of compounds to help differentiate among species such as adulterants or substitutes that illustrate the applied use of chemosystematics are welcome. In contrast, studies solely employing macromolecular phylogenetic techniques (gene sequences, RAPD studies etc.) will be considered out of scope. Discouraged are manuscripts that report known or new compounds from a single source taxon without addressing a systematic hypothesis. Also considered out of scope are studies using outdated and hard to reproduce macromolecular techniques such as RAPDs in combination with standard chemosystematic techniques such as GC-FID and GC-MS.

In the Biochemical Ecology subject area, studies addressing the role compounds play in the ecology of the organisms producing them are invited. Moreover, manuscripts that address hypothesis associated with the influence of factors such as altitude, geography, and seasonal variation on the expression of primary and secondary metabolites are encouraged.Research papers should generally represent a complete investigation and not preliminary data. Preliminary reports will only be considered where findings are of sufficient interest to justify rapid publication. New Source Reports will onlybe considered in cases where a significant chemosystematic or ecological finding is reported. New Source Reports have to be written in a standard format (Example).

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Biochemical Systematics and Ecology is devoted to the publication of original papers and reviews, both submitted and invited, in two subject areas: (i) the application of biochemistry to problems relating to systematic biology of organisms (biochemical systematics); (ii) the role of biochemistry in interactions between organisms or between an organism and its environment (biochemical ecology).

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Contributions will be accepted in English as Reviews, Research Papers or New Source Reports. Reviews which survey important and developing areas of biochemical systematics and ecology are encouraged but authors are advised to consult the Editor before preparing such articles. Research papers should generally be of completed investigations. Preliminary reports will be published where findings are considered to be of sufficient interest to justify rapid publication. In addition, short reports of new sources of known compounds (New Source Reports) will be accepted where they can be justified in terms of systematic or ecological significance. These reports must be written to a standard format (see below). Papers will be grouped in each issue according to subject area.

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Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/ aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003). Reference to a dataset: [dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/ xwj98nb39r.1.

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