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GABRIELA WEMILLY BARROS DA SILVA

HETEROGENEIDADE MOLECULAR EM *Atelopus hoogmoedi* (ANURA: BUFONIDAE) NO LESTE DA AMAZÔNIA, PARÁ - BRASIL

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Trabalho de Conclusão de Curso apresentado à Faculdade de Ciências Biológicas da Universidade Federal do Pará, Campus Universitário de Altamira, como requisito parcial para obtenção do grau de Licenciado em Ciências Biológicas.

Orientador: Dr. Emil José Hernández-Ruz
Coorientador: MSc. Elciomar Araújo de Oliveira

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“Quando tudo tiver parecendo ir contra você,
lembre-se que o avião decola contra o vento,
e não a favor dele”.

Henry Ford

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SUMÁRIO

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Heterogeneidade molecular em *Atelopus hoogmoedi* (Anura: Bufonidae) no Leste da Amazônia, Pará - Brasil.

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RESUMO

SILVA, G.W.B. **Heterogeneidade molecular em *Atelopus hoogmoedi* (Anura: Bufonidae) no Leste da Amazônia, Pará - Brasil.** Trabalho de Conclusão de Curso, Universidade Federal do Pará, Altamira, 2018, 43 p.

O gênero *Atelopus* é um dos mais diversos da família Bufonidae e devido à cor brilhante são referidas como rãs harlequin. Por ocorrerem em áreas de florestas maduras 97% das espécies estão em risco de extinção. Uma das espécies, *Atelopus hoogmoedi*, ocorre no norte e sul do rio Amazonas e que provavelmente representa mais de uma unidade evolutiva. Baseado nessa premissa, nosso objetivo foi comparar os indivíduos de *A. hoogmoedi* do sul e norte do rio Amazonas. O DNA foi extraído pelo método fenol clorofórmio de oito indivíduos de *A. hoogmoedi*, sendo sete de Monte Alegre (norte do rio Amazonas) e um de Anapu (sul do rio Amazonas) e em seguida foi amplificado via Reação em Cadeia da Polimerase (PCR) utilizando o marcador mitocondrial 16S rRNA. Na análise filogenética de Máxima Verossimilhança para a espécie *A. hoogmoedi* revelou um grupo parafilético com três linhagens: Guiana Francesa 1 e 2; Guiana e Monte Alegre; e Anapu. A distância genética entre Anapu e Monte Alegre foi de 2.9%. De acordo com o Automatic Barcode Gap Discovery (ABGD) em ambos os modelos Simple Distance e Kimura 2 Parameters, *A. hoogmoedi* coletada em Anapu foi reconhecido como uma espécie distinta daquelas do Escudo das Guianas. Dessa forma *Atelopus hoogmoedi* ao sul do rio Amazonas foi classificada como espécie candidata não confirmada, necessitando de mais coletas e acesso aos indivíduos das outras localidades de sua ocorrência.

Palavras- Chaves: ABGD, Bacia do Xingu, Escudo das Guianas, Espécies candidatas, Espécies Endêmicas.

ABSTRACT

SILVA, G.W.B. **Molecular heterogeneity in *Atelopus hoogmoedi* (Anura: Bufonidae) in Eastern Amazonia, Pará - Brazil.** Course Completion Work, Universidade Federal do Pará, Altamira, 2018, 43 p.

The genus *Atelopus* is one of the most diverse of the Bufonidae family and because of the bright color, they are referred to as harlequin frogs. As they occur in mature forest areas, 97% of species are in extinction risk. One of the species, *Atelopus hoogmoedi*, occurs in the north and south of the Amazon River and probably represents more than one evolutionary unit. Based on this premise, our objective was to compare the individuals of *A. hoogmoedi* of the south and north of the Amazon river. The DNA was extracted by the phenol chloroform method from eight individuals of *A. hoogmoedi*, seven from Monte Alegre (north of Amazon River) and one from Anapu (south of Amazon River) and then amplified via Polymerase Chain Reaction (PCR) using the mitochondrial 16S rRNA marker. In the phylogenetic analysis of maximum likelihood for *A. hoogmoedi* revealed a paraphyletic group with three lines: French Guiana 1 and 2; Guyana and Monte Alegre; and Anapu. The genetic distance between Anapu and Monte Alegre was 2.9%. According to the Automatic Barcode Gap Discovery (ABGD) in both Simple Distance and Kimura 2 Parameters models, *A. hoogmoedi* collected in Anapu was recognized as a distinct species from those of the Guiana Shield. Thus *Atelopus hoogmoedi* to the south of the Amazon River was classified as an unconfirmed candidate species, requiring more collections and access to individuals from other localities of its occurrence.

Keywords: ABGD, Candidate Species, Endemic Species, Guiana Shield, Xingu Basin.

Introdução

O gênero *Atelopus*, Duméril e Bibron 1841, é um dos mais diversos da família Bufonidae Gray 1825, com 96 espécies, distribuídas desde a Costa Rica até à Bolívia e Guiana Francesa (Frost 2018). As espécies do gênero possuem atividade diurna e atividade terrestre na serapilheira de florestas primárias associadas a corpos d'água (Lima et al. 2006, Lötters 2007, Coloma et al. 2000, Rueda-Almonacid et al. 2005) devido à cor brilhante são referidas como rãs harlequin. A maioria das espécies vive em montanhas acima de 1500m (Lötters 1996). A reprodução ocorre nas margens de córregos (Lötters 2007). Devido as espécies ocorrerem em áreas de florestas maduras, 97% estão em risco de extinção (Stuart et al. 2008), com muitas populações diminuindo drasticamente (Marca et al. 2005).

Atelopus é dividido em dois principais clados: Amazônia-Guiana (clado *flavescens-spumarius* + clado *tricolor*) e América Central-Andes-Chocó (clado *varius* mais todos os outros *Atelopus*) (Lötters et al. 2011). O primeiro mencionado apresenta 12 espécies (*A. carbonerensis* Rivero 1974, *A. chrysocorallus* La Marca 1996, *A. cruciger* Lichtenstein and Martens 1856, *A. mucubajiensis* Rivero 1974, *A. oxyrhynchus* Boulenger 1903, *A. pinangoi* Rivero 1982, *A. sorianoi* La Marca 1983, *A. tamaense* La Marca, García-Pérez, and Renjifo 1990, *A. vogli* Muller 1934, *A. flavescens* Duméril and Bibron 1841, *A. spumarius* Cope 1871 e *A. hoogmoedi* Lescure 1974). Destas, apenas três ocorrem na Amazônia brasileira: *A. hoogmoedi*, *A. spumarius* e *A. flavescens* (Amphibia Web 2018). Devido à conservação fenotípica em espécies deste gênero (Lötters et al. 2011), é provável que outras espécies podem está sendo mascaradas na bacia Amazônica.

A espécie *Atelopus hoogmoedi*, está distribuída no sul e oeste da Guiana Francesa, Suriname, sul da Guiana e regiões adjacentes do Brasil: estados do Amapá, Pará e Roraima (Frost 2018). Lötters et al. (2011) propuseram a filogenia do gênero *Atelopus* com base nos marcadores 12S e 16S e indicaram o posicionamento filogenético de *A. hoogmoedi*, do norte do rio Amazonas no Escudo das Guianas. A população de *Atelopus hoogmoedi* no sul do rio Amazonas representa um grupo isolado daqueles do Escudo das Guianas, cujo *status* taxonômico encontra-se incerto, assim como muitas espécies do gênero (Noonan e Gaucher 2005).

Segundo Lötters et al. (2011), a espécie *A. hoogmoedi* é um grupo parafilético, com três linhagens (duas para Guiana Francesa e uma para Guiana): a linhagem da Guiana Francesa 1, é a espécie irmã de *A. spumarius barbotini* Lescure 1981, da Guiana Francesa, região de Saul, enquanto que as outras duas linhagens da Guiana Francesa 2, região de Saul e da Guiana, região de Mabura Hill, representam um grupo externo da primeira. De acordo com Zink (1997), linhagens agrupadas numa espécie biológica não compartilham o mesmo ancestral recente comum (não são monofiléticas), o que leva a existência de espécies parafiléticas e ao isolamento reprodutivo.

A utilização de dados moleculares aplicados a resolução de problemas taxonômicos em espécies amplamente distribuídas, pode ser uma poderosa ferramenta na identificação de linhagens (Fouquet et al. 2007, Funk et al. 2012), proporcionando uma revisão dos caracteres morfológicos e bioacústicos (Elmer e Cannatella 2008). As linhagens identificadas com os marcadores mitocondriais 16S e Citocromo Oxidase Subunidade I (COI) são consideradas como sendo espécies candidatas não confirmadas (Ortega-Andrade et al. 2017, Padial et al. 2010), aguardando uma revisão de outros caracteres (morfologia, bioacústica, distribuição, ecologia, entre outros), de acordo com a proposta da taxonomia integrativa (Dayrat 2005).

Portanto esse trabalho trata da heterogeneidade molecular em *Atelopus hoogmoedi* que provavelmente representa mais de uma unidade evolutiva, no qual o objetivo foi comparar os indivíduos de *A. hoogmoedi* do sul e norte do rio Amazonas. Sendo assim utilizamos métodos para delimitação taxonômica molecular como o Automatic Barcode Gap Discovery, ABGD (Puillandre et al. 2012), evidenciamos que a linhagem de *A. hoogmoedi* do sul do rio Amazonas representa uma espécie candidata não confirmada. Dados morfológicos e bioacústicos devem ser incorporados em trabalhos futuros.

Metodologia

Área de estudo

O trabalho foi desenvolvido com indivíduos de *A. hoogmoedi* coletados em dois municípios: Monte Alegre- PA (3°01’28.11”S; 53°15’26.17” O) na região do Escudo das Guianas que possui cobertura vegetal que abrange desde as florestas aluviais ao sul (percorrendo o rio Amazonas), Savanas Amazônicas (Campos de Monte Alegre) na parte central, floresta ombrófila densa submontana e topografia acidentada (Fróis et al. 2018). Já no município de Anapu-PA (3°09’01.59”S; 51°29’37.08” O) as coletas foram realizadas no Projeto de Desenvolvimento Sustentável (PDS) Virola-Jatobá, um assentamento do Instituto de Colonização de Reforma Agrária (INCRA) com área territorial de 41.153,31 hectares e cerca de 180 famílias assentadas que vivem do extrativismo e agricultura familiar que possui floresta ombrófila densa com árvores de médio e grande porte (Fig.1).

Extração e amplificação do DNA

O DNA foi extraído de oito indivíduos de *A. hoogmoedi*, sendo sete de Monte Alegre e um de Anapu. As amostras de tecidos do músculo da coxa ou do fígado foram acondicionadas em álcool 95% e os espécimes foram depositados na Coleção do Laboratório de Zoologia-Adriano Giorgi da Faculdade de Ciências Biológicas na Universidade Federal do Pará-UFPA/Campus de Altamira.

O DNA total de cada indivíduo foi extraído pelo método fenol clorofórmio (Sambrook et al. 1989). A qualidade das amostras foi observada por meio de eletroforese em gel de agarose 1%, visualizado em trans luminador UV 302 nm, corado com Gel Red.

O DNA foi amplificado via Reação em Cadeia da Polimerase (PCR) utilizando-se um par de iniciadores (*primers* 16Sa e 16Sb) Reverse e Forward para o marcador 16S (Palumbi et al. 1991) com as seguintes condições da PCR: temperatura inicial de 92°C (60 sec.), seguidos por 35 ciclos de 92°C (60 sec.), 50°C (50 sec.) e 72°C (1.5 min.) e extensão final a 72 °C por 7 min. A amplificação foi verificada por meio de eletroforese em gel de agarose 1%.

A precipitação das amostras foi purificada seguida da leitura em sequenciador ABI 3500xl (Life Techologies) usando o protocolo do fabricante. Após a leitura das sequências pelo sequenciador, estas foram alinhadas usando o algoritmo CLUSTAL W (Thompson et al. 1994) implementado no programa BIOEDIT (Hall 1999) e editadas manualmente. As sequências geradas e/ou analisadas neste estudo estão disponíveis no GenBank (os números de acesso estão listados na Tabela 1).

Análise filogenética

O modelo molecular evolutivo GTR + G foi escolhido através do software JMODEL TEST (Darriba et al. 2012). A árvore de Máxima Verossimilhança (ML) foi construída no software TREEFINDER (Jobb 2011) com 20.000 réplicas de bootstrap utilizando *Melanophryniscus stelzneri* e *Melanophyniscus sp.* como grupo externo seguindo (Lötters et al. 2011). A distância genética par a par não corrigida (distância-p) entre as espécies foi calculada no software MEGA 6.0 (Tamura et al. 2011).

Delimitação de espécies

Automatic Barcode Gap Discovery ou método ABGD (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) foi utilizado para identificar grupos de sequências que podem corresponder a mais de uma espécie com base na distribuição de distâncias genéticas entre as sequências de DNA alinhadas (Guarnizo et al. 2015, Vacher et al. 2017). Este método estatisticamente infere várias lacunas ou limiares de códigos de barras em potencial, particionando assim as sequências de modo que a distância entre duas sequências tomadas a partir de clusters distintos será maior que o gap de código de barras (Puillandre et al. 2012).

O alinhamento do gene mitocondrial 16S foi processado em ABGD em dois métodos de substituição de nucleotídeos, Kimura 2 Parameters - K2P (Kimura 1980) e Simple Distance, sob as seguintes configurações: Pmin: 0.001, Pmax: 0.1, steps: 10, Nb bins: 20 e (X) relative gap width: 1.5. O K2P é o modelo padrão de substituição de DNA por estudos de código de barras, obtendo bom desempenho por ser semelhante a outros modelos mais complexos na identificação de espécies (Collins e Cruickshank 2013), apesar de estudos sugerir que a taxa de sucesso de identificação de espécies não é afetada pelo modelo (Collins et al. 2012).

Resultados

A análise filogenética de ML de *Atelopus hoogmoedi* revelou um grupo parafilético com três linhagens: Guiana Francesa 1 e 2; Guiana e Monte Alegre; e Anapu. As duas primeiras estão no norte do rio Amazonas enquanto a última para o sul do rio Amazonas (Fig.2). *Atelopus hoogmoedi*, *A. flavescens*e *A. barbotini* da Guiana Francesa representam espécies irmãs de *A. hoogmoedi* da Guiana e Monte Alegre, com alto suporte de bootstrap 93, no entanto o relacionamento filogenético entre *A. hoogmoedi*, *A. barbotini* e *A. flavescens* na Guiana Francesa aparece mal resolvido, com polítomias presentes. A linhagem de *A. hoogmoedi* de Anapu (sul do rio Amazonas) aparece como irmã de *A. hoogmoedi*, *A. flavencens*, *A. barbotini* da Guiana Francesa e *A. hoogmoedi* da Guiana e Monte Alegre como valor de bootstrap de 57.

A distância genética entre as linhagens de *A. hoogmoedi* da Guiana Francesa 1 e 2 foi de 0%, o mesmo valor foi encontrado entre Monte Alegre e Guiana. A distância genética entre as linhagens Monte Alegre/Guiana e Guiana Francesa 1 e 2 foi de 0.5%. A linhagem de Anapu, do sul do rio Amazonas, apresentou uma distância genética de 2.3% para a linhagem da Guiana Francesa 1 e 2 e 2.9% para Monte Alegre/Guiana. Diversas espécies válidas de *Atelopus* (*A. hoogmoedi* Guiana, *A. barbotini* Guiana Francesa, *A. flavescens* Guiana Francesa, *A. seminiferus* Peru, *A. pulcher* Peru, *A. spumarius* Peru) apresentaram distâncias genéticas variando de 0 – 2.6% entre si, enquanto que as mesmas espécies apresentaram valores variando de 2.3 – 3.2% para a linhagem de Anapu no sul do rio Amazonas (Tabela 2).

De acordo com o ABGD, o modelo Simple Distance indicou que *A. hoogmoedi* da Guiana Francesa 1, 2, Guiana, Monte Alegre, *A. flavescens* e *A. barbotini* representam uma única espécie, enquanto que *A. hoogmoedi* de Anapu representou uma espécie distinta. O modelo K2P mostrou que *A. hoogmoedi* de todas as localidades citadas acima, *A. flavescens* e *A. barbotini* representam espécies distintas. Assim, em ambos os modelos, *A. hoogmoedi* de Anapu (sul do rio Amazonas) é reconhecido como uma espécie distinta daquelas do Escudo das Guianas (Fig. 2).

Discussão

No sul do rio Amazonas, *Atelopus hoogmoedi* tem sido registrado em cinco localidades: Tucuruí e Serra de Carajás (interflúvio Xingu/Tocantíns-Araguaia), Itaituba e Santarém (Interflúvio Xingu/Tapajós) (Avila-Pires et al. 2010) e margem direita do baixo rio Xingu. Essas populações estão separadas daquelas do Escudo das Guianas pelo rio Amazonas e entre si pelo rio Xingu, delimitando duas áreas de endemismo (Tapajós e Xingu) (Silva et al. 2002). Os resultados encontrados neste trabalho demostram que *A.hoogmoedi* de Anapu pertence a uma linhagem diferente das do Escudo das Guianas. Outros trabalhos apontam que linhagens de *A. hoogmoedi* do sul do rio Amazonas representam linhagens distintas daquelas do Escudo das Guianas, como proposto por Noonan e Gaucher (2005).

Nos últimos anos, o marcador mitocondrial 16S tem sido utilizado para a descrição de diversas espécies de anuros neotropicais (De Oliveira et al. 2017, De Oliveira e Hernández-ruz 2017, Rojas et al. 2016). A proposta de um valor limite (>3%) para separar espécies com o marcador 16S vem sendo defendido por diversos autores (Fouquet et al. 2007, Lyra et al. 2017, Moraes et al. 2016, Padial et al. 2010, Vences et al. 2005, Vieites et al. 2009). No entanto, algumas espécies são descritas com valores inferiores a 3%, como por exemplo *Pseudopaludicola jaredi* (De Andrade et al. 2016) e *Proceratophrys ararype* (Mângia et al. 2018). Os resultados mostram que diversas espécies válidas de *Atelopus* apresentam distâncias genéticas entre si variando de 0 – 2.6% (*A. hoogmoedi*, *A. barbotini*, *A. flavescens, A. seminiferus, A. pulcher, A. spumarius*), enquanto que as mesmas apresentaram distâncias genéticas variando de 2.3 – 3.2% para *A. hoogmoedi* de Anapu. Valores abaixo de 3% é observado em complexo de espécies crípticas de anuros, como *Engystomops* e *Hypsiboas* (Funk et al. 2012), *Physalaemus* (Funk et al. 2007), *Ameerega* (Lötters et al. 2009).

Neste trabalho foi constatada a linhagem de *Atelopus hoogmoedi* ao sul do rio Amazonas, classificada como espécie candidata não confirmada, necessitando de mais coletas e acesso aos indivíduos das outras localidades de sua ocorrência. É fortemente encorajado a integração de dados morfológicos, bioacústicos e outros marcadores moleculares para uma taxonomia integrativa (Padial e De La Riva 2009, Vieites et al. 2009), para a resolução do status taxonômico. Devido a espécie candidata ser encontrada em um Projeto de Desenvolvimento Sustentável, a necessidade de estabelecer áreas prioritárias para a conservação é recomendado, até a confirmação da ocorrência em outras localidades.

Conclusão

Portanto foram observadas grandes distâncias genéticas entre as populações do sul e norte do rio Amazonas em que *Atelopus hoogmoedi* do sul do rio Amazonas representa uma espécie candidata não confirmada. Assim, é de extrema importância sua conservação devido à ausência de estudos desta população em relação as do Escudo das Guianas e pelas espécies deste gênero estar em risco de extinção. E possivelmente dados morfológicos e bioacústicos devem ser incorporados em trabalhos futuros.

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Legendas das figuras

Figura 1: Localidades de *A. hoogmoedi* utilizadas neste estudo. Os círculos azul, amarelo, laranja e vermelho representam localidades ao Norte do rio Amazonas e o círculo roxo ao Sul do rio Amazonas.

Figura 2: Árvore ML da população de *Atelopus* na Amazônia. Abreviações: Equador (Equ); Costa Rica (CoR); Panamá (Pan); Colômbia (Col); Peru (Per); Bolívia (Bol); Guiana Francesa 1 (GF1); Guiana Francesa 2 (GF2); Guiana Francesa (GuF); Guiana (Gui); Monte Alegre (Mon); Anapu (Ana).

Tabelas

Tabela 1. Lista de espécimes utilizados para análise molecular.

Espécies	Localidade	GenBank
Atelopus hoogmoedi	Anapu- PA	MK166205
Atelopus hoogmoedi	Monte Alegre- PA	MK166206
Atelopus hoogmoedi	Monte Alegre- PA	MK166207
Atelopus hoogmoedi	Monte Alegre- PA	MK166208
Atelopus hoogmoedi	Monte Alegre- PA	MK166209
Atelopus hoogmoedi	Monte Alegre- PA	MK166210
Atelopus hoogmoedi	Monte Alegre- PA	MK166211
Atelopus hoogmoedi	Monte Alegre- PA	MK166212
Atelopus hoogmoedi	Guiana Francesa	EU672972
Atelopus hoogmoedi	Guiana Francesa	DQ283260
Atelopus hoogmoedi	Guiana	EU672974
Atelopus barbotini	Guiana Francesa	EU672971
Atelopus spumarius	Peru	EU672977
Atelopus bomolochos	Equador	AF375508
Atelopus bomolochos	Equador	GU252225
Atelopus bomolochos	Equador	GU252226
Atelopus bomolochos	Equador	GU252227
Atelopus bomolochos	Equador	GU252231
Atelopus bomolochos	Equador	GU252232
Atelopus bomolochos	Equador	AF375509
Atelopus varius	Costa Rica	AY325996
Atelopus varius	Panamá	U52779
Atelopus peruensis	Peru	GU252229
Atelopus peruensis	Peru	GU252230
Atelopus halihelos	Equador	AF375510
Atelopus longirostris	Equador	AF375511
Atelopus zeteki	Panamá	DQ283252
Atelopus flavescens	Guiana Francesa	EU672970
Atelopus pulcher	Peru	EU672973
Atelopus spurrelli	Colômbia	EU672975
Atelopus seminiferus	Peru	EU672976
Atelopus tricolor	Bolívia	EU672978
Atelopus oxapampae	Peru	EU672979
Atelopus sp2	Peru	EU672980
Atelopus nanay	Equador	GU252228
Atelopus chiriquiensis	Panamá	U52780
Melanophryniscus stelzneri	Brasil	U52782
Melanophryniscus sp.	Brasil	KM204371

Tabela 2. Distância Genética. **1)** *Atelopus hoogmoedi* (Anapu); **2)** *A. hoogmoedi* (Monte Alegre); **3)** *A. hoogmoedi* (Guiana Francesa 1); **4)** *A. hoogmoedi* (Guiana Francesa 2); **5)** *A. hoogmoedi* (Guiana); **6)** *A. barbotini* (Guiana Francesa); **7)** *A. flavescens* (Guiana Francesa); **8)** *A. seminiferus* (Peru); **9)** *A. pulcher* (Peru); **10)** *A. spumarius* (Peru); **11)** *A. nanay* (Equador); **12)** *A. bomolochos* (Equador); **13)** *A. halihelos* (Equador); **14)** *A. peruensis* (Peru); **15)** *A. tricolor* (Bolívia); **16)** *A. oxapampae* (Peru); **17)** *A. longirostris* (Equador); **18)** *A. sp.2* (Peru); **19)** *A. zeteki* (Panamá); **20)** *A. varius* (Costa Rica); **21)** *A. varius* (Panamá); **22)** *A. spurrelli* (Colômbia); **23)** *A. chiriquiensis* (Panamá).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1																							
2	2.9																						
3	2.3	0.5																					
4	2.3	0.5	0.0																				
5	2.9	0.0	0.5	0.5																			
6	2.3	0.5	0.0	0.0	0.5																		
7	2.3	0.5	0.0	0.0	0.5	0.0																	
8	2.6	2.6	2.0	2.0	2.6	2.0	2.0																
9	2.9	2.3	1.7	1.7	2.3	1.7	1.7	1.7															
10	3.2	2.0	1.4	1.4	2.0	1.4	1.4	1.7	1.7														
11	8.8	9.4	9.1	9.1	9.4	9.1	9.1	8.8	7.6	8.2													
12	9.1	9.8	9.5	9.5	9.8	9.5	9.5	8.9	8.1	8.7	0.9												
13	9.1	9.7	9.4	9.4	9.7	9.4	9.4	9.1	7.9	9.1	2.3	2.9											
14	9.4	10.2	10.0	10.0	10.2	10.0	10.0	9.1	9.1	9.1	2.6	2.2	3.8										
15	10.5	10.8	10.5	10.5	10.8	10.5	10.5	10.5	9.4	10.0	9.7	10.1	10.8	11.4									
16	10.5	9.1	9.4	9.4	9.1	9.4	9.4	9.4	7.6	8.8	8.8	9.2	10.0	11.1	4.4								
17	10.8	11.4	11.1	11.1	11.4	11.1	11.1	10.5	10.2	10.2	3.2	3.0	4.7	2.6	10.8	10.0							
18	10.8	10.5	10.2	10.2	10.5	10.2	10.2	9.7	8.5	10.2	8.5	8.9	8.8	10.8	6.4	5.5	8.8						
19	11.1	12.0	11.7	11.7	12.0	11.7	11.7	11.1	10.8	11.4	5.2	5.4	7.0	5.2	11.4	11.1	4.1	9.7					
20	12.0	12.9	12.6	12.6	12.9	12.6	12.6	12.0	11.7	12.3	5.5	5.7	7.3	5.5	12.3	12.0	4.4	10.5	0.8				
21	12.3	13.2	12.9	12.9	13.2	12.9	12.9	12.3	12.0	12.6	5.8	6.0	7.6	5.8	12.6	12.3	4.7	10.8	1.1	0.8			
22	12.6	12.9	12.6	12.6	12.9	12.6	12.6	12.3	11.7	11.7	4.7	4.6	5.8	4.4	12.0	11.1	2.9	9.7	3.2	3.5	3.8		
23	12.6	13.2	12.9	12.9	13.2	12.9	12.9	12.3	11.4	12.6	5.0	4.7	6.7	5.0	12.0	11.7	3.8	10.2	1.4	1.7	1.4	2.9	

Figuras

Figura 1.

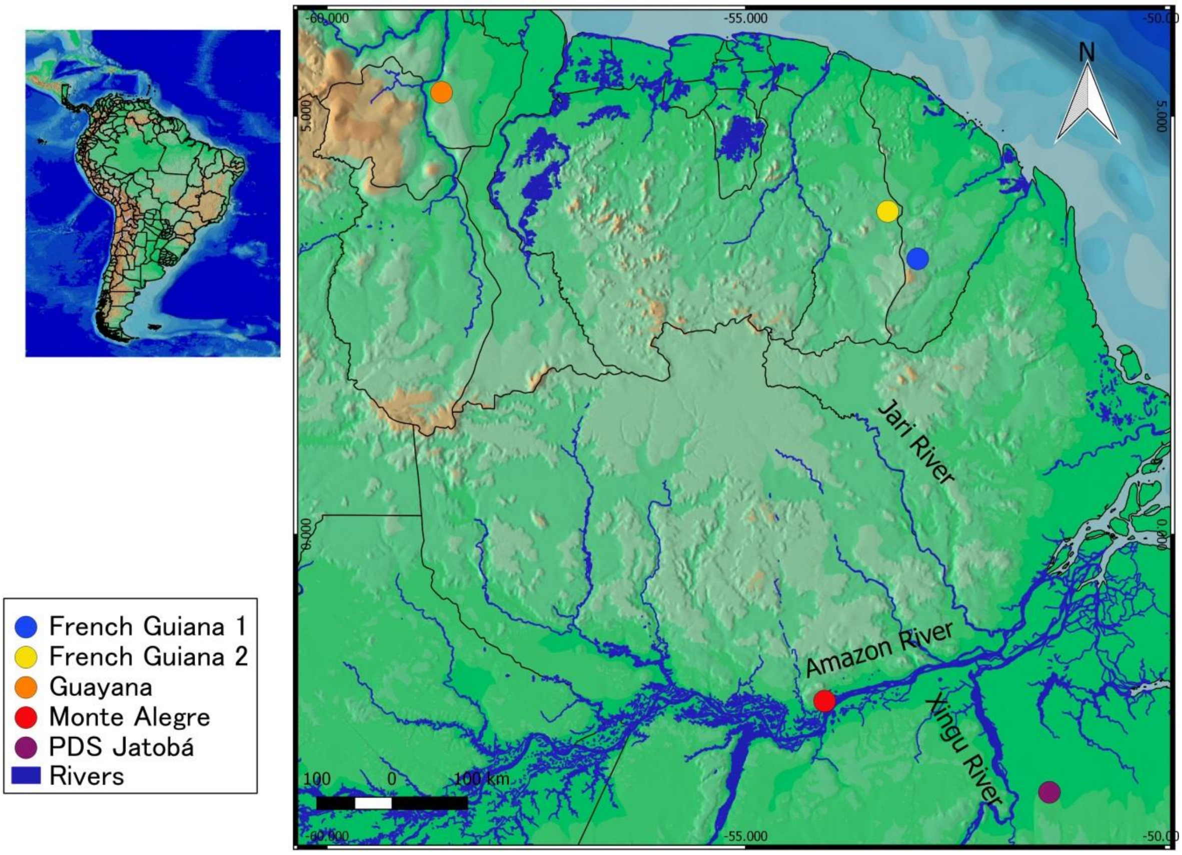
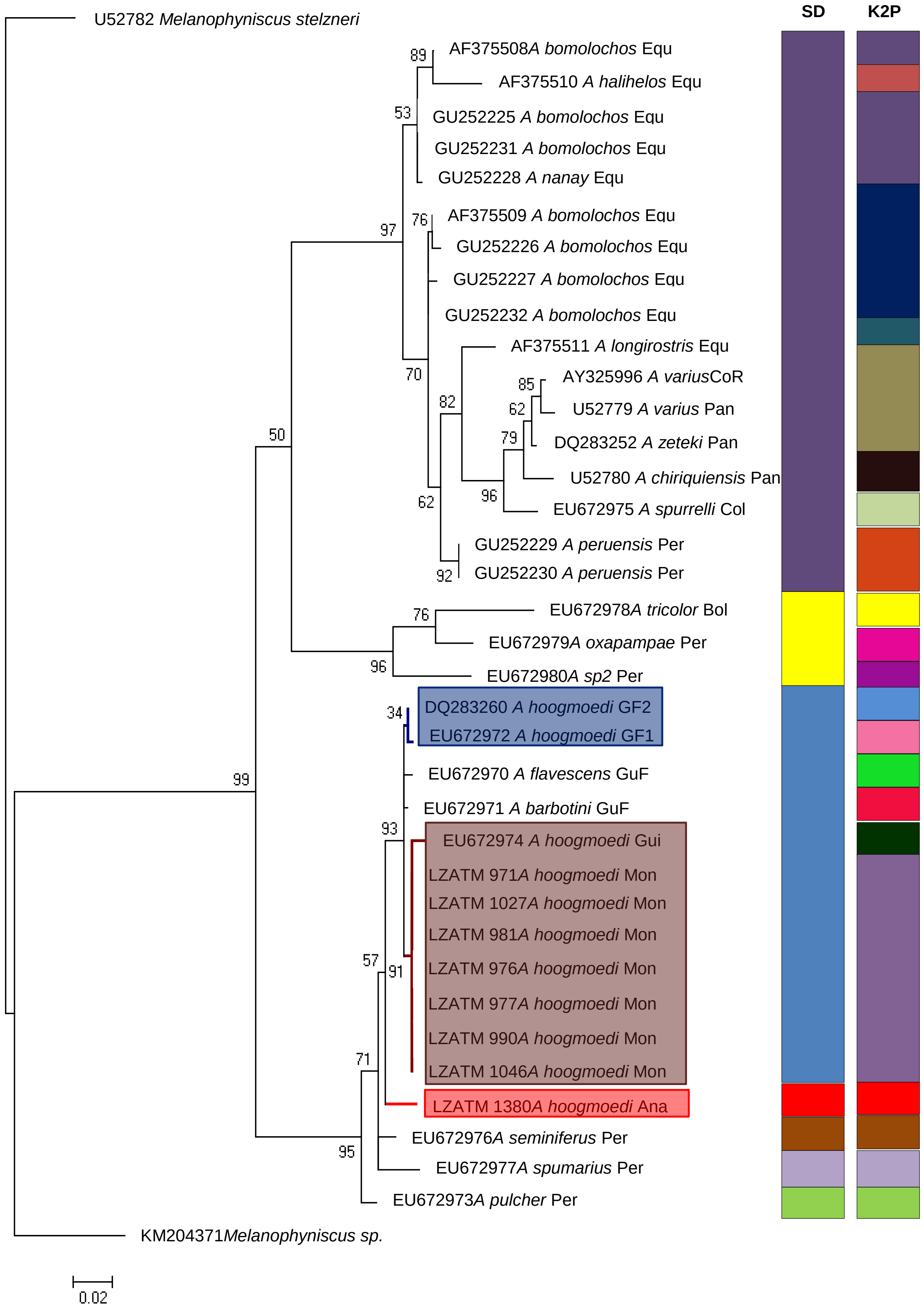


Figura 2.


SD
K2P
U52782 *Melanophyniscus stelzneri*
89
AF375508*A bomolochos* Equ
AF375510 *A halihelos* Equ
53
GU252225 *A bomolochos* Equ
GU252231 *A bomolochos* Equ
GU252228 *A nanay* Equ
76
AF375509 *A bomolochos* Equ
97
GU252226 *A bomolochos* Equ
GU252227 *A bomolochos* Equ
GU252232 *A bomolochos* Equ
AF375511 *A longirostris* Equ
70
85
AY325996 *A varius*CoR
82
62
U52779 *A varius* Pan
79
DQ283252 *A zeteki* Pan
50
U52780 *A chiriquiensis* Pan
96
62
EU672975 *A spurrelli* Col
GU252229 *A peruensis* Per
92
GU252230 *A peruensis* Per
76
EU672978*A tricolor* Bol
EU672979*A oxapampae* Per
96
EU672980*A sp2* Per
34
DQ283260 *A hoogmoedi* GF2
EU672972 *A hoogmoedi* GF1
99
EU672970 *A flavescens* GuF
EU672971 *A barbotini* GuF
93
EU672974 *A hoogmoedi* Gui
LZATM 971*A hoogmoedi* Mon
LZATM 1027*A hoogmoedi* Mon
LZATM 981*A hoogmoedi* Mon
57
91
LZATM 976*A hoogmoedi* Mon
LZATM 977*A hoogmoedi* Mon
LZATM 990*A hoogmoedi* Mon
71
LZATM 1046*A hoogmoedi* Mon
LZATM 1380*A hoogmoedi* Ana
EU672976*A seminiferus* Per
95
EU672977*A spumarius* Per
EU672973*A pulcher* Per
KM204371*Melanophyniscus sp.*
0.02

ANEXO I

NORMAS DE PUBLICAÇÃO DA REVISTA

ZooKeys

An International Journal
ISSN 1313–2970 (online)
ISSN 1313–2989 (print)

ZooKeys publishes papers in systematic zoology containing taxonomic/faunistic data on any taxon of any geological age from any part of the world with no limit to manuscript size. To respond to the current trends in linking biodiversity information and synthesizing the knowledge through technology advancements, *ZooKeys* also publishes papers across other taxon-based disciplines, such as ecology, molecular biology, genomics, evolutionary biology, paleontology, behavioural science, bioinformatics, etc.

Journal Impact Factor (JIF): 1.079

Authors Guidelines

MAIN TEXT

Title: The title should be in a sentence case (only scientific, geographic or person names should be with a first capital letter, i.e. *Elater ferrugineus* L., Germany, etc.), and should include an accurate, clear and concise description of the reported work, avoiding abbreviations. The higher taxa within the title should be separated with commas and not with a semicolon, e.g.: (Coleoptera, Elateridae, Elaterini).

Authors and Affiliations: Provide the complete names of all authors, and their addresses for correspondence, including e.g., institutional affiliation (e.g. university, institute), location (street, boulevard), city, state/province (if applicable), and country. One of the authors should be designated as the corresponding author. It is the corresponding author's responsibility to ensure that the author list, and the individual contributions to the study are accurate and complete. If the article has been submitted on behalf of a consortium, all consortium members and their affiliations should be listed after the **Acknowledgements section**.

Abstract and Keywords: Please have your abstract and keywords ready for input into the submission module. Keywords should be in alphabetical order and ideally differ from the words used in the title.

Body Text: All papers should be in grammatically correct English. Non-native English speaking authors are required to have their manuscripts checked by a native English speaker prior to submission. Use either British/Commonwealth or American English provided that the language is consistent within the paper. A manuscript must be written with precision, clarity, and economy. The voice - active or passive - and the tense used should be consistent throughout the manuscript. Avoid the use of parenthetical comments and italics or bold for emphasis. This journal discourages the use of quotation marks except for direct quotations, words defined by the author, and words used in unusual contexts. Short quotations should be embedded in the text and enclosed in double quotation marks ("). Long quotations should be on a separate line, italicized, but without quotation marks. Single quotation marks are to be used only for a quotation that occurs within another quotation.

Spacing, Fonts, and Page Numbering: Single-space all material (text, quotations, figure legends, tables, references, etc.). Separate paragraphs with a blank line. Use a 12-point font (preferably Times New Roman or Arial).

Capitals: First capital letters should be used only in the beginning of a sentence, in proper names and in headings and subheadings, as well as to indicate tables, graphs and figure/s within the text. Software programmes should be written with capital letters (e.g., ANOVA, MANOVA, PAUP).

Italicization/Underlining: Scientific names of species and genera, long direct quotations and symbols for variables and constants (except for Greek letters), such as p, F, U, T, N, r, but not for SD (standard deviation), SE (standard error), DF (degrees of freedom) and NS (non significant) should be italicized. These symbols in illustrations and equations should be in italics to match the text. Italics should not be used for emphasis, and not in abbreviations such as e.g., i.e., et al., etc., cf. Underlining of any text is not acceptable.

Abbreviations: Abbreviations should be followed by ‘.' (full stop or period; for instance: i.e., e.g., cf., etc.). Note that you shouldn't add a full stop at the end of abbreviated words if the last letter of the abbreviation is the same as the last letter of the full word. For example, you should abbreviate "Eds", "Dr", "Mr" without full stop at the end. All measures, for instance mm, cm, m, s, L, should be written without full stop.

On the use of dashes: **(1)** Hyphens are used to link words such as personal names, some prefixes and compound adjectives (the last of which vary depending on the style manual in use) **(2)** En-dash or en-rule (the length of an 'n') is used to link spans. In the context of our journal en-dash should be used to link numerals, sizes, dates and page numbers (e.g., 1977–1981; figs 5–7; pp. 237–258); geographic or name associations (Murray–Darling River; a Federal–State agreement); and character states combinations such as long–pubescent or red–purple. **(3)** Em-dash or em-rule (the length of an 'm') should be used rarely, only for introducing a subordinate clause in the text that is often used much as we use parentheses. In contrast to parentheses an em-dash can be used alone. En-dashes and em-dashes should not be spaced.

Footnotes: Avoid footnotes in the body text of the manuscript. It is always possible to incorporate the footnote into the main text by rewording the sentences, which greatly facilitates reading. Additionally, footnotes are not always handled well by the journal software, and their usage may cause a failure of submission. Footnotes are acceptable only below tables; instead of numbers, please use (in order): †, ‡, §, |, ¶, #, ††, ‡‡, §§, ||, ¶, ##.

Geographical coordinates: It is strongly recommended to list geographical coordinates as taken from GPS or online gazetteer, or georeferencer. Geographical coordinates must be listed in one of the following formats:

Definition: The locality consists of a point represented by coordinate information in the form of latitude and longitude. Information may be in the form of

- Degrees, Minutes and Seconds (DMS),
- Degrees and Decimal Minutes (DDM), or
- Decimal Degrees (DD).

Records should also contain a hemisphere (E or W and N or S) or, with Decimal Degrees, minus (–) signs to indicate western and/or southern hemispheres.

Examples:

- Example 1: 36° 31' 21" N; 114° 09' 50" W (DMS)
- Example 2: 36° 31.46’N; 114° 09.84’W (DDM)
- Example 3: 36.5243° S; 114.1641° W (DD)
- Example 4: −36.5243; −114.1641 (DD using minus signs to indicate southern and western hemispheres)

Note on accuracy: Because GPS units are very commonly used today to record latitude/longitude, many authors simply give the GPS readings for their localities. However, these readings are much too accurate. For example, a GPS unit might give the latitude in decimal seconds as 28°16'55.87"N. Since one second of latitude is about 30 m on the ground, the second figure after the decimal in 55.87 represents 30 cm, yet a typical handheld GPS unit is only accurate at best to a few metres.

We therefore recommend two ways to report GPS-based locations. If you give the GPS reading without rounding off, make sure you include an uncertainty figure as a context for the over-accurate GPS reading. We recommend the Darwin Core definition of uncertainty (http://rs.tdwg.org/dwc/terms/index.htm#coordinateUncertaintyInMeters):

"The horizontal distance (in meters) from the given decimalLatitude and decimalLongitude describing the smallest circle containing the whole of the Location."

If you only give the GPS reading, please round it off to an implied precision appropriate to the error in the measurement, or to the extent of the area sampled. We suggest rounding off

- to the nearest second in degree-minute-second format (28°16'56"N), which implies roughly ± 25-30 m at middle latitudes
- to four decimal places in decimal degree format (28.2822°N), which implies roughly ± 10-15 m at middle latitudes
- to two decimal places in decimal minute format (28°16.93'N), which implies roughly 15-20 m at middle latitudes

Altitude: Many GPS users simply record the elevation given by their GPS unit. However, GPS elevation is NOT the same as elevation above sea level. GPS units record the elevation above a mathematical model of the earth's surface. The difference between this elevation and elevation above sea level can be tens of metres. In any case, the accuracy of a GPS elevation is often the same as the usual accuracy in horizontal position, so a GPS elevation such as '753 m' is much too accurate and should be rounded off to 'ca 750 m'.

We **strongly recommend** the use of Example 2 (the DDM format). The other three are also possible but will be recalculated to DDM during the process of online mapping from the HTML version of the paper.

The only restriction on format is in creating a KML (Keyhole Markup Language) file. KML latitudes and longitudes must be in the DD format shown above in Example 4.

Please also consider submitting a **table of localities** with your manuscript, either as a spreadsheet or in CSV text format. By doing so you will make your specimen localities much more easily available for use in biodiversity databases and geospatial investigations. The geospatial table will be put online as supplementary material for your paper. A minimum table will have three fields: species (or subspecies) name, latitude and longitude. A full table will have the same data for each specimen lot as appears in the text of your paper. Please check latitude/longitude carefully for each entry.

Units: Use the International System of Units (SI) for measurements. *Consult Standard Practice for Use of the International System of Units*(ASTM Standard E−380−93) for guidance on unit conversions, style, and usage.

Statistics: Use leading zeroes with all numbers, including probability values (e.g., $P < 0.001$). For every significant F−statistic reported, provide two df values (numerator and denominator). Whenever possible, indicate the year and version of the statistical software used.

Web (HTML) links: Authors are encouraged to include links to other Internet resources in their article. This is especially encouraged in the reference section. When inserting a reference to a web-page, please include the **http://** portion of the web address.

Supplementary files: Larger datasets can be uploaded separately as Supplementary Files. Tabular data provided as supplementary files can be uploaded as an Excel spreadsheet (.xls), as an OpenOffice spreadsheets (.ods) or comma separated values file (.csv). As with all uploaded files, please use the standard file extensions.

Headings and subheadings: Main headings: The body text should be subdivided into different sections with appropriate headings. Where possible, the following standard headings should be used: **Introduction**, **Methods**, **Results**, **Discussion**, **Conclusions**, **Acknowledgements**, **References**. These headings need to be in bold font on a separate line and start with a first capital letter. Please do not number headings or subheadings.

- **Introduction** − The motivation or purpose of your research should appear in the Introduction, where you state the questions you sought to answer, and then provide some of the historical basis for those questions.
- **Methods** − Provide sufficient information to allow someone to repeat your work. A clear description of your experimental design, sampling procedures, and statistical procedures is especially important in papers describing field studies, simulations, or experiments. If you list a product (e.g., animal food, analytical device), supply the name and location of the manufacturer. Give the model number for equipment used. Supply complete citations, including author (or editor), title, year, publisher, and version number, for computer software mentioned in your article.
- **Results** − Results should be stated concisely and without interpretation.
- **Discussion** − Focus on the rigorously supported aspects of your study. Carefully differentiate the results of your study from data obtained from other sources. Interpret your results, relate them to the results of previous research, and discuss the implications of your results or interpretations. Point out results that do not support speculations or the findings of previous research, or that are counter-intuitive. You may choose to include a Speculation subsection in which you pursue new ideas suggested by your research, compare and contrast your research with findings from other systems or other disciplines, pose new questions that are suggested by the results of your study, and suggest ways of answering these new questions.
- **Conclusion** −This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.
- **References** − The list of References should be included after the final section of the main article body. A blank line should be inserted between single-spaced entries in the list. Authors are requested to include links to online sources of articles, whenever possible!

Where possible, the standard headings should be used in the order given above. Additional headings and modifications are permissible.

Subordinate headings: Subordinate headings (e.g. *Field study and Simulation model or Counts*, *Measurements and Molecular analysis*), should be left-justified, italicized, and in a regular sentence case. All subordinate headings should be on a separate line.

ENGLISH LANGUAGE EDITING

This journal has well-defined policies for English language editing. Involving mandatory outsourced language editing services would considerably increase the price of the Article Processing Charges, which would become an additional obstacle for persons and institutions to publish in the journal. Therefore we rely both on the conscience of our authors to provide stylistically written texts and our editors and reviewers to filter out badly written manuscripts.

Authors are required to have their manuscripts edited by a native English speaker **BEFORE** submission. Authors have to confirm by checking a tick box in the submission process that they have followed the above requirement:

The text is checked by a native English speaker, duly acknowledged in the manuscript. I am aware that non-edited manuscripts could be rejected prior to peer-review.

The submission process includes an option to request a professional linguistic and copy editing at a price of **EURO 15 per 1800 characters**:

The text has not been checked by a native speaker and I request thorough editing prior to peer review at a price. I agree to cover the costs even if my manuscript is not accepted for publication.

The authors are **NOT** obliged to use our linguistic services, but they must ensure that their manuscripts have been checked by a native speaker.

CITATIONS AND REFERENCES

Citations within the text: Before submitting the manuscript, please check each citation in the text against the References and vice-versa to ensure that they match exactly. Citations in the text should be formatted as follows: Smith (1990) or (Smith 1990), Smith et al. (1998) or (Smith et al. 1998) and (Smith et al. 1998, 2000, Brock and Gunderson 2001, Felt 2006).

References: It is important to format the references properly, because all references will be linked electronically as completely as possible to the papers cited. It is desirable to add a DOI (digital object identifier) number for either the full-text or title and abstract of the article as an addition to traditional volume and page numbers. If a DOI is lacking, it is recommended to add a link to any online source of an article. Please use the following style for the reference list (or download the *Pensoft EndNote style*): here

Published Papers: Polaszek A, Alonso-Zarazaga M, Bouchet P, Brothers DJ, Evenhuis NL, Krell FT, Lyal CHC, Minelli A, Pyle RL, Robinson N, Thompson FC, van Tol J (2005) ZooBank: the open-access register for zoological taxonomy: Technical Discussion Paper. Bulletin of Zoological Nomenclature 62: 210-220.

Accepted Papers: Same as above, but "in press" appears instead the year in parentheses.

Electronic Journal Articles: Mallet J, Willmott K (2002) Taxonomy: renaissance or Tower of Babel? Trends in Ecology and Evolution 18 (2): 57-59. https://doi.org/10.1016/S0169-5347(02)00061-7.

Paper within conference proceedings: Orr AG (2006) Odonata in Bornean tropical rain forest formations: Diversity, endemicity and applications for conservation management. In: Cordero Rivera A (Ed.) Forest and Dragonflies. Fourth WDA International Symposium of Odonatology, Pontevedra (Spain), July 2005. Pensoft Publishers, Sofia-Moscow, 51-78.

Book chapters: Mayr E (2000) The biological species concept. In: Wheeler QD, Meier R (Eds) Species Concepts and Phylogenetic Theory: A Debate. Columbia University Press, New York, 17-29.

Books: Goix N, Klimaszewski J (2007) Catalogue of Aleocharine Rove Beetles of Canada and Alaska. Pensoft Publishers, Sofia-Moscow, 166 pp.

Book with institutional author: International Commission on Zoological Nomenclature (1999) International code of zoological nomenclature. Fourth Edition. London: The International Trust for Zoological Nomenclature.

PhD thesis: Dalebout ML (2002) Species identity, genetic diversity and molecular systematic relationships among the Ziphiidae (beaked whales). PhD thesis, Auckland, New Zealand: University of Auckland.

Link/URL: BBC News: Island leopard deemed new species http://news.bbc.co.uk/

Citations of Public Resource Databases: It is highly recommended all appropriate datasets, images, and information to be deposited in public resources. Please provide the relevant accession numbers (and version numbers, if appropriate). Accession numbers should be provided in parentheses after the entity on first use. Examples of such databases include, but are not limited to:

- ZooBank (www.zoobank.org)
- Morphbank (www.morphbank.net)
- Genbank (www.ncbi.nlm.nih.gov/Genbank)
- BOLD (www.barcodinglife.org)

Providing accession numbers to data records stored in global data aggregators allows us to link your article to established databases, thus integrating it with a broader collection of scientific information. Please hyperlink all accession numbers through the text or list them directly after the References in the online submission manuscript.

All journal titles should be spelled out completely and should **NOT** be italicized.

Provide the publisher's name and location when you cite symposia or conference proceedings; distinguish between the conference date and the publication date if both are given. Do not list abstracts or unpublished material in the References. They should be quoted in the text as personal observations, personal communications, or unpublished data, specifying the exact source, with date if possible. When possible, include URLs for articles available online through library subscription or individual journal subscription, or through large international archives, indexes and aggregators, e.g., PubMedCentral, Scopus, CAB Abstracts, etc. URLs for pdf articles that are posted on personal websites only should be avoided.

Authors are encouraged to cite in the References list the publications of the original descriptions of the taxa treated in their manuscript.

ILLUSTRATIONS, FIGURES AND TABLES

Figures and illustrations are accepted in the following image file formats:

- **EPS** (preferred format for diagrams)
- **TIFF** (at least 300dpi resolution, with LZW compression)
- **PNG** (preferred format for photos or images)
- **JPEG** (preferred format for photos or images)

- **GIF**
- **BMP**
- **SVG**

Should you have any problems in providing the figures in one of the above formats, or in reducing the **file below 20 MB**, please contact the Editorial Office at journals@pensoft.net

Figure legends: All figures should be referenced consecutively in the manuscript; legends should be listed consecutively immediately after the References. For each figure, the following information should be provided: Figure number (in sequence, using Arabic numerals − i.e. Figure 1, 2, 3 etc.); short title of figure (maximum 15 words); detailed legend, up to 300 words.

Illustrations of measurable morphological traits should bear mute scale bars, whose real size is to be given in the figure captions.

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Tables: Each table should be numbered in sequence using Arabic numerals (i.e. Table 1, 2, 3 etc.). Tables should also have a title that summarizes the whole table, maximum 15 words. Detailed legends may then follow, but should be concise.

Small tables can be embedded within the text, in portrait format (note that tables on a landscape page must be reformatted onto a portrait page or submitted as additional files). These will be typeset and displayed in the final published form of the article. Such tables should be formatted using the 'Table object' in a word processing program to ensure that columns of data are kept aligned when the file is sent electronically for review. Do not use tabs to format tables or separate text. All columns and rows should be visible, please make sure that borders of each cell display as black lines. Colour and shading should not be used; neither should commas be used to indicate decimal values. Please use a full stop to denote decimal values (i.e., 0.007 cm, 0.7 mm).

Larger datasets can be uploaded separately as Supplementary Files. Tabular data provided as supplementary files can be uploaded as an Excel spreadsheet (.xls), as an OpenOffice spreadsheets (.ods) or comma separated values file (.csv). As with all uploaded files, please use the standard file extensions.

TAXONOMIC TREATMENTS

General guidelines

By publishing in this journal you are already creating a modern taxonomic product that is more accessible than previous print only works. The following guidelines are provided to ensure that other elements of the work follow modern standards and enable the full advantage of the ARPHA platform.

- Include unique specimen identifiers for type material. Unique identifiers are for example museum collections specimen IDs. Unique identifiers can be provided also by international taxon-based databases that do not indicate ownership, such as AntWeb.org for ants, for example.
- Holotype should not deposited in private collections.
- Include images of type material or representative species. Imaging is not a technical problem anymore and is provided by many institutional collections or international taxon-based services (again,AntWeb.org is a good example as they will provide free imaging of ant type material if necessary).
- Specimen data of material examined provided as auxiliary file as a .txt or .cvs file or table at end of document, based on the Darwin Core standard. Specimen file should include unique specimen identifiers when possible.
- Include latitude, longitude, elevation, habitat, microhabitat information of primary type material. For format of geographical coordinates see section "Main text" above.
- Provide dichotomous key of taxa or related taxa (i.e. species group) or links to online-based keys.
- Single species descriptions should be clearly justified with regard as to why a more detailed larger scale, comparative revision was not conducted. For descriptions of single species see also section "Focus and Scope".

Sequence data

Manuscripts containing novel amino acid sequences (e.g. primer sequences) will only be accepted if they carry an International Nucleotide Sequence Databases (INSD) accession number from the European Biology Laboratory (EMBL), GenBank Data Libraries (GenBank) or DNA Data Bank of Japan (DDBJ). We strongly recommend that authors include institutional catalog numbers for specimens preserved in collections, and information identifying sequences that are derived from type specimens (see below) when they deposit data in genetic databanks. A summary table with the INSD accession [catalog] numbers should be included in either Materials and Methods or Data Resources section of the paper. If specimens were not vouchered (tissued specimens should be vouchered whenever possible!), collection locality data and possibly photographs of tissued specimens must be provided. A nomenclature for genetic sequences for types and confidently identified nontype specimens has been proposed by Chakrabarty et al. (2013); a sequence from a holotype is identified as genseq-1, one from a paratype is identified as genseq-2, one from a topotype is genseq-3, etc. The genetic marker(s) used should also be incorporated into the nomenclature (e.g. genseq-2 COI).

Examples

Table 1. Ranking Sequence Reliability. Ranking of source materials of genetic sequences based on reliability of taxonomic identification. Examples of the source material are listed in the third column with the last column providing the corresponding GenSeq nomenclature (after Chakrabarty et al. (2013)).

Reliability Ranking	**Source Materials**	**Examples**	**Corresponding GenSeq Nomenclature**
Highest 1st	Primary Types	Holotype, Lectotype, Syntype, Isosyntype, Neotype, Isotype	genseq-1
2nd	Secondary Types	Paratype, Paralectotypes, etc.	genseq-2
3rd	Topotypes (vouchered), or	Topotype, Non-type	genseq-3

	non-type specimens listed in original description or redescription	specimen listed in original description or redescription	
4th	Collections-vouchered non-types (not from original description or redescription)	Vouchered specimen	genseq-4
5th	Photo voucher only	No specimen voucher but photo voucher available	genseq-5
Lowest	No voucher	Non-vouchered	No classification

Table 2. Example Reporting Table. Examples of how links between genetic sequences and vouchers in institutional collections could be displayed as a table in publications reporting new sequences.

Species	**Specimen Catalog #**	**GenBank #**		**GenSeq Nomenclature**
		COI	**ND1**	
Typhleotris mararybe	LSUMZ 13636 (holotype)	HM590594	HM590606	genseq-1 COI, ND1
Paretroplus tsimoly	AMNH 229558 (paratype)	JZ590596	NA	genseq-2 COI
Nandopsis haitiensis	UMMZ 236321 (topotype)	BK590595	BK590607	genseq-3 COI, ND1
Halieutichthys intermedius	FMNH 96353 (non-type specimen voucher)	AY722169	AY722306	genseq-4 COI, ND1
Equulites absconditus	NMNH 12345PV2 (photo voucher)	NA	BG34621	genseq-5 ND1

International Code of Zoological Nomenclature

This journal will publish papers that strictly adhere to the rules of the last edition of the International Code of Zoological Nomenclatureand its amendment. Authors are also advised to follow all recommendations of the Code and to consult the guidelines below, as well as ICZN's manual Best practice in the use of the scientific names of animals prior to submitting the manuscript.

General: Each **first mentioning** of an animal species name within the text must be provided with author(s)' name(s). **Year of publication** of an animal species should be given in taxonomic revisions with quotation of the work providing the original species' description in the list of references.

New names: When new taxonomic acts are proposed, they should be explicitly indicated as being new by adding the respective abbreviation after the taxon name i.e., sp. n., comb. n., nomen n. Authors of newly described taxa should be given any time the taxon is mentioned, if different from the publication authors.

Examples:

- Genus *X-us* Smith, new genus (author(s) of the publication and authority (-ies) of the taxon is/are identical);
- *X-us albus* Jones & Peters, new species (the publication is authored by persons different in composition or combination from the authority (-ies) of the taxon itself, e.g. Smith, Jones & Peters or Peters & Jones).

We highly recommend that authors of new species are also included as co-authors of the work where the taxa are described. If the authors of the work do not want to include the authors of the taxonomic name then to be absolutely certain that the authority for the name is unequivocal there should be a statement in the work saying that these authors (of the name) are responsible for making the name available under the code (Article 50.1.2, etc.) i.e. they are responsible for coining the name and for satisfying all other criteria for availability.

New family-group names: Although all family group names are derived/based on their type genus, the type genus is to be compulsorily designated in any description of a family-group name published after 31st December 1999 (Article 16.2). It is not sufficient that the type genus is mentioned as belonging to the new family-group name; it must be stated that this is the type genus. We recommend a single type line as: Type-genus: *Musca* Linnaeus, 1758.

New genus-group names: The origin ("etymology", or "derivatio nominum") of name and its gender should be indicated. The type-species and the character of the proposed taxonomic act should be specified for new genus-group names. The type species name should be given in its original combination with an author and year. If the type species is now considered a junior synonym there need to be a clear mention of that. The fixation type should derive from the International Code of Zoological Nomenclature (see Articles 68 & 69; original designation, monotypy, absolute tautonymy, Linnaean tautonymy, subsequent monotypy, subsequent designation).

Example:

- *Sympycnus* Loew

Type-species: *Porphyrops annulipes* Meigen, 1824 by subsequent designation of Coquillett (1910: 610) =*pulicarius* Fallen, 1823.

New species-group names: According to the ICZN Art. 11.9, but also Art. 11.3 the origin "etymology", or "derivatio nominum") new species-group names should be supplemented by information on whether the epithet is an 1) adjective or participle in the nominative singular; 2) noun in the nominative singular; 3) a noun in the genitive case; 4) an adjective used a substative in the genitive case; or 5) an arbitrary combination of letters (ICZN Art. 11.3). For **species-group names**, there are two separate statements of type information that are needed:

- the **statement of species' type locality** – that is the exact place whence the primary type origins, including exact collecting dataplace with geographical coordinates, geographical or political unit (Area/ District/ State) and country;also, if possible, supplementary locality information should be included – habitat type, method of collecting, date, collector's names, host name (for parasites), etc.
- there should be a separate statement about the **type specimen**, exact quotation of its original label, condition of specimen (dry pinned, in alcohol, slide, fossil, etc.) and repository (organization's name and city).

Examples:

For a **new species**:

- **Type-locality:** USA, Viriginia: Fairfax County, Kingstowne, 38°46'N, 77°07'W, broad-leaf forest, under bark, 10 July 2000, J. Smith leg.
- **Type-specimen:** Holotype male, pinned, with genitalia in a separate microvial. Original label: "USA, VA, Fairfax, Kingstowne, 38°46'N, 77°07'W, 12 Oct 2003, BJ & FC Thompson" "USNM ENT 00033805" [Code 49 barcode], "HOLOTYPE / Xylota / x-us / Thompson [red handwritten label].

For a **previously described species**:

Lectotype male, pinned … [details] here designated to fix the concept of *X-us albus* Jones and to ensure the universal and consistent interpretation of the same. Or … [details then] by designation of Smith (1976: 999).

Previously published names: For a **previously published name**, please provide the year of description. Also use the parentheses convention for subsequent new combinations.

[Etymology]

Authors of new species name should state exactly what the epithet is in terms of the ICZN, as outlined in Article 11.9.1.1 to 11.9.1.4 as well as 11.3. A name may be a word in or derived from Latin, Greek or any other language (even one with no alphabet), or be formed from such a word. In short, a name can be declared as arbitrary combination (the best solution) or must be or be treated as:

I) a word of two or more letters, or a compound word, and, if a Latin or latinized word must be, or be treated as:

1. an adjective or participle in the nominative singular (as in *Echinus esculentus*, *Felis marmorata*, *Seioptera vibrans*), or
2. a noun in the nominative singular standing in apposition to the generic name (as in *Struthio camelus*, *Cercopithecus diana*), or

3. a noun in the genitive case (e.g. *rosae*, *sturionis*, *thermopylarum*, *galliae*, *sanctipauli*, *sanctaehelenae*, *cuvieri*, *merianae*, *smithorum*), or
4. an adjective used as a substantive in the genitive case and derived from the specific name of an organism with which the animal in question is associated (as in *Lernaeocera lusci*, a copepod parasitic on *Trisopterus luscus*).

II) An adjectival species-group name proposed in Latin text but written otherwise than in the nominative singular because of the requirements of Latin grammar is available provided that it meets the other requirements of availability, but it is to be corrected to the nominative singular if necessary.

Arranging sections within species treatments (sections in square brackets are requested for new descriptions only!):

[Name]
[Material]
  - [Type material]
  - Other material
[Diagnosis]
[Description]
[Etymology]
Distribution
Ecology (including phenology)
Conservation status (optional, we encourage authors to follow the IUCN categories and criteria, please see http://www.iucnredlist.org/static/categories_criteria_3_1#critical))
Discussion (optional, but very desirable)

MATERIALS AND METHODS

In line with responsible and reproducible research, as well as FAIR (Findability, Accessibility, Interoperability and Reusability) data principles, we highly recommend that authors describe in detail and deposit their science methods and laboratory protocols in the open access repository protocols.io.

Once deposited on protocols.io, protocols and methods will be issued a unique digital object identifier (DOI), which could be then used to link a manuscript to the relevant deposited protocol. By doing this, authors could allow for editors and peers to access the protocol when reviewing the submission to significantly expedite the process.

Furthermore, an author could open up his/her protocol to the public at the click of a button as soon as their article is published.

Stepwise instructions:

1. Prepare a detailed protocol via protocols.io.
2. Click **Get DOI** to assign a persistent identifier to your protocol.
3. Add the DOI link to the Methods section of your manuscript prior to submitting it for peer review.
4. Click **Publish** to make your protocol openly accessible as soon as your article is published (optional).
5. Update your protocols anytime.

SUPPLEMENTARY FILES

Online publishing allows an author to provide datasets, tables, video files, or other information as supplementary information, greatly increasing the impact of the submission. Uploading of such files is possible in Step 6 of the submission process.

The maximum file size for each Supplementary File is 20 MB.

The Supplementary Files will not be displayed in the printed version of the article but will exist as linkable supplementary downloadable files in the online version.

While submitting a supplementary file the following information should be completed:

- File format (including name and a URL of an appropriate viewer if format is unusual)
- Title of data
- Description of data

All supplementary files should be referenced explicitly by file name within the body of the article, e.g. 'See supplementary file 1: Movie 1" for the original data used to perform this analysis.

Ideally, the supplementary files should not be platform-specific, and should be viewable using free or widely available tools. Suitable file formats are:

For supplementary documentation:

- **PDF** (Adobe Acrobat)

For animations:

- **SWF** (Shockwave Flash)

For movies:

- **MOV** (QuickTime)
- **MPG** (MPEG)

For datasets:

- **XLS** (Excel spreadsheet)
- **CSV** (Comma separated values)
- **ODS** (OpenOffice spreadsheets)

As for images, file names should be given in the standard file extensions. This is especially important for Macintosh users, since the Mac OS does not enforce the use of standard file extensions. Please also make sure that each additional file is a single table, figure or movie (please do not upload linked worksheets or PDF files larger than one sheet).