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DELIMITANDO ESPÉCIES DA ORDEM ORTHOPTERA: FERRAMENTAS E FILOGENIA

PORTO ALEGRE
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Orientador: Profª Drª Maríndia Deprá

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SUMÁRIO

LISTA DE FIGURAS

LISTA DE TABELAS

RESUMO

Orthoptera é uma das linhagens mais antigas dentro dos insetos, com cerca de 350 milhões de anos. Atualmente conta com cerca de 29.500 espécies válidas distribuídas pelo mundo. Recentemente tem sido classificado como o terceiro inseto domesticado para pesquisa científica devido a uma série de características que facilitam, principalmente, sua criação em laboratório e em larga escala. Entretanto, muitas questões taxonômicas ainda são obscuras em alguns grupos, o que leva a classificações e identificações errôneas. Para mitigar esses problemas e elucidar questões evolutivas, a taxonomia integrativa tem sido utilizada mais intensivamente nas pesquisas mais atuais, principalmente com dados moleculares e sonoros. Neste âmbito, os dois trabalhos realizados nesta tese estão diretamente relacionados com dados moleculares, com o segundo tendo associação com dados sonoros. No primeiro trabalho (Capítulo II) foi feita uma avaliação do gene citocromo c oxidase subunidade I (COI) para testar sua eficiência como DNA Barcode dentro da ordem Orthoptera. Para isso, utilizamos todas as sequências de COI depositadas no banco de dados *Barcode of Life Data System* (BOLD) e as submetemos a duas análises baseadas em distâncias intra e interespecíficas, *barcode gap* e probabilidade de identificação correta (PCI), além de estimarmos a riqueza de espécies através das ferramentas *Automatic Barcode Gap Discovery* (ABGD) e *Assemble Species by Automatic Partitioning* (ASAP). Nesse trabalho, foram utilizadas 11.605 sequências englobando 1.132 espécies de 226 gêneros. Baseado na quantidade de gêneros que apresentaram resultados de *barcode gap* aceitáveis, no valor geral da PCI e nas estimativas de riqueza de espécies, concluímos que o COI é efetivo como DNA Barcode em Orthoptera. No segundo trabalho (Capítulo III) foi realizada uma filogenia do gênero *Gryllus* utilizando sequências obtidas de espécimes previamente classificadas em sonotipos com o objetivo de esclarecer suas relações. Para isso, foi realizada estimativa de divergência genética, reconstrução filogenética por inferência bayesiana (IB) e máxima verossimilhança (MV) e teste de introgressão baseado em dados de 16S rRNA, COI e 12S rRNA. Foram analisados no total 32 espécimes (19 sequenciadas neste trabalho e 13 sequências baixadas do GenBank). A divergência genética deu indícios que o valor arbitrário de 3% para delimitação de espécies pode não ser o ideal para todas as espécies de *Gryllus*. As árvores consenso de IB e MV não mostraram diferenças significantes, porém serviram para confirmarmos as

hipóteses de duas novas espécies, além de refutarmos uma possível espécie nova hipotética e a existência de espécies crípticas dentro de um sonotipo. Além disso, destacamos a possibilidade de zonas de hibridação no Brasil baseado em dados de teste de introgressão.

Palavras-chave: filogenia, hibridação, introgressão, marcador mitocondrial, sonotipos, taxonomia integrativa

ABSTRACT

Orthoptera is one of the oldest lineages within insects, dating back approximately 350 million years. Currently, it comprises around 29.500 valid species distributed worldwide. Recently, it has been classified as the third insect species domesticated for scientific research, owing to a series of characteristics that primarily facilitate its breeding in laboratory and large-scale settings. Nevertheless, many taxonomic questions remain obscure in some groups, leading to erroneous classifications and identifications. To mitigate these issues and shed light on evolutionary questions, integrative taxonomy has been more intensively employed in recent research, especially using molecular and acoustic data. In this context, the two studies conducted in this thesis are directly related to molecular data, with the second one having an association with acoustic data. In the first study (Chapter II), an assessment of the cytochrome c oxidase subunit I (COI) gene was conducted to test its efficiency as a DNA Barcode within the Orthoptera order. For this purpose, all COI sequences deposited in the Barcode of Life Data System (BOLD) database were utilized, and they underwent two analyses based on intra- and interspecific distances, barcode gap, and the probability of correct identification (PCI). Additionally, species richness was estimated using the Automatic Barcode Gap Discovery (ABGD) and Assemble Species by Automatic Partitioning (ASAP) tools. In this work, 11.605 sequences encompassing 1.132 species from 226 genera were used. Based on the number of genera that exhibited acceptable barcode gap results, the overall PCI value, and species richness estimates, we concluded that COI is effective as a DNA Barcode in Orthoptera. In the second study (Chapter III), a phylogeny of the *Gryllus* genus was conducted using sequences obtained from specimens previously classified as sonotypes with the aim of clarifying their relationships. To achieve this, genetic divergence estimation, phylogenetic reconstruction through Bayesian inference (BI) and maximum likelihood (ML), and introgression testing based on 16S rRNA, COI, and 12S rRNA data were performed. In total, 32 specimens were analyzed (19 sequenced in this study and 13 sequences downloaded from GenBank). Genetic divergence indicated that the arbitrary 3% threshold for species delimitation might not be ideal for all *Gryllus* species. The BI and ML consensus trees did not show significant differences but helped confirm the hypotheses of two new species while refuting a hypothetical new species and the existence of cryptic

species within a sonotype. Additionally, we highlighted the possibility of hybridization zones in Brazil based on introgression test data.

Keywords: phylogeny, hybridization, introgression, mitochondrial marker, sonotypes, integrative taxonomy

CAPÍTULO I

Introdução Geral

Orthoptera

A Ordem Orthoptera é uma das linhagens mais antigas dentro dos insetos, datando cerca de 350 milhões de anos e, atualmente, conta com aproximadamente 29.500 espécies válidas com registros pelo mundo inteiro (Grimaldi & Engel 2005; Song *et al*. 2020; Cigliano *et al*. 2023).

Essa Ordem engloba os insetos saltadores, nome dado pela adaptação do terceiro par de pernas ao salto, sendo os grilos (Grylloidea), os gafanhotos (Acridoidea) e as esperanças (Tettigonioidea) os representantes mais conhecidos. Também estão dentro dessa ordem as paquinhas (Gryllotalpoidea), seres que também possuem adaptações para escavação no seu primeiro par de pernas e pronoto, devido ao seu hábito escavador (Grimaldi & Engel 2005; Sperber *et al*. 2021; Cigliano *et al*. 2023).

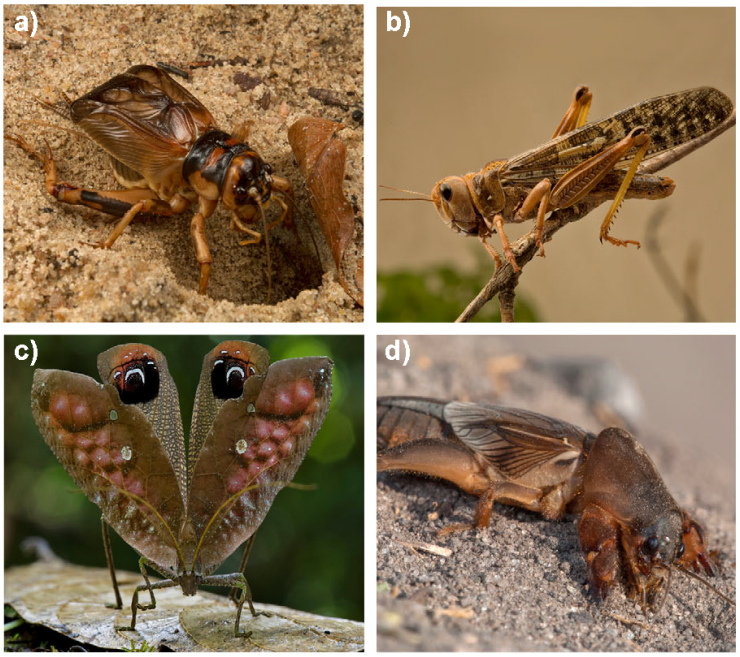

FIGURA 1. Principais representantes da Ordem Orthoptera. a) Grylloidea; b) Acridoidea; c) Tettigonioidea; d) Gryllotalpoidea. Imagens retiradas de Song *et al*. 2015 (Fotos de Piotr Naskrecki).

Orthoptera está dividida em duas subordens bem definidas filogeneticamente conforme Song *et al*. (2015): (1) Ensifera, onde estão incluídos os grilos, paquinhas e esperanças, caracterizada pela presença de antenas longas (mais de 30 antenômeros, sendo ela geralmente maior que o corpo do indivíduo), ovipositor em forma de espada ou agulha e tímpano localizado na tíbia do primeiro par de pernas; e (2) Caelifera, onde estão incluídos os gafanhotos, caracterizada pelas antenas curtas (menos de 30 antenômeros), ovipositor com apenas dois pares valvares e tímpano disposto no primeiro tergito do abdômen (Sperber *et al*. 2021).

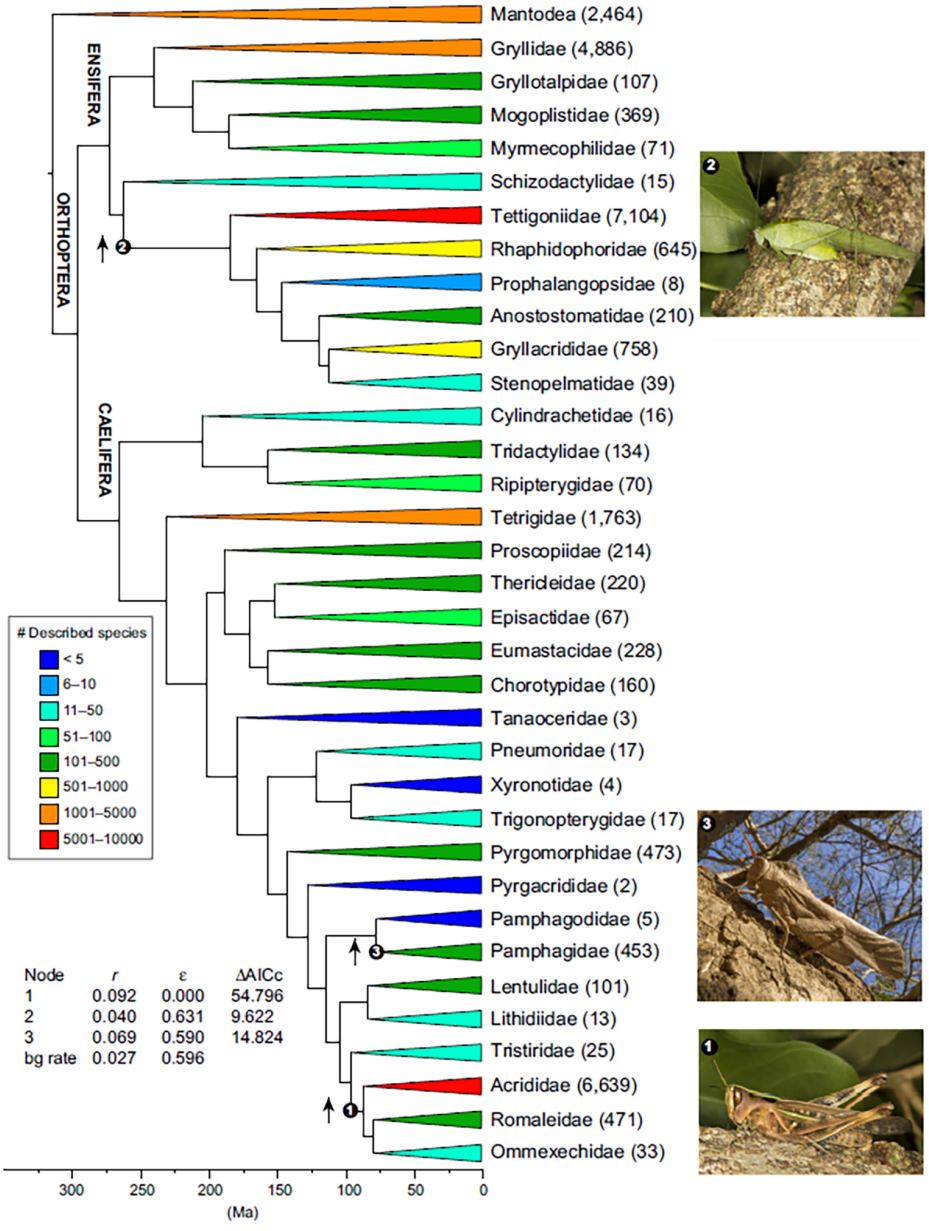

FIGURA 2. Filogenia da Ordem Orthoptera. Imagem adaptada de Song *et al*. 2015.

Ambas as subordens têm representantes amplamente distribuídos por todos os continentes e algumas ilhas, que apresentam espécies endêmicas (Otte & Peck 1997; Cigliano *et al*. 2023). Em geral, Ensifera tem hábitos noturnos, sendo encontrados caminhando ou pulando em diferentes substrato em busca de alimentos, como em troncos de árvores e serrapilheira, cavernas, tocas ou escondidos entre pedras, como é o caso da família Phalangopsidae (Desutter-Grandcolas 1995; Bidau 2014; Sperber *et al*. 2021). Já Caelifera, tem maior atividade durante o dia, possuindo hábito arbóreo, sendo encontrados desde o solo em gramíneas até o dossel e até mesmo associados a macrófitas aquáticas (Bidau 2014; Sperber *et al*. 2021).

Do ponto de vista alimentar, o aparelho bucal encontrado em Orthoptera é do tipo mastigador e a ordem é classificada com omnívora, embora existam espécies que vão desde exclusivamente predatórias até exclusivamente herbívoras, como é o caso de Caelifera (Sperber *et al*. 2021). Essa diversificação no hábito alimentar facilita sua criação em laboratório para utilização dos indivíduos em experimentos, podendo ser a dieta dos criadouros baseada em ração de animais domésticos, vegetais, frutas, dentre outras (Bidau 2014).

Com isso, esses insetos têm ganhado cada vez mais importância como modelo de estudo em diversas linhas de pesquisas. No último século, os grilos ganharam muita atenção, servindo como modelo em estudos que vão desde a pesquisa básica até a pesquisa aplicada. Isso está atrelado à facilidade de obtenção, criação e manutenção em grande quantidade e custo baixo, ganhando o *status* de terceiro inseto domesticado ao longo da história para pesquisa científica (Mito *et al*. 2022).

Além disso, alguns grupos da ordem assumem grande importância econômica e ecológica, como é o caso de gafanhotos *Locusta migratoria* (Linnaeus, 1758) e *Schistocerca gregaria* (Forskål, 1775), que acabam por danificar plantações diversas e causam milhões de dólares de prejuízo aos países produtores (Belayneh 2005; Tokuda *et al*. 2010; Peng *et al*. 2020; Roussi 2020; Singh & Kumari 2021).

FIGURA 3. Plantação devastada por nuvem do gafanhoto adulto *Schistocerca gregaria*. Imagem retirada de Singh & Kumari 2021.

Uma adaptação bastante difundida em Orthoptera, principalmente entre as esperanças e os gafanhotos, é a coloração. Por apresentarem maior atividade durante o dia, seus corpos apresentam uma gama de cores e formas diferenciadas que auxiliam a evitar predação, mantendo-os assim, vivos. Como exemplo, as esperanças da subfamília Conocephalinae são geralmente verdes ou marrons, cor predominante do substrato em que são encontradas (Sperber *et al*. 2021).

Características de cor e forma podem ser caracteres taxonômicos utilizados para identificação e/ou delimitação de espécies para alguns grupos (Weissman & Gray 2019), embora não seja tão relevante dentro de Grylloidea. Por apresentarem hábito noturno, a coloração não é tão marcante e, na maioria dos casos, dificilmente é possível utilizar como caracter taxonômico. Isto é visto claramente dentro do gênero *Gryllus*, um dos mais complexos no campo da sistemática. Por apresentar um conjunto de espécies cosmopolita e crípticas, existe uma longa história - desde o início do século XX - de dificuldades em sua classificação. Isso pode ser devido a identificação e classificação por diferentes pesquisadores, seguindo padrões taxonômicos diferentes (David *et al*. 2003) e pela própria ausência de caracteres morfológicos informativos para delimitação das espécies desse gênero.

O gênero *Gryllus* Linnaeus, 1758, também conhecido como grilos-de-campo (do inglês *field crickets*) são grilos bastante notáveis devido ao seu tamanho e que produzem sons com grande intensidade (dB). Medem aproximadamente 25 a 30 mm, têm cor escura não chamativa e podem se tornar abundantes em diversos habitats (Weissman & Gray 2019), além de serem ótimos colonizadores, até mesmo de ilhas (Otte & Peck 1997). Essa habilidade de colonização e crescimento populacional em diferentes habitats se dá pelo fato de apresentarem oviposição generalizada e serem bons voadores, sendo comum morfotipos de asa longa (Otte & Peck 1997).

FIGURA 4. Fotos de *Gryllus* spp. adaptadas de Zefa *et al*. 2022. (Fotos de Edison Zefa).

Por serem abundantes em diferentes tipos de habitats, chamam a atenção de pesquisadores de diferentes áreas para estudos em diferentes contextos (Weissman & Gray 2019; Gray *et al*. 2020; Cigliano *et al*. 2023).

Atualmente o gênero apresenta 102 espécies válidas, com ampla distribuição na América do Sul e África, ao sul da América do Norte e sudoeste da Ásia, além de algumas ilhas nos oceanos Pacífico e Atlântico (Otte & Peck 1997; Cigliano *et al*. 2023). Dentro do gênero são encontradas muitas espécies crípticas (Weissman *et al*. 1980, Weissman & Gray 2019) e sua taxonomia ainda hoje é bastante confusa por apresentarem genitálias pouco variáveis, o que facilita intercruzamentos e a formação de híbridos (Randell 1964; Weissman *et al*. 1980; Cade & Tyshenko 1990; Panagiotopoulou *et al*. 2016; Weissman & Gray 2019).

Contudo, o panorama taxonômico e sistemático do gênero *Gryllus* apresenta-se mais claramente definido na América do Norte, onde Weissman & Gray (2019) fizeram uma extensa revisão das espécies encontradas nesse continente. Os autores redigiram uma monografia com todas as informações contidas na literatura

sobre os indivíduos norte-americanos, discussões sobre biologia, distribuição, bioacústica, análises genéticas e relações entre as espécies.

Já na América do Sul o panorama é outro. A falta de pesquisadores, descrições antigas e a maioria delas baseadas em espécimes de museus tornam o conhecimento sobre *Gryllus* no continente bastante defasado (Zefa & Fontanetti 2002). Até o momento apenas 13 espécies foram registradas no continente e cinco delas tiveram informações de som de chamado adicionadas à sua descrição. Além disso, existem 14 espécies endêmicas dos arquipélagos do Caribe e Galápagos (Cigliano *et al*. 2023). Martins & Zefa (2011) discutem algumas dúvidas sobre o material tipo de algumas dessas espécies, o que dificulta, ainda mais, o conhecimento sobre a real quantidade de espécies existentes no continente.

Marcadores moleculares

Os marcadores moleculares são sequências de DNA altamente conservadas ou variáveis que podem ser utilizadas para identificar e comparar a diversidade genética entre diferentes organismos. Em insetos, diferentes marcadores são utilizados em diferentes propósitos. O DNA mitocondrial (mtDNA) é o mais comumente utilizado em estudos de variações genéticas e história evolutiva de espécies relacionadas (Behura 2006).

O uso de sequências de DNA como parte das pesquisas de identificação de espécies teve seu início na década de 1980 (DeSalle & Goldstein 2019). O DNA Barcode é um dos métodos moleculares mais conhecidos para a identificação e delimitação de espécies e vem sendo cada vez mais utilizado em pesquisas de seres vivos em geral (Hebert *et al*. 2003). No Reino Animal, a sequência que caracteriza o barcode é uma região de 648 pares de base (pb) do gene citocromo c oxidase subunidade I (COI) (Hebert *et al*. 2003; Savolainen *et al*. 2005; Ratnasingham & Hebert 2007). Essa região apresenta boa sensibilidade para identificação e delimitação de espécies próximas, auxiliando na descoberta de espécies crípticas e análises filogenéticas e, com isso, tem sido cada vez mais incorporada na taxonomia integrativa (DeSalle & Goldstein 2019).

O DNA barcode atua em duas linhas fundamentais: no diagnóstico de espécies (identificação de espécimes) e na descoberta de novas espécies

(delimitação de espécies) (DeSalle *et al*. 2005). A primeira ocorre de forma comparativa, na qual a sequência genética do espécime estudado é comparada com sequências de um banco de dados de referência; enquanto a segunda utiliza de valores limites baseados em distâncias intra e interespecíficas, isto é, a existência de um “barcode gap”, termo cunhado por Meyer & Paulay (2005) (DeSalle & Goldstein 2019).

Nos insetos, os genes mitocondriais são atrativos por serem bastante conservados dentro de espécies, mas com taxa evolutiva suficiente para distinção entre espécies, além de serem transmitidos exclusivamente pelas mitocôndrias maternas e facilmente amplificados usando *primers* universais (Behura 2006).

Em Orthoptera, os marcadores moleculares têm sido aplicados para resolver questões taxonômicas, determinar relações filogenéticas entre espécies e populações, investigar padrões de migração e fluxo gênico, além de desvendar processos evolutivos que moldaram a diversidade genética dentro da Ordem. Entre os marcadores moleculares mais comumente utilizados nos estudos envolvendo Orthoptera, destacam-se os genes mitocondriais, como o citocromo c oxidase subunidade I (COI) e o gene 16S rRNA.

O gene COI, principalmente, vem sendo cada vez mais utilizado como DNA barcode e se mostrando uma grande ferramenta molecular para soluções de identificação e delimitação de espécies (Huang *et al*. 2013; Zhao *et al*. 2015; Chapuis *et al*. 2016; Guo *et al*. 2016; Chen *et al*. 2018; Zhou *et al*. 2019; Kim *et al*. 2020; Wang *et al*. 2021). Além disso, trabalhos com diversos outros marcadores, como os mitocondriais 12S, 16S, citocromo b, citocromo c oxidase subunidade II, e os nucleares 18S, 28S, H3, ITS1, ITS2 também são corriqueiramente usados para construção de filogenias e análises variadas (Flook *et al*. 1999; Robillard & Desutter-Grandcolas 2006; Nattier *et al*. 2011; Chintauan-Marquier *et al*. 2016; Song *et al*. 2018; Wang *et al*. 2021).

Compreender a filogenia e a diversidade genética de alguns grupos de Orthoptera tem sido um desafio para os pesquisadores devido à sua similaridade morfológica e à ocorrência de convergência evolutiva. Nesse contexto, os marcadores moleculares têm se mostrado ferramentas poderosas para investigar a história evolutiva e a relação de parentesco dentro dessa ordem (Flook & Rowell 1997; Flook *et al*. 1999; Jost & Shaw 2006; Song *et al*. 2015).

Bioacústica

Orthoptera é um grupo de insetos que produz som com padrões de sinais acústicos diversificados e frequências que variam de 0.6 a 130 kHz (Heller 1995; Montealegre *et al*. 2006). Os sons são classificados em *chirp*, sinal intervalado de curta duração, e *trill*, sinal contínuo de longa duração, de acordo com o ritmo da emissão (Otte 1992).

O som é composto por elementos básicos, as notas, que são facilmente vistas através de um sonograma e identificáveis por sua estrutura espectral e temporal, além de sua posição no som (Desutter-Grandcolas & Robillard 2003).

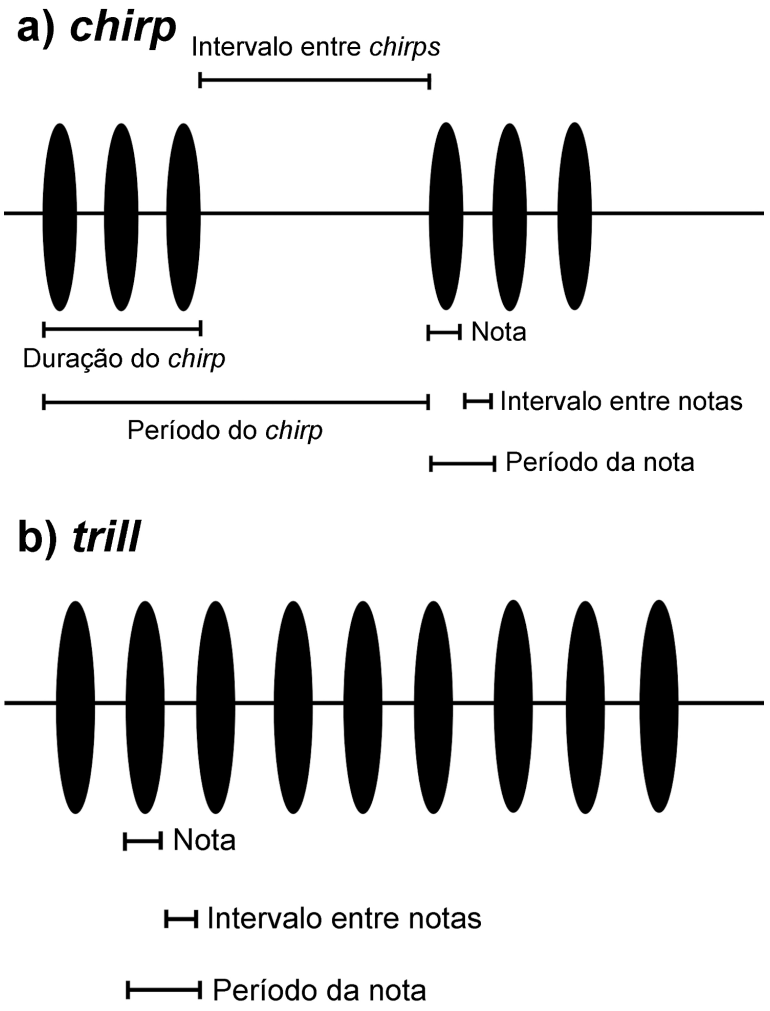

FIGURA 5. Esquema adaptado de Weissman & Gray 2019 mostrando as duas classificações de som, *chirp* e *trill*, e seus elementos básicos.

Nas espécies em que existe comunicação acústica, como na maioria dos ortópteros, os sinais acústicos apresentam grande importância no processo de formação de casais e, consequentemente, na reprodução das mesmas (Otte 1992; Brandbury & Vehrencamp 1998). Dentro de todo o repertório acústico já conhecido, os mais destacados e importantes são o som de chamado e de corte. O som de chamado, emitido pelo macho, tem a função de reconhecimento e atração a longa distância de indivíduos coespecíficos (Alexander 1962; Desutter-Grandcolas 1995; Bailey & Zuk 2008).

Em geral, os principais métodos para elucidar problemas de identificação, distinção e reconhecimento de espécies são através de dados morfológicos e, mais atualmente, dados moleculares, como por exemplo, sequências de DNA. Contudo, dentro de espécies que produzem som, dados acústicos têm sido também de grande importância para solucionar questões específicas (Tan *et al*. 2018).

Os grilos (Grylloidea) produzem sinais acústicos por meio da raspagem das tégminas. Em cada fechamento de asas, uma estrutura chamada *plectrum* (“palheta”) da tégmina esquerda raspa em uma fileira de dentes (*scraper*) presente na tegmina direita, criando pulsos de ondas sonoras (Alexander 1966; Otte 1992). Os sinais sonoros emitidos pelos grilos podem ser usados na delimitação de espécies, justamente por fornecerem informações indiretas de isolamento reprodutivo (Walker 1964; Jaiswara *et al*. 2012).

Dentro do gênero *Gryllus*, a análise do sonograma/oscilograma, somado às características morfológicas de produção do som (por exemplo: número de dentes, dentes por milímetro, etc) geralmente são suficientes para separação de machos, porém, de acordo com Weissman *et al*. (1980), informações da localidade, tamanho e padrões de coloração não devem ser descartados para a identificação definitiva. Por outro lado, as fêmeas e ninfas não são distinguíveis através destes caracteres, mas sim por associação com os machos (Weissman *et al*. 1980, Weissman *et al*. 2009), características morfológicas específicas da espécie, quando existente, comparação de DNA com outros machos já determinados e eclosão de ovos e posterior análise de caracteres do macho adulto (Weissman & Gray 2019)

Contudo, *Gryllus* é um gênero que apresenta bastante variação de ritmo na emissão sonora, existindo espécies que emitem tanto *chirps*, com variação na velocidade e quantidade de notas, quanto *trills*, lentos ou mais acelerados, além de

espécies com som irregular e que não emitem nenhum sinal (Weissman & Gray 2019).

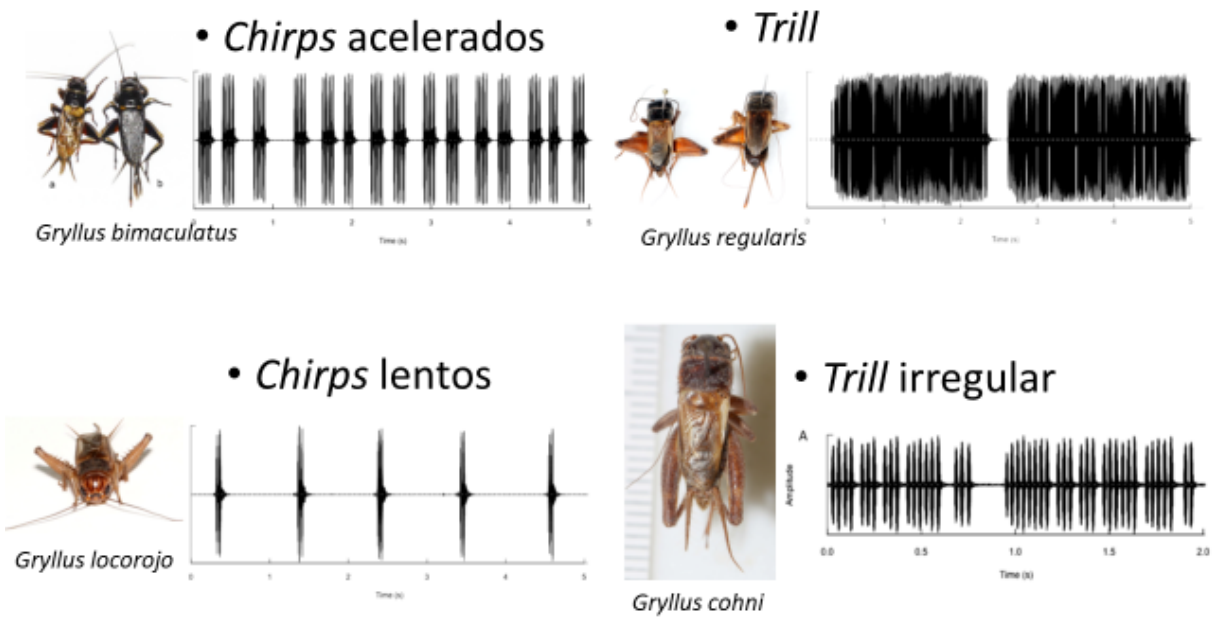

FIGURA 6. Diversidade de emissão sonora dentro do gênero *Gryllus*. Imagens adaptadas de Weissman & Gray 2019.

Justificativa

A escassez de taxonomistas para a ordem Orthoptera é evidente, visto a riqueza e complexidade do grupo, além da falta de investimentos para estudos taxonômicos (Cigliano & Eades 2010). Erros de identificação são comuns na literatura e em materiais depositados em banco de dados, sejam museus ou repositórios de dados genéticos, o que posteriormente retorna no aumento no número de sinonímias, que já é bastante significativo dentro do grupo (Cigliano *et al*. 2023). Uma análise do estado da arte e do desempenho do DNA Barcode com sequências depositadas em um banco de dados pode ser útil para orientar estudos futuros com grupos específicos.

Somado a isso, o gênero *Gryllus* é considerado um dos mais complexos grupos dentro de Grylloidea à luz da sistemática. Por apresentar características pouco variáveis, grande variação de ritmos de som de chamado, além de ampla

distribuição e casos de hibridação entre espécies, a utilização da biologia molecular associada à bioacústica é uma alternativa que pode auxiliar na discriminação das espécies dentro do gênero.

Objetivo Geral

Avaliar a eficácia do gene mitocondrial citocromo c oxidase subunidade I (COI) na identificação e delimitação de espécies de Orthoptera e as relações evolutivas no gênero *Gryllus* associadas a sonotipos empregando marcadores mitocondriais.

Objetivos Específicos

- Fornecer uma visão geral das sequências do gene COI depositadas no banco de dados Barcode of Life Data System (BOLD);
- Testar a eficiência do gene COI como DNA barcode dentro de Orthoptera;
- Destacar os principais gêneros que apresentam inconsistência taxonômica que podem ser favorecidos com o advento de estudos integrativos;
- Elaborar uma reconstrução filogenética de espécies do gênero *Gryllus* baseado nos genes 16S, COI e 12S;
- Estabelecer relações filogenéticas entre sonotipos classificados por Zefa *et al*. 2022;
- Buscar a possibilidade de novas espécies dentro dos sonotipos citados acima, baseado em dados moleculares;

Estrutura da tese

A presente tese foi estruturada em quatro capítulos.

O primeiro, recém apresentado, contém uma introdução geral que servirá como base referente aos temas dos dois capítulos seguintes que compõem os trabalhos desenvolvidos durante o doutorado.

O segundo consta um artigo já publicado na revista ***Canadian Journal of Zoology***, onde foi testada a eficiência de um marcador molecular através de quatro ferramentas para identificação e delimitação de espécies dentro da ordem Orthoptera. Este trabalho teve de ser realizado remotamente, utilizando informações depositadas em um banco de dados, devido à pandemia da COVID-19 que se estendeu por dois anos, metade do período de realização desta tese.

O terceiro trata de um artigo - em preparação para ser submetido - que consiste na reconstrução filogenética de espécies do gênero *Gryllus* com o emprego de marcadores moleculares de indivíduos deste gênero previamente classificados em sonotipos, com base em seu som de chamado por Zefa *et al*. (2022). A reconstrução filogenética se deu pela geração e comparação de árvores consenso por inferência bayesiana e máxima verossimilhança de dados concatenados dos genes 16S rRNA, citocromo c oxidase subunidade I (COI) e 12S rRNA.

Já no último capítulo, são apresentadas as considerações gerais referentes aos trabalhos realizados nessa tese.

Por fim, durante o período de realização do doutorado que resultou nesta tese, foram realizadas colaborações com outros pesquisadores que resultaram em sete artigos publicados, que estão listados na seção Anexos deste documento.

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CAPÍTULO II

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The efficiency of the *COI* gene as a DNA barcode and an overview of Orthoptera (Caelifera and Ensifera) sequences in the BOLD System

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Abstract

Orthoptera, among the oldest and most numerous insect lineages, is an excellent model for evolutionary studies but has numerous taxonomic problems. To mitigate these issues, the cytochrome *c* oxidase subunit I (*COI*), standardized with the DNA barcode for Metazoa, is increasingly used for specimen identification and species delimitation. We tested the performance of *COI* as a DNA barcode in Orthoptera, using two analyses based on intra- and interspecific distances, barcode gap and Probability of Correct Identification (PCI); and estimated species richness through Automatic Barcode Gap Discovery (ABGD) and Assemble Species by Automatic Partitioning (ASAP). We filtered all sequences of Orthoptera available in Barcode of Life Data System (BOLD) and used 11,605 *COI* sequences, covering 1,132 species, 226 genera, and 18 families. The overall average PCI was 73.86%. For 82.2% of genera the barcode gap boxplots were classified as *good* or *intermediate*, indicating that *COI* can be effective as a DNA barcode in Orthoptera, although with varying efficiency depending on the need for more information. ABGD and ASAP inferred species richness similar to labels informed by BOLD for the suborders Caelifera and Ensifera. The representation of Orthoptera in the BOLD database and the results of these analyses are discussed.

Key words: Orthoptera, Caelifera, Ensifera, integrative taxonomy, specimen identification, species delimitation

Introduction

Orthoptera is one of the oldest lineages of insects (350 million years of diversification) (Song et al. 2020) and comprises around 29,000 described species worldwide, except for the polar regions (Grimaldi and Engel 2005; Cigliano et al. 2022). The order is subdivided into two suborders, Caelifera (grasshoppers and similar), which groups 35 families, 2,525 genera, and almost 12,400 species; and

Ensifera (crickets, katydids, and similar), with 41 families, 2,684 genera, and about 16,800 species, differentiated by the number of antennomeres, tympanum location, and ovipositor shape (Grimaldi and Engel 2005; Sperber et al. 2021). Orthopterans occur in most terrestrial habitats and a few species are semiaquatic (Bidau 2014*a*). Most species are omnivorous, although strictly predatory and phytophagous species exist (Sperber et al. 2021). Several species are major agricultural pests (Singh and Kumari 2021); for instance, locust and grasshopper swarms are known for their ability to wipe out crop fields in a single day (Zhang and Hunter 2017; Peng et al. 2020).

Orthopteran taxonomy is based primarily on adult morphology, and the male genitalia plays a prominent role in species delimitation studies (Alexander and Otte 1967; Desutter 1987). However, morphology alone may be misleading, and even expert taxonomists may struggle with species diagnoses. Plastic phenotypes, lack of descriptions for immature stages, and cryptic species complexes also hamper accurate specimen identification in this group (e.g., Song and Wenzel 2008; Guo et al. 2016). Such taxonomic puzzles may benefit from integrative approaches incorporating additional evidence, including acoustic profiling (Riede 2018), cytogenetic characters (Bidau and Martí 2010), and DNA markers (Cigliano and Eades 2010).

DNA barcoding (Hebert et al. 2003), perhaps one of the most emblematic contemporary techniques for molecular taxonomy, since its advent has been applied in a myriad of scientific disciplines (DeSalle and Goldstein 2019). For metazoans, the canonical DNA barcode sequence is a region of 648 base pairs (bp) located at the 5' end of the mitochondrial gene cytochrome *c* oxidase subunit I (*COI-5P*) (Hebert et al. 2003; Savolainen et al. 2005; Ratnasingham and Hebert 2007). Because it is a rapid and relatively inexpensive method with overall good identification accuracy, DNA barcoding can be employed in large-scale biodiversity surveys (Huang et al. 2013; Hawlitschek et al. 2017), as well as in identification of environmental samples (Hardulak et al. 2020) and even centenarian archived specimens (Françoso and Arias 2013; Raxworthy and Smith 2021). DNA barcoding has also been implemented in species discovery and delimitation, often based on the difference between the maximum intraspecific and the minimum interspecific genetic distance of barcode sequences (the "*barcoding gap*"; Meyer and Paulay 2005).

The *COI* gene has been increasingly used as a DNA barcode for orthopterans and is proving to be a useful complementary tool in diagnosing and delimiting species (Huang et al. 2013; Zhao et al. 2015; Chapuis et al. 2016; Guo et al. 2016; Chen et al. 2018; Zhou et al. 2019; Kim et al. 2020; Wang et al. 2021). While the number of barcodes is increasing in public databases (such as the Barcode of Life Data System, BOLD), orthopteran taxonomy suffers from a shortage of taxonomists and lack of sufficient funding for taxonomic studies (Cigliano and Eades 2010). Hence, misidentifications permeate the literature and the material entered in databases and deposited in museums, a scenario further complicated by the significant number of synonymies for some groups (Cigliano et al. 2022). Because correct identification of reference sequences is essential for the success of DNA barcoding (Ekrem et al. 2007), an analysis of the state-of-the-art and the performance of orthopteran *COI* barcodes available in BOLD could be useful to guide future studies with these organisms.

Here, we datamined all the orthopteran *COI* sequences available in BOLD, aiming to: i) provide an overview of the sequences available in BOLD; ii) assess the existence of barcoding gaps in the available genera; iii) test the specimen identification efficiency of *COI* through the Probability of Correct Identification (PCI); and iv) point out orthopteran genera with inconsistent taxonomy that may benefit from integrative studies implementing DNA barcodes.

Materials and methods

Data obtention and filtering

We retrieved all sequences of Orthoptera available in BOLD (https://www.boldsystems.org; Ratnasingham and Hebert 2007) in April 2021, using as query the 82 valid orthopteran family names (Cigliano et al. 2022) and generating a separate FASTA file for each family. To ensure the quality of our final dataset, sequences were filtered following the downstream workflow described by Gonçalves et al. (2021). Briefly, we retained only sequences labeled as *COI-5P* (the standard DNA barcode of metazoans; Hebert et al. 2003) and identified at species level.

A total of 34,549 sequences were aligned using MAFFT 7.0 (Katoh et al. 2019) with the default parameters. AliView (Larsson 2014) was used to inspect alignments,

verify stop codons and reading frames, and remove sequences with insertions and deletions. Then, alignments were trimmed to restrict our analysis to the canonical 658 bp barcode region (Hebert et al. 2003) and we removed sequences shorter than 400 bp. Finally, species labels of the remaining sequences were cross-validated with the Orthoptera Species File (http://orthoptera.speciesfile.org; Cigliano et al. 2022).

Data analyses

For the barcoding gap analysis, we generated separate FASTA files for each genus. To allow both intra- and interspecific comparisons, the analyses described below comprise only genera featuring at least two species, with at least one of the species represented by two or more barcodes. For each genus, the R package APE (Paradis and Schliep 2019) was used to estimate intraspecific and interspecific pairwise p-distances through the function “dist.dna()”. The values generated were visualized as boxplots, which were used to test the performance of *COI* for each genus, following the visual classification proposed by Badotti et al. (2017): *good*, *intermediate*, and *poor*. Performance was considered *good* when there was a clear gap between boxplots of intra- and interspecific distances; *intermediate* when the whiskers of the boxplots overlapped; and *poor* when the boxes of the boxplots overlapped (for more details see Badotti et al. 2017).

DNA barcoding can be useful for specimen identification even in the absence of a barcoding gap (see Collins and Cruickshank 2012). Therefore, we calculated the Probability of Correct Identification (PCI) (Hollingsworth et al. 2009) to measure the discriminative effectiveness of *COI* in specimen identification. We used the R package SPIDER (Brown et al. 2012) to estimate the maximum intraspecific distance and minimum interspecific distance (or nearest-neighbor distance) for each species. If the maximum intraspecific distance was less than the minimum interspecific distance, identification was considered a success (Hollingsworth et al. 2009). Species with only one sequence were not analyzed, as it would not be possible to obtain intraspecific distances. The PCI values obtained were graphically represented in scatter plots, as suggested by Collins and Cruickshank (2012). All analyses were conducted in R v. 4.0.5 (R Core Team 2018).

Finally, we estimated the species richness in the dataset compiled for each suborder, using the Automatic Barcode Gap Discovery (ABGD; Puillandre et al. 2012) and the Assemble Species by Automatic Partitioning (ASAP; Puillandre et al.

2021). Both methods cluster barcode sequences into hypothetical species based on pairwise distances. The analyses were run in their respective web interfaces (ABGD: bioinfo.mnhn.fr/abi/public/abgd/; ASAP: bioinfo.mnhn.fr/abi/public/asap/), using default parameters and simple distances. We interpreted the ABGD results using a prior intraspecific divergence limit of P = 0.01 (Puillandre et al. 2012; Gonçalves et al. 2021). The best ASAP partition was selected based on the lowest *asap-score* (Puillandre et al. 2021). We compared the ABGD and ASAP estimates with the number of species labels (species names) according to BOLD.

Results

Of the 82 valid orthopteran families, 47 lack sequence data in BOLD. We started our analysis with 34,549 sequences, encompassing 35 orthopteran families. After the filtering steps to ensure a robust dataset (see Materials and Methods), our final dataset consisted of 11,605 *COI* sequences, representing 18 families (21.9% of the total valid families), 226 genera (4.3% of the total valid genera), and 1,132 species (3.8% of the total valid species) (Table 1; for a complete list see Supplementary Table S1). We changed the labels of 809 sequences (7% of the dataset) belonging to 98 species, due to invalid or misspelled names (Supplementary Table S2). Most of the sequences belong to suborder Caelifera (55.2%). Regarding family coverage, our sample was heterogeneous. Families that contributed most to our dataset were Acrididae (46.4%), Tettigoniidae (27.7%), and Gryllidae (7.9%); in contrast, Phalangopsidae and Prophalangopsidae were represented by one genus and two species each. Sequence coverage by species ranged from 1 to 355, with 77% of the species represented by fewer than 10 sequences. The sampled sequences cover roughly 4% of the valid species of Orthoptera.

Among the 226 genera evaluated, *COI* performance was ranked *good* for 139 (61.5%), *intermediate* for 47 (20.8%), and *poor* for 40 (17.7%). The rank system is illustrated in Fig. 1, and an overall classification of each family is provided in Table 1. Data for each genus are appended in Supplementary Table S1. Intra- and interspecific distances varied considerably among genera (Supplementary Table S1), with an average of 3.78 and 6.06%, respectively.

The average PCI of Orthoptera was 73.86% (Fig. 2), but this value ranged dramatically among families and genera. We found a consistent relationship between the barcoding gap and the PCI results: genera with *good* performance showed a higher PCI, whereas genera with *poor* performance showed a lower PCI (Fig. 3). Detailed PCI values for each family and genus are provided in Supplementary Table S1.

The number of hypothetical species estimated by ABGD and ASAP was higher than the number of species labels in our dataset, for both suborders. For Caelifera, our dataset comprised 546 species labels, but ABGD and ASAP estimated 1,173 and 584 hypothetical species, respectively. For Ensifera, our dataset comprised 586 species labels, but ABGD and ASAP estimated around 607 and 570 hypothetical species, respectively. These results suggest potential cryptic diversity among the *COI* sequences of BOLD, particularly for Caelifera.

Discussion

Database

The standard DNA barcode for metazoans is *COI*, but validating its efficacy and searching for appropriate threshold values is mandatory for accurate specimen identification and species delimitations (Hebert et al. 2003). Here, we aimed to test the efficiency of *COI* as a DNA barcode for Orthoptera, using the barcoding gap and PCI analyses. Our results showed that *COI* performs well and that BOLD displays a taxonomic consistency, because we found overall high PCI values and *good* barcoding gap performance for most of the genera analyzed. Low PCI values and overlapping intra- and interspecific distances for genera indicate potential mislabeling in reference databases or complex biological scenarios, such as non-monophyletic taxa, which should be assessed with more detail in future studies.

We started our filtering with 34,549 sequences. However, almost 23,000 sequences were discarded. Most of the sequences removed lack species-level identification, showing that these data were generated and published without a strong taxonomic basis. As a result, such sequences with incomplete taxonomic labels affect the efficacy of DNA barcoding. We emphasize the importance of depositing sequences obtained from rigorously identified specimens to increase the efficiency

and accuracy of the method, besides improving studies using molecular data such as phylogenetic (Gu et al. 2020; Shen et al. 2020; Wang et al. 2021) and phylogeographic (Ma et al. 2012) in their analyses.

The use of molecular data in taxonomic studies of Orthoptera is still incipient for many families. So far, only eight genomes have been sequenced, and about 250 complete mitogenomes are known (Song et al. 2020). More extensive work has recently emerged, focused on studies of genome-size evolution (Mao et al. 2020; Ylla et al. 2021; Yuan et al. 2021), mitogenomics (Ma et al. 2019; Li et al. 2019), and phylogenomics associated with the evolution of acoustic communication (Song et al. 2020). However, these studies have investigated only a tiny fraction of orthopteran diversity.

For some families a large number of sequences were available, such as Acrididae (5,386), Tettigoniidae (3,221), and Gryllidae (913); whereas other families lack sequence data in BOLD. We somewhat expected this contrast because these families attract more researchers due to their economic and social importance (Bidau 2014*b*; Zhang and Hunter 2017; Song 2018; Singh and Kumari 2021), besides being easier to collect (Sperber et al. 2021). Surprisingly, groups such as Phalangopsidae had few sequences even though they occur across the Neotropical region (Cigliano et al. 2022). We believe that this reflects the difficulty of collecting specimens (Desutter-Grandcolas 1995).

Although orthopteran diversity is higher in Neotropical regions (Song 2018; Cigliano et al. 2022), many of the available sequences are from species of the Oriental region. The Acrididae (suborder Caelifera), considered by several authors as the family with the most orthopteran crop pests (Bidau 2014*b*; Zhang and Hunter 2017; Peng et al. 2020; Singh and Kumari 2021), is the best represented in BOLD. Their reputation as “agricultural pests” is caused mainly by the outbreaks that result in devastated crops, lending this family high economic importance (Chatterjee 2022; Singh and Kumari 2021). Additionally, the ancient cultural practices in Asia revolving around Orthoptera (Jin and Yen 1998; Costa-Neto 2003; Bidau 2014*b*) explain the high abundance in sequences from native Asian families and the interest of local researchers in using these organisms as study models.

Barcoding gap

The overall *good* performance found for the genera analyzed shows that *COI* is an effective marker for studying the diversity of Orthoptera. Intra- and interspecific distances varied considerably. The majority of genera showed interspecific distances ranging from 7 to 15% and intraspecific distances less than 5%. Previous studies suggested that a threshold value of 3% could be used to delimit arthropod species (Hebert et al. 2003; Huang et al. 2013), and this value has been much used for Orthoptera (Huang et al. 2013; Zhao et al. 2015; Wang et al. 2019; Zhou et al. 2019; Gu et al. 2020; Kim et al. 2020; Kundu et al. 2020). However, we must consider that this is an average value for a broad taxonomic scope, which could well have a wider genetic diversity. Moreover, evolution rates and coalescence times may vary among taxa (Wiemers and Fiedler 2007; Meier et al. 2008). Studies with other arthropod groups show that there is no universal threshold to distinguish between intra- and interspecific distances (Zimmermann et al. 2015; Poppe et al. 2017, 2019), and that a specific value should be assessed whenever possible.

The best-represented families in terms of sequence abundance (Acrididae, Tetiigoniidae, and Gryllidae) displayed a *good* barcoding performance (see Table 1). Furthermore, our analysis at genus level agrees with the results from previous studies that validated the efficiency of the method (Huang et al. 2013, for *Spathosternum*; Wang et al. 2019, for *Sinocyrtaspis*; Gu et al. 2020, for *Fruhstorferiola*; Kim et al. 2020, for *Tettigonia* and *Paratlanticus*; Wang et al. 2021, for *Tonkinacris*). However, for groups with few sequences or inadequate sampling, DNA barcoding is less accurate, as reported by Trewick (2008) for *Sigaus*.

Biological factors can also affect the efficacy of DNA barcoding (Song et al. 2008; Moulton et al. 2010), such as heteroplasmy (White et al. 2008) and nuclear-mitochondrial pseudogenes (numts) (Song et al. 2008). Moreover, incomplete lineage sorting, hybridization between closely related species, and *Wolbachia* infections are also reported as interfering with DNA barcoding efficacy in orthopteran specimen identification (Hawlitschek et al. 2017). A solution to these biases is to obtain several barcode sequences from the same specimen, in order to determine a dominant haplotype and assume that it is representative of the species (Kang et al. 2016).

Several genera studied here had boxplots with a prominent number of outlier comparisons. These atypical values may be related to the above scenarios, resulting

in molecular distances outside the average for their species. Outliers can also result from population under-sampling, cryptic species complexes, or operational biases such as mislabeling.

Probability of correct identification (PCI)

The global PCI value for Orthoptera was 73.86%, and PCI values were generally positively related to the barcoding gap rank system. Genera with higher PCI values overall were classified as *good*, whereas those with low PCI values received a *poor* classification. Previous studies using the same metrics to evaluate barcodes available in public databases found similar values for nematodes (72.72%; Gonçalves et al. 2021), true bugs (74.33%; Bianchi and Gonçalves 2021), and apid bees (77.02%; Gonçalves et al. *in press*). For other taxonomic groups, identification success was closer to 100%, such as in spiders (Blagoev et al. 2009), lepidopterans (Pérez-Asso et al. 2016), and dipterans (Bakhoum et al. 2018).

Considering Acrididae and Tettigoniidae, the two best-represented families in terms of sequence abundance, we observed PCI values of 46.43% and 75.39%, respectively. Although well-characterized families in general yield more robust and reliable results, the results for Acrididae were not expected. Although the abundance of studies minimizes taxonomic errors, more extensive specimen sampling by multiple research groups may actually increase the number of errors. Half of the sequences sampled here belong to this family, and as seen from the barcode gap boxplots, most genera (62%) showed several outliers. These outliers may account for the low PCI values (Badotti et al. 2017).

Hawlitschek et al. (2017) analyzed sequences from 127 orthopteran species from Central Europe and found 100% identification success for species of Ensifera, whereas for Caelifera only 59.1% of species were successfully identified. Our results agree with these findings when considering only the best-represented families for species and sequences (Gryllidae+Tettigoniidae for Ensifera, with an average PCI of 74%; Acrididae for Caelifera, with only 46.43%). We believe that the comprehensive sampling lowered our PCI values due to the increase in errors, but the general context is proportionally similar. We emphasize that there is no consensus about the definition and calculation of PCI (Martín et al. 2020) or about threshold values for splitting intra- and interspecific distances (Will and Rubinoff 2004; DeSalle et al. 2005), and therefore these metrics will vary among different taxa and approaches.

ABGD and ASAP

ABGD and ASAP species estimates were incongruent with the number of species labels in our dataset, especially considering the ABGD estimates for Caelifera. Because ABGD is heavily affected by the sequences used as input (Puillandre et al. 2012), these inconsistencies may be due to the barcode gap performance of Caelifera, in which 50% of the genera showed *intermediate* or *poor* results. In contrast, around 75% of the genera of Caelifera displayed a *good* barcode gap performance, which could explain the higher congruency of ABGD and ASAP estimates with the number of species labels. Further research may investigate possible cryptic species complexes in Orthoptera, especially in Caelifera.

Comments on certain genera

Some of the genera studied here showed conflicting results between the barcoding gap and PCI analyses. The overlapping of intra- and interspecific distances (the lack of a barcode gap) does not necessarily limit the use of DNA barcoding for specimen identification (see Collins and Cruickshank 2012), and these patterns may reflect the differences among species in coalescence times (Virgilio et al. 2010). Thus, the intraspecific distances of a species can often overlap the interspecific distances of other species without compromising the success of identification, here evaluated using the PCI.

We have demonstrated the efficiency of *COI* as a DNA barcode in 226 genera (see Supplementary Table S1). Our sample encompassed genera with hundreds of described species, such as *Anaxipha*, as well as others subdivided into subgenera or species groups such as *Schistocerca*, and even synonymies such as *Neoconocephalus* (Cigliano et al. 2022). The large number of species, as well as the emergence of new morphological characters used to designate groups as new species are discovered, has made the taxonomy of certain genera increasingly complex.

Anaxipha, for instance, although under-sampled, showed a PCI of 100% and a *good* barcoding gap performance, showing that DNA barcoding may aid in the resolution of taxonomic problems concerning this group. Similarly, for *Gryllus*, which consists of 102 valid species (Cigliano et al. 2022), many of them with outdated

descriptions based solely on body color and external morphology, we found a PCI of 75% and a clear barcoding gap. Richness estimates for both genera were largely congruent with the taxonomic labels of BOLD.

On the other hand, the performance of *COI* for *Schistocerca* was insufficient. Its PCI was only 5% and the barcode gap was ranked as *intermediate*, showing several outliers with intra- and interspecific distances above 25%. Moreover, the ABGD and ASAP estimates for this genus were highly incongruent with the number of BOLD species labels. This wide variation shows that there are probably several identification errors in the sequences, possibly caused by the phenotypic plasticity found for the genus (Song and Wenzel 2008), the presence of nuclear mitochondrial pseudogenes (numts) (Moulton et al. 2010), and/or operational biases, such as inefficient taxonomic reference, alignment errors, and sample contamination, among others (Mutanen et al. 2016), given the relatively large number of sequences available in BOLD.

Hawlitschek et al. (2017) highlighted hybridization and incomplete lineage sorting as the main factors that reduce the DNA barcode efficiency, especially in Caelifera. For instance, hybridization has been reported and speciation is still recent for some genera such as *Chorthippus*, *Stenobothrus*, *Omocestus* (Caelifera), and *Teleogryllus* (Ensifera). On the other hand, the genus *Gryllus*, for which hybridization has also been studied, had a PCI of 75% and *good* barcode gap performance, besides having several outliers. In this case, the outliers may have occurred because of misidentifications rather than hybridization.

Heteroplasmy and high prevalences of numts have reported in previous studies with Orthoptera. *Ognevia* (Acrididae), *Podisma* (Acrididae) (Bensasson et al. 2000), *Anaposdisma* (Acrididae) (Kang et al. 2016), *Ellipes* (Tridactylidae), and *Taeniopoda* (Romaleidae) (Jesús-Bonilla et al. 2017) showed here a *poor* resolution, with PCI values lower than 20%. These low values may be related to fluctuation in intraspecific distances due to erroneous numt amplification. On the other hand, genera with previously reported numts, such as *Arcyptera* (Acrididae) (Bensasson et al. 2000), *Anabrus* (Tettigoniidae), and *Myrmecophilus* (Myrmecophilidae) (Moulton et al. 2010) showed an *intermediate COI* performance and PCI around 50%, with well-defined intra- and interspecific distances.

Final comments

We have shown here that DNA barcoding is, overall, a valuable tool for orthopteran specimen identification and species delimitation. Genera with *poor* barcode gap performance and/or low PCI values should be further investigated: are these results a consequence of operational biases, or is DNA barcoding not sufficient for these groups? We advocate for the use of *COI* together with other taxonomic characters (e.g., morphology, bioacoustics, behavior, ecology, other genetic data) to study the Orthoptera, under an integrative taxonomy framework.

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Competing interests statement

The authors declare that there are no competing interests.

Contributors' statement

MD conceived the research. VFT and LTG collected the data and conducted the formal analyses. VFT wrote the original draft of the manuscript. All authors revised the manuscript. MD and VLSVG supervised the project.

Data availability statement

All data used for analyses are available at BOLD – Barcode of Life Data System https://www.boldsystems.org.

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Tables

Table 1. Families of Orthoptera with the number of genera, species, and sequences (percentage shows the proportion of each family of the total sequences), number of genera with the barcode gap classification according to Badotti *et al.* (2017), and Probability of Correct Identification (PCI) values. Total values are provided for the Orthoptera.

Family	**Genera**	**Species**	**Sequences (%)**	**Barcode gap boxplot classification**			**PCI (%)**
				good	***intermediate***	***poor***	
Caelifera							
Acrididae	95	477	5386 (46.41)	44	30	21	46.43
Dericorythidae	1	5	8 (0.06)	1	-	-	100
Morabidae	2	4	179 (1.54)	1	1	-	75

Pamphagidae	3	13	22 (0.18)	2	-	1	85.71
Pyrgomorpgidae	4	15	229 (1.97)	3	1	-	84.61
Romaleidae	1	11	335 (2.88)	1	-	-	27.27
Tetrigidae	3	16	222 (1.91)	3	-	-	38.46
Tridactylidae	2	5	23 (0.19)	1	-	1	33.33
Ensifera							
Anostostomatidae	3	7	73 (0.62)	3	-	-	100
Gryllidae	17	122	913 (7.86)	13	1	3	72.72
Gryllotalpidae	2	7	42 (0.36)	1	1	-	71.42
Mogoplistidae	1	13	41 (0.35)	1	-	-	88.88
Myrmecophilidae	1	3	76 (0.65)	1	-	-	66.66
Phalangopsidae	1	2	9 (0.07)	-	-	1	50
Prophalangopsidae	1	2	43 (0.37)	-	-	1	0
Rhaphidophoridae	9	88	533 (4.59)	8	1	-	82.19
Tettigoniidae	69	305	3221 (27.75)	48	10	11	75.39
Trigonidiidae	11	37	250 (2.15)	8	2	1	75.75
				139	**47**	**40**	
TOTAL	**226**	**1132**	**11605**	**(61.5%)**	**(20.7%)**	**(17.7%)**	**73.86**

Figure captions

Fig. 1. Examples of the barcode gap classification according to Badotti et al. (2017) used in this study. Classification was *good* when boxplots showed a gap between

intra- and interspecific distances; *intermediate* when the whiskers of intra- and interspecific distances overlapped; and *poor* when the boxes of intra- and interspecific distances overlapped.

Fig. 2. Probability of Correct Identification (PCI) of Orthoptera. Each point represents a species and is coded by suborder (Caelifera, blue dots; Ensifera, red triangles). For each species, the maximum intraspecific distance was compared to the minimum interspecific distance (nearest-neighbor distance). Species above the central diagonal line were successfully identified; species below the line were identification failures.

Fig. 3. Violin plot showing the correlation between barcode gap classification and Probability of Correct Identification (PCI) values in our analysis.

Supplementary material

Table S1. Taxa of Orthoptera analyzed and their Probability of Correct Identification (PCI) values, number of species and sequences, barcoding gap performance, maximum intraspecific distance (limit of upper intraspecific whisker), and minimum interspecific distance (limit of lower interspecific whisker). PCI = Probability of Correct Identification; Max intra = maximum intraspecific distance; Min inter = minimum interspecific distance. Values of PCI and distances are in percentages.

Table S2. Species list of Orthoptera, with labels updated or changed due to invalid or misspelled names. BOLD – Barcode of Life Data System <https://www.boldsystems.org>; OSF – Orthoptera Species File online catalogue <http://orthoptera.speciesfile.org>. Seq = number of sequences.

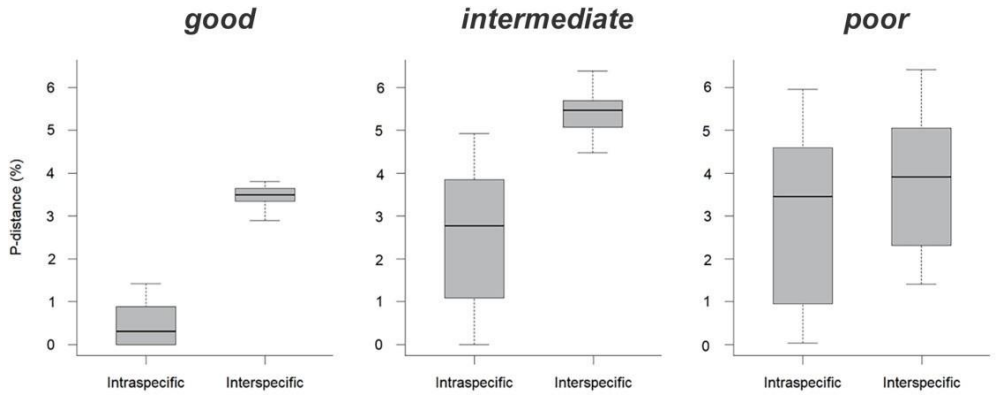

Fig. 1. Examples of the barcode gap classification according to Badotti et al. (2017) used in this study. Classification was good when boxplots showed a gap between intra- and interspecific distances; intermediate when the whiskers of intra- and interspecific distances overlapped; and poor when the boxes of intra- and interspecific distances overlapped.

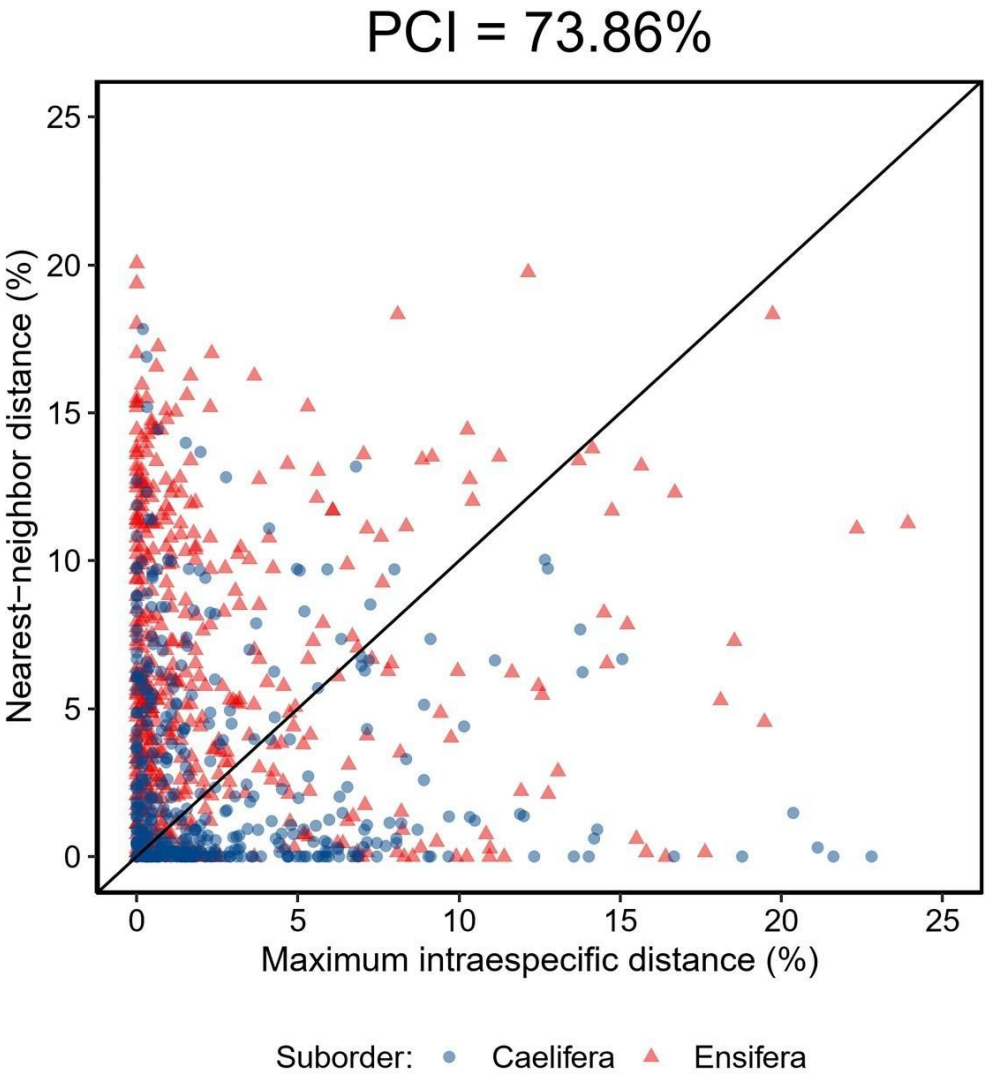

Fig. 2. Probability of Correct Identification (PCI) of Orthoptera. Each point represents a species and is coded by suborder (Caelifera, blue dots; Ensifera, red triangles). For each species, the maximum intraspecific distance was compared to the minimum interspecific distance (nearest-neighbor distance). Species above the central diagonal line were successfully identified; species below the line were identification failures.

431x479mm (300 x 300 DPI)

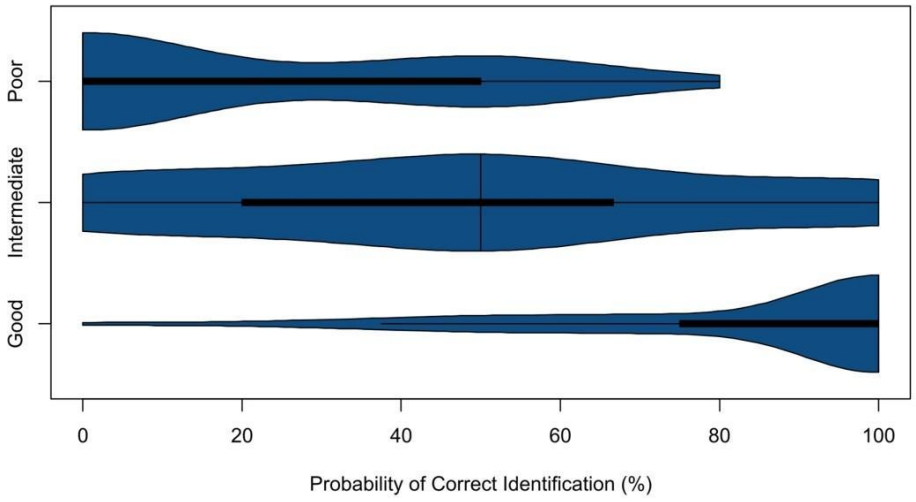

Fig. 3. Violin plot showing the correlation between barcode gap classification and Probability of Correct Identification (PCI) values in our analysis.

Table S1 - Taxa of Orthoptera analyzed and their Probability of Correct Identification (PCI) values, number of species and sequences, barcoding gap performance, maximum intraspecific distance (limit of upper intraspecific whisker), and minimum interspecific distance (limit of lower interspecific whisker). PCI = Probability of Correct Identification; Max intra = maximum intraspecific distance; Min inter = minimum interspecific distance. Values of PCI and distances are in percentages.

Genera (PCI)	Species	Sequences	Barcode gap boxplot classification	Max intra	Min inter
Caelifera					
Acrididae (46,43)					
Acrida (50)	7	99	*intermediate*	2,89	0,40
Acrotylus (33.33)	4	175	*good*	2,73	8,14
Aeoloplides (100)	2	3	*good*	0,91	4,71
Aeropedellus (66.67)	3	50	*good*	0,76	1,61
Aiolopus (33.33)	3	71	*poor*	17,53	7,35
Anacridium (100)	2	23	*good*	1,56	3,18
Anapodisma (0)	2	68	*intermediate*	4,60	4,30
Angaracris (50)	2	5	*poor*	4,50	2,27
Arcyptera (50)	5	87	*good*	1,96	2,73
Arphia (66.67)	3	48	*intermediate*	3,93	1,52
Atrachelacris (0)	2	5	*poor*	5,18	3,96
Aulocara (100)	2	7	*good*	1,47	9,71
Booneacris (100)	3	10	*good*	1,25	3,33
Boopedon (100)	2	3	*good*	1,51	13,98
Bruneria (0)	2	10	*poor*	1,20	0,15
Bryodema (0)	3	6	*poor*	5,20	2,03
Bryodemella (0)	3	14	*poor*	4,39	0,64
Buckellacris (33.33)	3	13	*intermediate*	0,60	0,00
Calliptamus (33.33)	6	189	*intermediate*	13,88	0,89
Caryanda (100)	3	7	*good*	1,38	6,73
Catantops (100)	2	12	*good*	1,07	8,74
Ceracris (60)	5	431	*good*	5,62	9,87
Chloealtis (100)	2	37	*good*	1,06	11,48
Choroedocus (50)	4	34	*intermediate*	2,70	0,31
Chorthippus (17.65)	30	387	*intermediate*	2,61	0,00
Chortophaga (0)	2	12	*poor*	3,85	0,32
Circotettix (0)	4	25	*poor*	2,70	0,00
Conozoa (100)	3	75	*good*	0,74	4,58
Cratypedes (100)	2	13	*good*	1,21	9,98

Cyrtacanthacris (0)	2	10	*intermediate*	3,04	2,12
Diabolocatantops (50)	2	17	*good*	0,75	3,29
Dichromatos (100)	2	3	*good*	1,49	3,47
Dichromorpha (50)	2	41	*intermediate*	13,67	12,47
Dichroplus (20)	15	21	*intermediate*	8,90	3,97
Dissosteira (100)	2	39	*good*	2,88	7,49
Dociostaurus (80)	13	70	*good*	2,58	9,06
Epacromius (50)	3	10	*intermediate*	11,39	7,44
Euchorthippus (50)	4	32	*intermediate*	3,83	0,71
Fruhstorferiola (20)	5	55	*intermediate*	1,97	0,15
Gastrimargus (0)	3	23	*poor*	2,88	1,47
Gomphocerus (50)	3	16	*poor*	1,87	0,45
Gonista (100)	2	4	*good*	0,00	8,81
Hesperotettix (0)	2	8	*poor*	7,15	6,73
Heteracris (100)	2	5	*good*	7,15	13,18
Heteropternis (0)	2	5	*poor*	0,00	0,00
Hieroglyphus (80)	6	38	*good*	1,16	6,45
Kosciuscola (60)	5	57	*good*	2,71	4,31
Lactista (100)	2	14	*good*	1,61	6,25
Leptopternis (100)	3	8	*good*	0,51	0,68
Melanoplus (33.33)	52	461	*intermediate*	3,90	0,87
Mermiria (100)	2	11	*good*	1,24	3,30
Myrmeleotettix (0)	3	90	*poor*	7,91	0,00
Notostaurus (100)	3	8	*intermediate*	1,56	1,43
Oedaleonotus (100)	2	6	*good*	0,91	2,88
Oedaleus (33.33)	6	60	*good*	3,07	5,97
Oedipoda (33.33)	5	38	*good*	3,61	9,55
Ognevia (0)	2	9	*intermediate*	2,50	1,79
Omocestus (25)	7	84	*poor*	2,95	0,00
Orotettix (100)	4	35	*good*	6,26	6,83
Oxya (28.57)	8	166	*good*	1,53	4,26
Pardalophora (100)	2	19	*intermediate*	2,47	2,12
Paroxya (50)	2	22	*good*	1,41	2,89
Patanga (50)	3	23	*intermediate*	1,25	0,45
Pedopodisma (50)	2	9	*poor*	2,12	0,60
Pezotettix (100)	2	3	*good*	3,70	7,88
Phlaeoba (60)	5	27	*intermediate*	2,76	0,45
Podisma (0)	4	8	*poor*	3,40	0,00
Podismopsis (0)	5	10	*poor*	6,07	1,38
Prumna (0)	2	8	*poor*	3,19	0,89

Pseudochorthippus (0)	3	128	*intermediate*	4,28	0,00
Psoloessa (100)	2	22	*good*	6,23	9,66
Pternoscirta (50)	2	8	*intermediate*	1,06	0,75
Qinlingacris (100)	3	4	*good*	0,00	0,46
Ramburiella (50)	3	325	*intermediate*	12,97	8,52
Ronderosia (100)	6	7	*intermediate*	2,89	2,48
Schistocerca (5)	24	696	*intermediate*	9,14	1,51
Scotussa (100)	4	5	*good*	5,62	5,69
Shirakiacris (0)	2	23	*good*	1,21	1,51
Sinipta (100)	2	7	*good*	0,84	1,95
Sinopodisma (14.29)	9	68	*intermediate*	2,43	2,43
Spathosternum (100)	2	13	*good*	1,82	9,72
Spharagemon (60)	6	27	*intermediate*	8,83	3,96
Sphingonotus (31.58)	34	153	*intermediate*	3,27	2,64
Stenobothrus (12.5)	14	56	*poor*	1,74	0,00
Stenocatantops (50)	3	12	*good*	4,17	7,29
Stethophyma (50)	4	23	*intermediate*	2,12	1,12
Syrbula (100)	2	13	*good*	0,75	9,42
Thalpomena (50)	4	11	*intermediate*	1,02	0,34
Tonkinacris (100)	5	38	*good*	3,19	4,86
Trilophidia (100)	2	50	*good*	8,03	8,29
Trimerotropis (23.08)	15	166	*intermediate*	1,44	0,00
Vosseleriana (100)	3	6	*good*	1,87	5,46
Xanthippus (50)	2	11	*poor*	6,16	0,60
Xenocatantops (0)	2	19	*poor*	3,78	0,15
Yunnanacris (100)	2	3	*good*	0,00	2,43
Dericorythidae (100)					
Conophyma (100)	5	8	*good*	2,27	8,24
Morabidae (75)					
Vandiemenella (50)	2	126	*good*	6,44	7,07
Warramaba (100)	2	53	*intermediate*	10,67	9,71
Pamphagidae (85.71)					
Asiotmethis (50)	5	9	*poor*	2,12	1,27
Beybienkia (100)	2	4	*good*	0,00	6,07
Filchnerella (100)	6	9	*good*	0,00	0,45
Pyrgomorphidae (84.61)					

Atractomorpha (50)	5	56	*good*	2,88	8,66
Mekongiella (100)	2	5	*good*	0,00	4,86
Poekilocerus (100)	2	6	*good*	0,30	16,89
Sphenarium (100)	6	162	*intermediate*	1,48	1,28
Romaleidae (27.27)					
Taeniopoda (18.18)	11	335	*good*	2,43	2,76
Tetrigidae (38.46)					
Euparatettix (100)	2	6	*good*	0,84	14,77
Paratettix (75)	4	15	*good*	2,21	13,67
Tetrix (12.5)	10	201	*good*	6,68	12,50
Tridactylidae (33.33)					
Ellipes (0)	2	19	*poor*	1,95	0,00
Xya (100)	3	4	*good*	0,18	17,02
Ensifera					
Anostostomatidae (100)					
Anabropsis (100)	2	4	*good*	0,00	6,07
Hemiandrus (100)	3	66	*good*	9,16	13,60
Hemideina (100)	2	3	*good*	8,09	18,33
Gryllidae (72,72)					
Agnotecous (50)	15	31	*good*	1,82	2,12
Cardiodactylus (92.31)	36	106	*good*	3,18	3,34
Gryllodes (0)	2	28	*poor*	1,58	0,00
Gryllus (75)	8	102	*good*	3,42	3,49
Hemiphonus (100)	2	4	*good*	0,35	14,28
Lebinthus (100)	4	5	*good*	0,30	10,94
Loxoblemmus (25)	4	45	*poor*	18,69	0,15
Malgasia (100)	3	4	*good*	0,00	20,22
Melanogryllus (100)	2	5	*good*	2,43	9,72
Microbinthus (0)	3	4	*intermediate*	1,21	1,21
Mitius (33.33)	3	9	*poor*	11,09	0,00
Modicogryllus (100)	2	6	*good*	1,36	12,74
Oecanthus (57.14)	18	148	*good*	2,75	7,14
Orocharis (100)	2	21	*good*	1,82	10,23
Teleogryllus (50)	6	16	*good*	3,64	4,55
Velarifictorus (75)	9	373	*good*	3,19	9,38

Xenogryllus (100)	3	6	*good*	3,05	8,99
Gryllotalpidae (71.42)					
Gryllotalpa (60)	5	35	*intermediate*	14,19	6,27
Neoscapteriscus (100)	2	7	*good*	7,14	15,34
Mogoplistidae (88.88)					
Ornebius (88.89)	13	41	*good*	1,60	16,02
Myrmecophilidae (66.67)					
Myrmecophilus (66.67)	3	76	*good*	0,60	15,19
Phalangopsidae (50)					
Cacoplistes (0)	2	9	*poor*	0,30	0,00
Prophalangopsidae (0)					
Cyphoderris (0)	2	43	*poor*	6,99	3,80
Rhaphidophoridae (82.19)					
Ceuthophilus (69.23)	15	127	*good*	2,11	5,28
Daihinibaenetes (100)	2	7	*good*	0,45	6,38
Diestrammena (100)	2	3	*good*	0,00	12,48
Dolichopoda (80.56)	40	182	*good*	1,43	2,31
Euhadenoecus (100)	2	5	*good*	0,30	13,66
Hadenoecus (100)	2	20	*good*	1,49	8,83
Pristoceuthophilus (100)	3	93	*good*	3,50	9,68
Talitropsis (100)	3	13	*intermediate*	2,12	1,49
Troglophilus (76.92)	19	83	*good*	4,71	7,23
Tettigoniidae (75.39)					
Aerotegmina (100)	5	57	*good*	2,65	15,60
Altihoratosphaga (50)	2	10	*good*	0,54	1,44
Amblycorypha (100)	3	20	*good*	2,12	8,22
Amytta (100)	11	30	*good*	0,00	0,15
Anabrus (50)	2	94	*intermediate*	15,26	7,90
Antaxius (100)	2	5	*good*	0,30	15,50
Arantia (66.67)	3	7	*good*	11,46	14,44
Atlanticus (100)	9	37	*good*	6,83	10,18
Banza (88.89)	9	19	*intermediate*	5,02	3,50
Barbitistes (100)	2	10	*good*	4,39	11,55

Calliphona (66.67)	3	22	*intermediate*	6,81	6,10
Chortoscirtes (100)	4	13	*good*	0,45	5,77
Conanalus (100)	4	71	*good*	5,31	6,68
Conocephalus (77.27)	24	422	*good*	9,95	14,36
Ducetia (100)	5	193	*good*	3,64	8,47
Elimaea (50)	5	39	*good*	1,82	2,50
Eobiana (50)	2	5	*poor*	5,33	0,66
Eoxizicus (54.55)	12	76	*poor*	7,29	2,88
Eremopedes (100)	2	4	*good*	0,15	15,95
Euconocephalus (0)	2	21	*poor*	3,49	0,00
Eupholidoptera (66.67)	7	19	*good*	2,91	4,12
Euxiphidiopsis (66.67)	5	128	*good*	0,75	11,09
Gampsocleis (50)	6	235	*poor*	7,45	0,00
Grigoriora (100)	2	69	*good*	0,00	4,10
Hemielimaea (100)	2	25	*good*	1,06	5,01
Hexacentrus (33.33)	6	184	*good*	3,34	10,94
Holochlora (100)	3	5	*good*	0,15	8,96
Horatosphaga (100)	4	20	*intermediate*	10,27	7,05
Idiostatus (100)	4	8	*good*	9,00	12,31
Isophya (100)	5	20	*intermediate*	3,49	2,02
Isopsera (100)	4	54	*good*	4,40	16,41
Kuwayamaea (0)	2	43	*poor*	2,73	1,36
Leptophyes (100)	3	17	*good*	3,13	11,94
Meconema (0)	2	30	*intermediate*	11,92	7,43
Mecopoda (0)	2	128	*intermediate*	11,45	5,80
Melanoscirtes (100)	4	18	*good*	2,88	3,64
Metrioptera (100)	2	13	*good*	2,74	10,48
Modestana (100)	2	4	*good*	0,66	17,24
Montana (100)	2	5	*good*	2,73	5,76
Monticolaria (100)	3	10	*good*	0,72	2,70
Neoconocephalus (75)	6	25	*intermediate*	6,12	6,04
Nigrimacula (50)	2	5	*poor*	8,51	1,51
Orchelimum (66.67)	6	22	*good*	0,91	5,65
Pachytrachis (100)	2	6	*good*	1,13	14,43
Palaeoagraecia (100)	2	14	*good*	1,97	10,03
Parapsyra (100)	3	45	*good*	1,51	5,16
Paratlanticus (50)	3	21	*poor*	13,12	0,91
Paraxantia (100)	3	6	*good*	0,15	3,49
Peronura (100)	2	23	*good*	1,44	6,48
Phaneroptera (66.67)	3	72	*poor*	16,13	13,37

Pholidoptera (100)	4	63	*good*	1,99	11,26
Phyllomimus (0)	3	38	*good*	7,59	12,31
Platycleis (66.67)	4	12	*poor*	20,06	5,28
Poecilimon (100)	4	16	*good*	0,15	9,87
Pseudocosmetura (100)	2	4	*good*	2,27	10,79
Pseudokuzicus (0)	2	4	*poor*	13,06	2,88
Pseudorhynchus (50)	4	23	*good*	0,91	11,24
Pyrgocorypha (100)	3	10	*good*	0,60	8,05
Ruidocollaris (100)	2	44	*good*	6,68	11,70
Ruspolia (44.44)	9	229	*intermediate*	3,64	0,00
Saga (71.43)	9	28	*good*	1,21	7,29
Scudderia (33.33)	6	59	*good*	4,20	6,67
Sinochlora (0)	3	69	*poor*	7,59	0,15
Sinocyrtaspis (100)	7	21	*good*	1,06	4,86
*Teneraspha*ga (100)	2	4	*good*	1,98	7,22
Tessellana (100)	3	10	*good*	4,98	10,18
Tettigonia (71.43)	10	76	*intermediate*	4,40	2,27
Xiphidiopsis (75)	7	19	*good*	2,58	11,55
Xizicus (71.43)	8	63	*good*	8,64	11,39
Trigonidiidae (75.75)					
Allonemobius (40)	5	106	*good*	1,67	11,13
Amusurgus (100)	2	3	*good*	0,00	9,87
Anaxipha (100)	6	14	*good*	3,79	5,81
Anaxiphomorpha (100)	2	5	*good*	0,75	1,97
Homoeoxipha (50)	4	43	*intermediate*	3,64	0,00
Natula (100)	2	3	*good*	0,00	8,81
Neonemobius (0)	2	19	*intermediate*	15,19	10,41
Paratrigonidium (100)	2	7	*good*	0,60	11,24
Pteronemobius (100)	3	23	*good*	1,93	12,47
Svistella (100)	4	11	*good*	0,91	12,00
Trigonidium (80)	5	16	*poor*	10,83	0,75

Table S2 - Species list of Orthoptera, with labels updated or changed due to invalid or misspelled names. BOLD – Barcode of Life Data System <https://www.boldsystems.org>; OSF – Orthoptera Species File online catalogue <http://orthoptera.speciesfile.org>. Seq = number of sequences.

BOLD (seq)	OSF	Problem	Situation
Caelifera			
Acrididae			
Aeropus armeniacus (1)	*Gomphocerus armeniacus*	taxon change	changed to current
Aeropus sibiricus (1)	*Gomphocerus sibiricus*	taxon change	changed to current
Arciptera fusca (1)	*Arcyptera fusca*	wrong spelling	adjusted
Arcotylus insubricus (5)	*Acrotylus insubricus*	wrong spelling	adjusted
Arcotylus longipes (145)	*Acrotylus longipes*	wrong spelling	adjusted
Atrachelacris grammineus (1)	*Atrachelacris unicolor*	synonym	changed to senior
Calliptamus blaucha blaucha (26)	*Calliptamus balucha balucha*	wrong spelling	adjusted
Caryanda elegans (3)	*Caryanda neoelegans*	taxon change	changed to current
Chorthippus montanus (25)	*Pseudochorthippus montanus*	taxon change	changed to current
Chorthippus parallelus (37)	*Pseudochorthippus parallelus*	taxon change	changed to current
Chorthippus parallelus erythropus (5)	*Pseudochorthippus parallelus erythropus*	taxon change	changed to current
Chorthippus parallelus parallelus (6)	*Pseudochorthippus parallelus parallelus*	taxon change	changed to current
Cyratanrhairis tatarica tatarica (4)	*Cyrtacanthacris tatarica taratica*	wrong spelling	adjusted
Diabolocatantops pinguis pinguis (1)	*Diabolocatantops pinguis*	taxon change	changed to current
Dociostaurus crassiusculus nigrogeniculatus (1)	*Dociostaurus kraussi nigrogeniculatus*	taxon change	changed to current
Glyptobothrus jacobsi (1)	*Chorthippus jacobsi*	taxon change	changed to current
Hieroglyphus nigroreplatus (8)	*Hieroglyphus nigrorepletus*	wrong spelling	adjusted
Leptoternis gracilus (3)	*Leptopternis gracilus*	wrong spelling	adjusted
Longgenacris rufiantennus (2)	*Fruhstorferiola tonkinensis*	synonym	changed to senior
Melanoplus turnidicercus (3)	*Melanoplus tumidicercus*	wrong spelling	adjusted
Nomadacris apicerca (1)	*Patanga apicerca*	taxon change	changed to current
Nomadacris japonica (2)	*Patanga japonica*	taxon change	changed to current
Oedaleus decorus asiaticus (8)	*Oedaleus asiaticus*	taxon change	changed to current
Omocestus burri (3)	*Omocestus minutissimus*	taxon change	changed to current
Omocestus navasi (1)	*Omocestus antigai*	taxon change	changed to current
Oxya fuscuvittata (27)	*Oxya fuscovittata*	wrong spelling	adjusted
Oxya hyla hyla (2)	*Oxya hyla*	taxon change	changed to current
Oxya hyla intricata (1)	*Oxya intricata*	taxon change	changed to current
Podisma pedeitris (1)	*Podisma pedestris*	wrong spelling	adjusted
Podisma tyatiensis (1)	*Podisma sapporensis*	synonym	changed to senior
Ronderosia bergi (2)	*Ronderosia bergii*	wrong spelling	adjusted
Schistocerca nitens caribbeana (1)	*Schistocerca caribbeana*	taxon change	changed to current
Sinopodisma funiushana (4)	*Pedopodisma funiushana*	taxon change	changed to current
Sinopodisma wudangshanensis (6)	*Pedopodisma wudangshanensis*	taxon change	changed to current
Stenobothrus brunneus (1)	*Bruneria brunnea*	taxon change	changed to current
Stenobothrus eurasius hyalosuperficies (1)	*Stenobothrus hyalosuperficies*	taxon change	changed to current
Trimerotropis pallidipennis salina (2)	*Trimerotropis salina*	taxon change	changed to current
Xenocatantops humilis humilis (4)	*Xenocatantops humilis*	taxon change	changed to current
Dericorythidae			
Conophyma kuznetzovi (1)	*Conophyma kusnezovi*	wrong spelling	adjusted
Pamphagidae			

Pseudotmethis rubimarginis (2)	*Filchnerella rubimarginis*	taxon change	changed to current
Sinotmethis amicus (2)	*Beybienkia amicus*	taxon change	changed to current
Sinotmethis brachypterus (2)	*Beybienkia brachypterus*	taxon change	changed to current
Pyrgomorphidae			
Sphenarium mexicanum histrio (6)	*Sphenarium histrio*	taxon change	changed to current
Sphenarium mexicanum mexicanum (4)	*Sphenarium mexicanum*	taxon change	changed to current
Sphenarium purpurascens minimum (8)	*Sphenarium minimum*	taxon change	changed to current
Sphenarium purpurascens purpurascens (132)	*Sphenarium purpurascens*	taxon change	changed to current
Ensifera			
Gryllidae			
Lebinthus lifouensis (1)	*Microbinthus lifouensis*	taxon change	changed to current
Lebinthus nattawa (1)	*Microbinthus nattawa*	taxon change	changed to current
Lebinthus santoensis (3)	*Microbinthus santoensis*	taxon change	changed to current
Mundeicus brunneovariegatus (3)	*Hemiphonus brunneovariegatus*	taxon change	changed to current
Mundeicus longifemur (1)	*Hemiphonus longifemur*	taxon change	changed to current
Gryllotalpidae			
Scapteriscus acletus (1)	*Neoscapteriscus borellii*	taxon change	changed to current
Scapteriscus borellii (4)	*Neoscapteriscus borellii*	taxon change	changed to current
Scapteriscus vicinus (2)	*Neoscapteriscus vicinus*	taxon change	changed to current
Rhaphidophoridae			
Dolichopoda ligustica (11)	*Dolichopoda azami ligustica*	taxon change	changed to current
Tettigoniidae			
Conocephalus discolor (4)	*Conocephalus fuscus fuscus*	synonym	changed to senior
Conocephalus gladiatus (40)	*Conocephalus exemptus*	synonym	changed to senior
Euconocephalus indicus (6)	*Ruspolia indica*	synonym	changed to senior
Eupholidoptera karatolosi (1)	*Eupholidoptera megastyla*	synonym	changed to senior
Hexacentrus japonicus hareyamai (9)	*Hexacentrus hareyamai*	taxon change	changed to current
Hexacentrus japonicus japonicus (6)	*Hexacentrus japonicus*	taxon change	changed to current
Horatosphaga meruensis (1)	*Terenasphaga meruensis*	taxon change	changed to current
Horatosphaga montivaga (7)	*Altihoratosphaga montivaga*	taxon change	changed to current
Horatosphaga nou (3)	*Altihoratosphaga nou*	taxon change	changed to current
Horatosphaga tenera (3)	*Terenasphaga tenera*	taxon change	changed to current
Meconemopsis quadrinotata (1)	*Nigrimacula quadrinotata*	taxon change	changed to current
Metrioptera engelhardti (3)	*Eobiana engelhardti*	taxon change	changed to current
Metrioptera japonica (2)	*Eobiana japonica*	taxon change	changed to current
Platycleis montana (3)	*Montana montana*	taxon change	changed to current
Platycleis tessellata (2)	*Tessellana tessellata*	taxon change	changed to current
Platycleis veyseli (2)	*Tessellana veyseli*	taxon change	changed to current
Ruspolia indicus (7)	*Ruspolia indica*	wrong spelling	adjusted
Saga campbelli campbelli (3)	*Saga campbelli*	taxon change	changed to current
Saga campbelli gracilis (3)	*Saga gracilis*	taxon change	changed to current
Sinocyrtaspis brachycerca (4)	*Sinocyrtaspis huangshanensis brachycerca*	taxon change	changed to current
Tettigonia dolichoptera dolichoptera (3)	*Tettigonia dolichoptera*	taxon change	changed to current
Tettigonia dolichoptera maritima (1)	*Tettigonia uvarovi*	taxon change	changed to current

Xiphidiopsis cheni (6)	*Grigoriora cheni*	taxon change	changed to current
Xiphidiopsis gurneyi (6)	*Euxiphidiopsis gurneyi*	taxon change	changed to current
Xiphidiopsis maculatus (1)	*Xizicus maculatus*	taxon change	changed to current
Xiphidiopsis protensus (1)	*Euxiphidiopsis protensus*	taxon change	changed to current
Xiphidiopsis quadrinotata (2)	*Nigrimacula quadrinotata*	taxon change	changed to current
Xizicus concavilaminus (3)	*Eoxizicus concavilaminus*	taxon change	changed to current
Xizicus coreanus (5)	*Eoxizicus coreanus*	taxon change	changed to current
Xizicus divergentis (10)	*Eoxizicus divergentis*	taxon change	changed to current
Xizicus howardi (30)	*Eoxizicus howardi*	taxon change	changed to current
Xizicus kulingensis (1)	*Eoxizicus kulingensis*	taxon change	changed to current
Xizicus kweichowensis (62)	*Grigoriora kweichowensis*	taxon change	changed to current
Xizicus laminatus (7)	*Eoxizicus laminatus*	taxon change	changed to current
Xizicus magnus (4)	*Eoxizicus magnus*	taxon change	changed to current
Xizicus rehni (4)	*Eoxizicus rehni*	taxon change	changed to current
Xizicus sinuatus (5)	*Eoxizicus sinuatus*	taxon change	changed to current
Xizicus tinkhami (2)	*Eoxizicus tinkhami*	taxon change	changed to current
Xizicus transversus (2)	*Eoxizicus transversus*	taxon change	changed to current
Xizicus xiai (3)	*Eoxizicus xiai*	taxon change	changed to current
Trigonidiidae			
Metioche japonica (3)	*Trigonidium japonicum*	taxon change	changed to current
Trigonidium nitidum (5)	*Paratrigonidium nitidum*	taxon change	changed to current
Trigonidium venustulum (2)	*Paratrigonidium venustulum*	taxon change	changed to current

CAPÍTULO III

Artigo em preparação para submissão ao periódico ***Zootaxa***
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FILOGENIA MOLECULAR DO GÊNERO *Gryllus* Linnaeus, 1758 (ORTHOPTERA, GRYLLIDAE) ASSOCIADA A SONOTIPOS

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CAPÍTULO IV

Considerações finais

Nesta tese utilizamos ferramentas moleculares com o intuito de adicionar informações a ordem Orthoptera e a grupos específicos dentro dela, visto que muitos ainda apresentam lacunas de conhecimento, que vão desde a falta de estudos até a complexidade biológica.

No primeiro artigo, já publicado no periódico ***Canadian Journal of Zoology***, buscamos testar a eficiência do gene COI, amplamente utilizado como DNA Barcode em metazoários, como identificador e delimitador de espécies dentro de Orthoptera. Como resultado das análises empregadas, mostramos que o COI, de modo geral, é eficiente para alguns grupos, embora para outros nem tanto, mostrando que mais estudos devem ser realizados no âmbito taxonômico, principalmente através da taxonomia integrativa. Esse estudo servirá de base principalmente para o delineamento de futuras pesquisas, auxiliando com informações prévias de DNA barcode em diferentes grupos.

Já no segundo artigo, em preparação para publicação, realizamos uma filogenia do gênero *Gryllus*, um dos mais complexos dentro de Grylloidea, com o objetivo de estabelecer as relações filogenéticas de espécimes coletados no Brasil e associá-las com sonotipos previamente definidos. Através da análise de distâncias genéticas e reconstruções filogenéticas baseadas em três marcadores moleculares, conseguimos responder quatro hipóteses acerca de possíveis espécies novas, além de espécies crípticas com pouca variação sonora. A importância desse trabalho se dá pelo acréscimo de informações genéticas sobre o grupo que ainda é pouco estudado na América do Sul. Além disso, também levantamos a hipótese de possíveis zonas de hibridação que possam ocorrer entre populações no Brasil.

Por fim, vale ressaltar que à medida que os estudos avançam, a abordagem da taxonomia integrativa se torna cada vez mais essencial para novas descobertas, seja ela de identificação de espécie ou análises mais complexas.

ANEXOS

Seguem outras publicações, mesmo que sem relação à tese, as quais tive participação durante o período de realização do Doutorado, mesmo com os impasses impostos pela pandemia do COVID-19.

1. Acosta, R.C., **Timm, V.F.**, Szinwelski, N., Da Costa, M.K.M. & Zefa, E. (2020) Mating behavior and acoustic communication of the long-legged cricket *Endecous* (*Notendecous*) *onthophagus* (Berg, 1891) from Southern Brazil (Orthoptera: Grylloidea: Phalangopsidae). *Zootaxa*, 4743 (3), 427–437. https://doi.org/10.11646/zootaxa.4743.3.10

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