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REVISÃO TAXONÔMICA DO COMPLEXO *ARICORIS CONSTANTIUS* (FABRICIUS, 1793) (LEPIDOPTERA: RIODINIDAE)

PORTO ALEGRE
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Dissertação apresentada ao Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Mestre em Biologia Animal.

Área de concentração: Biologia Comparada

Orientador: Dr. Lucas Augusto Kaminski

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Aprovada em__ de __________ de_____.

BANCA EXAMINADORA

Dr. Luis Alexandre Campos

Dr. Diego Rodrigo Dolibaina

Dra. Rosângela Brito

“A ciência, meu rapaz,

é feita de erros,

mas de erros benéficos,

já que conduzem

pouco a pouco à verdade”

(Júlio Verne)

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SUMÁRIO

RESUMO

Aricoris Westwood, 1851 é um gênero Neotropical cujas larvas são mirmecófilas e que até o presente momento é constituído por 27 espécies. Dentro do gênero, o grupo *constantius* é representado por borboletas de médio a pequeno porte, de coloração marrom e facilmente confundidas com Satyrinae (Nymphalidae). Por formarem um complexo de espécies crípticas, apresentam uma história taxonômica confusa e tortuosa. Dessa maneira, aqui é proposta uma revisão taxonômica com abordagem integrativa, utilizando-se um gene mitocondrial (*citocromo c oxidase subunidade* I -*COI*) aliado a análise morfológica. O complexo *constantius* é recuperado como uma linhagem monofilética constituída de seis espécies: *A. constantius* (Fabricius, 1793), *A. monotona* (Stichel, 1910), *A. tutana* (Godart, [1824]), *A.* sp. 1 sp. nov. (Brasil: Distrito Federal, Goiás, Minas Gerais, São Paulo), *A.* sp. 2 sp. nov. (Brasil: Distrito Federal, Mato Grosso do Sul e Paraná) e *A.* sp. 3 sp. nov. (Brasil: Bahia e Minas Gerais). As espécies novas aqui descritas eram tratadas até então como *A. tutana*. Os resultados moleculares corroboram com os caracteres morfológicos. Neótipos são designados para *Papilio constantius* Fabricius, 1793 e *Erycina tutana* Godart, [1824], cujos tipos foram perdidos. Todas as espécies podem ser identificadas através de uma combinação de características de padrão alar e genitálias. Para auxiliar na identificação destas espécies, foram elaboradas diagnoses, ilustrações dos caracteres diagnósticos e uma chave de identificação. Os indivíduos adultos apresentam óleo em suas asas, indicando hábitos afitófagos no estágio larval. Além disso, *A. constantius* foi observada na natureza se alimentando de *honeydew* de hemípteros atendentes de formigas. Esse trabalho contribui com o conhecimento de Riodinidae, uma das famílias de borboletas menos estudadas, e ilustra a necessidade de uma abordagem integrativa na taxonomia deste grupo.

ABSTRACT

Aricoris Westwood, 1851 is a Neotropical genus whose larvae are myrmecophilous and, until the present moment, is constituted of 27 species. Within the genus, the group *constantius* is represented by medium to small butterflies of brown color and easily confused with Satyrinae (Nymphalidae). Because these butterflies form a complex of cryptic species, they present a confusing and tortuous taxonomic history. Thus, a taxonomic revision with an integrative approach is proposed herein, using a mitochondrial gene (*cytochrome c oxidase subunit I – COI*) allied to the morphological analysis. The complex *constantius* is recovered as a monophyletic lineage consisting of six species: *A. constantius* (Fabricius, 1793), *A. monotona* (Stichel, 1910), *A. tutana* (Godart, [1824]), *A.* sp. 1 sp. nov. (Brazil: Distrito Federal, Goiás, Minas Gerais and São Paulo), *A.* sp. 2 sp. nov. (Brazil: Distrito Federal, Mato Grosso do Sul and Paraná) e *A.* sp. 3 sp. nov. (Brazil: Bahia and Minas Gerais). The new species described herin were previously treated as *A. tutana*. The molecular results corroborate with the morphological characters. Neotypes are assigned to *Papilio constantius* Fabricius, 1793and *Erycina tutana* Godart, [1824], whose types were lost. All species can be identified through a combination of characteristics in the wing pattern and of genitalia. To assist in the identification of these species, diagnoses, illustrations of the diagnostic characters and an identification key were elaborated. Adult individuals have greasy in their wings, indicating aphytophagy in the larval stage. Furthermore, *A. constantius* was observed in nature feeding on honeydew produced by ant-tended Hemiptera. This work contributes to the knowledge of Riodinidae, one of the less studied butterfly families, and illustrates the need for an integrative approach in the taxonomy of this group.

CAPÍTULO I

INTRODUÇÃO GERAL

1.1. Taxonomia: importância, desafios e novas tendências

A sistemática biológica ocupa função central no estudo e percepção da biodiversidade, configurando um crítico pilar para todas as outras disciplinas da biologia (CRACRAFT 2002; BOERO 2010). Essa ciência busca entender a origem, evolução, diversidade e distribuição dos seres vivos (MAYR 1969; HUBNER & LANGOR 2004). Sistemática e taxonomia estão extremamente relacionadas e difíceis de separar, podendo ser definidas em conjunto como a teoria e prática de descobrir, identificar, descrever, nomear e classificar os organismos (HUBER & LANGOR 2004).

Desde a primeira publicação do *Systema Naturae* por Carolus Linnaeus, em meados de 1700, aproximadamente 1,5 a 1,8 milhões de espécies já foram descritas. O número total de espécies no planeta tem sido estimado por diferentes métodos, e as estimativas variam de 2 a mais de 100 milhões de espécies viventes, embora a maioria dos métodos sugira algo em torno de 10 milhões (WILSON 2003; WILSON 2004; COSTELLO, MAY & STORK 2013).

Existem muitos motivos importantes para se descobrir, delimitar e descrever espécies para a ciência. Espécies constituem uma das unidades fundamentais da biologia, comparáveis em importância com genes, células e organismos, outras unidades menores de organização biológica (DE QUEIROZ 2007). Uma vez que uma espécie é descrita, estudos mais detalhados em ecologia, genética, fisiologia e bioquímica podem ser conduzidos. Além disso, identificar espécies é necessário em termos de conservação para a garantia de bons estudos em ciências biológicas, de ecossistemas e no gerenciamento de recursos naturais. Espécies fornecem uma medida prática para diferenciar habitats e acompanhar os efeitos da atividade humana sobre a biodiversidade do planeta (WILSON 2004; COSTELLO, MAY & STORK 2013).

Mesmo com o mundo passando por constantes cenários de mudanças (climáticas, ambientais e tecnológicas) e de desenvolvimento acelerado, tornando a necessidade de identificação de espécies cada vez mais importante para a compreensão da biodiversidade, a taxonomia atualmente é uma ciência subfinanciada e subestimada (GODFRAY 2002; WILSON 2003; DREW 2011). Além de ser vista equivocadamente por muitos como uma ciência meramente descritiva e de pouco conteúdo intelectual (AGNARSSON & KUNTNER 2007), um dos principais motivos para a chamada “crise na taxonomia” é o reduzido incentivo para que pesquisadores sigam nesse ramo. Como a maioria dos grupos taxonômicos são estudados por poucas pessoas, trabalhos de taxonomia são usualmente pouco citados, diminuindo o fator de impacto de revistas especializadas na publicação desse assunto e, por consequência, a atratividade em publicar esse tipo de estudo. Além disso, tradicionalmente a primeira citação da espécie em um texto deveria ser seguida pelo nome dos autores e o ano da publicação dessa espécie, porém isso nem sempre é exigido pelas revistas. Para agravar o cenário, esse tipo de citação não é normalmente referenciado na listagem de literatura consultada (WERNER 2006; AGNARSSON & KUNTNER 2007).

Apesar das dificuldades acima mencionadas, taxonomia por si só é mantida como uma disciplina confusa devido à problemática do próprio conceito de espécie. MAYDEN (1997) listou 22 conceitos diferentes, dentre os quais podem ser citados: o morfológico, biológico, cladístico, ecológico e evolucionário, genético. Muitos desses conceitos e suas definições apresentam incongruências e podem levar a diferentes conclusões no que diz respeito a limites e ao número total de espécies (DE QUEIROZ 2007).

Esse último autor propõe uma alternativa para esse impasse: manter o elemento comum de todos conceitos contemporâneos de espécies e eliminar os conflitos entre conceitos rivais. Segundo DE QUEIROZ, 2007, linhagens não precisam ser fenéticamente diferentes, diagnosticáveis, monofiléticas, reprodutivamente isoladas ou ecologicamente diferentes, apenas precisam estar evoluindo separadamente de outras linhagens. Dessa maneira, todas essas características citadas podem ser consideradas propriedades que podem ou não ser adquiridas durante o curso da existência da espécie. Entretanto, mesmo existindo vários conceitos diferentes, existe um consenso com relação às questões formais da taxonomia, tais como: táxons em nível de espécie devem possuir um nome formal em latim ou latinizado; há necessidade de seguir as regras padronizadas de nomenclatura regidas pelos códigos internacionais e deve haver espécimes vouchers para documentar as decisões e descrições taxonômicas (CRACRAFT 2002).

Tradicionalmente, a taxonomia é baseada principalmente em caracteres morfológicos, o que representa algumas limitações de identificação, já que discrimina apenas morfoespécies (sensu MAYDEN 1997). Isso não quer dizer que uma morfoespécie não seja uma espécie válida, mas sim que são hipóteses que podem ser testadas através de diferentes abordagens e com diferentes tipos de dados (DAYRAT 2005). O problema maior aparece quando estamos lidando com complexos de espécies crípticas, ou seja, duas ou mais espécies classificadas com um único nome, pois são no mínimo superficialmente indistinguíveis morfologicamente (BICKFORD *et al.* 2007). Outros problemas enfrentados pela taxonomia morfológica são a dificuldade de lidar com a plasticidade fenotípica dentro de uma mesma espécie, a falta de chaves taxonômicas para muitos grupos e o alto grau de especialização que um taxonomista deve ter para trabalhar com os diferentes grupos (PIRES & MARINONI 2010). No entanto, cabe ressaltar que outras abordagens além da morfológica podem auxiliar significantemente para acelerar o processo de identificação de espécies (SCHLICK-STEINER *et al.* 2010).

A descoberta do DNA por James Watson e Francis Crick em 1953 contribuiu relevantemente com os progressos no campo da genética, auxiliando na compreensão de processos biológicos inerentes à evolução das espécies. Com o desenvolvimento de métodos rápidos e efetivos de extração, purificação e sequenciamento de DNA, surgiu uma nova linha de pesquisa em biologia, a taxonomia molecular (BUENO-SILVA 2012). Nesse sentido, HEBERT *et al.* (2003) identificaram e propuseram a utilização de um marcador mitocondrial, o gene *citocromo c oxidase subunidade I* (*COI*), como um código de barras genético para animais, conhecido como DNA *barcode*. Segundo os autores, o gene apresenta *primers* universais muito robustos, possibilitando a recuperação do terminal 5’ de representantes de praticamente, se não todos, filos de animais. Além disso, a taxa de evolução deste gene ocorre num limiar ideal para discriminar, na maioria das vezes, espécies próximas e grupos filogeográficos dentro de uma mesma espécie.

Uma das principais vantagens da taxonomia molecular é o acesso a um maior conjunto de dados (sequências de nucleotídeos) em relação ao número de caracteres que a análise morfológica pode conseguir (HILLIS 1987). Outras grandes vantagens são o reconhecimento e delimitação de espécies crípticas, identificação de indivíduos imaturos, usualmente diferentes dos adultos e muitas vezes sem características externas diagnósticas para separação de espécies, e clarificação de problemas taxonômicos, tais como sinonímias (HEBERT *et al*. 2004; PIRES & MARINONI 2010). Entretanto, é importante salientar que o DNA *barcode* sozinho gera apenas informação, e não conhecimento, já que as sequências devem estar relacionadas com um espécime descrito e depositado em alguma coleção (EBACH & HOLDREGE 2005). Além disso, o DNA *barcode*, assim como a morfologia, deve ser utilizado para delimitar hipoteticamente a espécie testada, visto que ele não é útil na separação de todas as linhagens de espécies existentes (ver ELIAS *et al*. 2007; SANTOS, FRANCO & MANFRIN 2009; JUSTI, DALE & GALVÃO 2014). Assim como a taxonomia molecular, a identificação morfológica também tem suas vantagens, como por exemplo, a aplicabilidade em indivíduos preservados em museus há muitos anos, já que o DNA é uma molécula que se degrada com o passar do tempo, aplicabilidade em fósseis e um custo usualmente reduzido em relação a taxonomia molecular, embora alguns trabalhos sugerem que com os avanços na tecnologia, a identificação molecular tende a baixar seus custos (HILLIS 1987; LINDAHL 1993; HEBERT & GREGORY 2005; ZIMMERMANN *et al*. 2008; SUTRISNO 2012).

A taxonomia integrativa utiliza-se de ferramentas de diferentes origens que se complementam em diversas disciplinas, tais como o estudo de moléculas, filogeografia, ecologia, comportamento, história de vida e morfologia dos estágios imaturos (DAYRAT 2005; SCHLICK-STEINER *et al*. 2010). Como as abordagens morfológica e molecular quando usadas sozinhas apresentam vantagens e desvantagens, a taxonomia integrativa é uma ferramenta promissora para descrevermos a biota ainda não conhecida, bem como para lidarmos com problemas taxonômicos causados pelas fracas e incompletas descrições de espécies antigas. Além disso, táxons propostos por dados integrados são hipóteses mais bem definidas e melhor suportadas para o desenvolvimento de outros estudos posteriores (DAYRAT 2005; PIRES & MARINONI 2010).

A abordagem integrativa é muito importante para resolver problemas taxonômicos envolvendo espécies crípticas, já que a utilização do DNA pode dar luz a características morfológicas antes ignoradas pelos especialistas (DAYRAT 2005; PIRES & MARINONI 2010). Nas últimas décadas, muitas dessas espécies ditas crípticas vêm sendo reconhecidas e descritas graças a taxonomia integrativa (HEBERT *et al*. 2004; SCHLICK-

STEINER *et al.* 2006; BURNS *et al.* 2008; MILANKOV *et al.* 2008; VAGLIA *et al.* 2008; YANG *et al.* 2012; PRITI *et al.* 2016).

A taxonomia integrativa pode ser conduzida através de dois protocolos básicos: integração por congruência e integração por acumulação; cada apresentando vantagens e desvantagens (PADIAL *et al.* 2010). No primeiro caso, é desejável que exista concordância em diferentes conjuntos de dados informativos, pois seria muito improvável que um padrão coerente de concordância de caracteres tenha surgido por acaso. Desta maneira, a integração por congruência assume que quando diferentes grupos de caracteres (e.g., morfológicos, moleculares, ecológicos, comportamentais) concordam, as linhagens evolutivas estão totalmente separadas e podem ser classificadas como espécies diferentes (PADIAL *et al.* 2010). Embora essa abordagem garanta mais confiança na informação taxonômica e, consequentemente, estabilidade taxonômica, a principal desvantagem é que a taxonomia integrativa por congruência é mais rigorosa e, portanto, pode levar a uma subestimação de espécies. Por exemplo, algumas linhagens de peixes ciclídeos apresentam diferenças morfológicas que se originaram devido a uma rápida seleção divergente associada com transições ecológicas, porém demonstram fraco isolamento reprodutivo, baixo agrupamento genotípico e pouca diferenciação genética neutra (KOCHER 2004; PADIAL *et al.* 2010).

A integração por acumulação, por outro lado, é baseada no princípio que divergência em qualquer carácter pode ser tomada como uma evidência para a existência de espécies diferentes. Em outras palavras, o acúmulo de todas as fontes de características diagnosticas é analisado, e mesmo quando uma única característica é interpretada como suficiente para justificar o status de espécie, ela é considerada. Concordâncias e discrepâncias são explicadas a partir de uma perspectiva evolutiva das populações estudadas e a decisão é tomada baseada na informação disponível (PADIAL *et al.* 2010). A principal vantagem dessa abordagem, é que taxonomistas podem selecionar e dar ênfase aos conjuntos de informações de carácteres que julgam ser mais apropriadas para cada grupo de organismos. Porém, a taxonomia baseada em acumulação pode levar a uma superestimação de espécies. Por exemplo, duas populações coespecíficas podem apresentar diferenças morfológicas que poderiam ser interpretadas como pontos importantes para, erroneamente, delimitá-las em duas espécies (PADIAL *et al.* 2010).

1.2. Riodinidae

Lepidoptera corresponde às borboletas e mariposas, sendo a segunda ordem mais diversa dentro de Insecta, atrás apenas de Coleoptera. Possui cerca de 160 mil espécies descritas e algumas estimativas sugerem que pode chegar a 500 mil espécies (MUTANEN *et al*. 2010; KRISTENSEN *et al*. 2007). Essa diversidade é especialmente alta na região Neotropical, quando comparada com outras regiões biogeográficas, sendo que no Brasil são conhecidas aproximadamente 26 mil espécies, embora estimativas indiquem valores entre 60 e 80 mil (DUARTE *et al.* 2012; BRITO *et al*. 2016).

Dentre as 133 famílias que compõem a ordem, Pieridae, Nymphalidae, Lycaenidae, Riodinidae, Papilionidae e Hesperiidae representam o que popularmente chamamos de borboletas. Classificações mais antigas dividiam as borboletas em duas superfamílias, Hesperioidea, incluindo apenas Hesperiidae, e Papilionoidea englobando as demais (MINET 1991; ROE *et al.* 2009; KIM *et al.* 2010). Entretanto, estudos filogenéticos recentes demonstram que essa classificação não é natural e atualmente todas essas famílias, juntamente com Hedylidae, constituem o clado Papilionoidea (MUTANEN *et al.* 2010; HEIKKILÄ *et al.* 2012; REGIER *et al.* 2013; KAWAHARA & BREINHOLT 2014).

Riodinidae ocorre quase que exclusivamente na região Neotropical, onde são registradas aproximadamente 1300 espécies (95% da riqueza da família) (DEVRIES *et al*. 1992; HALL 2002; DUARTE *et al*. 2012; SIEWERT *et al*. 2014; SERAPHIM *et al.* 2018). Propostas filogenéticas recentes para a família são baseadas em caracteres moleculares (ESPELAND *et al.* 2015; SERAPHIM *et al.* 2018). Esse último propõe a divisão de Riodinidae em duas subfamílias: Nemeobiinae, contendo as tribos Nemeobiini (que inclui todos os gêneros do Velho Mundo) e Euselasiini, e Riodininae, contendo as tribos Eurybiini, Nymphidiini, Calydnini, Symmachiini, Helicopini, Emesidini, Sertaniini, Dianesiini e Riodinini.

Na Região Neotropical, as espécies de Riodinidae apresentam diversos padrões de coloração e formas, com tamanho variando de pequeno a médio comparado com representantes de outras famílias de borboletas (BROWN *et al*. 2012). Apesar de sua grande riqueza, Riodinidae tem sido pouco estudada quando comparada com as demais famílias de Papilionoidea, tanto em aspectos básicos de biologia como em história natural (KAMINSKI 2008a; BROWN *et al*. 2012; SIEWERT *et al*. 2014).

Existiram divergências entre autores em considerar Riodinidae uma subfamília de Lycaenidae ou ambas as famílias separadas (DEVRIES 1991, CAMPBELL *et al*. 2000). Estudos moleculares envolvendo genes como *wingless*, *COI*, *EF-1α* e outros, sugerem que os dois táxons são duas famílias irmãs (CAMPBELL *et al*. 2000; WAHLBERG *et al*. 2005; HEIKKILA *et al.* 2012). Recentemente CONG *et al.* (2017) sequenciaram os primeiros genomas completos para a família e demonstram que Riodinidae e Lycaenidae são mais relacionadas uma a outra que qualquer outro par de famílias de Papilionoidea. Além disso, o estudo sugere que o tempo de divergência entre elas (~87 milhões de anos atrás) é compáravel com o tempo de divergência de subfamílias em outras famílias de borboletas. Dessa maneira, CONG *et al.* (2017) levantam novamente a hipótese de Riodinidae deveria ser tratada como uma subfamília de Lycaenidae.

Riodinidae caracteriza-se por apresentar antena com clava afilada; perna anterior reduzida nos machos; coxa protorácica do macho prolongada em forma de espinho após articulação com o trocânter e genitália masculina com o unco relativamente desenvolvido. Essas duas últimas características separam Riodinidae Lycaenidae (DUARTE *et al*. 2012). Conhecidas popularmente como borboletas-metálicas, os adultos apresentam uma miríade de padrões de coloração e formas, especialmente na região Neotropical (BROWN *et al.* 2012; ESPELAND *et al.* 2015). A distribuição espacial é restrita em várias espécies; as populações apresentam menos indivíduos quando comparadas com outras famílias e tendem a grandes oscilações (BATES 1859; CALLAGHAN 1983). A riqueza de espécies parece responder ao gradiente latitudinal, sendo que em temperaturas mais elevadas a riqueza tende a ser maior (FRANCINI *et al.* 2011; SIEWERT *et al*. 2014).

Embora existam vários registros de interações entre lepidópteros e formigas (HÖLLDOBLER & WILSON 1990), Lycaenidae e Riodinidae são as duas principais linhagens de lepidópteros mirmecófilos (DEVRIES 1997). Essas associações variam de facultativas a obrigatórias, e do mutualismo ao parasitismo. Geralmente essas interações são consideradas mutualísticas: as formigas se alimentam de secreções nutritivas produzidas por glândulas especializadas nas larvas dos lepidópteros, enquanto estas recebem proteção contra predadores e parasitoides mediado pelo espaço livre de inimigos gerado pelo patrulhamento das formigas próximas as larvas (FIEDLER 1991; KAMINSKI *et al.* 2010). Nas interações facultativas não há especificidade em relação à espécie de formiga e as larvas são capazes de sobreviverem tanto na ausência como na presença da formiga atendente. Já nas interações obrigatórias, há especificidade com relação à formiga atendente e as larvas sempre são encontradas associadas a estas formigas (FIEDLER 1991; FIEDLER *et al.* 1992; PIERCE *et al.* 2002; KAMINSKI 2008b; KAMINSKI *et al*. 2009).

A mirmecofilia em borboletas está associada com a grande variedade de hábitos alimentares encontrados nas larvas, incluindo especializações incríveis com o abandono do hábito ancestral herbívoro típico de Lepidoptera. Apenas 1% das espécies de Lepidoptera são afitófagas, ou seja, não se alimentam de tecido vegetal, sendo esse o caso de algumas linhagens de Lycaenidae e Riodinidae que desenvolveram hábitos carnívoros (PIERCE 1995). O melhor caso conhecido é de *Phengaris* Doherty, 1891 (= *Maculinea* (van Eecke, 1915)) (Lycaenidae) que parasita ninhos de formigas (ALS *et al*. 2004; THOMAS & SETTELE 2004). Entretanto, pouco se conhece sobre a biologia desse tipo de interação em riodinídeos quando comparado com seu grupo irmão (DEVRIES 1991; D'ABRERA 1994).

1.3. *Aricoris* Westwood, 1851 e o grupo *constantius*

Nymphidiini é a maior das nove tribos de Riodininae, sendo composta por aproximadamente 300 espécies e abrigando a maioria das espécies de Riodinidae mirmecófilas (HALL & HARVEY 2002). Dentro da tribo, *Aricoris* Westwood, 1851 contém 27 espécies, encontradas tipicamente em ambientes abertos da América do Sul (DEVRIES 1997; CALLAGHAN & LAMAS 2004; KAMINSKI & CARVALHO-FILHO 2012). A riqueza deste gênero neotropical ainda é considerada subestimada, devendo dobrar o número de espécies (C. Callaghan & L. A. Kaminski, com. pess.).

Aparentemente, a maioria das espécies de *Aricoris* é herbívora, porém existem relatos de larvas ocorrendo no interior de ninhos de formigas e/ou se alimentando de exudatos de cochonilhas (Hemiptera: Sternorrhyncha: Coccidae) (DEVRIES *et al.* 1992; VOLKMANN & BUSTOS 2010; KAMINSKI *et al.*, em prep.). Além disso, a presença de óleo, frequentemente visualizado nas asas dessas borboletas, é tido como um indício de afitofagia (DEVRIES 1997; HALL & HARVEY 2002; HALL & WILLMOTT 1995). Todas as espécies de *Aricoris* conhecidas mantém interações obrigatórias ou facultativas com formigas (KAMINSKI & CARVALHO-FILHO 2012; L.A. Kaminski, dados não publicados).

HALL & HARVEY (2002) propuseram uma filogenia da tribo Nymphidiini utilizando caracteres morfológicos e recuperaram *Aricoris* como monofilético, apresentando as seguintes sinapomorfias: profundo espaço em forma de “U” entre os lobos do uncus em vista dorsal que atinge a margem posterior do tégumen e espinhos proeminentes na extremidade das valvas. Muitas espécies, atualmente inseridas em *Aricoris*, foram descritas em outros gêneros, como *Eiseleia* L. & J. Miller, 1972 e *Audre* Hemming, 1934 (anteriormente *Hamearis* Hübner, [1819]). Por muitos anos, acreditou-se que *Aricoris* era um gênero monotípico, já que a espécie-tipo *A. constantius* (Fabricius, 1793) apresenta um padrão alar altamente modificado. HALL & HARVEY (2002) sinonimizaram *Eiseleia* e *Audre* com *Aricoris* e propuseram cinco grupos de espécies: "*aurinia*", "*chilensis*", "*colchis*", "*constantius*" e "*epulus*". Atualmente uma ampla revisão taxonômica de *Aricoris* está sendo realizada e a monofilia destes grupos de espécies está sendo testada com base em caracteres moleculares e morfológicos (CALLAGHAN & KAMINSKI, em prep.).

O grupo *constantius* (*sensu* Hall & Harvey, 2002) é constituido por espécies que usualmente habitam áreas de campos abertos e cerrado e formam um complexo de espécies crípticas, com padrão alar marrom sendo facilmente confundidas com Satyrinae (Nymphalidae), com excessão de *Aricoris terias* (Godman, 1903) que possui padrão de coloração amarelada único no gênero (DOLIBAINA *et al.* 2010). Resultados preliminares baseados em dados moleculares e morfológicos indicam que *A. terias* é relacionado ao grupo *colchis* (CALLAGHAN & KAMINSKI, em prep.), nesse sentido trataremos neste trabalho apenas das espécies que compoem o complexo *constantius*. A história taxonômica deste complexo é caótica (ver comentários em CALLAGHAN 2010). Neste complexo, ocorrem as espécies primariamente descritas em *Aricoris*, sendo *A. tisiphone* Westwood, 1851 a espécie tipo do gênero. Em pouco tempo, muitas espécies foram adicionadas ao gênero, até que STICHEL (1911) moveu a maioria delas para *Orimba* Herrich-Schäfer, [1853]. Posteriormente, HEMMING (1967) realocou as espécies de *Orimba* para *Setabis* Westwood, 1851, já que considerou o anterior como *nomen nudum*. Em *Aricoris* permaneceu *A. tisiphone*, *A. tutana* (Godart, [1824]), e *A. bahiana* C. Felder & R. Felder, 1865. STICHEL (1910) descreveu *A. monotona* e sinonimizou *A. tisiphone* e *A. bahiana* com *A. tutana*.

FABRICIUS (1793) descreveu *Papilio constantius* de maneira concisa e sem ilustrações. Além disso, não há informações sobre a localização do tipo dessa espécie, que é dado como perdido. William Jones compilou em sua monografia nunca publicada, conhecida como JONES' ICONES (1745-1818), várias ilustrações dos tipos das espécies descritas por Fabricius, onde consta uma representação de *Papilio constantius* (Vol. 5(6), pl. L). Os caracteres alares da ilustração indicam que essa espécie érelacionada com *A. tutana*, tanto que D'ABRERA (1994) ilustrou *Aricoris constantius* como o macho de *A. tutana*. HALL & HARVEY (2002) foram os primeiros autores a aplicarem o nome *A. constantius* para esse táxon, e, além disso, sinonimizaram *A. monotona* e *A. tutana* com *A. constantius*. Ainda, a proposta filogenética de HALL & HARVEY (2002) associou ao grupo *constantius* a espécie *A. terias*, previamente descrita em *Eiseleia* L. & J. Miller, 1972.

CALLAGHAN (2010), baseado em diferenças morfológicas no padrão das asas e genitália, realizou a mais recente revisão do complexo *constantius,* e revalidou as espécies *A. monotona* e *A. tutana,* embora nenhum neótipo tenha sido designado. As diagnoses e descrições propostas por CALLAGHAN (2010) mostraram fortes evidências de que a decisão taxonômica de HALL & HARVEY (2002) em sinonimizar as espécies do complexo *constantius* foi precipitada. Atualmente o grupo é formado por três espécies: *A. constantius*, *A. monotona* e *A. tutana*. Como parte de um projeto mais amplo de revisão e filogenia de *Aricoris* (CALLAGHAN & KAMINSKI, em prep.), amostras de espécímes do grupo *constantius* foram sequenciadas e análises preliminares confirmam a validade das três espécies. Além disso, os dados moleculares indicaram a existência de uma diversidade críptica escondida que inclui pelo menos três táxons sem nome, até aqui identificados como *A. tutana* (CAPITULO 2).

O objetivo desta dissertação foi elucidar os problemas taxonômicos envolvendo esse complexo de espécies crípticas. Levando em consideração a dificuldade de identificação dessas espécies; o fato de serem borboletas que formam populações pequenas e geograficamente restritas, usualmente relacionadas a ambientes abertos que sofrem forte pressão antrópica; e a possibilidade de um número maior de espécies existentes; utilizou-se uma abordagem integrativa por concordância, aliando o uso de morfologia com caracteres moleculares, para identificar adequadamente as espécies que compõem o grupo *Aricoris constantius*. Para tanto, foi utilizado o conceito de espécie proposto por De Queiroz (2007): espécies são metapopulações que evoluem independentemente uma das outras. Dessa maneira, esse é outro fator que justifica a utilização de uma abordagem de taxonomia integrativa neste estudo.

Referências

AGNARSSON, I. & KUNTNER, M. 2007. Taxonomy in a Changing World: Seeking Solutions for a Sciense in Crisis. **Systematic Biology 56**: 531-539.

ALS, T.D.; VILA, R.; KANDUL, N.P.; NASH, D.R.; YEN, S.-H; HSU, Y.-F; MIGNAULT, A.A.; BOOMSMA, J.J. & PIERCE, N.E. 2004. The evolution of alternative parasitic life histories in large blue butterflies. **Nature 432**: 386-390.

BATES, H.W. 1859. Notes on South American butterflies. **Transactions of the Entomological Society of London, new series 5**: 1-11.

BICKFORD, D.; LOHMAN, D.J.; SODHI, N.S.; NG, P.K.L.; MEIER, R.; WINKER, K.; INGRAM, K.K. & DAS, I. 2007. Cryptic species as a window on diversity and conservation. **Trends in Ecology and Evolution 22**: 148-155.

BOERO, F. 2010. The Study of Species in the Era of Biodiversity: A Tale of Stupidity. **Diversity 2**: 115-126.

BRITO, R.; DE PRINS, J.; DE PRINS, W.; MIELKE, O.H.H.; GONÇAVEZ, G.L. & MOREIRA, G.R.P. 2016. Extant diversity and estimated number of Gracillariidae (Lepidoptera) species yet to be discovered in the Neotropical region. **Revista Brasileira de Entomologia 60**: 275-283.

BROWN, K.S. JR.; VON SCHOULTZ, B.; SAURA, A.O. & SAURA, A. 2012. Chromosomal evolution in the South American Riodinidae (Lepidoptera: Papilionoidea). **Hereditas 149**: 128-138.

BUENO-SILVA, M. 2012. Genética molecular e sistemática animal: Um breve histórico, contribuições e desafios. **Estudos de Biologia, Ambiente e Diversidade 34**: 157-163.

BURNS, J.M.; JANZEN, D.H.; HAJIBABAEIM, M.; HALLWACHS, W. & HEBERT, P.D.N. 2008. DNA barcodes and cryptic species of skipper butterflies in the genus *Perichares* in Area de Conservación Guanacaste, Costa Rica. **Proceedings of the National Academy of Sciences 105**: 6350-6355.

CALLAGHAN, C.J. & LAMAS, G. 2004. Riodinidae. *In*: LAMAS, G. (Ed.), **Checklist: Part 4A. Hesperioidea—Papilionoidea**, Gainesville, Fla: USA Association for Tropical Lepidoptera. p. 141-170.

CALLAGHAN, C.J. 1983. A study of isolating mechanisms amoung neotropical butterflies of the subfamily Riodininae. **The Journal of Research on the Lepidoptera 21**: 159-176.

CALLAGHAN, C.J. 2010. A re-evaluation of the *Aricoris constantius* group with the recognition of three species (Lepidoptera: Riodinidae). **Zoologia 27**: 395-402.

CAMPBELL, D.L.; BROWER, A.V.Z.; PIERCE, N.E. 2000. Molecular evolution of the wingless gene and its implications for the phylogenetic placement of butterfly family Riodinidae (Lepidoptera: Papilionoidea). **Molecular Biology and Evolution 17**: 684-696.

CONG, Q.; SHEN, J.; LI, W.; BOREK, D.; OTWINOWSKI, Z. & GRISHIN, N.V. 2017. The first complete genomes of Metalmarks and the classification of butterfly families. **Genomics 109**: 485-493.

COSTELLO, M.J.; MAY, R.M. & STORK, N.E. 2013. Can We Name Earth's Species Before They Go Extinct? **Science 339**: 413-416.

CRACRAFT, J. 2002. The Seven Great Questions of Systematic Biology: An Essential Foundation for Conservation and the Sustainable Use of Biodiversity. **Annals of the Missouri Botanical Garden 89**: 127-144.

D'ABRERA, B. 1994. **Butterflies of the Neotropical Region. Part VI Riodinidae**. Victoria, Black Rock: Hill House.

DAYRAT, B. 2005. Towards integrative taxonomy. **Biological Journal of the Linnean Society 85**: 407-415.

DE QUEIROZ, K. 2007. Species Concepts and Species Delimitation. **Systematic Biology 56**: 879-886.

DEVRIES, P. J.; CHACON, I. A. & MURRAY, D. 1992. Toward a better understanding of host use and biodiversity in riodinid butterflies (Lepidoptera). **Journal of Research on the Lepidoptera 31**: 103-126.

DEVRIES, P.J. 1991. Ecological and evolutionary patterns in myrmecophilous riodinid butterflies. *In*: Huxley, C.R. & Cutler, D.F. (Eds.), **Ant-plant interactions**. Oxford University, Oxford, p. 143-156.

DEVRIES, P.J. 1997. **The butterflies of Costa Rica and their natural history II: Riodinidae.** (Vol. 2): Princeton University Press.

DOLIBAINA, D.R.; DIAS, F.M.S.; UEHARA-PRADO, M.; MIELKE, O.H.H. & CASAGRANDE, M.M. 2010. Insecta, Lepidoptera, Riodinidae, Nymphidiini, *Aricoris terias* (Godman, 1903): First records from Brazil and updated geographic distribution map. **Check List 6**: 637-638.

DREW, L.W. 2011. Are We Losing the Science of Taxonomy? **BioScience 61**: 942-946.

DUARTE, M.; MARCONATO, G.; SPECHT, A. & CASAGRANDE M.M. 2012. Lepidoptera Linnaeus, 1758. *In:* RAFAEL, J.A.; MELO, G.A.R.; CARVALHO, C.J.B.; CASARI, S.A. & CONSTANTINO, R. **Insetos do Brasil: diversidade e taxonomia.** Ribeirão Preto, Holos. p. 626-682

EBACH, M.C. & HOLDREGE, C. 2005. DNA barcoding is no substitute for taxonomy. **Nature 434**: 697.

ELIAS, M.; HILL, R.I.; WILLMOTT, K.R.; DASMAHAPATRA, K.K.; BROWER, A.V.Z.; MALLET, J. & JIGGINS, C.D. 2007. Limited performance of DNA barcoding in a diverse community of tropical butterflies. **Proceedings of The Royal Society B 274**: 2881-2889.

ESPELAND, M.; HALL, J.P.W.; DEVRIES, P.J.; LEES, D.C.; CORNWALL, M.; HSU, Y-F.; WU, L-W.; CAMPBELL, D.L.; TALAVERA, G.; VILA, R.; SALZMAN, S.; RUEHR, S.; LOHMAN, D.J. & PIERCE, N.E. 2015. Ancient Neotropical origin and recent recolonisation: Phylogeny, biogeography and diversification of the Riodinidae (Lepidoptera: Papilionoidea). **Molecular Phylogenetics and Evolution 93**: 296-306.

FABRICIUS, J.C. 1793. **Entomologia systematica emendata et aucta. Secundum classes, ordines, genera, species, adjectis synonimis, locis, observationibus, descriptionibus Tom. III, Pars I.** C.G. Proft, Fil. et Soc., Hafniae.

FIEDLER, K. 1991. Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionoidea). **Bonner Zoologische Monographien 31:** 1-210p.

FIEDLER, K.; SEUFERT, P.; PIERCE, N.E.; PEARSON, J.G. & BAUGARTEN, H. 1992. Exploitation of lycaenid-ant mutualisms by braconid parasitoids. **Journal of Reaserch on the Lepidoptera 31**: 153-168.

FRANCINI, R.B.; DUARTE, M.; MIELKE, O.H.H.; CALDAS, A. & FREITAS, A.V.L. 2011. Butterflies (Lepidoptera, Papilionoidea and Hesperioidea) of the "Baixada Santista" region, coastal São Paulo, southeastern Brazil. **Revista Brasileira de Entomologia 55**: 55-68.

GODFRAY, H.C. 2002. Challenges for taxonomy. **Nature 417**: 17-19.

HALL, J. P. W. & WILLMOTT, K. R. (1995). Five New Species and a New Genus of Riodinid From the Cloud Forests of Eastern Ecuador (Lepidoptera: Riodinidae). **Tropical Lepidoptera 6**: 131-135.

HALL, J. P. W. 2002. Phylogeny of the riodinid butterfly subtribe Theopeina (Lepidoptera: Riodinidae: Nymphidiini). **Systematic Entomology 27**: 139-167.

HALL, J.P.W. & HARVEY, D.J. 2002. Basal subtribes of the Nymphidiini (Lepidoptera: Riodinidae): phylogeny and myrmecophily. **Cladistics 18**: 539-569.

HEBERT, P.D.N. & GREGORY, T.R. 2005. The Promise of DNA Barcoding for Taxonomy. **Systematic Biology 54**: 2005.

HEBERT, P.D.N.; CYWINSKA, A.; BALL, S.L. & DE WAARD J.R. 2003. Biological identifications through DNA barcodes. **Proceedings of the Royal Society B: Biological Sciences 270**: 313-321.

HEBERT, P.D.N.; PENTON, E.H.; BURNS, J.M.; JANZEN, D.H. & HALLWACHS. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. **Proceedings of the National Academy of Sciences 101**: 14812-14817.

HEIKKILÄ, M.; KAILA, L.; MUTANEN, M.; PEÑA, C. & WAHLBERG, N. 2012. Cretaceous origin and repeated tertiary diversification of the redefined butterflies. **Proceedings of The Royal Society**: 1-7.

HEMMING, A.F. 1967. The generic names of the butterflies and their type species (Lepidoptera: Rhopalocera). **Bulletin of the British Museum of Natural History (Entomology) 9**: 1-509

HILLIS, D.M. 1987. Molecular Versus Morphological Approaches to Systematics. **Annual Review of Ecology and Systematics 18**: 23-42.

HÖLLDOBLER, B. & E. O. WILSON. 1990. **The Ants.** Massachussets, The Belknap Press of Harvard University Press, 732p.

HUBNER, J.T. & LANGOR, D.W. 2004. Systematics: Its role in supporting sustainble forest management. **The Forestry Chronicle 80**: 451-457.

JONES, W. 1745-1818. Willian Jones Collection [Jones' Icones]. Manuscrito não publicado. Oxford, United Kingdom: Hope Entomological Collection, Oxford University Museum of Natural History,

JUSTI, S.A.; DALE, C. & GALVÃO. 2014. DNA barcoding does not separate South American *Triatoma* (Hemiptera: Reduviidae), Chagas Disease vectors. **Parasites & Vectors 7**: 1-4.

KAMINSKI, L.A. 2008a. Immature stages of *Caria plutargus* (Lepidoptera: Riodinidae), with discussion on the behavioral and morphological defensive traits in nonmyrmecophilous riodinid butterflies. 2008. **Annals of the Entomological Society of America 101**: 906-914.

KAMINSKI, L.A. 2008b. Polyphagy and Obligate Myrmecophily in the Butterfly *Hallonympha paucipuncta* (Lepidoptera: Riodinidae) in the Neotropical Cerrado Savanna. **Biotropica 40**: 390-394.

KAMINSKI, L.A.; SENDOYA, S.F.; FREITAS, A.V.L & OLIVEIRA, P.S. 2009. Ecologia comportamental na interface formiga-planta-herbívoro: interações entre formigas e lepidópteros. **Oecologia Brasiliensis 13**: 27-44.

KAMINSKI, L.A.; FREITAS, A.V.L. & OLIVEIRA, P.S. 2010. Interaction between mutualisms: ant-tended butterflies exploit enemy free space provided by ant-treehopper associations. **American Naturalist 176**: 322-334.

KAMINSKI, L.A. & CARVALHO-FILHO, F.S. 2012. Life history of *Aricoris propitia* (Lepidoptera: Riodinidae) - A Myrmecophilous Butterfly Obligately Associated with Fire Ants. **Psyche**: 1-10.

KAWAHARA, A.Y. & BREINHOLT, J.W. 2014. Phylogenomics provides strong evidence for relationships of butterflies and moths. **Proceedings of the Royal Society B 281**: 1-8.

KIM, M.I.I.; WAN, X.; KIM, M.J.; JEONG, H.C.; AHN, N.-H.; KIM, K.-G.; HAN, Y. S. & KIM, I. 2010. Phylogenetic Relationships of True Butterflies (Lepidoptera: Papilionoidea) Inferred from COI, 16S rRNA and EF-1α Sequences. **Molecules and Cells 30**: 409-425.

KOCHER, T.D. 2004. Adaptive Evolution and Explosive Speciation: The Cichlid Fish Model. **Nature 5**: 288-298.

KRISTENSEN, N.P.K., SCOBLE, M.J. & KARSHOLT, O. 2007. Lepidoptera phylogeny and systematics: the state of inventorying moth and butterfly diversity. **Zootaxa 1668:** 699-747.

LINDAHL, T. 1993. Instability and decay of the primary structure of DNA. **Nature 362**: 709-715.

MAYDEN, R.L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. 381-424 *in* **Species: The Units of Biodiversity**. M.F. CLARIDGE, H.A. DAWAH & M.R. WILSON eds. Chapman and Hall, London.

MAYR, E. 1969. **Principles of Systematic Zoology.** McGraw-Hill, New York, NY, 428p.

MILANKOV, V.; STAHLS, G.; STAMENKOVIC, J. & VUJIC, A. 2008. Genetic diversity of *Merodon aureus* and *M. cinereus* species complexes (Diptera, Syrphidae): integrative taxonomy and implications for conservation priorities on the Balkan Peninsula. **Conservation Genetics 9**: 1125-1137.

MINET, J. 1991. Tentative reconstruction of the ditrysian phylogeny (Lepidoptera: Glossata). **Scandinavian Entomology 22**: 69-95.

MUTANEN, M.; WAHLBERG, N. & KAILA, L. 2010. Comprehensive gene and taxon coverage elucidates radiation patterns in moths and butterflies. **Proceedings of the Royal Society:** 2839-2848.

PADIAL, J.M.; MIRALLES, A.; DE LA RIVA, I. & VENCES, M. 2010. The integrative future of taxonomy. **Frontiers in Zoology 7**: 1-14.

PIERCE, N.E. 1995. Predatory and Parasitic Lepidoptera: Carnivores Living on Plants. **Journal of the Lepidopterists' Society 49**: 412-453.

PIERCE, N.E.; BRABY, M.F.; HEATH, A.; LOHMAN, D.J.; MATHEW, J.; RAND, D.B. & TRAVASSOS, M.A. 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Annual Review Entomology 47: 733-771

PIRES, A.C. & MARINONI, L. 2010. DNA barcoding and traditional taxonomy unified through Integrative Taxonomy: a view that challenges the debate questioning both methodologies. **Biota Neotropica 10**: 339-346.

PRITI, H.; ROSHMI, R.S.; RAMYA, B.; SUDHIRA, H.S.; RAVIKANTH, G.; ARAVIND, N.A. & GURURAJA, K.V. 2016. Integrative Taxonomic Approach for Describing a New Cryptic Species of Bush Frog (*Raorchestes*: Anura: Rhacophoridae) from the Western Ghats, India. **PLoS ONE 11**: 1-21.

REGIER, J.C.; MITTER, C.; ZWICK, A.; BAZINET, A.D.; CUMMINGS, M.P.; KAWAHARA, A.Y.; SOHN, J.-C.; ZWICKL, D.J.; CHO, S.; DAVIS, D.R.; BAIXERAS, J.; BROWN, J.; PARR, C.; WELLER, S.; LEES, D.C. & MITTER, K.T. 2013. A Large-Scale, Higher-Level, Molecular Phylogenetic Study of the Insect Order Lepidoptera (Moths and Butterflies). **PlosOne 8**: 1-23.

ROE, A.D.; WELLER, S.J.; BAIXERAS, J.; BROWN, J.; CUMMINGS, M.P.; DAVIS, D.R.; KAWAHARA, A.Y.; PARR, C.S.; REGIER, J.C.; RUBINOFF, D.; SIMONSEN, T.J.; WAHLBERG, N. & ZWICK, A. 2009. Evolutionary Framework for Lepidoptera Model Systems. *In.* GOLDSMITH, M.R. & MAREC, F. (Eds.) **Molecular Biology and Genetics of the Lepidoptera.** CRC Press / Taylor & Francis (Contemporary Topics in Entomology series). Boca Raton, Florida, p. 1–24.

SANTOS, M.H.; FRANCO, F.F. & MANFRIN, M.H. 2009. The mitochondrial *COI* gene fails as DNA barcoding in the sibling species of *Drosophila buzzatii* cluster. **Drosophila Information Service 92**: 101-106.

SCHLICK-STEINER, B.C.; STEINER, F.M.; MODER, K.; SEIFERT, B.; SANETRA, M.; DYRESON, E.; STAUFFER, C. & CHRISTIAN, E. 2006. A multidisciplinary approach reveals cryptic diversity in Western Paleartic *Tetramorium* ants (Hymenoptera: Formicidae). **Molecular Phylogenetics and Evolution 40**: 259-273.

SCHLICK-STEINER, B.C.; STEINER, F.M.; SEIFERT, B.; STAUFFER, C.; CHRISTIAN, E. & CROZIER, R.H. 2010. Integrative Taxonomy: A Multisource Approach to Exploring Biodiversity. **Annual Review of Entomology 55**: 421-438.

SERAPHIM, N.; KAMINSKI, L.A.; DEVRIES, P.J.; PENZ, C.; CALLAGHAN, C.; WAHLBERG, N.; SILVA-BRANDÃO, K.L. & FREITAS, A.V.L. 2018. Molecular phylogeny and higher systematics of the metalmark butterflies (Lepidoptera: Riodinidae). **Systematic Entomology**: 1-19.

SIEWERT, R.R.; ISERHARD, C.A.; ROMANOWSKI, H.P.; CALLAGHAN, C.J. & MOSER, A. 2014. Distribution patterns of riodinid butterflies (Lepidoptera: Riodinidae) from southern Brazil. **Zoological studies 53:** 1-10.

STICHEL, H. 1910. Vorarbeiten zu einer Revision der Riodinidae Grote (Erycinidae Swains) (Lep Rhop). **Berliner Entomologische Zeitschrift 55:** 9-103.

STICHEL, H. 1911. Lepidoptera Rhopalocera. Fam. Riodinidae. *In*: WYTSMANN. Genera Insectorum 112B. p. 113-720.

SUTRISNO, H. 2012. The Impact of Storage Times of Museum Insect Specimens on PCR Success: Case Study on Moth Collections in Indonesia. **HAYATI Journal of Biosciences 19**: 99-104.

THOMAS, J.A. & SETTELE, J. 2004. Butterfly mimics of ants. **Nature 432**: 283-284.

VAGLIA, T.; HAXAIRE, J.; KITCHING, I.J.; MEUSNIER, I. & ROUGERIE, R. 2008. Morphology and DNA barcoding reveal three cryptic species within the *Xylophanes neoptolemus* and *loelia* species-groups (Lepidoptera: Sphingidae). **Zootaxa** 1923: 18-36.

VOLKMANN, L. & BUSTOS, E.N. 2010. Mariposas Serranas de Argentina Central. Tomo I. Papilionidae, Pieridae, Lycaenidae, Riodinidae. (1 ed.). Córdoba, Argentina: Equipo Gráfico, Huerta Grande. 140p.

WAHLBERG, N.; BRABY, M.F.; BROWER, A.V.Z.; JONG, R.; LEE, M.; NYLIN S.; PIERCE, N.E.; SPERLING, F.A.H.; VILA, R.; WARREN, A.D. & ZAKHAROV, E. 2005. Synergistic effects of combining morphological and molecular data in resolving the phylogeny of butterflies and skippers. **Proceedings of the Royal Society of London B 272**: 1577-1586.

WERNER, Y.L. 2006. The case of impact factor versus taxonomy: a proposal. **Journal of Natural History 40**: 1285-1286.

WILSON, E.O. 2003. The encyclopedia of life. **TRENDS in Ecology and Evolution 18**: 77-80.

WILSON, E.O. 2004. Taxonomy as a fundamental discipline. **Philosophical Transactions of the Royal Society B 359**: 739.

YANG, Z.; LANDRY, J.F.; HANDFIELD, L.; ZHANG, Y.; SOLIS, M.A.; HANDFIELD, D.; SCHOLTENS, B.G.; MUTANEN, M.; NUSS, M. & HEBERT, P.D.N. 2012. DNA barcoding and morphology reveal three cryptic species of *Anania* (Lepidoptera: Crambidae: Pyraustinae) in North America, all distinc from their European conterpart. **Systematic Entomology 37**: 686-705.

ZIMMERMANN, J.; HAJIDABAEI, M.; BLACKBURN, D.C.; HANKEN, J.; CANTIN, E.; POSFAI, J. & EVANS JR., T.C. 2008. DNA damage in preserved specimens and tissue samples: a molecular assessment. **Frontiers in Zoology 5**: 1-13.

CAPÍTULO II

Hidden diversity in aphytophagous butterflies: Integrative taxonomy reveals new cryptic species in the *Aricoris constantius* complex (Lepidoptera: Riodinidae)

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Research Article

Hidden diversity in aphytophagous butterflies: Integrative taxonomy reveals new cryptic species in the *Aricoris constantius* complex (Lepidoptera: Riodinidae)

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Short running title (Hidden diversity in aphytophagous butterflies)

Abstract

Butterflies in the *Aricoris constantius* complex have an obscure taxonomy history with the existence of various synonyms and difficulty to delimit species. So far, there are three species in this complex: *A. constantius* (Fabricius, 1793), *A. monotona* (Stichel, 1910) and *A. tutana* (Godart, [1824]). DNA barcode sequencing (*cytochrome c oxidase subunit I*, *COI*), however, pointed to the existence of a hidden diversity in this group. Here, we present morphological and molecular evidence to describe three new species, so far erroneously named as *A. tutana*. We designate neotype for both *Papilio constantius* and *Erycina tutana*. All species in the *A. constantius* group are illustrated, diagnosed and a revised geographic distribution is presented. Adults have wings with greasy, a possible indicative of larval aphytophagy, and at least one species (*A. constantius*) feeds on honeydew secretions produced by ant-tended Hemiptera in the early instars and completes its cycle within ant-nests. Genetic distances between species are markedly high (3.91 - 5.00%) and all species can be identified by combination of characteristics of male and/or female genitalia. This study highlights the importance of an integrative taxonomy approach to delimitate cryptic species in metalmark butterflies.

Key words: Caatinga, Cerrado, DNA barcoding, myrmecophily, Nymphidiini, Pampa

Introduction

When two or more species are at least superficially morphologically indistinguishable and are classified wrongly as a single nominal species, they are considered cryptic species (Bickford et al., 2007). As traditional taxonomy based on morphological characters is not always adequate to distinguish cryptic species, it is indicated an approach of an integrative taxonomy, which is based on the use of different disciplines on an integrated manner, such as the study of molecules, phylogeography, ecology, behaviour, life history and immature morphology (Dayrat, 2005; Schlick-Steiner et al., 2010). Knowledge of Lepidoptera diversity is increasing rapidly on the past two decades because of researches based on integrative taxonomy, which has accelerated discoveries and description of complexes of cryptic species (e.g., Burns, Janzen, Hajibabaeim, Hallwachs, & Hebert, 2008; Dinca, Lukhtanov, Talavera, & Vila, 2011; Hebert, Penton, Burns, Jazen, & Hallwachs, 2004; Lumley & Sperling, 2010; Vaglia, Haxaire, Kitching, Meusnier, & Rougerie, 2008; Yang et al., 2012).

The metalmark butterflies (Riodinidae) occur almost exclusively in the Neotropics, where about 1,300 species are recorded representing 95% of the family richness (Callaghan & Lamas, 2004; Espeland et al., 2015). Riodinids butterflies show a wide morphological variation and, with its sister family, Lycaenidae, embrace the greatest diversity of life histories among all butterflies (DeVries, 1997; Fiedler, 1991; Kaminski, Iserhard, & Freitas, 2016; Pierce, Braby, Heath, Lohman, Mathew, Rand, & Travassos, 2002). Despite the significant number of species already identified, several new Riodinidae species are waiting to be described, since the true diversity in the family can be considered underestimated (Kaminski et al., 2016; Seraphim et al., 2018).

One of the most distinctive characteristic in Riodinidae species is the larval symbiotic association with ants (myrmecophily), also present in the sister family Lycaenidae (DeVries, 1997; Fiedler, 1991; Pierce et al., 2002). Based on the costs/benefits balance between species, the myrmecophilous interactions can be divided into three types: commensalism, mutualism or parasitism (Pierce & Young, 1986). In commensalist interactions, caterpillar of these families receives indirect protective benefits by occupying locals frequently visited by ants (enemy-free space) or by feeding on their discards (e.g. Kaminski, Freitas, & Oliveira, 2010). In mutualist interactions, on the other hand, mutual benefits exist between butterflies and ants, that is, caterpillars produce reward secretions through glandules that release nutritive substances, such as carbohydrates and amino acids, used as feed by ants (Pierce et al., 1987; DeVries, 1991a). From an ancestral commensal or mutualist, the parasitism can evolve, such cases occur when at least in one ontogenetic stage, the caterpillar feed on ant brood inside ant nests, or the caterpillars are fed through trophallaxis by their host ants (Fiedler, 2012; Pierce, 1995). The Blue butterflies *Phengaris* Doherty, 1891 (= *Maculinea* van Eecke, 1915) are the most known lycaenid lineage that exhibits this kind of behaviour (Als et al., 2004; Thomas & Settele, 2004). Some authors suggest that the caterpillar-ant associations may accelerate diversification of species (Eastwood et al., 2006; Fiedler, 1997; Pierce, 1984). However, this is still an open question and little is known about Riodinidae evolutionary biology when compared to other butterfly groups (D’Abrera, 1994; DeVries, 1991b).

Aricoris Westwood, 1851 is an interesting model for studying the transitions between mutualism to parasitism and consequences to diversification (Kaminski et al., in prep.). It belongs to the entire myrmecophilous tribe Nymphidiini and contains currently 27 species, found typically in open and dry environments of South America

(Callaghan & Lamas, 2004; DeVries, 1997; Kaminski & Carvalho-Filho 2012). Hall & Harvey (2002) reviewed *Aricoris* and proposed a phylogenetic hypothesis based on adult morphology. Five species groups are recognized in this review, including the *Aricoris constantius* group which includes the *constantius* complex more *Aricoris terias* (Godman, 1903) with an unusual yellowish colour pattern (sensu Hall & Harvey, 2002). Adults in the *constantius* complex are represented by brown cryptic butterflies, size ranging from small to medium (forewing size ranging from 15 to 25 mm), easily confused with Satyrinae (Nymphalidae). These riodinids inhabit mainly open grassland areas, from southern to northeast of Brazil, including some regions of Argentina and Paraguay.

The taxonomic history of the *constantius* complex is obscure and confused, mainly due to its high morphological similarity and sympatric distribution of some species. Previously, the complex was formed by *A. constantius* (Fabricius, 1793), *A. tutana* (Godart, [1824]), *A. bahiana* C. Felder & R. Felder, 1865, *A. tisiphone* Westwood, 1851 and *A. monotona* (Stichel, 1910). Stichel (1910) synonymized *A. tisiphone* and *A. bahiana* with *A. tutana*. Afterwards, all remaining species were synonymized with *A. constantius* by Hall & Harvey (2002). Recently, Callaghan (2010) did the late taxonomic revision of the complex, reinstating the species status for *A. tutana* and *A. monotona* based on consistent morphological differences in adults (wings and genitalia). We present herein molecular and morphological evidence demonstrating that the *A. constantius* group is formed by at least six species, including three new ones. Also, we present first evidence that this species group has aphytophagous larvae. Additionally, this work provides an update of the geographic area occupied by the *A. constantius* species group and contribute to the molecular and morphological differences the species.

Material and methods

A total of 196 specimens were analyzed, deposited in the following entomological collections: **CJC,** Curtis Callaghan Collection, Bogotá, Colombia, **CLDZ,** Coleção de Lepidoptera do Departamento de Zoologia, Porto Alegre, Rio Grande do Sul, Brazil; **CSTR,** Centro de Saúde e Tecnologia Rural, Universidade Federal de Campina Grande, Patos, Paraíba, Brazil; **DD,** Diego Dolibaina Collection, Curitiba, Paraná, Brazil; **DZUP,** Entomological Colecction Padre Jesus Santiago Moure, Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil; **EF,** Eurides Furtado Collection, Diamantino, Mato Grosso, Brazil; **ENB,** Colección de Lepidoptera de Argentina Ezequiel Núñez Bustos, Laboratorio Barcode, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Ciudad de Buenos Aires, Argentina; **OM,** Olaf Hermann Hendrik Mielke Collection, Curitiba, Paraná, Brazil; **MGCL,** McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, USA; **MNRJ,** Museu Nacional do Rio de Janeiro, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; **MZUSP,** Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil; **ZUEC,** Museu de Zoologia Adão José Cardoso, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil. Additional data was provided from literature.

The male and female genitals of some specimens (n = 42, 25 males and 17 females) were studied by removing and clearing the abdomens in a 10% potassium hydroxide (KOH) solution and, then, stored in glycerol. Photographs were taken using a Nikon AZ100M stereomicroscope and NIS-Elements Advanced Research software. The terminology of the genitalia follows Klots (1970) and the venation from Miller (1970). The distribution map was produced with aid of the website SimpleMappr (Shorthouse, 2010).

Molecular phylogeny inference

Genetic distance within and between species in the *Aricoris constantius* group and the phylogenetic relationships among them were estimated to evaluate the molecular variability for those taxa. Genomic DNA was extracted from two legs of adults by using Chelex 100 resin, 100–200 mesh, sodium form (Bio-rad), under the following protocol: one leg was removed and introduced into 100 µl of Chelex 10% and 5 µl of Proteinase K (20 mg ml^{-1}) were added. The samples were incubated overnight at 55 °C and were subsequently incubated at 100 °C for 15 min. Afterwards they were centrifuged for 10 s at 3000 rpm. Primers were used for the amplification of the mitochondrial *Cytochrome Oxidase subunit I* (*COI*) (558 bp total). Double-stranded DNA was amplified in 25 µl volume reactions: 13.22 µl ultra pure (HPLC quality) water, 2.5 µl 10 buffer, 4.5 µl 25 mM MgCl2, 0.25 ll 100 mM dNTP, 1.2 ll of each primer (10 mM), 0.13 ll Taq DNA Gold Polymerase (Qiagen) and 2 ll of extracted DNA. The typical thermal cycling profile for *COI* was 95 C for 60 s, 44 C for 60 s and 72 C for 90 s, for 40 cycles. PCR products were purified and sequenced by Macrogen Inc. Sequences were aligned with *Aricoris* species from other group of species (Table 1). The final matrix comprised 29 individuals; *Ariconias glaphyra* (Westwood, 1851), *Aricoris middletoni* (Sharpe, 1890) and *Aricoris terias* were used as outgroup. Bayesian analyses (Huelsenbeck et al., 2001, 2002) were carried using the program MrBayes 3.2 (Ronquist, & Huelsenbeck, 2003). Four simultaneous chains were run for 10×106 generations for two runs, sampling trees every 1,000 cycles. The first 10 percent trees were discarded as fixed “burn in”.

Only dissected and/or molecularly sequenced specimens were designated for paratypes for the new species. Other analysed specimens were included in lists of additional material examined.

Table 1. Specimens of sequenced *Aricoris* for the evolutionary history inference with codes, sampling sites data and GenBank accession numbers.

Species	**Locality**	**GenBank codes**
Ariconias glaphyra (LAK-133)	Campinas, São Paulo, Brazil	
Aricoris middletoni (LAK-163)	Pirenópolis, Goiás, Brazil	
Aricoris terias (LAK-347)	Alto Paraíso, Paraná, Brazil	
Aricoris sp. (BLU-038)*	Parque Nacional das Sempre-Vivas, Minas Gerais, Brazil	
Aricoris constantius (LAK-356)	Dom Pedro de Alcântara, Rio Grande do Sul, Brazil	
Aricoris constantius (LAK-357)	Dom Pedro de Alcântara, Rio Grande do Sul, Brazil	
Aricoris constantius (LAK-438)	Dom Pedro de Alcântara, Rio Grande do Sul, Brazil	
Aricoris constantius (LAK-471)	Três Rios, Rio de Janeiro, Brazil	
Aricoris constantius (LAK-472)	Três Rios, Rio de Janeiro, Brazil	
Aricoris constantius (LAK-473)	Três Rios, Rio de Janeiro, Brazil	
Aricoris monotona (LAK-352)	Taim, Rio Grande do Sul, Brazil	
Aricoris monotona (LAK-469)	Parque Saint' Hilaire, Viamão, Rio Grande do Sul, Brazil	
Aricoris monotona (LAK-470)	Parque Saint' Hilaire, Viamão, Rio Grande do Sul, Brazil	
Aricoris monotona (LAK-501)	Morro do Araçatuba, Tijucas do Sul, Paraná, Brazil	
Aricoris tutana (BLU-019)	Serra do Cipó, Santana do Riacho, Minas Gerais, Brazil	
Aricoris tutana (LAK-380)	Parque Nacional Serra do Cipó, Santana do Riacho, Minas Gerais, Brazil	
Aricoris tutana (LAK-381)	Parque Nacional Serra do Cipó, Santana do Riacho, Minas Gerais, Brazil	
Aricoris tutana (LAK-382)	Parque Nacional Serra do Cipó, Santana do Riacho, Minas Gerais, Brazil	
Aricoris tutana (LAK-468)	Parque Nacional Serra do Cipó, Santana do Riacho, Minas Gerais, Brazil	
Aricoris sp. 1 sp. nov (LAK-165)	Pirenópolis, Goiás, Brazil	
Aricoris sp. 1 sp. nov (LAK-169)	Santo Antônio de Goiás, Goiás, Brazil	
Aricoris sp. 1 sp. nov (LAK-170)	Andorinhas, Goiás, Brazil	
Aricoris sp. 1 sp. nov (LAK-383)	Patrocínio Paulista, São Paulo, Brazil	
Aricoris sp. 1 sp. nov (LAK-467)	Parque Estadual das Furnas do Bom Jesus, Patrocínio Paulista, São Paulo, Brazil	
Aricoris sp. 3 sp. nov (LAK-479)	Parque Estadual Serra Nova, Porteirinha, Minas Gerais, Brazil	
Aricoris sp. 3 sp. nov (LAK-480)	Santo Antônio do Retiro, Minas Gerais, Brazil	
Aricoris sp. 3 sp. nov (LAK-481)	Parque Estadual Serra Nova, Porteirinha, Minas Gerais, Brazil	
Aricoris sp. 3 sp. nov (LAK-482)	Santo Antônio do Retiro, Minas Gerais, Brazil	
Aricoris sp. 3 sp. nov (LAK-483)	Santo Antônio do Retiro, Minas Gerais, Brazil	

*Material voucher lost.

Results

Molecular phylogeny inference

In accordance with our phylogenetic hypothesis, the validity of two new species with available sequences was strongly supported, as well as the validity of *Aricoris constantius*, *A. monotona* and *A. tutana*, with high support values (Fig. 1). In addition, *A. monotona* and *A. tutana* were recovered as sister species.

Taxonomy

Aricoris constantius **(Fabricius, 1793)**

(Figs. 2-5, 26, 32-33, 44-46, 57, 59 and 65)

Papilio constantius Fabricius, 1793: 152; Jone's Icones, 1745-1818: v. 5, plate L.

Aricoris constantius (Fabricius, 1793): Hall & Harvey, 2002: 540 (table), 542, 547 (table), 552-554, 560, 562, figs. 1C, 3B, 4B, 5; Carneiro, Mielke, & Casagrande, 2008: 265 (table); Callaghan, 2010: 395-397, 399-400, figs. 1-2, 7-8, 13; Bellaver et al., 2012: 186 (table); Orlandin, Favretto, Piovesan, & Santos, 2016: 156 (table); Palo Jr., 2017: 634, 722 (table).

Aricoris tutana (Godart, [1824]): D'Abrera, 1994: 985 (plate, ♂R). Penz & DeVries 1999: 6, 8 (table), 11, 14, 19, 21, 30-32 (appendixes), figs. 4I, 6, 7A-B, 14B, 16D.

Diagnosis. *Aricoris constantius* is easily differentiated from the other species by the presence of a very straight line on the hindwings separating the proximal and distal halves (Fig. 26) and the presence of a row of marginal conspicuous ocelli on the hindwings without a ring of yellowish scales surrounding them. Male genitalia present

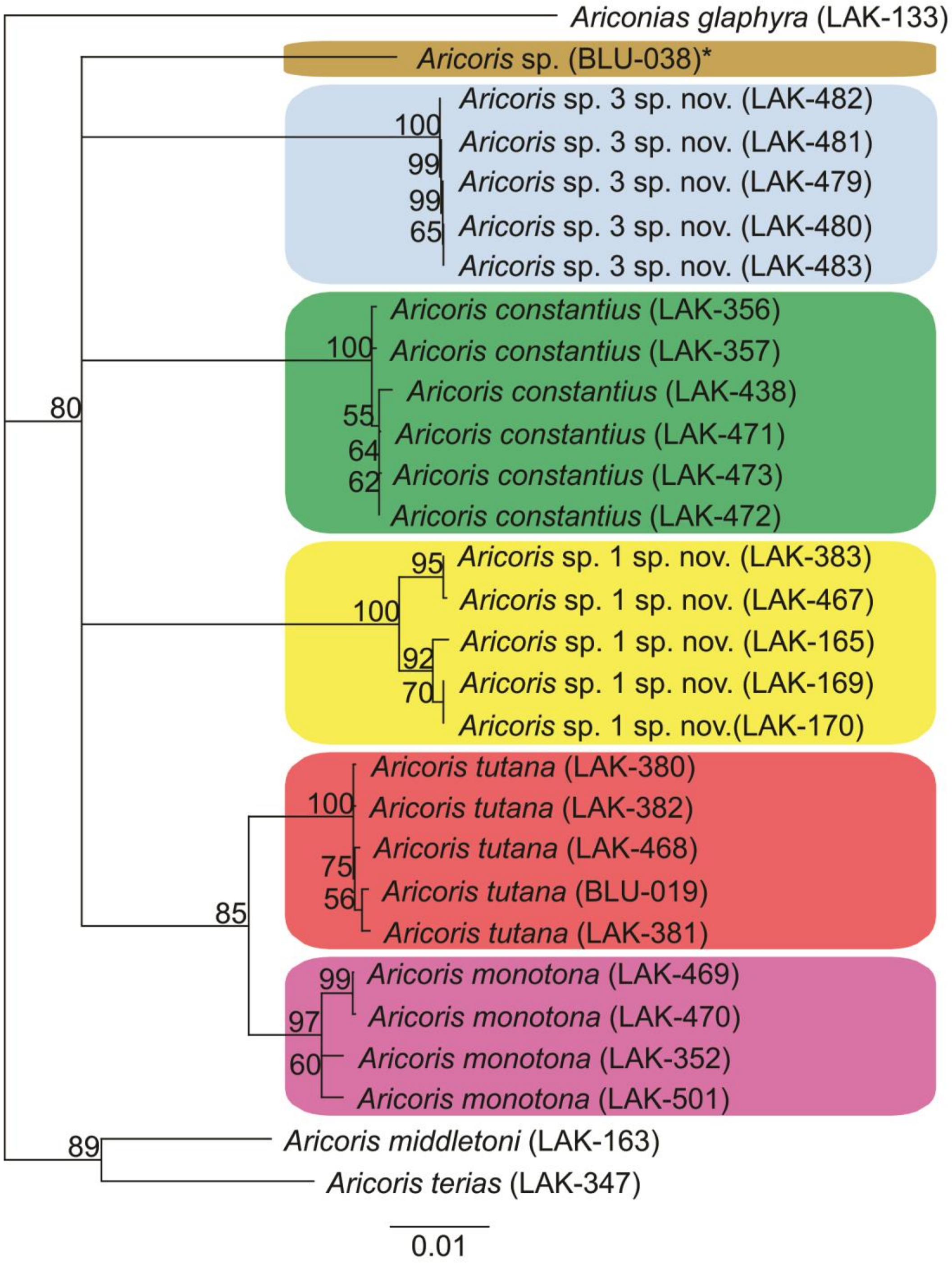


FIGURE 1. Relationships among species in the *Aricoris contantius* complex, and selected specimens of *Ariconias glaphyra*, *Aricoris middletoni* and *Aricoris terias*, based on DNA "Barcodes". Numbers near branch nodes are bootstrap branch support. Asterisk indicates voucher lost.

saccus with a sclerotic protuberance at the upper frontal region (Fig. 32). Female genitalia similar to *A. monotona*, but the invaginated pocket between ostium bursae and papillae anales is bilobed and parallel (Figs. 44-45), while in *A. monotona* this structure is bilobed and opposite. Ductus bursae with a sclerotized ring in upper margin (Fig. 45), as in *A. monotona* and *A. tutana*. This is the only species found in a forest habitat, associated with costal lowland Atlantic Forest in the southeast and southern Brazil (Fig. 58).

Distribution and biology (Figs. 58-59 and 65)**.** Brazil (Rio de Janeiro, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul). *Aricoris constantius* inhabits regions of the Atlantic Forest on the coastal Brazil, in open areas and clearings where the original forest cover has been removed (Callaghan, 2010). Larvae in the early instars exhibit semi-gregarious behaviour and feed on the honeydew secretions produced by ant-tended Hemiptera (Coccoidea), mature instar complete their development within ant nest. Apparently, larvae exhibit obligatory association with one unidentified *Camponotus* (Hymenoptera: Formicidae), as the female lay eggs only in the presence of this ant species (Neto et al. in prep.).

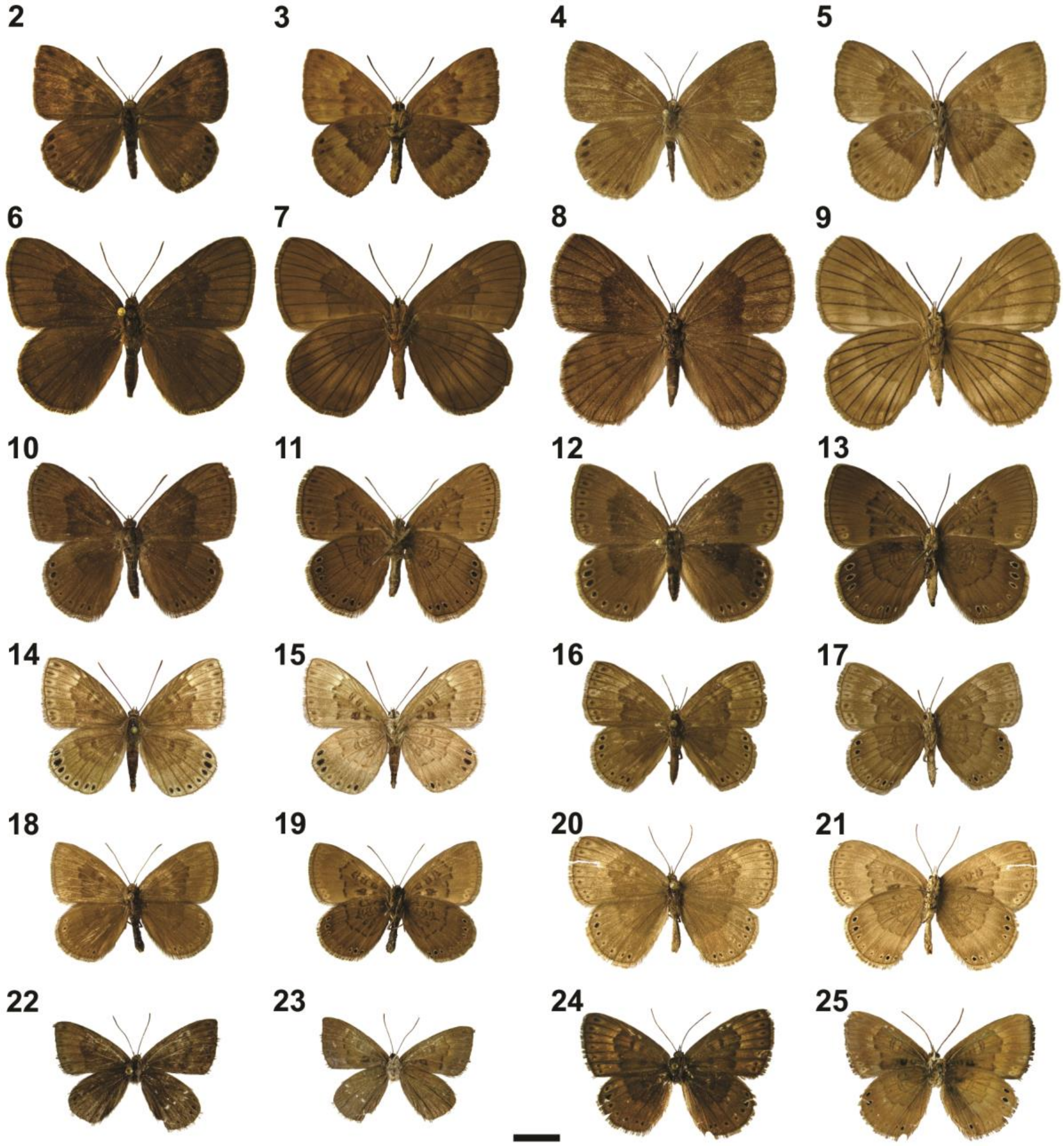
Original type. *Papilio constantius* Fabricius, 1793. Locality unknown. The holotype is lost, but there is an illustration in Jone’s Icones (v. 5, plate L) (Fig. 57).

Neotype here designated. Adult male (Figs. 2-3), DZ 16.381, deposited in Coleção Entomológica Padre Jesus Santiago Moure (DZUP), Federal University of Curitiba, Curitiba, Paraná, Brazil. *Type locality*: Guapimirim, Rio de Janeiro, Brazil; specimen collected on 17 February 1958 by P. Gagarin and H. Ebert. The neotype contains the following labels (labels separated by “/” and lines inside same label separated by “-”): /*Aricoris constantius* - (Fabricius, 1793) - NEOTYPUS♂/ GUAPY-

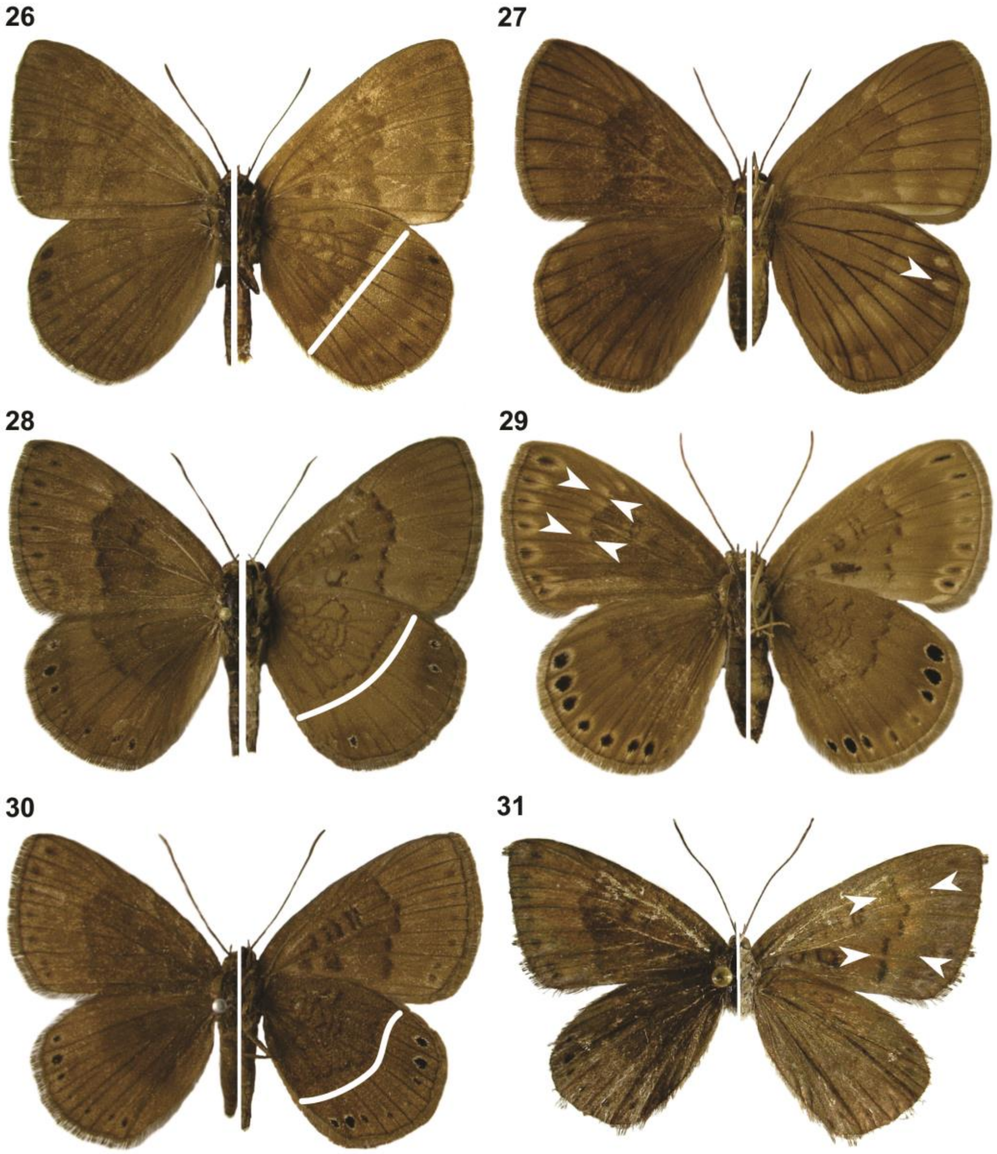
MIRIM – 17-II-1958 – GAGARIN - H. EBERT LEG/ Guapy-Mirin - P. Gagarin [*leg.*], H. Ebert [*leg.*] – 17-2-1958/ DZ 16.381/ Ex Col. Gagarin/.

Remarks. Callaghan (2010) did not designate a neotype for *A. constantius* in his revision. Therefore, it is herein designated the neotype of this species to define its nominal taxa objectively and clarifying its taxonomic status following the provisions of ICZN (1999; Article 75.3).

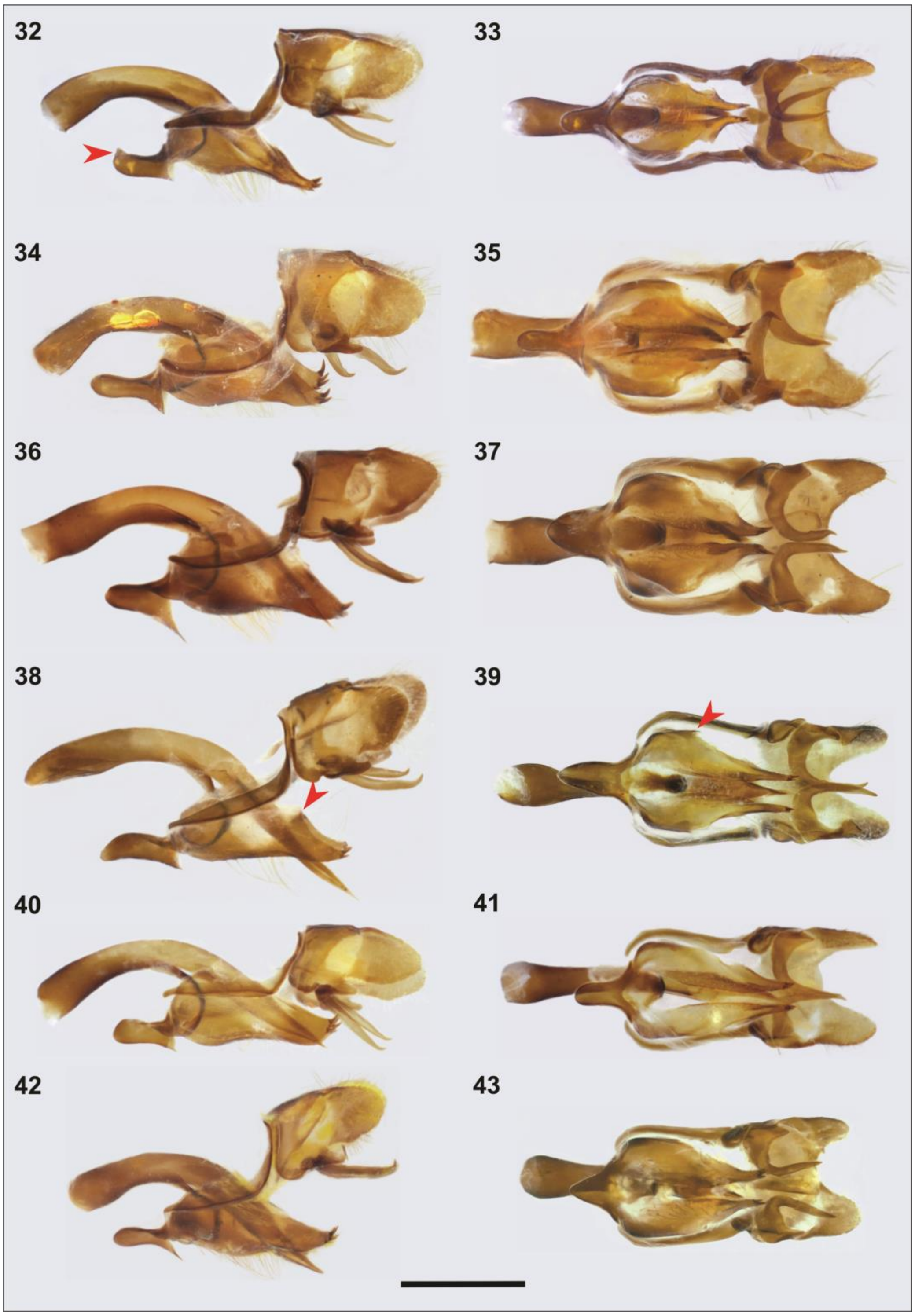
Although Fabricius did not illustrate *Papilio constantius* and its description was insufficient for identifying this species, the availability of the compilation of many types illustrations by William Jones, known as Jones' Icones, made possible the general idea of how *P. constantius*' type seemed. Hall & Harvey (2002) applied correctly the name *A. constantius* for the first time when referring to this taxon (Callaghan, 2010). The photography of *A. constantius* in Hall & Harvey (2002; Fig. 1C) demonstrates clearly the straight line on the hindwings separating the proximal and distal halves. However, the illustration of *P. constantius* on Jones' Icones (Fig. 57) demonstrates some inconsistencies with the reality. For example, the lateral view shows a very large fringe and it is represented a row of brown ocelli on the wings. Unlike expected for *A. constantius*, the *P. constantius*' illustration does not show any difference of colour tones between the proximal and distal halves on the dorsal view and there are represented some lighter scales above the dark ocelli of both wings. Also, the line that separates the halves of the hindwing is not straight as expected. However, in some few cases, we observed on specimens of *A. constantius* deposited on museums that the hindwing line is not always so straight. It is clearly that the illustration is not accurate, as Callaghan (2010) noticed, so we prefer to maintain the decision on name *A. constantius* for this species.



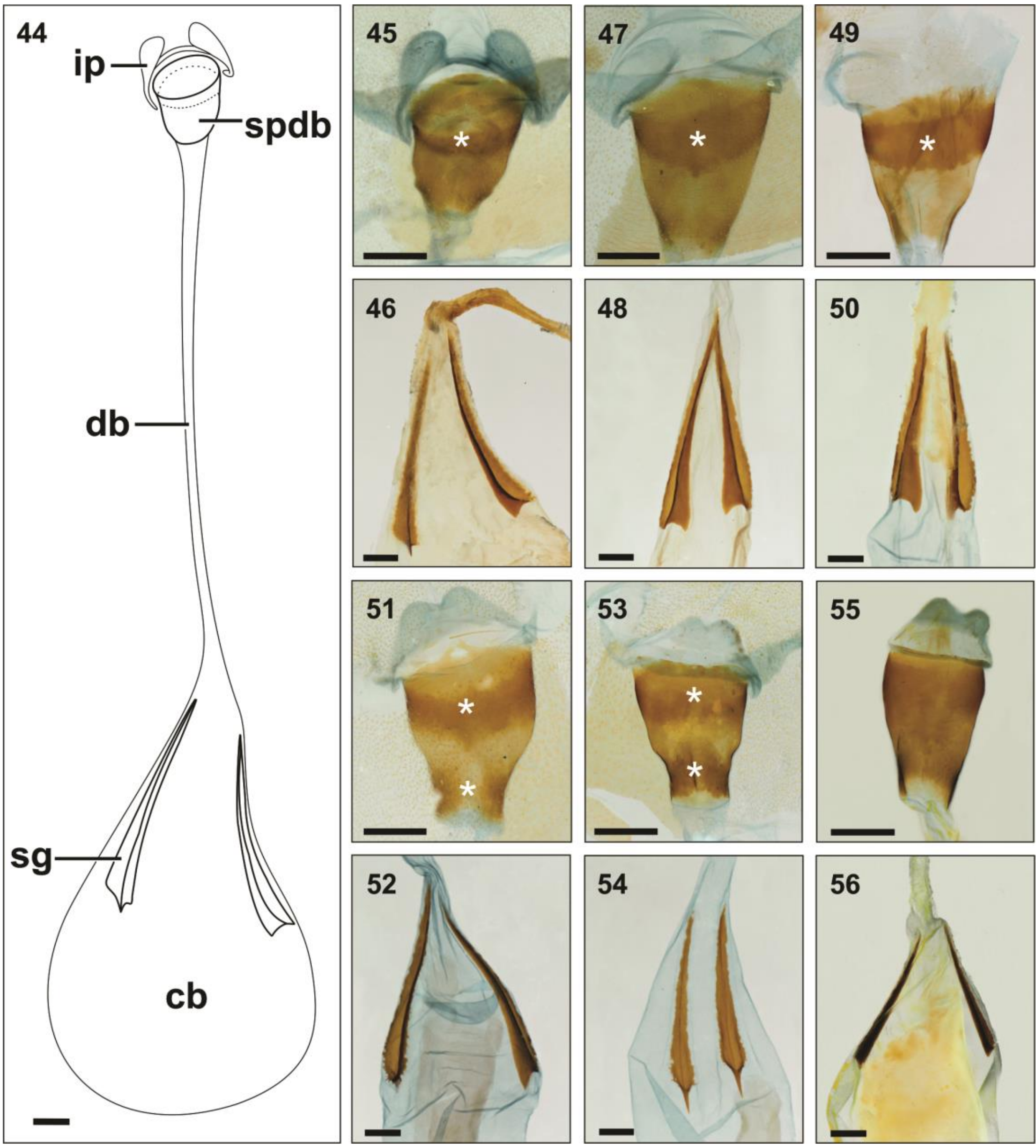
FIGURES 2-25. "*Aricoris constantius*" species group in dorsal and ventral views, respectively. **2-5.** *Aricoris constantius*: 2-3, male neotype; 4-5, female. **6-9.** *Aricoris monotona*: 6-7, male; 8-9, female; **10-13.** *Aricoris tutana*: 10-11, male neotype; 12-13, female; **14-17.** *Aricoris* sp. 1 sp. nov.: 14-15, male holotype; 16-17, female allotype; **18-21.** *Aricoris* sp. 2 sp. nov.: 18-19, male holotype; 20-21. female allotype; **22-25.** *Aricoris* sp. 3 sp. nov.: 22-23, male holotype; 24-25, female allotype. Scale bar = 10 mm.



FIGURES 26-31. Phenotypic variation in the wings elements of species of *Aricoris constantius* complex in dorsal and ventral views, respectively. **26.** *A. constantius*, male, Brazil (Santa Catarina), (DZ 16.706), (line indicates the straight line on hindwing). **27.** *A. monotona*, female, Brazil (Paraná), (DZ 16.453), (arrow indicates the presence of weakly ocelli present in some specimens). **28.** *A. tutana*, male, Brazil (Bahia), (DZ 16.727), (line indicates the rounded line on hindwing). **29.** *A.* sp. 1 sp. nov., female, Brazil (Distrito Federal), (DZ 16.306), (arrows indicate the band of light yellowish scales at the postdiscal area). **30.** *A.* sp. 2 sp. nov., male, Brazil (Distrito Federal), (DZ 16.313), (line indicates the irregular line on hindwing). **31.** *A.* sp. 3 sp. nov., holotype, Brazil (Minas Gerais), (arrows indicate the presence of splashed orange scales on discal and postdical area). Figures not in real scale.



FIGURES 32-43. Male genitalia of "*Aricoris constantius*" species group, in lateral and ventral views, respectively: **32-33.** *A. constantius* (red arrow indicates sclerotic protuberance at the upper frontal region); **34-35.** *A. monotona*; **36-37.** *A. tutana*; **38-39.** *Aricoris* sp. 1 sp. nov. (red arrow in Fig. 37 indicates the pointed projection at the upper medial region of valvae and in Fig. 38 indicates the lateral spine-shaped projection of valvae); **40-41.** *Aricoris* sp. 2 sp. nov.; **42-43.** *Aricoris* sp. 3 sp. nov. Scale bar = 1 mm.



FIGURES 44-56. Female genitalia of “*Aricoris constantius*” species group. **44.** General aspect of female genitalia of *Aricoris constantius*. Details of sclerotized portion of ductus bursae and signa, respectively: **45-46.** *A. contantius*; 45. signa. **47-48.** *A. monotona*; **49-50.** *A. tutana*; **51-52.** *A.* sp. 1 sp. nov.; **53-54.** *A.* sp. 2 sp. nov.; **55-56.** *A.* sp. 3 sp. nov. ip. invaginated pocket; spdb. sclerotized portion of ductus bursae; db. ductus bursae; cb. corpus bursae; sg. signum. Asterisks represent the most sclerotized area of the sclerotized portion of ductus bursae. Scale bar = 0.25 mm.

Recently, the publication of Palo Jr. (2017), a compilation of photos from Brazilian butterflies in nature, shows on page 635, a photo of an *A. tutana* group specimen wrongly identified as *A. constantius*. As most species of the *A. tutana* group are sympatric, and the identification through photos sometimes is not accurate, we cannot be sure about the real identity of that specimen.

Material examined. BRAZIL - *Paraná*: **Paranaguá**, Atami, 2♂, 21.iii.1991, 24.i.1993, OM 26.655, OM 33.967 (OM), Mielke *leg*.; *Rio de Janeiro*: **Angra dos Reis**, Jussaral, 1♂, 1♀, 16-19.iii.1934, 19.iii.1934, D'Almeida *leg.*, DZ 16.671, DZ 16.395 (DZUP); Jussaral, 1♂, 10.ii.1937, Costa, Travassos, and Oiticica *leg*. (MNRJ); **Guapimirim**, 2♂, 17.ii.1958, Gagarin & Ebert *leg.*, DZ 16.381, DZ 16.388 (DZUP); **Itatiaia**, Campo Bello, 1♀, 20.i.1936, Gagarin *leg*., DZ 16.367 (DZUP); (700 m), 1♀, 01.ii.1936, ex-coll. Gagarin, DZ 16.374 (DZUP); **Silva Jardim,** BR 101, Km 225, 2♀, 27.i.1986, C.J. Callaghan *leg*., GENITALIA #1035♀ and GENITALIA #1036♀ (CC); **Teresópolis**, Raiz da Serra de Teresa, 1♂, 22.ix.1960, D'Almeida *leg.*, DZ 16.650 (DZUP); **Três Rios**, 2♂, 1♀, 31.xii.2016, Carvalho *leg*., DNA-voucher LAK-471, LAK-472 and LAK-473, GENITALIA nº 2017-15♂ J. R. A. Lemes; *Rio Grande do Sul*: **Dom Pedro de Alcântara**, 1♂, 19.i.2011, A.K. Silva *leg*., DNA-voucher LAK-356, GENITALIA nº 2016-07♂ J. R. A. Lemes; 1♂, 01.iv.2011, N. Mega *leg*., DNA-voucher LAK-357, GENITALIA nº 2016-03♂ J. R. A. Lemes; **Mampituba**, 1♀, 01.iv.2011, N. Mega *leg*., DNA-voucher LAK-358, GENITALIA nº 2016-12♀ J. R. A. Lemes, CLDZ 7527 (CLDZ); **Morro Alto**, (Mata Paludosa),1♂, 27.iii.2010, P. Colombo *leg*., DNA-voucher LAK-355; *Santa Catarina*: **Barra do Sul**, 2♂, 05.iii.1978, Mielke *leg*., DZ 16.692 and DZ 16.706 (DZUP); **Bombinhas**, 1♂, 15.08.1995, A. Moser *leg*.; **Florianópolis**, Lago Petri, 2♀, 20.iii.2004, E. Santos *leg.*, DZ 16.657 and DZ 16.664 (DZUP); **Joinville**, 3♂, 12.iii.1967, Mielke *leg*., DZ 16.246, DZ 16.418 and

16.425 (DZUP); 1♂, 13.xii.1985, Mielke *leg*., OM. 62.388 (OM); (200 m), 1♂, 29.x.1978, Miers *leg*., DZ 16.699 (DZUP); (200 m), 1♂, 06.vii.1969, Mielke & Miers *leg*., DZ 16.678 (DZUP); (200 m), 1♀, 23.i.1984, Mielke & Miers *leg*., DZ 16.685 (DZUP); 2♂, 18.01.1981, C.J. Callaghan *leg*., GENITALIA nº #1033♂ and GENITALIA nº #1034♂, respectively (CJC); 1♂, 1♀, xii.1940, G. Pohl *leg*. (MZUSP); *São Paulo*: **Ubatuba,** Praia da Lagoinha, 1♀, 30.ix.1993, C.J. Callaghan *leg*., (CJC); 1♀, Coleção Julius ARP, nº 55/640, no collection data (MNRJ); 3♂, 1.x.1993, A. Freitas *leg*. (ZUEC); **Votorantim,** 1♀, 15.ii.2017, L. Braga *leg*. (ZUEC).

***Aricoris monotona* (Stichel, 1910)**

(Figs. 6-9, 27, 34-35, 47-48, 60 and 66)

Melanope (*Aricoris*) *monotona* Stichel, 1910: 13.

Aricoris monotona (Stichel, 1910): Biezanko, Mielke, Wedderhoff, 1978: 18; D'Abrera, 1994: 984, 985 (plate ♂R and ♂V); Callaghan, 2010 (reinstated status): 397, 399-400, figs. 5-6, 11-12, 13; Casagrande et al., 2012: 300 (table), 307, figs. 189-192; Núñez Bustos, 2015: 100; Orlandin et al., 2016: 156 (table); Palo Jr., 2017: 722 (table).

Diagnosis. It can be easily differentiated from other species by the absence of a row of dark ocelli at the marginal bands of the fore and hindwings (Figs. 6-9), although in some specimens, ocelli can be weakly present on the ventral side of the wings (Fig. 27). It presents an evident light-yellow band at the postdiscal regions of the fore and hindwings, and abundant orange scales ventrally on thorax and dorsally on the cervical area (Fig. 66). Male genitalia like *A. tutana*, differing from the other species by the wide tegumen in dorsal view and by the large width of the valve in ventral view (Fig. 35). The female genitalia are like *A. constantius*, differentiating by the direction of the opposite lobes of the invaginated pocket between ostium bursae and papillae anales (Fig. 47). It resembles also *A. constantius* and *A. tutana* by the sclerotized portion of ductus bursae with a sclerotized ring at the upper margin.

Distribution and biology (Fig. 58, 60 and 66)**.** Argentina (Misiones) and Brazil (São Paulo, Paraná, Santa Catarina and Rio Grande do Sul). This species inhabits areas of natural fields in the Pampa biome and also grassy natural clearings in rock outcrops in the Atlantic Forest biome (Biezanko et al., 1978; Callaghan, 2010). It is sympatric with *A.* sp. 2 sp. nov. in the natural grasslands in the Paraná state (Casagrande et al., 2012). Adults are quite local and can be seen year after year in the same location.

Holotype. *Melanope monotona* Stichel, 1910. Adult male, deposited in Humbold Universität, Berlin, Germany. *Type locality*: Casa Branca, São Paulo, Brazil; specimen collected by Standinger. The holotype contains the following labels (labels separated by "/" and lines inside same label separated by "-"): /Type/ Casa Br. – Bras. M. – G./ Type/ Coll. – Standinger/ *monotona* – Stich[el]/.

Remarks. Callaghan (2010) cites the presence of two specimens of *Aricoris monotona* from Bahia, deposited in the Muséum National d'Histoire Naturelle (MNHN) in Paris, France. However, we consulted the curators of the museum and these examples have not been found. Therefore, we do not include these specimens in the distribution map (Fig. 58). The disjunct distribution in Bahia needs confirmation, note that the sister species *A. monotona* and *A. tutana* present typical allopatric distributions, being the first with more austral distribution.

Material examined. ARGENTINA – *Misiones*: **San Ignacio**, Osununu Reserve (Elev. 569 f) (S 26º16.74' W 055º34.70'), 1♂, 18.x.2009, J.D. Turner *leg.*, 9941 (MGCL); **BRAZIL** – *Paraná*: **Foz do Iguaçu**, 1♂, 30.i.1973, Laroca & Jensen *leg.*,

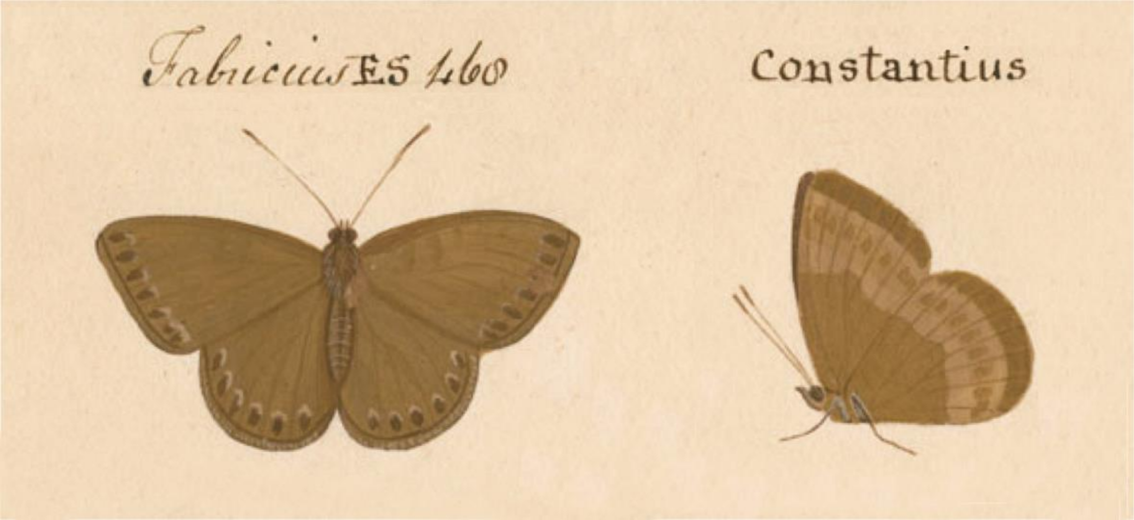


FIGURE 57. Representation of *Papilio constantius* (dorsal and lateral view) on page 156 (plate L, volume 5) of Jone’s Icones (1793).

DZ 16.481 (DZUP); **Jaguariaíva**, 1♀, v.1955, Bruhn *leg*., DZ 15.331 (DZUP); 1♂, 25.xi.1986, Miers & Casagrande *leg*., DZ 16.439 (DZUP); **Mandirituba**, 1♂, 24.i.1987, Mielke *leg*., OM 62.339 (OM); **Ortigueira**, (1260 m), 1♂, 30.xii.2003, Mielke *leg*., 12.974 (EF); **Palmeira**, 1♀, 01.iii.1987, Mielke *leg*., OM 62.451 (OM); **Ponta Grossa**, Vila Velha (900 m), 1♂, 10.iii.2013, Savaris & Lampert *leg*., DD 310 (DD); Vila Velha, 1♀, 07.iii.1973, Mielke *leg*., DZ 16.474 (DZUP); Vila Velha, 1♀, 15.x.1967, Mielke *leg*., DZ 16.467 (DZUP); Vila Velha (1000 m), 1♂, 22.iii.2015, C.J. Callaghan *leg*., GENITALIA #1031♂ (CJC); Vila Velha (1000 m), 1♂, 28.ii.1991, Mielke *leg*., OM 26.614 (OM); Vila Velha (1000 m), 5♂, 03.iii.2001, Mielke & Melo *leg*., OM 52.643, OM 52.699, OM 55.804, OM 55.846 and OM 55.853 (OM); (900 m), 1♂, 3♀, 05.ii.1984, Mielke *leg*., DZ 16.495, DZ 16.446, 16.453 and 16.460 (DZUP); 1♀, ii.1952, Coll. F. Justus Jor., DZ 16.432 (DZUP); **Tijucas do Sul**, Morro do Araçatuba (S 25º54’07’’ W 48º59’39’’), 1♂, 25.ii.2011, Carneiro, Grossi, Dolibaina & Zacca *leg*., DD310, (DD); *Rio Grande do Sul*: no location, 1♂, 1♀, Garbi *leg*. (MZUSP); **Ijuí**, 1♂, 12.xi.2002, L. Medeiros *leg*., DD 381 (DD); **Jari**, 5♂, 20.i.2017, C.J. Callaghan leg., (CJC); **Porto Alegre**, Morro do Osso, 1♂, 28.xi.2005, F. Camargo *leg*., GENITALIA nº 2016-04♂ J. R. A. Lemes; Morro do Osso, 1♂, 29.iii.2007, D. Castro *leg*., DNA-voucher LAK-354; Morro do Osso (Campo Platô), 1♀, 02.xii.2006, A.K. Silva *leg*., GENITALIA nº 2016-11♀ J. R. A. Lemes; Morro Santana, 1♂, 01.xii.2006, A.K. Silva *leg*., GENITALIA nº 2016-08♂ J. R. A. Lemes; **Taim**, 1♂, 26.xi.2013, H.P. Romanowski *leg*., DNA-voucher LAK-352; **Viamão,** Parque Saint’ Hilaire, 2♂, 02.xi.2016, L.A. Kaminski *leg*., DNA-voucher LAK-469 and LAK-470; *Santa Catarina*: **Curitibanos**, 2♂, 07.iii.1983, Miers *leg*., DZ 16.488 and DZ 21.565 (DZUP); 1♂, 1♀, 07.iii.1983, Miers *leg*., OM 67.574 and OM 67.462 (OM); **São Bento do Sul**, Rio Vermelho (850 m), 1♂, 07.xi.1985, Rank *leg*., OM 13.299 (OM); *São*

Paulo: **Campinas**, Mata Ribeirão Cachoeira, 1♂, 03.ii.2001, K.S. Brown, A.V. Freitas & M. Uehara-Prado *leg*., LEP 2142 (ZUEC); Mata Ribeirão Cachoeira, 1♂, no collection date, C.J. Callaghan *leg*., GENITALIA #1032♂ (CJC); Mata Ribeirão Cachoeira (S 22º50' W 46º50'), 1♂, 14.iv.2000, K. Brown *leg*., ZUEC-LEP 5214 (ZUEC); 1♂, 25.xii.2000, K. Brown *leg*., ZUEC-LEP 4265 (ZUEC); 1♂, 03.ii.2001, K. Brown *leg*., ZUEC-LEP 2101 (ZUEC); 1♂, 22.iv.2001, K. Brown *leg*., ZUEC-LEP 2833 (ZUEC); 1♀, 01.v.2000, A. Freitas *leg*. (ZUEC); 1♂, 05.v.2000, A. Freitas *leg*. (ZUEC); 1♂, 07-08.iv.2000, A. Freitas *leg*., (ZUEC); **São Paulo**, Santo Amaro, 1♀, 12.iii.1941, D'Almeida *leg*., DZ 16.636 (DZUP); 1♂, nº. 8093, no collection date (MNRJ); Bairro Ipiranga, 1♂, 08.ii.1927, R. Spitz *leg* (MZUSP); 1♀, 26.xi.1927, R. Spitz *leg*. (MZUSP); Mata do Governo, 1♂, 1♀, x.1913, G. Pohl *leg*. (MZUSP);

***Aricoris tutana* (Godart, [1824])**

(Figs. 10-13, 28, 36-37, 49-50, 61-62 and 67)

Erycina tutana Godart, [1824]: 295.

Aricoris tisiphone Westwood, 1851: 450.

Aricoris bahiana C. Felder & R. Felder, 1865: 295.

Aricoris tutana (Godart, [1824]): D'Abrera, 1994: 984, 985 (plate ♀V); Mielke, 1994: 768 (table); Callaghan, 2010: 397-400, figs. 3-4, 13; Palo, Jr., 2017: 722 (table); Pérez et al., 2017: 446 (table); Pires et al., in press: 7 (table)

Diagnosis. This species can be easily distinguished from *A. constantius* and *A. monotona* by the presence of a row of conspicuous ocelli on the fore and hindwings surrounded by yellow scales (Figs. 10-13 and 28). *Aricoris tutana* can be distinguished from others similar species in the *A. tutana* complex that have conspicuous ocelli by the more uniform brown ground colour pattern, general larger size, and by the orange scales at the cervical area and ventrally on thorax, similar to *A. monotona*. They usually have a nearly rounded line separating the proximal and distal halves of the hindwing in ventral view, less irregular than of *Aricoris* sp. 1 sp. nov., *A.* sp. 2 sp. nov and *A.* sp. 3 sp. nov. (Figs. 10-13 and 28). Valvae in lateral and ventral view without projected projections and enlarged in ventral view (Fig. 37) when compared to *A. constantius*, *A.* sp. 1 sp. nov. and *A* sp. 2 sp. nov. Female genitalia with sclerotized portion of ductus bursae with a sclerotized ring at the upper margin as in *A. contantius* and *A. monotona* (Fig. 49) and anterior region of the signa with short tips (Fig. 50).

Redescription

Male redescription.

Forewing size and shape: Average forewing length 21.72 mm, range 19-24 mm (n = 11). Costa curved to apex, and margin straight to tornus, distal margin straight, slightly curved to apex and tornus.

Dorsal forewing surface: Proximal half dark brown, distal half lighter, separated by an irregular line. Veins dark brown. Fringe brown as on the distal with a dark marginal line preceding the fringes. Presence of a row of seven ocelli at the marginal band, sometimes slightly surrounded of yellowish scales.

Ventral forewing surface: Ground colour light brown. Irregular line with dark brown scales separating proximal and distal half. Fringe as on the dorsal surface. Discal cell with three irregular spots surrounded by dark scales. Presence of two irregular dark spots at CuA_2. Presence of a row of seven ocelli at the marginal band of the forewing formed by brown scales, sometimes slightly surrounded of yellowish scales.

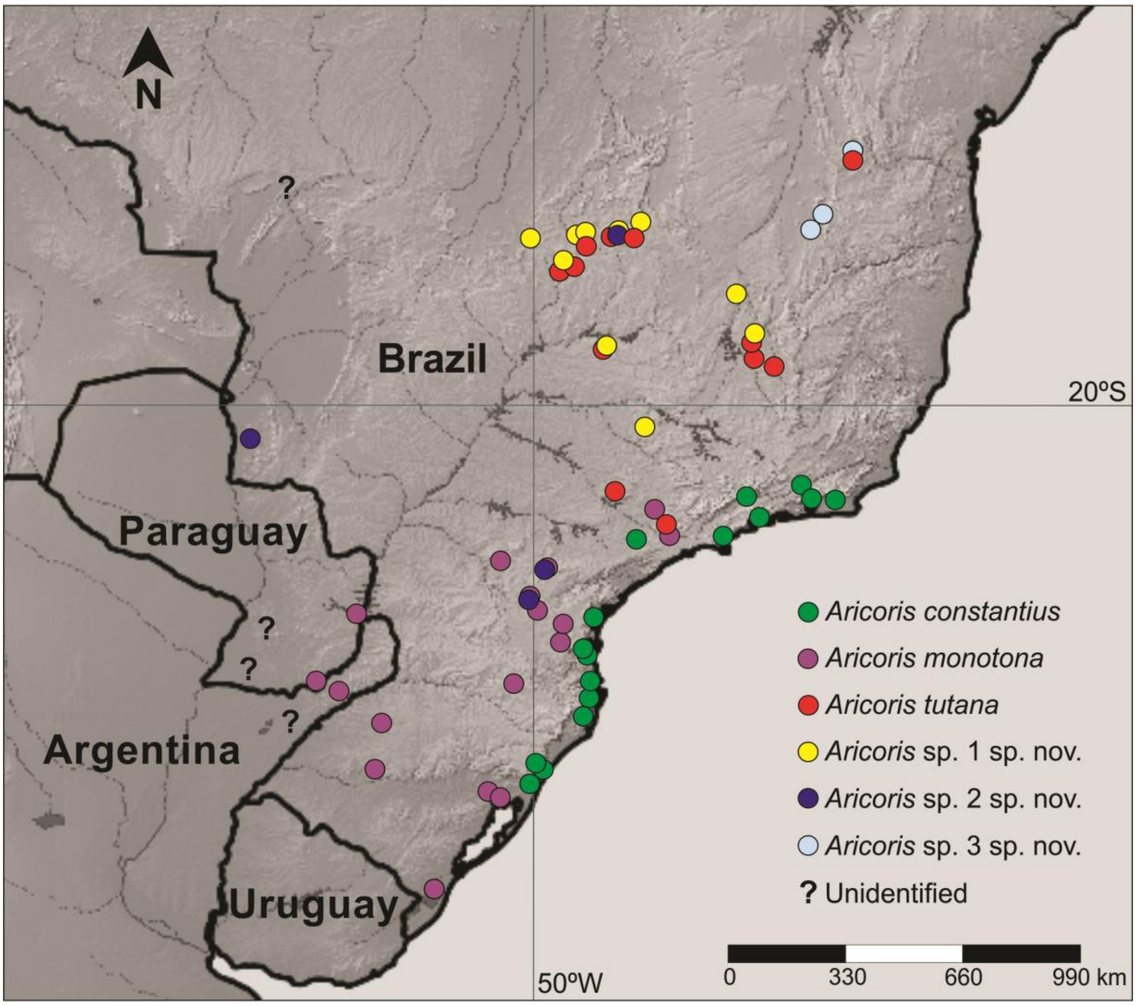


FIGURE 58. Geographical distribution of "*Aricoris constantius*" species group (based on examined material and additional records).

Hindwing size and shape: Average hindwing length 18.27 mm, range 15-21 mm (n = 11). Anal margin slightly curved to tornus, tornus rounded, distal margin and apex curved.

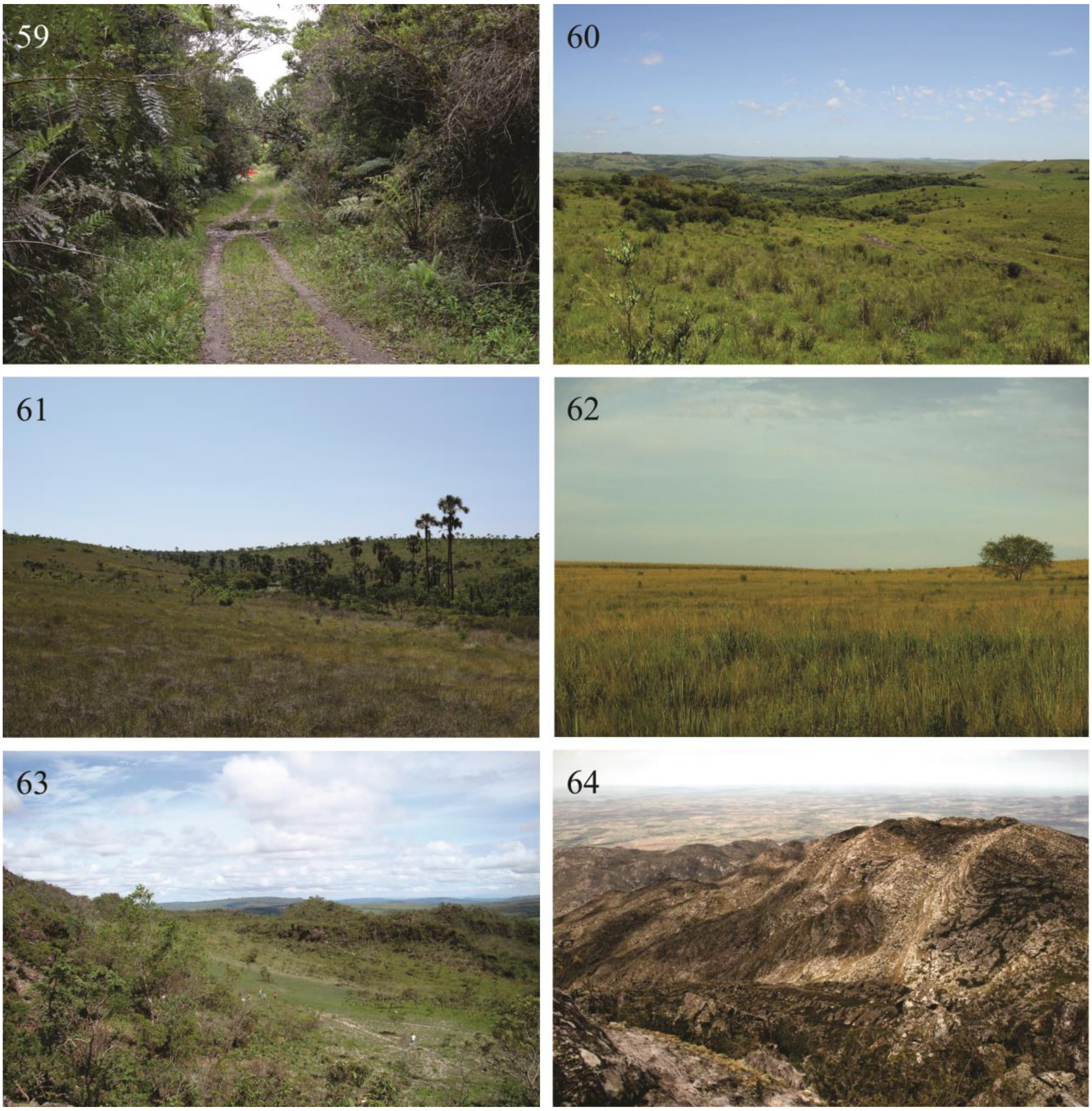
Dorsal hindwing surface: Proximal half dark brown, distal half lighter, separated usually by a uniform rounded line. Fringe brown as on the distal half of the forewing, with a dark marginal line basally. Presence of a row of seven black ocelli, clearly marked, surrounded nearly completely by yellowish scales. The fourth and the fifth ocelli between space M_2-CuA_1 and the last one at the region of the tornus are usually smaller than the others.

Ventral hindwing surface: Ground colour light brown. Presence of a uniform rounded line dark brown separating proximal and distal half. Fringe as on the dorsal surface. Presence of a row of eight black ocelli at the marginal band surrounded nearly completely by yellowish scales. The fourth and fifth ocelli, between space M_2-CuA_1 sometimes scarcely marked.

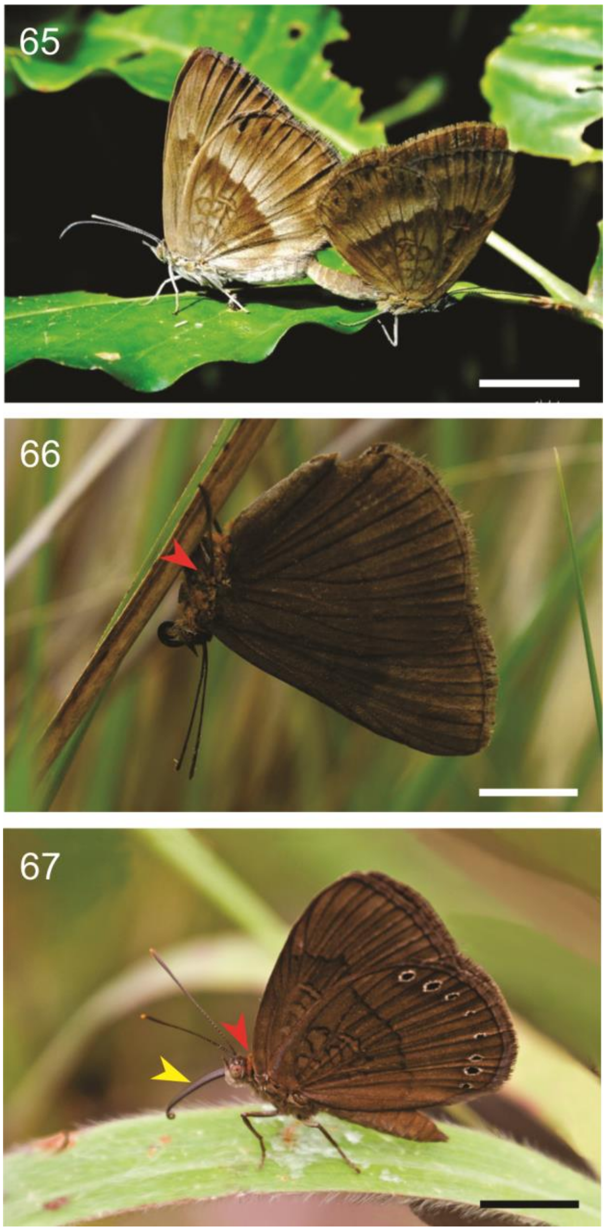
Head: Brown dorsally, long yellowish-brown scales on the frontoclypeus and labial palpi. Galea black and robust. Labial palpi flattened laterally, curved upwards. Antenna brown, cylindrical articles. Length of antenna surpassing more than half the length of the forewing.

Thorax: Presence of orange bristles dorsally on the cervical area (Fig. 67) and ventrally on the thorax. Dark brown, covered by bands of long yellowish-brown scales dorsally and long brown scales ventrally. Legs brown.

Genitalia (Fig. 36-37): Uncus in lateral view slightly rounded, slightly wider than long; in dorsal view lobes are widely separated with U-shaped area between them.



FIGURES 59-64. Overview of *Aricoris constantius* group habitats in South America. **59.** *A. constantius*, swamp forest in Balneário Barra do Sul, Santa Catarina, Brazil; **60.** *A. monotona*, natural grasslands in Jari, Rio Grande do Sul, Brazil; **61.** *A. tutana*, *A.* sp. 1 sp. nov. and *A.* sp. 2 sp. nov., “cerrado” area in Brasília, Distrito Federal, Brazil; **62.** *A. tutana*, flooded grasslands area in Corrientes, Argentina (photo by Ezequiel Núñez Bustos); **63.** *A.* sp. 1 sp. nov**.**, “cerrado rupestre” area in Parque Estadual dos Pireneus, Pirenópolis, Goiás, Brazil; **64.** *A.* sp. 3 sp. nov., “campo rupestre” in Pico da Formosa, Minas Gerais, Brazil (photo by Lucas Perillo).



FIGURES 65-67. Live adults of *Aricoris constantius* group in the field. **65.** *A. constantius*, couple in copula, Imbituba, Santa Catarina, Brazil (photo by Maurino André); **66.** *A. monotona*, male, resting on a grass leaf, Parque Natural Municipal Saint'Hilaire, Viamão, Rio Grande do Sul, Brazil (arrow indicates the orange bristles of the ventral part of thorax); **67.** *A. tutana*, male, resting on a grass leaf, Brasília, Distrito Federal, Brazil (red arrow indicates the orange bristles at the dorsal region of the prothorax and the yellow arrow indicates the robust black galea, typical of males of the *Aricoris constantius* group) (photo by Rodrigo Conte). Scale bar = 10 mm.

Scaphium long. Tegumen in lateral view wider than long, concave region at the posterior surface and upper margin elevated in relation to the uncus upper margin; inferior distal projection of the tegmen which connects with the gnathos long; gnathos with hook-shaped tips directed upwards; valvae in lateral view without a pointed projection in the superior medial region; extensive membranous area at the centre, posterior region surface wide and slightly inclined; in ventral view, anterior region slightly enlarged, without a lateral spine-shaped projection and posterior region thinner, with two to four spines at the tip. Saccus-shape variable,with short and pointed ventral projection, as well as the dorsal projection, which is connected with the anterior region of tegumen by a membranous area. Aedeagus long, pointed, three times the length of tegumen + uncus.

Female redescription.

Forewing size and shape: Forewing length 21 mm (n = 1). Like male with distal margins slightly rounded.

Dorsal forewing surface: Like males. Ocelli and yellowish area surrounding them are more conspicuous than in males.

Ventral forewing surface: Like males, but a background color lighter. Presence of a ring of yellowish scales surrounding the last two ocelli.

Hindwing size and shape: Hindwing length 20 mm (n = 1). Like male with distal margins slightly rounded.

Dorsal hindwing surface: Like males. The ocelli present approximately the same diameter.

Ventral hindwing surface: Like males, with a band of yellow scales at the postdiscal area.

Head: Like males, but the galea is brown and slender and labial palpi has yellow scales.

Thorax: Like males.

Genitalia (Fig. 49-50): Corpus bursae membranous, with a pair of two elongated signa of approximately same size. Signa pointed posteriorly, anterior region wider and with short tips. Ductus bursae membranous, length slightly less than twice the length of the corpus bursae. Sclerotized portion of ductus bursae funnel-shaped, with a sclerotized ring in upper margin. Invaginated pocket between ostium bursae and papillae anales absent. Papillae anales with seta at outer edge.

Distribution and biology (Figs. 58, 61-62 and 67)**.** Brazil (Bahia, Distrito Federal, Goiás, Minas Gerais and São Paulo). *A. tutana* occurs in natural grasslands areas of high altitudes (650 – 1600 m) and, apparently, has the widest distribution, occurring in the open physiognomies in the cerrado savanna (campo limpo and campo sujo) areas in Goias and Federal District and rocky montane fields (campos rupestres) in Minas Gerais and Bahia states. *A. tutana* is sympatric with *A.* sp. 1 sp. nov. in Distrito Federal, Goiás and Minas Gerais, and with *A.* sp. 2 sp. nov in Distrito Federal. There are some records of a putative *A. tutana* found in flooded grasslands in Paraguay and Argentina (Fig. 62) (D’Abrera, 1994; Contreras Chialchia, 2009; Núñez Bustos, 2017). Although the images examined from these specimens are externally similar with *A. tutana*, proper identification requires dissections, since the type of environment and altitude range is markedly different from that usually observed for Brazilian nominal populations of *A. tutana*.

Original types. *Erycina tutana* Godart, [1824]. A female from “Brésil”. The holotype is lost.

Junior synonyms:

Aricoris tisiphone Westwood, 1851. Adult male, deposited in The Natural History Museum, London, United Kingdom. *Type locality*: Minas Gerais, Brazil; specimen collected by Hewitson. The holotype contains the following label (lines inside same label separated by “-”): / Minas Geraes – Hewitson Coll. – 79-69. – Theope – *tisiphone* – *P. tisiphone* – in Coll./

Aricoris bahiana C. Felder & R. Felder, 1865. Adult male, deposited in The Natural History Museum, London, United Kingdom. *Type locality*: Bahia, Brazil; specimen collected by Felder. The holotype contains contains the following labels (labels separated by “/” and lines inside same label separated by “-”): Rothschild - Bequest - B.M.1993-1./ FELDER - COLL n./ aricoris bahiana - [unreadable]/ Bahia - [unreadable] - type/ Type/.

Neotype here designated. Adult male (Figs. 10-11), DZ 16.369, genitalia prepared by José Lemes (GENITALIA nº 2017-14♂ J. R. Lemes), deposited in Coleção Entomológica Padre Jesus Santiago Moure, Federal University of Curitiba, Curitiba, Paraná, Brazil. *Type locality*: Fazenda Água Limpa, Brasília, Distrito Federal; specimen collected on 27 January 1976 by Gifford. The neotype contains the following labels (labels separated by “/” and lines inside same label separated by “-”): /*Aricoris tutana* - (Godart, [1824]) - NEOTYPUS♂/ Faz.[enda] Água Limpa, - Brasília, DF - 27.I.1976 - Gifford leg./ Faz.[enda] Água Limpa - Brasília D.F. - 27.i.76 Cerrado/ *Aricoris tutana*/ ex coleção Gifford/ GENITALIA nº - 2017-14♂ - J. R. Lemes/ DZ 16.369/.

Remarks. Despite the original type of *A. tutana* is lost, the types of *A. tisiphone* and *A. bahiana* are found in the Natural History Museum of London and correspond to the same taxon. As this species is the most common and widely distributed we decided to associate it with the name *tutana* of Godart. Thus, we have designated the neotype of *A. tutana* for this species and maintained the names *tisiphone* and *bahiana* as junior synonyms.

Mistakenly, Callaghan (2010) cites, on his material examined of *A. tutana*, one male from Mato Grosso (Rio de Contas), deposited at DZUP. Actually, this specimen was collected in Rio de Contas, a locality of Bahia (not Mato Grosso). The “Mato Grosso” that Callaghan (2010) is referring is actually a suburb area of Rio de Contas (Bahia) (O. Mielke, personal communication).

Material examined. 1♀, no collection data (MZUSP); **BRAZIL** - *Bahia*: **Rio de Contas**, Pico das Almas (1400-1600 m), 1♂, 28.i.2005, Mielke & Casagrande *leg*., GENITALIA nº 2017-04♂ J. R. A. Lemes, DZ 16.748 (DZUP); Pico das Almas (1400-1600 m), 1♀, 28.i.2005, Mielke & Casagrande *leg*., GENITALIA nº 2017-09♀ J. R. A. Lemes, DZ 16.762 (DZUP); Pico das Almas (1400-1600 m), 1♂, 01.ii.2005, Mielke & Casagrande *leg*., GENITALIA nº 2017-06♂ J. R. A. Lemes, DZ 16.727 (DZUP); Pico das Almas (1400-1600 m), 1♂, 01.ii.2005, Mielke & Casagrande *leg*., DZ 16.755 (DZUP); *Distrito Federal*: **Brasília**, Cabeça do Veado, 1♂, 28.vi.1978, ex-coll. Gifford, D. Giford *leg*., GENITALIA nº 2017-07♂ J. R. A. Lemes, DZ 16.334 (DZUP); Fazenda Água Lima, 1♂, 27.i.1976, ex-coll. Gifford, D. Giford *leg*., DZ 16.369 (DZUP); Fazenda Água Limpa, 1♂, 11.xi.1976, ex-coll. Gifford, D. Giford *leg*., GENITALIA nº 2017-13♂ J. R. A. Lemes, DZ 16.376 (DZUP); Universidade de Brasília, Centro Olímpico, 1♂, 03.i.2000, E.O. Emery *leg*., (CJC); Universidade de Brasília, Centro Olímpico, 1♂, 24/iv/2004, E.O. Emery *leg*., GENITALIA #1026, (CJC); Universidade de Brasília, Centro Olímpico, 1♀, 24.ii.2002, E.O. Emery *leg*., (CJC); Universidade de Brasília, Centro Olímpico, 1♂, 15.x.2002, Ex-coll. Eduardo Emery, E.O. Emery *leg*., DZ 33.987 (DZUP); Reserva CO, 1♀, 20.x.2003, E.O. Emery *leg*., (CJC); Country Club, 1♂, 07.v.1968, C.J. Callaghan *leg*., (CJC); Poça Azul, 1♂, 01. i.2004, Ex-coll. Eduardo Emery, E.O. Emery *leg*., DZ 33.981 (DZUP); Parque Nacional de Brasília, 1♂, 22.xi.2009, Ex-coll. Eduardo Emery, E.O. Emery *leg*., DZ 33.973 (DZUP); **Planaltina** (1000 m), 1♂, 1♀, 27.ix.1984, V.O. Becker *leg*., Col. Becker n. 702 (CJC); Centro de Pesquisas Agropecuárias dos Cerrados, 4♂, 1♀, 09.v.2010, E.O. Emery *leg*., (CJC); Centro de Pesquisas Agropecuárias dos Cerrados, 9♂, 09.v.2010, Ex-coll. Eduardo Emery, E.O. Emery *leg*., DZ 33.975., DZ 33.976, DZ 33.977, DZ 33.978, DZ 33.982, DZ 33.983, DZ 33.984, DZ 33.985, DZ 33.986, (DZUP); *Goiás*: **Campinas [currently called Goiânia],** 2♂, 1♀, iii.1930, R. Spitz *leg*. (MZUSP); **Cocalzinho de Goiás**, (1320 m), 15º47’S 48º49’W, 3♂, 28.i.2012, C.J. Callaghan *leg*., GENITALIA #1000♂ and GENITALIA #1023♂, respectively (CJC); *Minas Gerais*: **Araguari,** ‘Engenheiro Bethould’, 1♂, xi.1927, B. Pohl *leg*. (MZUSP); **Corinto**, 1♂, 24.v.1979, C. Ellas *leg*., GENITALIA nº 2017-05♂ J. R. A. Lemes, DZ 16.615 (DZUP); **Curvelo**, Cabeceira do Córrego Lei, 1♂, 15.vi.1972, Mielke & Brown *leg*., GENITALIA nº 2017-03♂ J. R. A. Lemes, DZ 16.531 (DZUP); **Leopoldo de Bulhões**, 1♂, xi.1937, R. Spitz *leg*. (MZUPS); **Santana do Riacho**, Serra do Cipó, 3♂, 25.iii.2014, L.A. Kaminski *leg*., DNA-vouchers LAK-380, LAK-381 and LAK-382, GENITALIA nº 2016-05♂ J. R. A. Lemes, GENITALIA nº 2016-09♂ J. R. A. Lemes and GENITALIA nº 2016-01♂ J. R. A. Lemes, respectively; Parque Nacional Serra do Cipó, 1♂, 26-29.v.2013, J. Carrera *leg*., DNA-voucher LAK-468; 1♂, 13.v.2011, DNA-voucher BLU019, ZUEC-LEP 4279 (ZUEC); 1♂, 07-10.v.2002, A. Freitas *leg*. (ZUEC); 1♀, 04-07.xi.1991, A. Freitas *leg*. (ZUEC); *São Paulo*: **Atibaia,** Parque Municipal Grota Funda, 1♂, 14.xi.1998, A.

Freitas *leg*. (ZUEC); **Itirapina**, Estação Ecológica Itirapina (S 22º13’ W 47º54’), 1♂, 01.iv.2001, K. Brown *leg*., ZUEC-LEP 4279 (ZUEC).

***Aricoris* sp. 1** Callaghan, Lemes & Kaminski, sp. nov.

(Figs. 14-17, 29, 38-39, 51-52, 61 and 63)

Diagnosis. *Aricoris* sp 1. sp. nov. presents a row of ocelli at the marginal bands of the wings surrounded by yellow scales. A rounded line separates the proximal and distal halves of the hindwing. It differs from *A. tutana,* by having a band of light yellowish scales at the postdiscal area of wings, especially noticeable on the dorsal surface (Fig. 29). The valvae of the male is unique among the species of the *Aricoris constantius* group for having a pointed projection at the superior medial region in lateral view (Fig. 38), and with a lateral spine-shaped projection at the anterior region of valvae, in ventral view (Fig. 39). The sclerotized portion of ductus bursae is similar to *A*. sp. 2 sp. nov. (Figs. 51), but *A*. sp. 1 sp. nov. has short tips on the anterior region of signa (Fig. 52), unlike *A*. sp. 2 sp. nov., which has large ones.

Male description.

Forewing size and shape: Average forewing length 20.5 mm, range 20-21 mm (n = 2). Wing shape as in *Aricoris tutana*, but with costa margin straighter.

Dorsal forewing surface: Proximal half dark brown, distal half lighter, separated by an irregular line, bordered by a band of light yellowish scales at the postdiscal area. Veins dark brown. Fringe brown with a dark marginal line basally. Presence of a row of seven ocelli at the marginal band, formed by brown scales slightly darker than the region and slightly surrounded by yellowish scales.

Ventral forewing surface: Ground colour light brown. Irregular line with dark brown scales separating proximal and distal half, bordered by a subtle band of light yellowish scales at the postdiscal area. Fringe as on the dorsal surface. Discal cell with three irregular spots surrounded by dark scales. Presence of two darker scale spots at CuA_2. Presence of a row of seven ocelli at the marginal band formed by light brown scales and surrounded by slightly yellowish scales.

Hindwing size and shape: Average hindwing length 15.5 mm, range 15-16 mm ($n = 2$). Wing shape less elongated than in *A. tutana*.

Dorsal hindwing surface: Proximal half dark brown, distal half lighter, separated by an irregular line, as on forewing, bordered by a band of light yellowish scales at the postdiscal area. Fringe brown, with a dark marginal line, as on forewing. Presence of a row of seven large black ocelli, of approximately the same diameter, clearly marked, them all surrounded completely by yellowish scales at the marginal band, and an eighth ocellus at the region of the tornus, that is remarkably smaller than the others.

Ventral hindwing surface: Ground colour light brown. Irregular line with dark brown scales separating basal and distal half, bordered by a subtle band of light yellowish scales at the postdiscal area. Fringe as on the dorsal surface. Presence of a row of eight black ocelli at the marginal band surrounded by faint yellowish scales. The fourth and fifth ocelli, between space M_2-CuA_1 usually scarcely marked.

Head: Brown dorsally, long yellowish-brown scales at the frontoclypeus and labial palpi. Galea black and robust. Labial palpi flattened laterally, curved upwards. Antenna brown, cylindrical articles. Length of antenna surpassing half the length of the forewing.

Thorax: Dark brown, covered by bands of long yellowish-brown scales dorsally and ventrally. Legs brown.

Genitalia (Fig. 38-39): Uncus in lateral view slightly rounded, wider than long; in dorsal view lobes are widely separated with U-shaped area between them. Scaphium long. Tegumen in lateral view wider than long, concave region at the posterior surface and upper margin elevated in relation to the uncus upper margin; inferior distal projection of the tegumem which connects with the gnathos long. Gnathos with hook-shaped tips directed upwards. Valvae in lateral view with a pointed projection at the superior medial region and extensive membranous area at centre and posterior region surface thin and slightly inclined; in ventral view, anterior region slightly enlarged, with a lateral spine-shaped projection, and posterior region thinner, with two to four spines at the tip. Saccus-shape variable, connected with the anterior region of tegumen by a membranous area. Aedeagus long, pointed, three times the length of tegumen + uncus.

Female description.

Forewing size and shape: Average forewing length 18.71 mm, range 18-21 mm (n = 7). Like male, but with distal margin slightly more rounded.

Dorsal forewing surface: Like males, but with a background colour lighter. Yellowish scales surrounding ocelli more conspicuous than in males.

Ventral forewing surface: Like males, but with a background colour lighter.

Hindwing size and shape: Average hindwing length 16 mm, range 15-17 mm (n = 3). Like male.

Dorsal hindwing surface: Like males, but with a background colour lighter.

Ventral hindwing surface: Like males, but a background colour lighter.

Head: Like males, but the galea is brown and slender.

Thorax: Like males.

Genitalia (Fig. 51-52): Corpus bursae membranous, with a pair of two elongated signa of approximately same size. Signa pointed posteriorly, anterior region wider and concave with short tips. Ductus bursae membranous, length slightly less than twice the length of the corpus bursae. Sclerotized portion of ductus bursae funnel-shaped, with membranous region in the middle. Invaginated pocket between ostium bursae and papillae anales bifurcated. Papillae anales with setae at outer edge.

Distribution and biology (Fig. 58, 61 and 63)**.** This species is endemic of Brazilian cerrado (Distrito Federal, Goiás, Minas Gerais, São Paulo), inhabiting areas of rocky outcrops named as “cerrado rupestre”. This species is sympatric with *A. tutana* and *A.* sp. 2 sp. nov.

Holotype. Adult male (Figs. 14-15), DZ 33.967, deposited in Coleção Entomológica Padre Jesus Santiago Moure, Federal University of Curitiba, Curitiba, Paraná, Brazil. *Type locality*: Parque Nacional de Brasília, Brasília, Distrito Federal, Brazil; specimen collected on 22 November 2009 by Eduardo de Oliveira Emery. The holotype contains the following labels (labels separated by “/” and lines inside same label separated by “-”): /*Aricoris* sp. 1 - Callaghan, Lemes & Kaminski - HOLOTYPUS♂/ Brasil, Distrito Federal - Parque Nacional de Brasília - Data: 22/11/2009 - Eduardo de Oliveira Emery/ EX COLEÇÃO EDUARDO EMERY/ DZ 33.967/.

Allotype. Adult female (Figs. 16-17), DZ 16.643, deposited in Coleção Entomológica Padre Jesus Santiago Moure, Federal University of Curitiba, Curitiba, Paraná, Brazil. *Type locality*: Corinto, Minas Gerais, Brazil; specimen collected on 10 October 1979 by C. Elias. The allotype contains the following labels (labels separated by “/” and lines inside same label separated by “-”): /*Aricoris* sp. 1 - Callaghan, Lemes & Kaminski - ALOTYPUS♀/ Corinto, MG – 10.x.1979 – C. Elias leg/ GENITALIA nº - 2017-17♀ J. R. A. Lemes/ DZ 16.643/.

Paratypes (four males and eight females). **BRAZIL –** *Distrito Federal*: **Brasília**, Fazenda Água Limpa, 1♀, 21.ii.1976, Gifford *leg.*, DZ 16.306 (DZUP); *Goiás*: **Formosa**, Fazenda Itiguira, 1♀, 21.x.1971, K.S. Brown *leg.*, GENITALIA nº 2017-18♀ J. R. A. Lemes; **Goiás Velho,** Cachoeira das Andorinhas, 1♂, 27.iii.2013, J.Y.O. Carrera *leg.*, DNA-voucher LAK-170, GENITALIA nº 2016-06♂ J. R. A. Lemes; 1♀, xii.1976, ex-coll. Gifford, Gifford *leg.*, GENITALIA nº 2017-16♀ J. R. A. Lemes, DZ 16.348 (DZUP); **Pirenópolis,** 1♂, 11.v.2012, L.A. Kaminski *leg.*, DNA-voucher LAK-165, GENITALIA nº 2016-02♂ J. R. A. Lemes; **Santo Antônio de Goiás**, 1♀, 28.iii.2013, J.Y.O. Carrera *leg.*, DNA-voucher LAK-169, GENITALIA nº 2016-10♀; *Minas Gerais*: **Araguari,** 1♂, xii.1931, R. Spitz *leg.*, GENITALIA nº 2017-26♂ J. R. A. Lemes, MZUSP 46870 (MZUSP); **Diamantina,** (1300 m), 1♀, 26-28.xi.1988, O. and E.J. Mielke *leg.*, GENITALIA nº 2017-29♀ J. R. A. Lemes, OM 19.561 (OM); (1300 m), 1♀, 26-28.xi.1988, O. and E.J. Mielke *leg.*, GENITALIA nº 2017-30♀ J. R. A. Lemes, OM 19.562 (OM); **Pirapora,**); 1♂, no collection data, GENITALIA nº 2017-25♂ J. R. A. Lemes, MZUSP 54432 (MZUSP); *São Paulo*: **Patrocínio Paulista**, Parque Estadual das Furnas do Bom Jesus, 1♀, 22.ii.2001, K.S. Brown *leg.*, DNA-voucher LAK-467; 1♀, 22.ii.2001, K.S. Brown *leg.*, DNA-voucher LAK-383, GENITALIA nº 2017-10♀ J. R. A. Lemes.

Additional material examined. BRAZIL – *Goiás:* 1♀, no collection data (MZUSP); **BRAZIL** - *Distrito Federal*: **Brasília**, Jardim Zoológico, 1♀, 13.xi.1977, A. Negrett *leg.*, (CJC); Parque Nacional de Brasília, 2♂, 15.xi.2009, E.O. Emery *leg.*, genitalia #1024, (CJC); Parque Nacional de Brasília, 3♂, 15.xi.2009, Ex-coll. Eduardo

Emery, E.O. Emery *leg*., DZ 33.968, DZ 33.969, DZ 33.971 (DZUP); Parque Nacional de Brasília, 2♂, 22.xi.2009, E.O. Emery *leg*., genitalia #1139, (CJC); Parque Nacional de Brasília, 2♂, 22.xi.2009, Ex-coll. Eduardo Emery, E.O. Emery *leg*., DZ 33.970, DZ 33.972 (DZUP); *Goiás*: **Cocalzinho de Goiás**, Serra dos Pirineus (Cerrado Rupestre, 1320 m), 15º47'39.89''S 48º49'49.42''W, 1♀, 05.xii.2010, E.O. Emery *leg*., (CJC); 15º47'S 48º49'W, 1♂, 28.i.2012, C.J. Callaghan *leg*., genitalia #1140, (CJC); **Goiás Velho,** Serra Dourada, 1♂, 23-26.?.1973, C.J. Callaghan *leg*., (CJC); Parque Estadual dos Pirineus**,** 1♂, 20.xi.2010, A. Freitas *leg*. (ZUEC); **Pirenópolis**, (Cerrado Rupestre, 1290 m), 15º47'23.98''S 48º50'07.50''W, 1♀, 09.x.2010, E.O. Emery *leg*., genitalia #1025♀, (CJC); **Vianópolis**, 1♀, R. Spitz *leg*. (MZUSP); *Minas Gerais*: 1♀, R. Spitz *leg*. (MZUSP); **Pirapora,** 1♂, (MZUSP).

***Aricoris* sp. 2** Lemes & Kaminski, sp. nov.

(Figs. 18-21, 30, 40-41, 53-54 and 61)

Aricoris tutana (Godart, [1824]): Callaghan, 2010: 399, figs. 9-10; Casagrande et al, 2012: 300 (table), 307, figs. 193-196.

Diagnosis. *Aricoris* sp. 2 sp. nov. can be distinguished from the others by presenting an irregular line that separates the proximal and distal halves of the hindwing, as in *A.* sp. 1 sp. nov.(Fig. 30). It presents a row of reduced ocelli on the fore and hindwings surrounded by yellow rings, differently of *A. constantius*, *A. monotona* and *A.* sp. 1 sp. nov. Valvae without projections and thinner in the ventral view (Fig. 41). Male genitalia (Figs. 40-41) cannot be differentiated from *A.* sp. 3 sp. nov. Female genitalia with sclerotized portion of ductus bursae with membranous region in the middle (Fig. 53), like in *A.* sp. 1 sp. nov., and unlike the other species, anterior region of the signa with large tips (Fig. 54),

Male description.

Forewing size and shape: Average forewing length 16 mm (n = 3). Wing shape as in *A*. sp. 1 sp. nov. and *A*. sp. 3 sp. nov.

*Dorsal forewing surface***:** Proximal half dark brown, distal half lighter, separated by an irregular line. Veins dark brown. Fringe brown with a dark marginal line basally. Presence of a row of seven reduced ocelli at the marginal band, evidenced by the slightly yellowish scales surrounding them.

Ventral forewing surface: Ground colour light brown. Irregular line with dark brown scales separating basal and distal half. Fringe as on the dorsal surface. Discal cell with three irregular spots surrounded by dark scales. Presence of two irregular spots surrounded by dark scales at CuA_2. Presence of a row of seven ocelli at the marginal band formed of brown scales, evidenced by the region of slightly yellowish scales surrounding them, usually the seventh and eighth at the tornus region more marked.

Hindwing size and shape: Average hindwing length 13.6 mm, range 13-14 mm (n = 3). Wing shape as in *A.* sp.1 sp. nov. and *A.* sp.3 sp. nov.

Dorsal hindwing surface: Proximal half dark brown, distal half lighter, separated by an irregular line, as on forewing. Fringe brown, with a dark marginal line, as on forewing. Presence of a row of seven small ocelli, black, surrounded completely by yellowish scales, of approximately the same diameter at the marginal band, except for the last one at the region of the tornus.

Ventral hindwing surface: Ground colour light brown. Irregular line with dark brown scales separating proximal and distal half. Absence of a slightly band of light yellowish scales at the postdiscal area. Fringe as on the dorsal surface. Presence of a row of eight black ocelli at the marginal band surrounded by yellowish scales. The fourth and fifth ocelli, between space M_2-CuA_1, usually scarcely marked.

Head: Brown dorsally, with long yellowish-brown scales on the frontoclypeus and labial palpi. Galea black and robust. Labial palpi flattened laterally, curved upwards. Antenna brown, cylindrical articles. Length of antenna slightly more than half the length of the forewing.

Thorax: Dark brown, covered by bands of long yellowish-brown scales dorsally and ventrally. Legs brown.

Genitalia (Fig. 40-41): Uncus in lateral view slightly rounded, slightly wider than long; in dorsal view lobes are slightly separated with V-shaped area between them. Scaphium long. Tegumen in lateral view wider than long, concave region at the posterior surface and upper margin elevated in relation to the uncus upper margin; inferior distal projection of the tegumen which connects with the gnathos long. Gnathos with hook-shaped tips directed upwards. Valvae in lateral view sinuous; extensive membranous area at the centre, posterior region wide and slightly inclined; in ventral view, anterior region slightly enlarged, without projections, posterior region thinner, with two to four spines at the tip. Saccus-shape variable, connected with the anterior region of tegumen by a membranous area. Aedeagus long, pointed, three times the length of tegumen + uncus.

Female description. *Forewing size and shape*: Average forewing length 18.3 mm, range 17-21 mm (n = 3). Like male with distal margins slightly rounded.

Dorsal forewing surface: Like males, but with a background colour lighter. Yellowish area surrounding ocelli more conspicuous than in males.

Ventral forewing surface: Like males, but with a background colour lighter.

Hindwing size and shape: Average hindwing length 16.6 mm, range 16-18 mm (n = 3). Like male with distal margins slightly rounded.

Dorsal hindwing surface: Like males, but with a background colour lighter.

Ventral hindwing surface: Like males, but with a background colour lighter and presence of the yellowish band at the postdiscal area.

Head: Like males, but the galea is brown and slender and labial palpi has yellow scales.

Thorax: Like males, but pleura and ventral surface covered with yellowish scales.

Genitalia (Fig. 53-54): Corpus bursae membranous, with a pair of two elongated signa of approximately same size. Signa pointed posteriorly, anterior region wider and with large tips. Ductus bursae membranous, length slightly less than twice the length of the corpus bursae. Sclerotized portion of ductus bursae funnel-shaped, with a more sclerotized portion at the apex. Invaginated pocket between ostium bursae and papillae anales slightly bifurcated. Papillae anales with setae at outer edge.

Distribution and biology (Fig. 58 and 61)**.** Brazil (Distrito Federal, Mato Grosso do Sul and Paraná). This species is sympatric with *A. tutana* and *A.* sp. 1 sp. nov. in Distrito Federal and with *A. monotona* in Paraná, in a region where it is found the south “cerrado”, with different phytophysiognomies, ranging from “cerrado sensu stricto”, to grassland, “campo limpo” and “campo sujo” (Uhlmann, Galvão, & Silva, 1998).

Holotype. Adult male (Figs. 18-19), DZ 16.622, genitalia prepared by José Lemes (GENITALIA nº 2017-01♂ J. R. Lemes), deposited in Coleção Entomológica Padre Jesus Santiago Moure, Federal University of Curitiba, Curitiba, Paraná, Brazil. *Type locality*: Jaguariaíva, Paraná, Brazil; specimen collected on 17 Nov. 1976 by O. Mielke and Z. Buzzi. The holotype contains the following labels (labels separated by "/" and lines inside same label separated by "-"): /*Aricoris* sp. 2 – Lemes & Kaminski - HOLOTYPUS♂/ 17.XI-1976 - Jaguariaíva, PR - Mielke -Buzzi leg/ GENITALIA nº - 2017-01♂ - J. R. A. Lemes/ DNA-Voucher – LAK-502/ DZ 16.622/.

Allotype. Adult female (Figs. 20-21), DZ 13.390, genitalia prepared by José Lemes (GENITALIA nº 2017-19♀ J. R. A. Lemes), deposited in Coleção Entomológica Padre Jesus Santiago Moure, Federal University of Curitiba, Curitiba, Paraná, Brazil. *Type locality*: Brasília, Distrito Federal, Brazil; specimen collected on Nov. 1978 by Raw. The allotype contains the following labels (labels separated by "/" and lines inside same label separated by "-"): / *Aricoris* sp. 2 – Lemes & Kaminski - ALOTYPUS♀/ IX-1978 – Brasília, DF – Raw leg/ GENITALIA nº - 2017-19♀ - J. R. A. Lemes/ DNA-Voucher – LAK-504 - DZ 16.390/.

Paratypes (four males and four females)**. BRAZIL** - *Distrito Federal*: **Brasília**, Fazenda Água Lima, 1♂, 22.x.1976, ex-coll. Gifford, D. Giford *leg.*, GENITALIA nº 2017-11♂ J. R. A. Lemes, DZ 16.313 (DZUP); *Mato Grosso do Sul*: **Porto Murtinho, 1**♀, xi-xii.1929, R. Spitz *leg.*, GENITALIA nº 2017-27♀ J. R. A. Lemes, MZUSP 54434 (MZUSP); 1♀, xi-xii.1929, R. Spitz *leg.*, GENITALIA nº 2017-28♀ J. R. A. Lemes, MZUSP 54435 (MZUSP); *Paraná*: **Ponta Grossa**, Vila Velha (900 m), 1♂, 08.iii.1971, Mielke *leg.*, GENITALIA nº 2017-02♂ J. R. A. Lemes, DNA-Voucher LAK-503, DZ 16.545 (DZUP); Vila Velha (1000 m), 1♂, 1♀, 14.x.1967, Mielke *leg.*, GENITALIA nº 2017-12♂ J. R. A. Lemes, DZ 16.566 (DZUP); Vila Velha (1000 m), 1♀, 14.x.1967, Mielke *leg*., GENITALIA nº 2017-20♀ J. R. A. Lemes, DZ 16.573 (DZUP); Vila Velha (1000 m), 1♀, 14.x.1967, Mielke *leg*., genitalia 2017-08♀ J. R. A. Lemes, DZ 16.580 (DZUP).

Remarks. Although we could not obtain the barcode gene sequence for *A.* sp. 2 sp. nov.**,** we have found morphological characters on female genitalia and wing colour patterns that we consider sufficient to justify our taxonomic decision on describing this new species.

***Aricoris* sp. 3** Lemes & Kaminski, sp. nov.

(Figs. 22-25, 31, 42-43, 55-56 and 64)

Diagnosis. This species can be distinguished from the others by presenting an irregular line that separates the basal and distal halves of the hindwing, as in *A.* sp. 1 sp. nov. and *A.* sp. 2 sp. nov., but different from *A. tutana*. Discal area of the fore and hindwings with splashed orange scales (Fig. 31). Males hindwings in ventral view without ocelli on marginal area, unlike *A. constantius*, *A. tutana*, *A.* sp. 1 sp. nov. and *A.* sp. 2 sp. nov. Valvae in lateral and ventral view without projections and thinner in the ventral view when compared to *A. monotona* and *A. tutana*. Male genitalia (Figs. 42-43) cannot be differentiated from *A.* sp. 2 sp. nov., but female genitalia is distinct, presenting the sclerotized portion of ductus bursae without membranous region (Fig. 55) and the anterior region of the signa short (Fig. 56).

Male description.

Forewing size and shape: Average forewing length 15.5 mm, range 14-17 mm (n = 2). Wing shape as in *A.* sp. 1 sp. nov. and *A.* sp. 2 sp. nov.

Dorsal forewing surface: Proximal half dark brown, distal half lighter, separated by an irregular line. Veins dark brown. Fringe brown with a dark marginal line basally.

Presence of a row of seven ocelli at the marginal band of the forewing, formed by brown scales slightly darker than the distal half without a ring of yellowish scales surrounding them.

Ventral forewing surface: Ground colour light brown. Irregular line with dark brown scales separating proximal and distal half. Fringe as on the dorsal surface. Discal cell with three irregular spots surrounded by dark scales. Postdiscal and discal area of the wings with splashed orange scales. Presence of two darker scale spots at CuA_2. Row of seven ocelli at the marginal band of the wing weekly marked.

Hindwing size and shape: Average hindwing length 13 mm, range 12-14 mm (n = 2). Wing shape as in *A.* sp. 1 sp. nov. and *A.* sp. 2 sp. nov.

Dorsal hindwing surface: Proximal half dark brown, distal half lighter, separated by an irregular line, as on forewing. Fringe brown as on the distal half, with a dark marginal line basally, as on forewing. Presence of a row of seven ocelli at the margin of the wing, not surrounded by a yellowish scales ring.

Ventral hindwing surface: Ground colour light brown. Irregular line with dark brown scales separating proximal and distal half. Postdiscal and discal area with splashed orange scales. Fringe as on the dorsal surface. Row of ocelli at the marginal band absent or almost imperceptible.

Head: Brown dorsally, brownish scales at the frontoclypeus and labial palpi. Galea black and robust. Labial palpi flattened laterally, curved upwards. Antenna brown, cylindrical articles. Length of antenna slightly more than half the length of the forewing.

Thorax: Dark brown, covered by bands of brown scales and hair dorsally and long whitish hair ventrally. Legs brown.

Genitalia (Figs. 42-43): Uncus in lateral view slightly rounded, slightly wider than long; in dorsal view lobes are slightly separated with U-shaped area between them. Scaphium long. Tegumen in lateral view wider than long, concave region at the posterior surface and upper margin elevated in relation to the uncus upper margin; inferior distal projection of the tegumem which connects with the gnathos long. Gnathos with hook-shaped tips directed upwards. Valvae in lateral view without a pointed projection in the superior medial region; extensive membranous area at the centre, posterior region wide and slightly inclined; in ventral view, anterior region slightly enlarged, without a lateral spine-shaped projection, posterior region thinner, with two to four spines at the tip. Saccus-shape variable,, connected with the anterior region of tegumen by a membranous area. Aedeagus long, pointed, three times the length of tegumen + uncus.

Female description.

Forewing size and shape: Average forewing length 18.66 mm, range 18-19 mm (n = 3). Like male, but with distal margin slightly more rounded.

Dorsal forewing surface: Like males, but with a yellowish area surrounding the ocelli. Presence of a yellowish band of scales at the postdiscal area.

Ventral forewing surface: Like males, but with a background color lighter.

Hindwing size and shape: Average hindwing length 15.66 mm, range 15-17 mm (n = 3). Like male.

Dorsal hindwing surface: Like males, but with ocelli more conspicuous.

Ventral hindwing surface: Similar males, but a background color lighter and presence of a row of eight black ocelli surrounded slightly by a yellowish ring. The fourth and fifth ocelli, between space M_2-CuA_2, scarcely marked.

Head: Like males, but the galea is brown and slender. Labial palp with whitish scales at labial palp

Thorax: Like males.

Genitalia (Figs. 55-56): Corpus bursae membranous, with a pair of two elongated signa of approximately same size. Signa pointed posteriorly, anterior region wider and concave with short tips. Ductus bursae membranous, length slightly less than twice the length of the corpus bursae. Sclerotized portion of ductus bursae funnel-shaped, without any membranous region. Invaginated pocket between ostium bursae and papillae anales bifurcated. Papillae anales with setae at outer edge.

Distribution and biology (Fig. 58 and 63)**.** Brazil (Bahia and Minas Gerais). Apparently, this species is endemic of the mountain chain of “Serra do Espinhaço” in their northern portion, such as “Pico da Formosa” and in “State Park Serra Nova” in Minas Gerais.

Holotype. Adult male (Figs. 22-23 and 31), DNA-Voucher LAK-483, genitalia prepared by José Lemes (GENITALIA nº 2017-24♂ J. R. A. Lemes), deposited in Coleção Entomológica Padre Jesus Santiago Moure, Federal University of Curitiba, Curitiba, Paraná, Brazil. *Type locality*: Santo Antônio do Retiro, Minas Gerais, Brazil; specimen collected between 30 November and 06 December 2015 by Lucas Perillo (utilizing Malaise trap). The holotype contains the following labels (labels separated by “/” and lines inside same label separated by “-”): /*Aricoris* sp. 3– Lemes & Kaminski - HOLOTYPUS♂/ BRASIL, Minas Gerais – Santo Antônio do Retiro – 30.xi-06.xii.2016

– Lucas Perillo *leg.* – (Malaise trap)/ GENITALIA nº - 2017-24 ♂ - J. R. A. Lemes/ DNA-Voucher – LAK-483/.

Allotype. Adult female (Figs. 24-25), DNA-voucher LAK-480, genitalia prepared by José Lemes (GENITALIA nº 2017-23♀ J. R. A. Lemes), deposited in Coleção Entomológica Padre Jesus Santiago Moure, Federal University of Curitiba, Curitiba, Paraná, Brazil. *Type locality*: Santo Antônio do Retiro, Minas Gerais, Brazil; specimen collected between 30 November and 06 December 2015 by Lucas Perillo (utilizing Malaise trap). The holotype contains the following labels (labels separated by "/" and lines inside same label separated by "-"): / *Aricoris* sp. 3 – Lemes & Kaminski - ALOTYPUS♀/ BRASIL, Minas Gerais – Santo Antônio do Retiro – 30.xi-06.xii.2016 – Lucas Perillo *leg./* GENITALIA nº - 2017-23♀ - J. R. A. Lemes/ DNA-Voucher – LAK-480/.

Paratypes (one male and two females)**. BRAZIL** – *Minas Gerais*: **Porteirinha**, 1♀, 08-14.xii.2015, A. Rosa leg., DNA-voucher LAK-479, GENITALIA nº 2017-21♀ J. R. A. Lemes; **Santo Antônio do Retiro**, 1♂, 30.xi.2015-06.xii.2015, L. Perillo leg., DNA-voucher LAK-482, GENITALIA nº 2017-22♂ J. R. A. Lemes; **Santo Antônio do Retiro**, 1♀, 30.xi.2015-06.xii.2015, L. Perillo leg., DNA-vouchers LAK-481.

Additional material examined. BRAZIL – *Bahia*: **Abaíra**, 1♂, 03.xi.2013, Kerpel & Ferreira Jr. leg. (CSTR).

Remarks. The material type of this species was collected with a Malaise trap and, therefore, the specimens remained for a few days in alcohol. There may have been some changes in the color tones of the wing scales, however, the visible wing characters are still informative. In addition, the characteristics of female genitalia as well as the molecular sequencing of *COI* justify the description of this new species.

Identification key to adults of the “*Aricoris constantius* species complex”

1. Uniform colour without the row of dark ocelli at the marginal bands of the fore and hindwings (Figs. 6-7 and 66), rarely few ocelli weakly present on the ventral side of the wings of a few individuals (Figs. 9 and 27). Presence of an evident light-yellow band at the postdiscal regions of wings, and orange bristles at the dorsal region of the prothorax and thorax ventrally (Fig. 66)……………………………. ***Aricoris monotona***

1’. Presence of a visible row of dark ocelli, at least on hindwing. Light yellow band at the postdiscal regions of wings and orange bristles at the dorsal region of the prothorax and thorax ventrally can or not be presented……………………………..2

2. Presence of a very straight line on the hindwings separating the basal and distal halves (Figs. 2-5, 26 and 65). Row of marginal ocelli on the hindwings without a ring of yellowish scales surrounding them (Figs. 2-5, 26 and 65)….***Aricoris constantius***

2’. Line that separates the basal and distal halves of the hindwings more rounded (Figs. 27-30). Marginal ocelli usually with a ring of yellowish scales surrounding them………………….………………………………………………….……..3

3. Presence of orange bristles at the dorsal region of the prothorax and thorax ventrally (Fig. 67). Presence of a line that separates the basal and distal halves of the hindwings nearly always uniformly rounded (Fig. 28)…....................***Aricoris tutana***

3’. Absence of range bristles at the dorsal region of the prothorax and thorax ventrally. Line that separates the basal and distal halves of the hindwings irregular (Fig. 30)……………………………………………………………………………..4

4. Presence of a row of large ocelli on the marginal area of hindwings (Figs. 14-17 and 29). Female wings with a yellowish tone background (Figs. 16-17). Male genitalia with valvae in lateral view with a pointed projection at the upper medial region (Fig. 38) and with a lateral spine-shaped projection on lateral view (Fig. 39). Female genitalia with anterior region of the signa with short tips (Fig. 52)…………………………………………………..……….***Aricoris* sp. 1 sp. nov.**

4’. Presence of a row of small ocelli on the marginal area of hindwings. Female wings without a yellowish tone background. Male genitalia without projections. Signa can present short or large tips...5

5. Wings without splashed orange scales on discal area (Figs. 18-21 and 30). Males forewings on ventral view with ocelli on marginal area (Fig. 19). Sclerotized portion of ductus bursae of the female genitalia with membranous region in the middle (Fig. 53) and signa with large tips (Fig. 54) and...........................***Aricoris* sp. 2 sp. nov.**

5’. Wings with splashed orange scales on discal and postdiscal areas (Figs. 22-25 and 31). Males ventral forewings without ocelli on marginal area (Figs. 23 and 31). Sclerotized portion of ductus bursae of the female genitalia without membranous region in the middle (Fig. 55) and signa with short tips (Fig. 56) and …………………….. ***Aricoris* sp. 3 sp. nov.**

Discussion

The *Aricoris constantius* group has passed through a very troubled taxonomic history. Cleary, Hall & Harvey (2002) were precipitated in considering all species names in this group as synonyms. So that, Callaghan (2010), based on classic morphological characteristics was able to identify three different taxa. In addition, our integrated taxonomy shows that the *Aricoris constantius* group is formed, at least, by six species, with strong correlation between morphology and molecular data. In fact, we consider the possibility that additional new species belonging to the *Aricoris constantius* group are to be described in the next years. The records of *A. tutana* from Paraguay and Argentina (Contreras Chialchia, 2009; Núñez Bustos, 2017) need to be dissected and sequenced for a proper identification, as those populations inhabit environments distinct from observed in nominal Brazilian populations. Also, we had access to barcode region of a specimen (BLU-038) from Minas Gerais that belongs to an unidentified species in our phylogenetic analysis (Fig. 1; Table 1). Unfortunately, this voucher has been lost and its identity remains unknown. In addition, an unpublished photographic record indicates the occurrence of an unidentified species of *A. tutana* complex in the Pampas del Heath in Peru.

Our molecular data shows that *A. monotona* and *A. tutana* are close related species, agreeing with morphological characters. The pattern of spots on the wings in the new species herein described is very similar to each other plus *A. tutana* and the intraspecific variation of these species sometimes overlaps. Furthermore, more than one species may occur in sympatry (Fig. 58). For this reason, we suggest the denomination *A. tutana* complex for include *A. tutana* and the three new species. Though this complex is formed by species hard to identify, the combination of male and/or female genitalia are useful to distinguish species, that jointly with the molecular analyses justify the delimitation of these species as different lineages and the necessity of describing them.

Aricoris constantius is the only species of the group inhabiting forest environments, while the other species live only in open grassland areas. The open questions that remain are: how these species have evolved? Do they use the same resources and have evolved by resource partitioning those, or have they speciated by allopatric processes, and then have expanded their distributions and now they co-exist together? We have observed that *A. constantius* is an obligated associated-ant that feeds on honeydew-producing hemipterans (Neto et al., in prep.). Despite we know nothing about the biology of the other species of the group, the presence of greasy on the wings indicates carnivory in the larval stages (DeVries 1997; Hall & Harvey, 2002). Moreover, Callaghan (2010) mentions that there is evidence of adults of one of the species belonging to the *Aricoris tutana* complex emerging from ant's nets. There are records of *Aricoris* species living inside ants' nests, being an indication that some species in this genus can be social parasites (Kaminski et al. in prep.). Studies on natural history of these butterflies are extremely necessary and, possibly, will help us to understand the speciation processes within the group, besides contributing with one more dimension of the integrative taxonomy.

Even with few details about the biology of the species in the *A. constantius* group, we presume that some of these butterflies may be threatened. In general, Riodinids are known for their restricted spatial distribution and low population levels (Bates 1859; Callaghan 1983), being thus very susceptible to extinction risks (Wootton, & Pfister, 2013). The open vegetation environments that most of these species inhabit in the Cerrado and Pampa are among the most threated and neglected biomes in Brazil, needing urgently the development of conservation strategies for sustainable management (Overbeck et al., 2007; Veldman et al., 2015). Anthropic actions such as extensive agriculture of soya, forestry, and livestock farming constitute major threats of these environments (Casagrande et al., 2012; Ganem, Drummond, Franco, 2013; Klink & Machado, 2005; Overbeck et al., 2007).

The fact that *Aricoris constantius* complex species are probably social parasites makes them even more vulnerable to environmental changes, due to the high specificity between larvae and ants in this type of myrmecophily (e.g. Ueda, Komatsu, Itino, Arai,

& Sakamato, 2016). Moreover, this risk is amplified if we consider that these species depend on a complex network of interactions involving hemipterans, ants and plants. As there is evidence of aphytophagy and social parasitism among the species of the *Aricoris constantius* group, more attention is required in what concerns the conservation status of these species. All these facts suggest important criterions to categorize some of these species as threatened under the criteria of the IUCN (2012).

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References

Als, T. D., Vila, R., Kandul, N. P., Nash, D. R., Yen, S.-H, Hsu, Y.-F, ... Pierce, N. E. (2004). The evolution of alternative parasitic life histories in large blue butterflies. *Nature, 432*, 386-390.

Bates, H. W. (1859). Notes on South American butterflies. *Transactions of the Entomological Society of London, new series*, *5*: 1-11.

Bellaver, J., Iserhard, C. A., Santos, J. P., Silva, A. K., Torres, M., Siewert, R. R., ... Romanowski, H. P. (2012). Borboletas (Lepidoptera: Papilionoidea e Hesperioidea) de Matas Paludosas e Matas de Restinga da Planície Costeira da região Sul do Brasil [Butterflies (Lepidoptera: Papilionoidea and Hesperioidea) from Matas Paludosas and Matas de Restinga of the Planície Costeira of Southern Brazil]. *Biota Neotropica*, *12*(4): 181-190.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., ... Das, I. (2007). Cryptic species as window on diversity and conservation. *Trends in Ecology and Evolution*, *22*(3), 148-155.

Biezanko, C. M., Mielke, O. H. H., & Vedderhoff, A. (1978). Contribuição ao estudo faunístico dos Riodinidae do Rio Grande do Sul, Brasil (Lepidoptera) [Contribution to the faunistic study of the Riodinidae of Rio Grande do Sul, Brazil]. *Acta Biológica Paranaense*, *7*(1, 2, 3, 4): 7-22.

Burns, J. M., Janzen, D. H., Hajibabaeim, M., Hallwachs, W., & Hebert, P. D. N. (2008). DNA barcodes and cryptic species of skipper butterflies in the genus *Perichares* in Area de Conservación Guanacaste, Costa Rica. *PNAS*, *105*(17), 6350-6355.

Callaghan, C. J. (1983). A study of isolating mechanisms among Neotropical butterflies of the subfamily Riodininae. *The Journal of Research on the Lepidoptera*, *21*: 159-176.

Callaghan, C. J. (2010). A re-evaluation of the *Aricoris constantius* group with the recognition of three species (Lepidoptera: Riodinidae). *Zoologia*, *27*(2), 395-402.

Callaghan, C. J., & Lamas, G. (2004). Riodinidae. In Lamas, G. (Ed.), *Checklist: Part 4A. Hesperioidea—Papilionoidea* (pp. 141–170), Gainesville, Fla: USA Association for Tropical Lepidoptera.

Carneiro, E., Mielke, O. H. H., & Casagrande, M. M. (2008). Borboletas do sul da ilha de Santa Catarina, Florianópolis, Santa Catarina, Brasil (Lepidoptera: Hesperioidea e Papilionoidea) [Southern butterflies of the island of Santa Catarina, Florianópolis, Santa Catarina, Brazil (Lepidoptera: Hesperioidea and Papilionoidea]. *SHILAP Revista de Lepidopterología*, *36*(142), 261-271.

Casagrande, M. M., Dolibaina, D. R., Carneiro, E., Dias, F. M. S., Leite, L. A. R., & Mielke, O. H. H. (2012). Borboletas (Hesperioidea e Papilionoidea) de Jaguariaíva, Paraná, Brasil: Inventário em um enclave de Cerrado Meridional [Butterflies (Hesperioidea and Papilionoidea) from Jaguariaíva, Paraná, Brazil: Inventory in a Southern Cerrado enclave]. In Carpanezzi O. T. B. & Campos J. B. (Org.), *Coletânea de Pesquisa do Parques Estaduais de Vila Velha, Cerrado e Guartelá* [Collection of Research of Parques Estaduais de Vila Velha, Cerrado and Guartelá]. (1st ed., Vol. 1, pp. 295-308), Curitiba: Idealle Editora e Publicidade Ltda.

Contreras Chialchia, A. O. (2009). Ocurrencia de *Aricoris tutana* (GODART, 1824) (Lepidoptera: Riodinidae) en la Ecorregión del Ñeembucú, en el Paraguay

Oriental [Occurrence of *Aricoris tutana* (GODART, 1824) (Lepidoptera: Riodinidae) in the Ñeembucú Ecoregion, in Eastern Paraguay]. *Azariana (Universidad Nacional de Pilar), 1*(12): 115-118.

D'Abrera, B. (1994). *Butterflies of the Neotropical Region. Part VI Riodinidae.* Victoria, Black Rock: Hill House.

Dayrat, B. (2005). Towards integrative taxonomy. *Biological Journal of the Linnean Society*, *85*(3), 407–415.

DeVries, P. J. (1991a). Mutualism between *Thisbe irenea* butterflies and ants, and the role of ant ecology in the evolution of larval–ant associations. *Biological Journal of the Linnean Society, 43,* 179–195.

DeVries, P. J. (1991b). Ecological and evolutionary patterns in myrmecophilous riodinid butterflies. In Huxley, C, & Cutler, D. F. (Eds.), Ant-Plant Interactions, (Chapter 12, pp. 143-156). New York, NY: Oxford University Press.

DeVries, P. J. (1997). *The butterflies of Costa Rica and their natural history II: Riodinidae*. (Vol. 2): Princeton University Press.

Dinca, V., Lukhtanov, V.A., Talavera, G., & Vila, R. (2011). Unexpected layers of cryptic diversity in wood white *Leptidea* butterflies. *Nature Communications*, *2*, 324.

Eastwood, R., Pierce, N. E., Kitching, R. L., & Hughes, J. M. (2006). Do ants enhance diversification in lycaenid butterflies? Phylogeographic evidence from a model myrmecophile, *Jalmenus evagoras*. *Evolution*, *60*(2), 315-327.

Espeland, M., Hall, J. P. W., DeVries, P. J., Lees, D. C., Cornwall, M., Hsu, T.-F., ... Pierce, N. E. (2015). Ancient Neotropical origin and recent recolonisation: Phylogeny, biogeography and diversification of the Riodinidae (Lepidoptera: Papilionoidea). *Molecular Phylogenetics and Evolution*, *93,* 296-306.

Fabricius, J.C. (1793). *Entomologia systematica emendata et aucta. Secundum classes, ordines, genera, species, adjectis synonimis, locis, observationibus, descriptionibus* [*Systematic entomologic amended and augmented. According to the classes, ordens, generos, espécies, synonyms added, locals, observations, descriptions*]. *Tom. III, Pars I,* C.G. Proft, Fil. et Soc., Hafniae.

Felder, C., & Felder, R. (1865-74). *Reise derösterreichischen Fregatte Novara um die Erde in den Jaren 1857, 1858, 1859 unter den Befehlen des Commodore B. von Wüllerstorf-Urbair. Zoologischer Theil. Zweiter Band. Zweite Abtheilung: Lepidoptera* [*Travel of the Austrian Fregatte Novara around the earth in the years 1857, 1858, 1859 under the orders of the Commodore B. of Wüllerstorf-Urbair. Zoological part. Second volume. Second Division: Lepidoptera*]. Carl Gerold's Sohn, Wein 1-4.

Fiedler, K. (1991). Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionoidea). *Zoologisches Forschungsinstitut und Museum Alexander Koenig Bonn - Bonner Zoologische Monographien, 31,* 1-211.

Fiedler, K. (1997). Life-history patterns of myrmecophilous butterflies and other insects: their implications on tropical species diversity. In Ulrich, H. (Ed.), Tropical biodiversity and systematics (pp. 71-92). - Pp. 71-92 in: ULRICH, H. (ed.): Tropical biodiversity and systematics. Bonn: Proceedings of the

International Symposium on Biodiversity & Systematics in Tropical Ecosystems, Museum Alexander Koenig.

Fiedler, K. (2012). The host genera of ant-parasitic Lycaenidae butterflies: A review. *Psyche*, *2012*, 1-10.

Ganem, R. S., Drummond, J. A., & Franco, J. L. de A. (2013). Conservations polices and control of habitat fragmentation in the Brazilian Cerrado Biome. *Ambiente & Sociedade*, *16*(3): 99-118.

Godart, J. B. (1824). In Latreille, P. A. & Godart, J. B. (Ed.). *Encyclopédie Méthodique* [*Methodical encyclopedia*], (pp. 329-828), 9(2) Paris: veuve Agasse.

Hall, J. P. W., & Harvey, D. J. (2002). Basal subtribes of the Nymphidiini (Lepidoptera: Riodinidae): phylogeny and myrmecophily. *Cladistics*, *18*(6), 539-569.

Hebert, P. D. N., Penton, E. H., Burns, J. M., Jazen, D. H., & Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *PNAS*, *101*(41), 14812-14817.

Huelsenbeck, J. P., Larget, B., Miller, R. E., & Ronquist, F. (2002). Potential applications and pitfalls of Bayesian inference of phylogeny. *Systematic Biology, 51*, 673–688.

Huelsenbeck, J. P., Ronquist, F., Nielsen, R. & Bollback, J. P. (2001). Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, *294,* 2310–2314.

International Commission on Zoological Nomenclature (1999). International Code of Zoological Nomenclature. International Trust for Zoological Nomenclature (4ed). Retrieved from http://www.iczn.org/

IUCN (2012). *IUCN Red List Categories and Criteria*, Version 3.1, (2nd ed.). IUCN, Gland, Switzerland.

Jones, W. (1745-1818). Willian Jones Collection [Jones' Icones]. Unpublished manuscript, Hope Entomological Collection, Oxford University Museum of Natural History, Oxford, United Kingdom.

Kaminski, L. A., & Carvalho-Filho, F. S. (2012). Life History of *Aricoris propitia* (Lepidoptera: Riodinidae) - A Myrmecophilous Butterfly Obligately Associated with Fire Ants. *Psyche*, *2012*, 1-10.

Kaminski, L. A., Freitas, A. V. L., & Oliveira, P. S. (2010). Interaction between Mutualisms: Ant-Tended Butterflies Exploit Enemy-Free Space Provided by Ant-Treehopper Associations. The American Naturalist, 176(3), 322-334.

Kaminski, L. A., Iserhard, C. A., & Freitas, A. V. L. (2016). *Thisbe silvestre* sp. nov. (Lepidoptera: Riodinidae): a new myrmecophilous butterfly from the Brazilian Atlantic Forest. *Austral Entomology*, *55*(2), 138-146.

Klink, C. A., & Machado, R. B. (2005). Conservation of the Brazilian Cerrado. *Conservation Biology*, *19*(3): 707-713.

Klots, A. (1970). *Taxonomist's glossary of genitalia in insects*. Munksgaard: Copenhagen.

Lumley, L. M., & Sperling, F. A. H. (2010). Integrating morphology and mitochondrial DNA for species delimitation within the spruce budworm (*Choristoneura fumiferana*) cryptic species complex (Lepidoptera: Tortricidae). *Systematic Entomology, 35,* 416-428.

Mielke, C. G. C. (1994). Papilionoidea e Hesperioidea (Lepidoptera) de Curitiba e seus arredores, Paraná, Brasil, com notas taxonômicas sobre Hesperiidae [Papilionoidea and Hesperioidea (Lepidoptera) of Curitiba and its surroundings, Paraná, Brazil, with taxonomic notes on Hesperiidae]. *Revista Brasileira de Zoologia, 11*(4): 759-776.

Miller, L. D. (1970). Nomenclature of wing veins and cells. *Journal of Research on the Lepidoptera, 8*(2), 37-48.

Núñez Bustos, E. (2017). Registros inéditos de mariposas diurnas (Lepidoptera: Papilionoidea) para Argentina III. Colección Núñez Bustos en el MACN [Unpublished records of diurnal butterflies (Lepidoptera: Papilionoidea) for Argentina III. Núñez Bustos Collection (MACN)]. *Tropical Lepidoptera Research, 27*(2): 78-85.

Núñez Bustos, E. (2015). Mariposas diurnas (Lepidoptera: Papilionoidea y Hesperioidea) del área de influencia del Embalse Yacyretá, Provincias de Corrientes y Misiones, Argentina [Daytime butterflies (Lepidoptera: Papilionoidea and Hesperioidea) from the area of influence of the Yacyretá Reservoir, Provinces of Corrientes and Misiones, Argentina]. In Baunl, V.; Homberg, M. & Capmourteres, V. *El Patrimonio Natural y Cultural en el área de influencia del Embalse de Yacyretá, Argentina* [The Natural and Cultural Heritage in the area of influence of the Yacyretá Reservoir, Argentina] (1st ed.). Ciudad Autónoma de Buenos Aires: Fundación de Historia Natural Félix de Azara.

Orlandin, E.; Favretto, M. A., Piovesan, M., & Santos, E. B. (2016). *Borboletas e Mariposas de Santa Catarina - Uma introdução* [Butterflies and Moths of Santa Catarina - An Introduction] (1st ed.). Campos Novos: Mario Arthur Favretto.

Overbeck, G. E., Müller, S. C., Fidelis, A., Pfadenhauer, J., Pillar, V. D., Blanco, C. C., ... Forneck, E. D. (2007). Brazil's neglected biome: The South Brazilian *Campos*. *Perspectives in Plant Ecology, Evolution and Systematics*, *9*: 101-116.

Palo Jr., H. (2017). *Borboletas do Brasil*. *Papilionidae, Pieridae, Lycaenidae e Riodinidae* [*Butterflies of Brazil. Papilionidae, Pieridae, Lycaenidae and Riodinidae*], (1st ed.) (Vol. 1.). São Carlos, SP, Brazil: Vento Verde Editora.

Penz, C. M., & DeVries, P. J. (1999). Preliminary Assessment of the Tribe Lemoniini (Lepidoptera: Riodinidae) Based on Adult Morphology. *American Museum of Natural History*, *3284*, 1-32.

Pérez, J. H., Gaviria-Ortiz, F. G., Santos, W. I. G., Carneiro, E., Mielke, O. H. H., & Casagrande, M. M. (2017). Long term survey of the butterfly fauna of Curitiba, Paraná, Brazil: How does a scientific collection gather local biodiversity information? (Lepidoptera: Papilionoidea). *SHILAP Revista de Lepidopterología*, *45*(179): 433-466.

Pierce, N. E. 1984. Amplified species diversity: a case study of an Australian lycaenid butterfly and its attendant ants. In R. I. Vane-Wright and P. R. Ackery (eds). The biology of butterflies (pp. 197-200). London: Academic Press.

Pierce, N. E. (1995). Predatory and Parasitic Lepidoptera: Carnivores Living on Plants. *Journal of the Lepidopterists' Society*, *49*(4), 412-453.

Pierce, N. E., Braby, M. F., Heath, A., Lohman, D. J., Mathew, J., Rand, D. B., & Travassos, M. A. (2002). The Ecology and Evolution of Ant Association in the Lycaenidae (Lepidoptera). *Annual Review of Entomology*, *47*, 733-771.

Pires, A. C. V., Beirão, M. V., Fernandes, G. W., Oliveira, I. F., Pereira, G. C. N., Silva, V. D., ... Duarte, M. (in press). Checklist of butterflies from the rupestrian grasslands of Serra do Cipó, Minas Gerais, Brazil (Lepidoptera: Papilionoidea). *SHILAP Revista de Lepidopterología*.

Pierce, N. E., Kitching, R. I., Buchley, R. C., Taylor, M. F. J., & Benbow, K. F. (1987). The costs and benifits of cooperation between the Australian lycaenid butterfly, *Jalmenus evagoras*, and its attendant ants. *Behavioral Ecology and Sociobiology*, *21*, 237-248.

Pierce, N. E., & Young, W. R. (1986). Lycaenid Butterflies and Ants: Two-Species Stable Equilibria in Mutualistic, Commensal, and Parasitic Interactions. *The American Naturalist*, *128*(2), 216-227.

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogeny inference under mixed models. *Bioinformatics 19*, 1572–1574.

Schlick-Steiner, B. C., Steiner, M. F., Seifert, B., Stauffer, C., Christian, E., & Crozier, R. H. (2010). Integrative Taxonomy: A Multisource Approach to Exploring Biodiversity. *Annual Review of Entomology, 55*, 421-438.

Seraphim, N., Kaminski, L. A., DeVries, P. J., Penz, C. M., Callaghan, C. J., Wahlberg, N., ... Freitas, A. V. L. (2018). Molecular phylogeny and higher systematics of metalmark butterflies (Lepidoptera: Riodinidae). *Systematic Entomology*.

Shorthouse, D. P. (2010). SimpleMappr, an online tool to produce publication-quality point maps. [Retrieved from http://www.simplemappr.net. Accessed May 22, 2017]

Stichel, H. (1910). Vorarbeiten zu einer Revision der Riodinidae Grote (Erycinidae Swains) (Lep Rhop) [Preliminary work on a revision of the Riodinidae Grote (Erycinidae Swains) (Lep Rhop)]. *Berliner Entomologische Zeitschrift 55*(1/2), 9-103.

Thomas, J. A., & Settele, J. (2004). Butterfly mimics of ants. *Nature, 432*, 283-284.

Ueda, S., Komatsu, T., Itino, T., Arai, R., & Sakamoto, H. (2016). Host-ant specificity of endangered large blue butterflies (*Phengaris* spp., Lepidoptera: Lycaenidae) in Japan. *Scientific Reports*, *6*(36364): 1-5.

Uhlmann, A.; Galvão, F., & Silva, S. M. (1998), Análise da estrutura de duas unidades fitofisionômicas de Savana (Cerrado) no sul do Brasil [Analysis of the structure of two phytophysiological units of Savana (Cerrado) in southern Brazil]. *Acta Botanica Brasilica 12*(3), 231-247.

Vaglia, T., Haxaire, J., Kitching, I. J., Meusnier, I., & Rougerie, R. (2008). Morphology and DNA barcoding reveal three cryptic species within the *Xylophanes neoptolemus* and *loelia* species-groups (Lepidoptera: Sphingidae). *Zootaxa, 1923*, 18-36.

Veldman, J. W., Overbeck, G. E., Negreiros, D., Mahy, G., Le Stradic, S., Fernandes, G. W., … Bond, W. J. (2015). Tyranny of trees in grassy biomes. *Science*, *347*(6221), 484-485.

Westwood, J. O. 1851. In Doubleday, E.; Westwood, J. O., & Hewitson, W. C. (1846-1852). *The genera of diurnal Lepidoptera, comprising their generic characters, a notice of their habits and transformations, and a catalogue of the species of each genus; illustrated with 86 plates by W. C. Hewitson*: London. Longman.

Wootton, J. T., Pfister, C. A. (2013). Experimental separation of genetic and demograpich factors on extinction risk in wild populations. *Ecology*, *94*(10): 2117-2123.

Yang, Z., Landry, J. F., Handfield, L., Zhang, Y., Solis, M. A., Handfield, D., … Hebert, P. D. N. (2012). DNA barcoding and morphology reveal three cryptic species of *Anania* (Lepidoptera: Crambidae: Pyraustinae) in North America, all distinc from their European conterpart. *Systematic Entomology*, *37*: 686-705.

CAPÍTULO III

CONSIDERAÇÕES FINAIS

Este estudo visou solucionar os problemas taxonômicos envolvendo as espécies que compõem o grupo *Aricoris constantius* (Lepidoptera: Riodinidae). Dessa maneira, foram designados neótipos para as espécies cujos tipos estão perdidos (*Papilio constantius* e *Erycina tutana*), já que a fixação de um nome zoológico com um espécime voucher é fundamental para o entendimento da taxonomia dos seres vivos. Além disso, foram descritas para a América do Sul três novas espécies, até então erroneamente classificadas como "*A. tutana*", bem como realizou-se a redescrição de *A. tutana*. Ressalta-se que com a separação dessas quatro espécies, há uma considerável redução na área de distribuição geográfica dessas, que quando considerado junto ao fato de serem espécies parasitas sociais, demonstram importantes argumentos para considerarmos algumas dessas espécies como ameaçadas de extinção pelos critérios da IUCN.

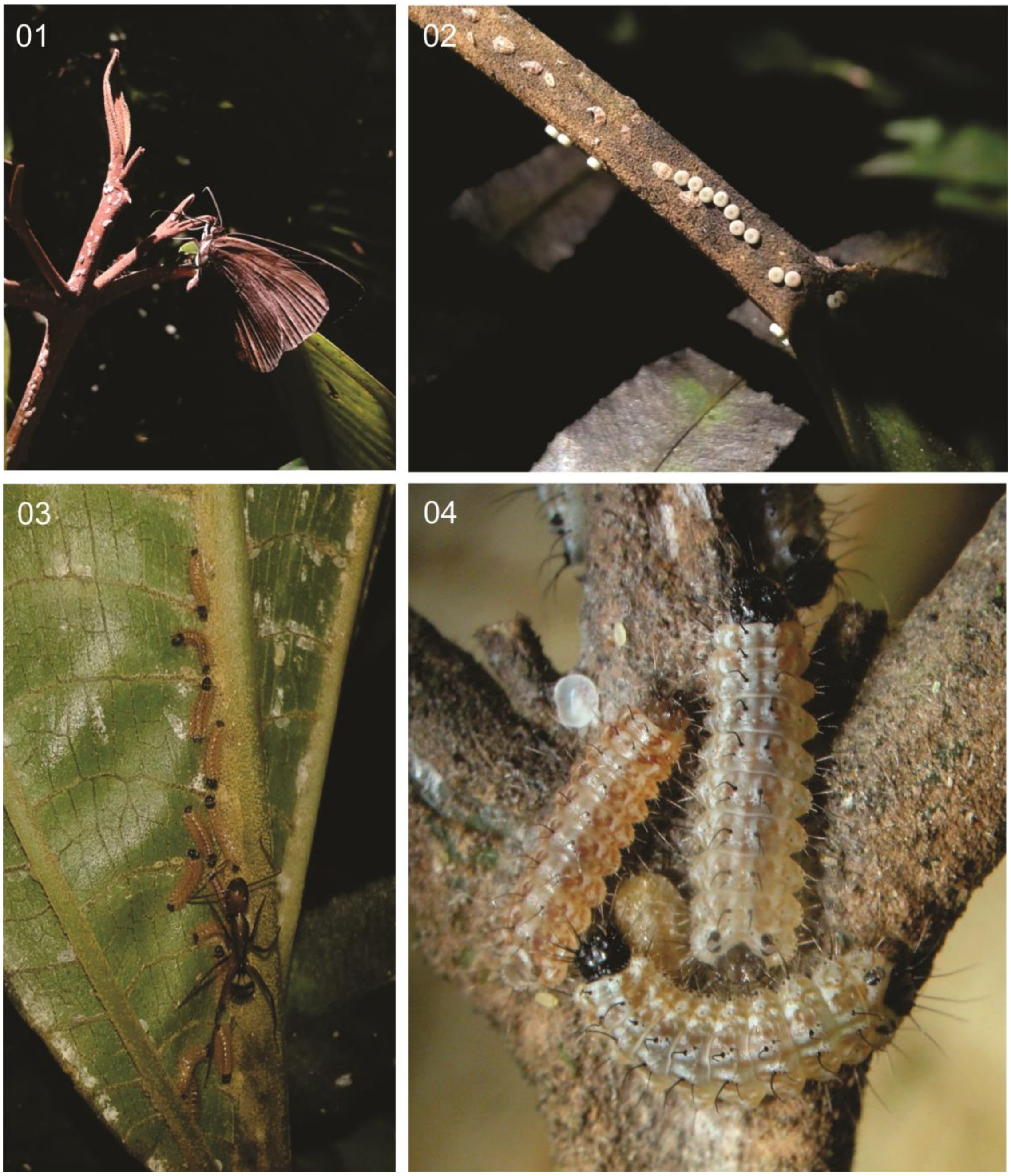
Através de uma abordagem integrativa utilizou-se de um marcador molecular, o gene mitocondrial *citocromo c oxidase subunidade I* (*COI*), bem como de caracteres morfológicos para desvendar a diversidade escondida dentro desse complexo de espécies crípticas. Para facilitar futuras identificações, foram elaboradas diagnoses, chave de identificação e ilustrações, especialmente nos padrões alares e das genitálias.

Nossa análise molecular utilizando o DNA *barcode* sugere que *A. monotona* e *A. tutana* são espécies relacionadas, o que é corroborado por algumas características morfológicas presentes nas genitálias, bem como pela presença abundante de cerdas laranja na região ventral do tórax e no pronoto. Entretanto, uma análise filogenética envolvendo mais genes é necessária para compreender as relações entre as demais espécies do grupo. Além disso, ressaltamos a possibilidade da existência de mais espécies ainda não descritas no grupo, pois há registros geográficos na Argentina, Paraguai e Peru de indivíduos que ainda precisam ser analisados. A sequência de um indivíduo não identificado (voucher perdido) sugere a existência de mais linhagens pertencentes ao complexo. *Aricoris* sp. 2 sp. nov. foi a única espécie não incluída na análise molecular, devido à dificuldade de acessar material genético de exemplares antigos de museu, entretanto, essa espécie apresenta importantes características morfológicas que justificam nossa decisão taxonômica.

Essas espécies habitam preferencialmente áreas de campo aberto e cerrado, com exceção de *A. constantius*, que habita formações de Mata Atlântica. Além disso, praticamente todas elas apresentam distribuição simpátrica. Há a presença de óleo nas asas dessas borboletas, um forte indicativo delas possuírem hábitos afitófagos quando larvas. Além disso, larvas de *A. constantius* foram encontradas em campo mantendo associação aparentemente obrigatória com uma espécie de formiga (*Camponotus* sp.) e se alimentando de secreções *honeydew* produzidas por cochonilhas (Hemiptera: Sternorrhyncha: Coccidae) (Apêndice). Afitofagia é considerada rara em Lepidoptera e a presença desse comportamento dentro de um gênero que possui outras espécies fitófagas demonstra a grande diversidade de hábitos dentro desse grupo.

Aricoris é um gênero interessante do ponto de vista ecológico e biogeográfico, e estudos em andamento demonstram que a riqueza do gênero, até então com 30 espécies, considerando as novas espécies descritas nesse trabalho, tende a duplicar. A taxonomia forma um pilar para as Ciências Biológicas, pois somente com a identificação correta das linhagens de espécies existentes podemos dar continuidades com estudos mais aprofundados, de cunho ecológico, genético, evolutivo, história natural, conservação, entre outros. A taxonomia integrativa será de contribuição essencial para estudos sistemáticos, pois assim como no grupo *constantius*, muitas outras linhagens de Riodinidae formam complexos de espécies crípticas. Como perspectivas futuras, visamos conseguir dados adicionais referentes a história natural dessas borboletas. Dessa maneira, conseguiremos inferir sobre processos de especiação e diversificação deste grupo e ampliar o conhecimento de Riodinidae, até então considerado um dos grupos de borboletas relativamente menos estudado.

ANEXO 1



Apêndice: História de vida de *Aricoris constantius* (Lepidoptera: Riodinidae). **01.** Fêmea ovipositando; **02.** Ovos; **03.** Larvas de primeiro instar sendo atendidas por *Camponotus* sp. (Hymenoptera: Formicidae: Formicinae); **04.** Larvas de segundo e terceiro instar próximas às cochonilhas (Hemiptera: Sternorrhyncha: Coccidae). Fotos 1-3 cedidas por José F. S. Neto.

ANEXO 2

NORMAS PARA PUBLICAÇÃO NA SYSTEMATICS AND BIODIVERSITY

Aims and scope

Systematics and Biodiversity is devoted to whole-organism biology. It is a quarterly, international, peer-reviewed, life science journal, without page charges, which is published by Taylor & Francis for The Natural History Museum, London. The criterion for publication is scientific merit. Systematics and Biodiversity documents the diversity of organisms in all natural phyla, through taxonomic papers that have a broad context (not single species descriptions), while also addressing topical issues relating to biological collections, and the principles of systematics. It particularly emphasises the importance and multi-disciplinary significance of systematics, with contributions which address the implications of other fields for systematics, or which advance our understanding of other fields through taxonomic knowledge, especially in relation to the nature, origins, and conservation of biodiversity, at all taxonomic levels.

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Each issue contains a main section devoted to formal peer-reviewed original research papers, and a shorter, more informal 'Perspective' section. As well as taxonomic discovery, description, revision and recording, the research section carries studies of adaptation, anatomy, biodiversity patterns in time and space (including response to environmental and human factors, and to global change), biogeography, coevolution, conservation biology, development, evolutionary biology, functional morphology, growth and form, molecular science, phylogenetics, cladistics, speciation, and systematic ecology. State-of-knowledge reviews and papers on the theory and practice of systematics are also welcome. There are no restrictions on the geographical location of authors, their material and study areas, or on the institutional locations of their studied collections. The 'Perspective' section covers a similar range of subjects to the main section, but gives scope for debate and comment as well as reviews of books.

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