UNIVERSIDADE ESTADUAL DE CAMPINAS

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EDUARDO DE PROENÇA BARBOSA

Phylogenetic relationships and biogeographic history of the clades *Yphthimoides* Forster, 1964 and *Pharneuptychia* Forster, 1964 (Nymphalidae: Satyrinae)

Relações filogenéticas e padrões de distribuição biogeográfica dos clados *Yphthimoides* Forster, 1964 e *Pharneuptychia* Forster, 1964 (Nymphalidae: Satyrinae)

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2016
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“Para minha família e amigos... obrigado.”
RESUMO

A alta diversidade de Satyrinae, um grupo com aproximadamente 2500 espécies, pode ser responsável pela incerteza e dificuldade taxonômica na classificação do grupo, que está entre os menos conhecidos entre as borboletas, principalmente na região Neotropical. Essa dificuldade pode ser atribuída também ao pouco conhecimento filogenético e biogeográfico do grupo nessa região. Entre os grupos que apresentam problemas destaca-se a subtribo Euptychiina, um grupo com vários gêneros ainda mal definidos (para e polifiléticos) e que só agora começa a ser melhor compreendido. De acordo com análises filogenéticas preliminares baseadas em dados moleculares, o gênero Ypthimoides aparece como monofilético, embora poucas espécies tenham sido estudadas. Já com relação ao gênero Pharneuptychia, análises filogenéticas preliminares apontam o mesmo como sendo não-monofilético, com muitas espécies sendo próximas às espécies de Moneuptychia e Euptychoïdes castrensis. Assim sendo, é imperativo que se use o máximo de espécies possíveis desses gêneros para tentar se traçar a história evolutiva e biogeográfica desses táxons na região Neotropical, bem como tentar entender os prováveis processos que levaram ao atual padrão de distribuição de Ypthimoides e Pharneuptychia na América do Sul. Este cenário reporta diretamente aos dois objetivos principais desse projeto, que são: 1) a obtenção de uma filogenia robusta para os gêneros Ypthimoides e Pharneuptychia com base em dados moleculares e 2) com base nessas filogenias, mapear os caracteres morfológicos e traçar a história biogeográfica de ambos para que se possa começar a entender a distribuição desses grupos nas diferentes unidades geográficas da região Neotropical. Os resultados apontam Ypthimoides, como atualmente classificado, como sendo um grupo não monofilético e algumas das espécies deveriam ser realocados em outros gêneros. Euptychoïdes castrensis aparece como um complexo de espécies crípticas que faz parte do gênero Moneuptychia, que por sua vez aparece como grupo-irmão do gênero Pharneuptychia. A atual distribuição geográfica de Ypthimoides poderia ser explicada em sua maior parte por eventos de dispersão na região Neotropical.
ABSTRACT

The high diversity of Satyrinae, a group containing approximately 2500 species, can be responsible for the uncertainty and taxonomic difficulty in the group’s classification, which is one of the lesser known butterfly groups, mainly in the Neotropical realm. This difficulty could be also due to the low phylogenetic and biogeographic knowledge of this group in the region. Among the Satyrinae groups that present some issues regarding this areas of knowledge it could be highlighted the subtribe Euptychiina, a particular group with several not well defined genera (para- and polyphyletic) and that just now begins to be better understood. According to previous phylogenetic analysis using molecular data, the genus *Yphthimoides* appears as monophyletic, although just a few species representing the genus had been used in the analysis. Another Euptychiina genus, *Pharneuptychia*, appears in preliminary phylogenetic analysis as been non-monophyletic, with several species appearing close to species of *Moneuptychia* and *Euptychoïdes castrensis*. So based on these information it is imperative to use the as many species of those genera as possible to generate a highly supported phylogenetic hypothesis to try and trace the evolutive and biogeographic history of these taxa in the Neotropical Realm, as well as try to understand the likely processes leading to the actual distribution pattern of *Yphthimoides* and the species of the *Pharneuptychia* clade in South America. This scenario reports directly to the two main objectives of this thesis, which are: 1) The acquisition of a robust phylogeny to the genera *Yphthimoides* and *Pharneuptychia* based on molecular data and 2) based on these phylogenies, to map the morphological characters and trace the biogeographic history of both genera so we can begin to understand the distribution patterns of these groups in the different geographic units of the Neotropical realm. The results show that *Yphthimoides* as currently conceived is not monophyletic and some of the species should be reassigned somewhere else. *Euptychoïdes castrensis* appeared as a complex of cryptic species being part of the *Moneuptychia* genus, which is sister to *Pharneuptychia* genus. The current geographical distribution of *Yphthimoides* could be explained mostly by dispersal events throughout the Neotropics.
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INTRODUÇÃO GERAL

Origem da Sistemática

Há muito tempo os seres humanos sentem essa enorme necessidade de se entender e de alguma forma organizar a biosfera, todos os organismos que fazem parte dela e como eles se interconectam. Com relação aos organismos, parte dessa necessidade vem da elevada diversidade de espécies e sua enorme variação morfológica, o que tem fascinado e intrigado os naturalistas desde muito antes de Aristóteles. Porém foi com Aristóteles que de certa maneira se deu o nascimento da área da ciência que hoje é conhecida como Biologia e que veio então a fortalecer os naturalistas que se viam na obrigação de entender como era e se havia alguma relação/conexão entre as mais variadas espécies e de organizar esse conhecimento coesamente (Papavero & Balsa 1986).

Um dos primeiros a tentar organizar as espécies animais foi Aristóteles, que criou um sistema de classificação funcional, binário e empírico (Amorim 2002; Wheeler 2012), embora haja divergências quanto a isso (ver Atran 1985). Dessa forma Aristóteles separou os animais optando por usar suas características funcionais como, por exemplo, a presença de asas em aves e insetos, ao invés de diferenças morfológicas (Lloyd 1961; Wheeler 2012).

Apesar de ter feito algumas das mais detalhadas observações acerca dos animais no seu tempo (Schuh & Brower 2009), a classificação criada por Aristóteles para os animais não era compreensiva ou consistente, mas ainda assim possuía uma hierarquia e, portanto, serviu de base para o sistema de classificação moderno (Wheeler 2012).

E foi o botânico e naturalista sueco Carolus Linnaeus, ou Carl von Linné, quem criou esse sistema de classificação moderno e que tem servido de base para a nomenclatura biológica por mais de 250 anos, tendo grande influência nos atuais códigos de nomenclatura zoológica e botânica (Amorim 2002; Schuh & Brower 2009; Wheeler 2012).

O sistema criado por Linnaeus tem por base uma nomenclatura binomial na forma de gênero e espécie, e cujos binômios são palavras em latim ou latinizadas (Amorim 2002). Esse sistema perdura até os dias atuais, sendo que os padrões hierárquicos contemporâneos incluem sete níveis (Reino, Filo, Classe, Ordem, Família, Gênero e Espécie) (Wheeler 2012), embora tenha havido a criação de outros níveis para que se
pudesse organizar mais precisamente a diversidade encontrada (e.g. McKenna and Bell, 1997).

Além de Aristóteles e Linnaeus, outros naturalistas e pesquisadores também tiveram sua contribuição para tentar entender e classificar corretamente a diversidade de espécies encontradas como, por exemplo, Jean-Baptiste Lamarck, Georges Louis Leclerc (Comte de Buffon), Georges Cuvier, Étienne Geoffroy Saint-Hilaire, Richard Owen e Charles Darwin (Wheeler 2012).

Todos tiveram uma parcela importante de contribuição, mas foi Darwin quem trouxe a maior contribuição (sem esquecer Alfred Russel Wallace, que chegou à mesma conclusão independentemente) para explicar a distribuição hierárquica da variação biológica, a teoria da origem das espécies por seleção natural (Darwin & Wallace 1858; Darwin 1859; Ridley 2006; Wheeler 2012).

A teoria proposta por Darwin-Wallace no final do século XIX deu aos pesquisadores uma base sólida para uma melhor e mais objetiva compreensão de como toda a diversidade biológica é originada e organizada (Amorim 2002; Ridley 2006), o que teve um impacto muito grande na forma como eram feitas as classificações dos organismos, servindo como justificativa para que essas classificações passassem a refletir as relações evolutivas entre as espécies, ou seja, sua genealogia (Wheeler 2012). De acordo com Hull (1988), Darwin foi capaz de criar um arcabouço intelectual, mas nenhum guia ou mapa mostrando como realmente acessar essas relações evolutivas, ou filogenéticas.

Entretanto, foi somente a partir do trabalho de Willi Hennig (1965), com o conceito de sistemática filogenética, que as relações evolutivas ou genealógicas das espécies se tornaram de certa maneira mais fáceis de serem compreendidas.

Para Hennig, as relações filogenéticas poderiam ser expressas em uma série de relações aninhadas no que ele chamou de grupos-irmãos (sister-groups, em inglês), nos quais dois taxa são mais proximamente relacionados entre si do que com um terceiro táxon, devido ao compartilhamento de um ou mais caracteres derivados, ou sinapomorfias (Hennig 1965; Kitching et al. 1998; Amorim 2002; Schuh & Brower 2009; Wheeler 2012).

De modo muito resumido, todo esse conhecimento forma a base da Sistemática biológica ou Biossistematique, que é a ciência da classificação e descrição da diversidade biológica e suas relações, cuja origem pode ser traçada desde a obra de Aristóteles até a proposição do Sistema Natura de Linnaeus (Amorim 2002; Schuh & Brower 2009;
Wheeler 2012).

No início a bio sistêmática era basicamente história natural, sendo durante muito tempo equiparada à taxonomia, a área da biologia responsável por organizar, classificar e nomear os táxons de modo coerente e regular. Subsequentemente, com o avanço em outras áreas da biologia, a bio sistema tática passou a ser mais inclusiva, englobando além da taxonomia, a filogenia, a biologia comparada, genética, ecologia e evolução (Schuh & Brower 2009; Wheeler 2012).

A incorporação de outras áreas da biologia consolidou em anos recentes uma nova abordagem dentro da bio sistema tática, chamada de Taxonomia integrativa (Dayrat 2005; Will et al. 2005; Padial et al. 2010). A taxonomia integrativa pode então ser entendida como a descrição/delimitação de espécies com base na integração entre diferentes fontes de evidência (morfologia, filogeografia, dados moleculares, comportamentais, ecológicos e químicos, dentre outros), trazendo uma maior robustez e um maior rigor para o processo de delimitação das espécies (Schlick-Steiner et al. 2010; Yeates et al. 2011; Pante et al. 2015).

A incorporação de novas fontes de dados, como no caso da taxonomia integrativa, fortalece a Sistemática de um modo geral, bem como seu principal objetivo que é o de se estudar e compreender a diversidade biológica (Savage 1995; Amorim 2002).

Os principais tópicos trabalhados por essa área das ciências biológicas podem ser divididos basicamente em três, sendo eles: 1) a descrição dessa diversidade, 2) a ordenação/classificação dessa diversidade e 3) a compreensão dos processos que geram essa diversidade (Amorim 2002; Schuh & Brower 2009).

De acordo com Schuh & Brower (2009), existe uma visão dominante entre o público em geral, e mesmo entre pesquisadores, de que a ciência é igual à experimentação. De fato muito da ciência é baseada em experimentos, mas muito do que a bio sistemática faz não tem uma base experimental, é puramente observação e comparação para se descrever um fenômeno natural, uma espécie ou mesmo um padrão geral, e isso não pode ser classificado como sendo não científico.

Para que questões complexas dentro das ciências naturais possam ser respondidas de maneira clara e concisa, é primeiramente necessário que se conheça o básico e é exatamente nesse ponto que a bio sistemática (história natural, taxonomia, filogenia) pode ajudar numa melhor compreensão da biodiversidade (Marques & Lamas 2006; Carbayo & Marques 2011), provando um arcabouço fundamental das relações entre os organismos e permitindo aos cientistas comparar seus resultados de maneira concisa e
coerente.

Em se tratando da filogenia, os seus princípios formam a base para que se possa reconhecer, caracterizar e posicionar os grupos naturais (clados) dentro de uma perspectiva que reflita sua história evolutiva e sua diversificação (Savage 1995).

Pode-se entender então que a biossistematica, como ciência fundamental da biodiversidade, é o alicerce de toda a biologia e tem contribuição enorme nos estudos evolutivos (Savage 1995).

**Sistemática e biodiversidade**

A diversidade biológica, ou biodiversidade, pode ser definida como a variedade total das formas de vida, tanto contemporânea como extintas, incluindo também variação genética, características funcionais, populações, riqueza de espécies e ecossistemas bem como os papéis ecológicos dos organismos nas comunidades biológicas (Savage 1995, Kim & Byrne 2006; Cardinale 2012).

Atualmente a biodiversidade está sendo perdida a taxas muito altas e a uma velocidade alarmante (Cardinale et al. 2012; Costello et al. 2013; Pimm et al. 2014) pela destruição, alteração e poluição de ambientes naturais (Savage 1995; Fahrig 2003; Mantyka-Pringle et al. 2012) e apesar dos seres humanos usarem os produtos e serviços derivados dessa biodiversidade (Kim & Byrne 2006), só recentemente o público em geral começou a perceber a importância fundamental da biodiversidade como um recurso global que mantém os ecossistemas e, portanto o planeta, saudáveis (Savage 1995, Kim & Byrne 2006; Cardinale 2012).

Tendo em vista essa perda acelerada da biodiversidade, é crucial que a taxa de obtenção de conhecimento da biodiversidade seja aumentada ao máximo para que políticas e decisões sobre como maximizar a preservação desses recursos naturais possam ser tomadas rapidamente (Myers et al. 2000).

E é exatamente nesse ponto que a biossistematica tem um papel fundamental a desempenhar nesse esforço para aumentar o conhecimento da biodiversidade (Savage 1995).

O número de espécies descritas para o planeta gira em torno de 1,4 a 1,75 milhões de espécies (Savage 1995; Kim & Byrne 2006; Zhang 2013), embora estimativas apontem que entre 5 a 30 milhões (algumas estimativas apontam mais de 100 milhões)
de espécies ainda precisam ser descobertas e formalmente descritas (Hammond 1995; May 2010; Mora et al. 2011).

Devido à taxa na qual as espécies estão sendo extintas e levando em consideração que existe uma falta de profissionais capacitados (taxonomistas) (Mora et al. 2011) para descrever novas espécies, é de se esperar que provavelmente muitas espécies sejam perdidas antes mesmo de serem conhecidas pela ciência (Costello et al. 2013).

Essa falta de profissionais em uma das principais áreas da Sistemática, a taxonomia, a qual é responsável pela descrição, nomeação e catalogação dos organismos nas unidades fundamentais da biologia - as espécies - está relacionada à importância que tem sido dada para essa área da ciência nos últimos anos, constantemente em declínio (Kim & Byrne 2006).

Dois dos principais motivos a afastar os pesquisadores interessados em seguir essa linha de pesquisa na área taxonômica são a falta de incentivos financeiro e de carreira (Wortley et al. 2002; Hopkins and Freckleton 2002; Wheeler 2004; Wheeler et al. 2004; Sodhi et al. 2004).

Pesquisadores com treinamento e experiência em taxonomia são de fundamental importância para reconhecer, descrever e nomear espécies, que são a base para qualquer estudo em qualquer área da biologia. Se as unidades biológicas sendo estudadas não tiverem um nome associado a elas o estudo não terá muita validade (Savage 1995).

E para que essas descrições de espécies, no caso espécies de animais, sejam uniformes e padronizadas dentro da comunidade científica, foi criada em 1895 a Comissão Internacional em Nomenclatura Zoológica, que é responsável por redigir, editar e publicar o Código Internacional de Nomenclatura Zoológica, garantindo assim que cada espécie tenha um nome científico único e universalmente aceito (ICZN 1999). Existe também um Código Internacional para Algas, Fungos e Plantas (ICN), publicado desde 1950 pela International Association for Plant Taxonomy (IAPT 2011).

Esse é o papel crucial e fundamental da biosistemática para a proteção da biodiversidade: prover o conhecimento básico sobre as unidades biológicas, quem são, quais são suas prováveis origens e suas relações de ancestralidade comum.

Um maior entendimento da situação atual da integração entre a sistemática e a biodiversidade pode ser visto em Kim and Byrne (2006) e referências.

**Sistemática, DNA barcode e delimitação de espécies**
A perda acelerada da biodiversidade aliada ao número cada vez menor de taxonomistas profissionais na sistemática torna urgente que aumentemos o conhecimento acerca da riqueza de espécies do planeta para que seja possível criar planos de manejo e conservação dessa biodiversidade remanescente.

Para tentar solucionar esse problema Herbert et al. (2003) propuseram um sistema de identificação de espécies baseado em DNA, mais especificamente em uma pequena porção um gene mitocondrial, o Citocromo c Oxidase subunidade I (COI ou CoxI), contendo aproximadamente entre 648 – 658 pares de base (Silva-Brandão et al. 2009; Dasmahapatra & Mallet 2006; Dasmahapatra et al. 2010) e que foi chamado de DNA Barcode ou código de barras de DNA e uma vantagem inquestionável seria a rápida aquisição de dados moleculares (Silva-Brandão et al. 2009).

Esse conceito se baseia no pressuposto de que cada espécie teria o seu próprio “DNA barcode” (Silva-Brandão et al. 2009) e que as variações genéticas intersetivas nesse gene superariam as intraespecíficas, o chamado “DNA gap” (um valor que estaria em torno de 2-3%) (Herbert et al. 2003a, b), facilitando assim a identificação de um indivíduo como sendo de uma ou outra espécie.

DeSalle et al. (2005) enfatizam que existem duas áreas distintas para as quais o DNA barcode está sendo usado: 1) a distinção (identificação) entre espécies e 2) a prospecção de novas espécies.

Os mesmos autores também apontam alguns problemas que poderiam limitar o uso do barcode, principalmente para a descoberta de novas espécies. Uma das limitações seria o uso de medidas de distância (número de nucleotídeos diferentes) (Herbert et al. 2003a) ao invés de caracteres quando da comparação entre as sequências de barcode. O principal problema aqui seria o fato de que a taxonomia se baseia em caracteres para a distinção e descrição de espécies e, com isso, o uso do barcode como medida de distância impediria a união com a taxonomia clássica (DeSalle et al. 2005).

Porém o uso do DNA barcode, mesmo quando analisado usando-se medidas de distância, tem se mostrado muito eficiente em algumas situações nas quais o uso da morfologia é difícil, como por exemplo, para espécies com ciclos de vida que incluem metamorfose, ou seja, quando imaturos e adultos são diferentes e identificá-los como uma mesma espécie é muito difícil (e.g., anfíbios e insetos), quando as espécies apresentam plasticidade fenotípica e, principalmente, na detecção de complexos de espécies cripticas (Dasmahapatra & Mallet 2006; Silva-Brandão et al. 2009 e referências incluídas).
Para alguns grupos de borboletas como a tribo Ithomiini (Danainae), cujos indivíduos em sua maioria apresentam padrões de coloração miméticos, e a subfamília Satyrinae, de coloração predominantemente marrom, o DNA *barcode* tem se mostrado muito útil para tanto distinguir quanto delimitar espécies (e.g., Dasmahapatra et al. 2010; Giraldo & Uribe 2012; Hill et al. 2012; Seraphim et al. 2014; Nakahara et al. 2015; Barbosa et al. 2015; Barbosa et al., submetido; Barbosa et al., in prep.), principalmente quando utilizado de forma integrativa com outras abordagens (Will et al. 2005), como morfologia e história natural (a chamada taxonomia integrativa) (Dayrat 2005; Padial et al. 2010; Schlick-Steiner et al. 2010; Yaetes et al. 2011; Pante et al. 2015).

Outra limitação proposta por DeSalle et al. (2005) seria quanto ao número de indivíduos usados nas análises para se delimitar/descrever espécies. Em teoria o ideal seria amostrar muitos indivíduos de cada espécie e de várias localidades para se tentar conseguir identificar toda a variação inerente à espécie. Alguns poucos indivíduos poderiam não ser representativos da espécie como um todo, principalmente de uma espécie com ampla distribuição, embora muito dificilmente haverá uma amostragem de indivíduos ideal ou razoável para todas as espécies (DeSalle et al. 2005).

Uma amostragem ampla nem sempre pode ser obtida e em algumas situações algumas espécies serão descritas com base em poucos indivíduos, o que pode estar relacionado ao fato de serem possíveis espécies raras. O ideal nesses casos é sempre empregar uma abordagem mais integrativa (Dayrat 2005), usando diversas fontes de dados para se chegar a uma conclusão quanto à descrição/delimitação de uma espécie.

Barbosa et al. (2015, 2016) descreveram cinco espécies de borboletas do gênero *Ypthimoides* (Satyrinae: Euptychiina) com base em uma abordagem integrativa, usando DNA *barcode* e morfologia, principalmente de genitália masculina. Duas dessas espécies, *Ypthimoides bella* e *Y. nareta*, tiveram poucos indivíduos amostrados, mas a combinação de dados permitiu uma correta delimitação/descrição dessas espécies, que aparentemente exibem uma distribuição geográfica restrita e bem localizada.

Em um estudo com algas vermelhas e usando DNA *barcode*, Saunders (2008) foi capaz de encontrar sete espécies em um complexo de espécies crípticas e foi capaz de corroborar essas espécies com base em outras fontes de dados. Em outro estudo, com moscas drosofilídeas, Yassin et al. (2008) descobriram duas espécies crípticas no que antes se acreditava ser somente uma única espécie.
Em outros casos, o DNA *barcode* pode detectar algumas espécies que são de fato espécies, porém também pode detectar espécies que não o são, como é o caso de estudo publicado por Dasmahapatra *et al.* (2010) com borboletas do gênero *Mechanitis*, que são conhecidas por fazer parte de anéis miméticos. Nesse estudo os autores usaram, além do *barcode*, também genes nucleares e genotipagem por AFLP’s para tentar investigar os limites específicos desse gênero. Os autores constataram que o DNA *barcode* apontava a existência de quatro novas espécies cripticas, mas os marcadores AFLP’s suportavam somente uma dessas como espécie nova. A conclusão do estudo foi a de que nem sempre o DNA *barcode* vai funcionar para delimitação de espécies e que outros marcadores moleculares deveriam ser usados para em conjunto para se testar esses limites específicos.

O uso do DNA *barcode* para delimitação de espécies nem sempre é 100% confiável e ainda conta com certa resistência por parte da comunidade científica (*e.g.*, Ebach & Holdrege 2005; Collins & Cruickshank 2013). Dentre as razões apontadas existem diversas relacionadas à própria natureza do *barcode*, como sua herança unicameral materna, o que poderia levar a hibridizações interespecíficas, infecções por bactérias endosimbiontes, como *Wolbachia* e a presença de DNA mitocondrial nuclear (NUMT’s – cópias do COI no genoma nuclear), entre outros (Dasmahapatra & Mallet 2006; Song *et al.* 2008; Silva-Brandão *et al.* 2009 e referências incluídas).

No entanto, apesar de algumas desvantagens no uso do DNA *barcode*, existem inúmeros exemplos de sucesso no uso dessa pequena porção de DNA mitocondrial na distinção de espécies e no descobrimento de complexos de espécies cripticas em diferentes *taxa*, como lepidópteros (Hebert *et al.* 2004a, Janzen *et al.* 2005, Hajibabaei *et al.* 2006, Burns *et al.* 2008), aranhas (Greenstone *et al.* 2005), aves (Hebert *et al.* 2004b, Kerr *et al.* 2007), gastrópodes (Remigio & Hebert 2003), dentre outros (Silva-Brandão *et al.* 2009).

Entretanto, mesmo possuindo uma grande eficiência, o DNA *barcode* não deve ser usado como única fonte de dados para a separação/identificação de espécies, pois erros podem acontecer (Hickerson *et al.* 2006; Dasmahapatra & Mallet 2006; Dasmahapatra *et al.* 2010) e o uso de uma abordagem mais holística deve ser preferida, integrando diferentes fontes de dados para uma melhor resolução na identificação, distinção e descrição de espécies e complexos de espécies cripticas.

O DNA *barcode* é mais uma ferramenta dentro da Sistemática que vem para auxiliar os estudos baseados em outras fontes de evidência já citadas anteriormente, e
não um substituto para a taxonomia tradicional, como alguns trabalhos apontaram (e.g., Ebach & Holdrege 2005).

Em suma, na atual situação pela qual passa a biodiversidade, com espécies sendo extintas a velocidades cada vez maiores, o emprego de técnicas moleculares como o DNA barcode para identificação, distinção e descrição de espécies só vem a somar esforços para que se possa aumentar cada vez mais o conhecimento acerca do real número de espécies existentes e talvez assim amenizar o impacto negativo que a biodiversidade vem sofrendo há tempos.

**O papel da Sistemática em estudos biogeográficos**

A biogeografia é uma área de pesquisa dentro da biologia que se desenvolveu tendo por base os fundamentos da filogenia, que se tornou a principal ferramenta para a análise de processos e padrões de história evolutiva (Quintero *et al.* 2015), e da ecologia, possuindo, portanto, uma relação muito próxima a essas duas áreas (Brown & Lomolino 1998). Entretanto apesar de ser considerada de fundamental importância para essas duas áreas, a biogeografia não tem sido utilizada como um elo entre a sistemática e a ecologia, ou porque a filogenia ignora aspectos ecológicos, ou porque a ecologia não leva em consideração a biogeografia histórica na resolução de suas questões (Wiens & Donoghue 2004).

Os padrões biogeográficos podem muitas vezes resultar de processos ecológicos e/ou históricos que influenciam a dispersão em diferentes escalas, tanto temporal quanto espacial (Wiens & Donoghue 2004). A vicariância, um processo histórico, é um exemplo disso, pois é considerada uma alternativa à dispersão, quando na verdade é uma consequência dos processos que restringem a dispersão dos indivíduos dentro da área originalmente ocupada pela espécie ancestral (Wiens & Donoghue 2004).

Geralmente nos estudos de biogeografia histórica a vicariância tem uma maior preferência em relação à dispersão para explicar padrões de distribuições disjuntas (Wiley 1988), com a dispersão aparecendo como uma segunda opção para se tentar explicar os padrões encontrados e que não podem ser explicados por eventos vicariantes (Kodandaramaiah & Wahlberg 2007). Porém, em diversos casos as distribuições dos organismos não sofreram de efeitos vicariantes em escala global, especialmente nos grupos que evoluíram após o Mioceno, que viveram em um período geológico relativamente estável. Nestes, as distribuições foram afetadas principalmente por fatores
como mudanças climáticas, e não por movimentação continental, enquanto que nos grupos que possuíam grande capacidade de dispersão os efeitos da vicariância podem ter sido mascarados pelas dispersões subsequentes (Kodandaramaiah & Wahlberg 2007).

Ao longo do tempo, diversos métodos de análise em biogeografia histórica foram desenvolvidos para se tentar entender os padrões de distribuição dos diversos grupos taxonômicos (ver Crisci et al. 2003; Schuh & Brower 2009; Carvalho & Almeida 2011) e esses métodos se baseiam principalmente na premissa de que combinando informação espacial e filogenética das linhagens um maior entendimento da história evolutiva dos grupos através do espaço e tempo pode ser alcançado (Lemmon and Lemmon 2008; Lemey et al. 2010; Ronquist and Sanmartín 2011).

Atualmente nos trabalhos de biogeografia histórica basicamente três métodos são usados com maior frequência: filogeografia, métodos baseados em eventos e métodos baseados em modelos.

Basicamente a filogeografia trata da relação entre genealogia e geografia (Avise et al. 1987), estudando os princípios e processos que determinam a distribuição geográfica das linhagens genealógicas, incluindo também o nível intraespecífico, com base no DNA mitocondrial (Crisci et al. 2003). De uma forma mais simples, a filogeografia estuda a distribuição espacial de alelos no espaço (Martins & Domingues 2011).

Com relação aos métodos de análise biogeográfica baseados em eventos, esses basicamente propõem a utilização de modelos dos processos (vicariância, dispersão, duplicação e extinção) que afetam a distribuição geográfica das linhagens e assim avaliar simultaneamente a influência desses vários eventos na distribuição dos grupos.


Recentemente os métodos baseados em modelos, que estimam parâmetros usando
a máxima verossimilhança ou estimativas bayesianas de distribuições posteriores usando Markov Chain Monte Carlo (MCMC), têm sido favorecidos em relação aos métodos que não são baseados em modelos (Beaumont et al. 2010; Bloomquist et al. 2010).

Matzke (2013) criou um pacote de análise biogeográfica para o programa estatístico R (R Development Core Team 2013) chamado BioGeoBEARS que permite uma inferência probabilística tanto da biogeografia histórica (amplitude geográfica ancestral em uma filogenia) quanto a comparação de diferentes modelos de evolução dessa amplitude de distribuição, além de uma série de outras funções para manipulação de filogenias datadas, dados de distribuição geográfica e também os resultados de outros programas de análise biogeográfica. De acordo com Matzke (2013, 2014) o pacote BioGeoBEARS tem grande potencial para aumentar a acessibilidade dos métodos de análise baseados em modelos probabilísticos e paramétricos em biogeografia histórica.

Atualmente a questão principal em análises biogeográficas é qual o método deve escolhido e que seja mais apropriado para uma determinada pesquisa. Não existe uma resposta padrão para essa pergunta e talvez a melhor maneira de se escolher seja testar os dados com os diferentes métodos analíticos para se chegar à conclusão de qual método escolher (Almeida 2011).

**Ordem Lepidoptera**

Apesar de ser um grupo taxônômico coeso com monofiletismo estabelecido há anos (Kristensen et al. 2007) e com a maioria dos taxa já terem sido previamente nomeados (Ackery et al. 1998), só recentemente a ordem Lepidoptera passou a contar com uma filogenia relativamente robusta (Regier et al. 2009; Regier et al. 2013; Mutanen et al. 2010; Heikkilä et al. 2015). Essa classificação dos organismos é muito importante para se tentar entender a diversidade existente (Wilson 2000) e deveria ser baseada nas relações de ancestralidade comum dos organismos, inserindo-os em um contexto evolutivo (Marín et al. 2011).

Portanto, o conhecimento da filogenia dos grupos é importante para a compreensão das suas relações internas e de que modo a diversificação dos grupos ocorreu. Além disso, hipóteses filogenéticas robustas são indispensáveis para o conhecimento dos padrões de distribuição biogeográfica, tanto no tempo histórico quanto no ecológico, permitindo a inferência dos processos que levaram os organismos
a ocupar as suas áreas de distribuição atual (ver seção anterior).

Contudo, a filogenia da maioria dos grupos de Lepidoptera permanece desconhecida e tem sido objeto de muita controvérsia (Ehrlich 1958; Ehrlich & Murphy 1981; Ackery 1988; Scoble 1992; de Jong et al. 1996), com uma falta de conhecimento que, segundo Wahlberg et al. (2005), chega a ser crítica e com muitas hipóteses que possuem pouco suporte empírico (Ehrlich 1958; de Jong et al. 1996; Vane-Wright 2003).

Embora algumas hipóteses filogenéticas recentes tenham sido propostas para as borboletas (Regier et al. 2009; Mutanen et al. 2010; Heikkilä et al. 2012), ainda não se compreende completamente os aspectos e detalhes de sua origem e evolução (Vane-Wright 2003). Com a publicação recente de diversas propostas filogenéticas (Penz 1999; Wahlberg & Zimmermann 2000; Freitas & Brown 2004; Peña et al. 2006; Willmott & Freitas 2006), as relações entre a grande maioria dos grupos de borboletas, que permaneciam desconhecidas até recentemente, começaram a ser compreendidas. Ainda assim, a maioria dos clados estabelecidos ainda não possui estudos de filogenia e tampouco de biogeografia, e a falta dessas informações, que poderiam ajudar a elucidar a sistemática e os padrões de especiação entre organismos, tem sido o entrave mais importante na compreensão da evolução das borboletas (Wahlberg & Freitas 2007).

Com relação à origem e idade do grupo, ainda pairam dúvidas se a idade do clado seria suficiente para que o mesmo tenha sido afetado pela separação do supercontinente Gondwana (de Jong 2003), já que o tempo, a origem geográfica, e o principal período de diversificação desse grupo, ainda são de certa forma amplamente debatidos na atualidade (Braby et al. 2005; Wahlberg 2006; Heikkilä et al. 2012).

Um estudo recente de datação molecular da origem das borboletas (Heikkilä et al. 2012) coloca a origem do grupo como tendo acontecido no início do Cretáceo, cerca de 110 milhões de anos atrás. Levando essa idade em consideração, é pouco provável que a separação de Gondwana da Laurásia, que aconteceu a cerca de 200 a 180 milhões de anos atrás, tenha afetado a diversificação do grupo do ponto de vista de eventos vicariantes dessa magnitude.

O que se pode dizer com certeza é que ainda existem lacunas a serem preenchidas tanto do ponto de vista filogenético quanto do biogeográfico e de diversificação para todos os grupos de borboletas, mesmo ao nível hierárquico de família.

Um exemplo típico é a família Nymphalidae; mesmo contando com cerca de 7200 espécies descritas, possuindo a maior diversidade entre os lepidópteros diurnos (Vane-
Wright 2003; Freitas & Brown 2004) e apresentando uma distribuição mundial, com seus representantes sendo encontrados em praticamente todos os continentes e biomas, exceto a Antártica (DeVries 1987; Shields 1989; Heppner 1991), ainda existem lacunas de conhecimento sobre esta família em praticamente em todas as áreas.


**Status taxonômico de Satyrinae**

Um dos grupos menos conhecidos de Nymphalidae é a subfamília Satyrinae. Este grupo, que teve origem aproximadamente no final do Cretáceo (cerca de 80 milhões de anos atrás), passou por uma grande diversificação entre 50 e 56 milhões de anos atrás, e conta atualmente com cerca de 2500 espécies organizadas em nove tribos (Peña et al. 2006; Peña & Wahlberg 2008) distribuídas em todos os continentes à exceção da Antártica (DeVries 2001).

Dentre todas as regiões biogeográficas, a Neotropical é a que abriga a maior diversidade de Satyrinae, com um número de espécies próximo a 1200, abrigadas em 137 géneros (Lamas 2004a) que se distribuem desde o nível do mar até grandes altitudes (DeVries 1987; DeVries et al. 1997; Brown & Freitas 2002).

Acredita-se que essa grande diversidade de Satyrinae seja responsável pelo alto grau de incerteza e dificuldade taxonômica na classificação do grupo (Marín et al. 2011), que está entre os menos conhecidos dentre as borboletas da região Neotropical (Peña & Lamas 2005).

Mesmo com trabalhos focando na revisão do grupo (Miller 1968) ou nas relações filogenéticas usando caracteres morfológicos (Freitas & Brown 2004) e moleculares (Wahlberg et al. 2003; Peña et al. 2006; Wahlberg et al. 2009), os satiríneos permanecem pouco compreendidos, tanto do ponto de vista filogenético quanto
biogeográfico (Peña et al. 2011).

Os trabalhos realizados por Murray & Prowell (2005), Peña et al. (2006), Peña & Wahlberg (2008), Wahlberg et al. (2009) e Peña et al. 2010 foram responsáveis pela compreensão inicial de como realmente são as relações internas de Satyrinae.


Com relação à distribuição biogeográfica, os eventos de dispersão parecem ter tido um papel muito importante para Euptychiina (Peña et al. 2010), muito provavelmente devido ao fato de que as plantas hospedeiras utilizadas por satiríneos em geral, as gramíneas, serem amplamente distribuídas, daí não impondo limites específicos para as espécies (Peña & Wahlberg 2008). Outro fator relevante pode ser a aparente baixa especificidade dos satiríneos pelas plantas hospedeiras, já que as mesmas não possuem compostos secundários de defesa (McNaughton & Tarrants 1983), tornando provável então o uso de diversas espécies alternativas.

Coincidentemente, o pico de diversificação de Euptychiina ocorreu paralelamente com o final do evento de soerguimento dos Andes, entre o final do Mioceno e o início do Plioceno (Gregory-Wodzicki 2000). Entretanto o efeito do soerguimento dos Andes, que em muitos grupos causou o que se costuma chamar de “bomba de espécies” (Hall 2005; Whinnett et al. 2005), parece não se aplicar a Euptychiina, uma vez que a grande maioria das espécies existentes habita terras baixas (Peña & Lamas 2005; Pulido & Andrade 2008).

De acordo com Peña et al. (2010), possivelmente a grande diversidade de Euptychiina esteja relacionada à história complexa da Amazônia, como por exemplo, incursões marínicas no Mioceno (Wesselingh et al. 2002) e a controversa teoria dos refúgios no Pleistoceno, com diminuição da temperatura e secas (Solomon et al. 2008), eventos esses que poderiam causar distúrbios às populações, iniciando assim o processo de diversificação.

Dois grupos pertencentes à subtribo Euptychiina e que carecem de estudos mais aprofundados de biogeografia histórica e que possuem pouca informação recente sobre

Os três gêneros acima foram propostos por Forster (1964) com base em espécimes bolivianos, porém as descrições foram baseadas em caracteres pouco informativos e não exclusivos (apomórficos), o que dificulta o correto delineamento de cada um desses gêneros.

O gênero *Yphthimoides* é altamente diversificado na região sudeste do Brasil e conta atualmente com 26 espécies descritas (Lamas 2004b; Freitas et al. 2012; Barbosa et al. 2015, 2016) e duas ainda não descritas (Lamas 2004b) (essas duas novas espécies não são *Yphthimoides* com base em análises de fotos dos adultos e das genitálias feitas por Shinichi Nakahara). De acordo com os únicos dois trabalhos de filogenia que incluíam espécies desse gênero, o mesmo pode ser monofiletico (Peña et al. 2010) ou não-monofiletico (Murray & Prowell 2005), embora em ambos os estudos apenas poucas espécies do gênero tenham sido incluídas na análise e em nenhum dos dois trabalhos a espécie-tipo do gênero, *Yphthimoides yphthima* (C. Felder & R. Felder, 1867), foi incluída.


Sendo assim, o estudo da filogenia e da biogeografia de gêneros bastante distintos em termos de distribuição geográfica, e para os quais ainda restam dúvidas sobre seu
monofilétismo, pode ajudar na compreensão dos padrões de diversificação desses gêneros e também dos diferentes processos responsáveis pela enorme diversidade biológica da região Neotropical.

**Por que ainda se estudar Nymphalidae e Satyrini?**

As borboletas da família Nymphalidae são possivelmente as mais reconhecidas entre o público em geral e mesmo entre grande parte dos pesquisadores. Elas são em sua maioria coloridas, grandes e com comportamento de voo que possibilita uma fácil observação (Wahlberg et al. 2009).

Devido a essa combinação de fatores que tornam fácil a localização, coleta e manipulação dessas borboletas, os ninfaídeos têm sido usados como modelos de estudo em pesquisas de carácter ecológico e evolutivo (Watt & Boggs 2003) e também em estudos de interação inseto-planta visando o entendimento dessas complexas relações (Ehrlich & Raven 1964). Porém os resultados desses estudos e suas interpretações só se tornam mais críveis quando relacionados a estudos filogenéticos robustos e que normalmente envolvam uma escala de tempo evolutivo (Vane-Wright 2004; Wahlberg 2006; Wahlberg et al. 2009).

Por ser o grupo mais estudado de borboletas, a família Nymphalidae já conta com diversas hipoteses filogenéticas propostas ao longo dos últimos anos (Brower 2000; Wahlberg et al. 2003; Freitas & Brown 2004; Wahlberg et al. 2005; Wahlberg et al. 2009), sendo a última delas (Wahlberg et al. 2009) muito robusta e tendo praticamente resolvido as relações de ancestralidade comum entre a maioria das subfamílias do grupo, porém as relações dentro das subfamílias ainda permanecem relativamente desconhecidas.


Dentre esses exemplos Satyrinae talvez seja o grupo mais problemático,
principalmente a tribo Satyrini, composta por espécies de borboletas de coloração predominantemente marrom e de tamanho relativamente pequeno e que, em muitos casos, fazem parte de complexos de espécies cripticas, o que torna a identificação das espécies somente com base nos padrões morfológicos e de coloração das asas quase impossível.

Mesmo contando com uma hipótese filogenética relativamente robusta para a tribo (Peña et al. 2011) e para uma das subtribos, Euptychiina (Peña et al. 2010), a taxonomia do grupo ainda é confusa e existem pouquíssimas hipóteses filogenéticas para os gêneros desses grupos (mas veja Matos-Maravi et al. 2013 e Seraphim et al. 2014).

Muitos dos gêneros de Euptychiina são agrupamentos não naturais de espécies e a sistemática filogenética é uma ferramenta poderosa que pode auxiliar numa melhor resolução taxonômica desses grupos, bem como prover meios para uma melhor compreensão dos padrões de diversificação das diversas linhagens do grupo na região Neotropical.

Assim sendo é importantíssimo que mais estudos filogenéticos, biogeográficos e de taxas de diversificação sejam feitos com borboletas neotropicais de modo geral, principalmente para os satiríneos, e especialmente a nível genérico, para que seja possível chegar a um maior entendimento de como essa região biogeográfica é tão rica e diversa em se tratando de espécies de borboletas.

**Objetivos da tese**

1) Provar uma hipótese filogenética robusta para o gênero *Yphthimoides* com base em caracteres moleculares, usando três marcadores (COI, GAPDH e RpS5) para se tentar corroborar o monofilétismo desse gênero;

2) Provar uma hipótese para se tentar explicar o padrão de distribuição geográfica atual do gênero *Yphthimoides* na região Neotropical;

3) Provar uma hipótese filogenética robusta para o clado *Pharneuptychia* (gêneros *Pharneuptychia*, *Moneuptychia* e o complexo de espécies cripticas *Euptychoides castrensis*) com base em caracteres moleculares, usando três marcadores (COI, GAPDH e RpS5);

4) Descrever as espécies novas que venham a ser identificadas durante a execução do trabalho filogenético;
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Capítulo 1: Relações filogenéticas e padrões de distribuição biogeográfica de *Yphthimoides* Forster, 1964

Este capítulo é composto por um único artigo, a saber:

1.1 - “*Systematics, diversification and biogeographic history of the Neotropical butterfly genus Yphthimoides (Nymphalidae: Satyrinae)*” – Este artigo trata das relações filogenéticas das espécies do gênero Yphthimoides, bem como da origem e diversificação do grupo na região neotropical e também dos padrões de distribuição biogeográfica.

**Resumo**

Este capítulo é composto por um único artigo contendo a filogenia usando marcadores moleculares, as estimativas de datação da diversificação do gênero *Yphthimoides* e as análises de biogeografia histórica.

Análises moleculares demonstraram que o gênero *Yphthimoides* não é um grupo natural como atualmente definido. Das espécies utilizadas nas análises, quatro não compartilham um ancestral em comum com o restante do grupo, sendo mais próximas de espécies identificadas como sendo do gênero *Paryphthimoides*. As espécies verdadeiras de *Yphthimoides* têm como grupo-irmão o gênero *Carminda*, do qual divergiram à aproximadamente 11.86 milhões de anos atrás, durante a época conhecida como Mioceno médio.
Systematics, diversification and biogeographic history of the Neotropical butterfly genus *Ypthimoides* (Nymphalidae: Satyrinae)

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Abstract. The systematics studies of butterflies had increased in the last years allowing a better understanding of evolutionary relationships among most groups and also bringing to light the huge amount of taxonomic mishmash pervading several groups. One of these groups is the nymphalid subtribe Euptuchiina which has recently been shown to comprise a great amount of non-monophyletic genera based on molecular phylogenetic analyses. Among these genera is *Ypthimoides*, whose distribution is widespread throughout the Neotropical region, from Mexico to Argentina. Most of its species occurs in the open areas of Southeastern Brazil. The present work using three molecular markers (COI, GAPDH and RpS5) corroborates the hypothesis that *Ypthimoides* as recognized today is a non-natural group with some of its currently assigned species been more closely related to some Paryphthimoides species. The analyses of male genitalia morphology also provided additional evidence to this hypothesis. The dating of divergences point out that the split between the ancestral lineage of *Ypthimoides* and its sister group, Carinda, is likely to have occurred around the middle to the end of Miocene, around 11.86 Mya.

Keywords. Lepidoptera, butterflies, phylogeny, species diversity, biodiversity loss
Running title: Systematic and biogeography of *Yphthimoides*
1. Introduction

Earth’s biodiversity is been lost at an exacerbated rate in the present days (Cardinale et al. 2012; Costello et al. 2013; Pimm et al. 2014) mostly due to habitat losses caused by mankind actions (Savage 1995; Fahrig 2003; Mantyka-Pringle et al. 2012). Species that are already known to science and those still in need to be discovered and described are being extinct in an increasing rate (Costello et al. 2013).

Species loss may also prevent scientists to study and understand the evolutionary or phylogenetic relationships among these species which would in a first moment allow the understanding of the patterns of origin and diversification of species through the tree of life. The knowledge of such patterns could help researchers to prevent or minimize species loss by identifying which lineages would have a higher or lower diversification rate and then help the political decisions in how to conserve these lineages in a better way (Myers et al. 2000).

Despite a substantial increase in phylogenetic studies in all domains of life (e.g., Ciccarelli et al. 2006; Regier et al. 2010; Ebersberger et al. 2012; Gatesy et al. 2013; Nosenko et al. 2013; Ruhfel et al. 2014; Whelan et al. 2015; Zuo et al. 2015) in the past years, there is still a lot to be done even in those groups that are apparently very well studied such Lepidoptera, especially the butterflies. Among butterflies one family has received a lot of attention in the past years, Nymphalidae, with several phylogenetic studies targeting almost all taxonomic levels from family to genera (e.g., Harvey 1991; Penz 1999; Brower 2000; Wahlberg et al. 2003; Willmott 2003; Freitas & Brown 2004; Willmott & Freitas 2006; Zhang et al. 2007; Wahlberg et al. 2009; Elias et al. 2009; Garzón-Orduña 2012; Murilo-Hiller 2012; Ortiz-Acevedo & Willmott 2013; Brower et al. 2014; Tóth et al. 2014).

Even having a great covering regarding phylogenetic studies the family Nymphalidae still has many taxa with a poor sampled phylogeny or lacking a phylogeny at all. And in those studies with well sampled and supported phylogenies some questions regarding taxonomic and phylogenetic issues still remain to be answered when looking at specific groups. One of these groups of Nymphalidae is the subfamily Satyrinae.

Notwithstanding these studies Satyrinae still remains with much taxonomic and phylogenetic mishmash (Marín et al. 2011) and the previous studies were of great value helping solve some of these issues and shedding light on other ones that still need to be solved.

The subtribes Pronophilina and Euptychina are two of these groups that still have to be thoroughly studied. The only phylogenetic treatment received by Pronophilina was in the phylogenetic studies of the subfamily Satyrinae and the tribe Satyrini by Peña et al. (2006, 2011) and more recently with Matz & Brower (2016) using South Temperate species.

Euptychina on the other hand has two phylogenetic studies (Murray & Prowell 2005; Peña et al. 2010) showing that this subtribe might not be monophyletic and also helping solve the major relationships among the groups revealing several non-natural genera spread throughout the entire subtribe.

These two works have disagreements regarding one particular genus, Yphantimoides Forster, 1964. Murray & Prowell (2005) found Yphantimoides as non-monophyletic and Peña et al. (2010) found Yphantimoides as monophyletic. As the aim of these studies was not the phylogeny of Yphantimoides, in both works the genus was under sampled. Murray & Prowell (2005) used only two species and Peña et al. (2010) used four species and neither work used the same species or the type species Yphantimoides yphantima (C. Felder & R. Felder, 1867) in their analyses.

Yphantimoides has 29 described species (Lamas 2004b; Freitas 2004; Freitas et al. 2012; Barbosa et al. 2015, 2016), and two non-described species (Lamas 2004b). These last two undescribed species are in fact not related to Yphantimoides based on the analysis of male’ genitalias and adults’ pictures. The genus is widespread in Neotropical region, distributed through Mexico to Argentina and is highly diverse in Southeastern Brazil occurring mainly in open habitats and forest edges.

Based on preliminary morphological analyses of male genitalia of several species currently assigned to Yphantimoides, a hypothesis of this genus as a non-monophyletic group has been raised, agreeing with Murray & Prowell (2005).

To test this hypothesis the present work grants a phylogenetic study of the genus Yphantimoides based on molecular data and sampling most of the species currently assigned to the genus. The work also deals with the biogeographic patterns of Yphantimoides in the Neotropical region to better understand how the diversification of this group occurred.

2. Material and methods
2.1 Taxon sampling.

Our dataset includes 461 terminal taxa representing 116 species of Satyrini from several localities in the Neotropical region and Southeast Asia (*Paleonympha opalina*). Most of them were used as outgroups to genus under study since our preliminary hypothesis based on morphology of male genitalia was that *Ypthimoides* was not a monophyletic group and therefore some of the species currently assigned to the genus would appear somewhere else in the phylogeny tree. All sequences have been deposited in GenBank. Table 1 shows the current classification of sampled species and the GenBank accession numbers. Taxonomic nomenclature for genera and species follows Lamas (2004), with additions by Freitas (2004, 2007), Freitas & Peña (2006), Peña et al. (2006, 2010, 2011) and Wahlberg et al. (2009).

*Ypthimoides* specimens were collected in the field by several researchers and send to us for molecular analyses and also examined in 10 public collections (see below). The Lamas collection of Neotropical butterfly type specimen photographs at the MUSM (also available online in Warren et al. 2013), representing most currently relevant names and recognized species of *Ypthimoides* (Lamas 2004).

The acronyms for the collections are: **DZUP** – Coleção Entomológica Padre Jesus de Santiago Moure, Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil; **IML** - Instituto Miguel Lillo, Tucumán, Tucumán, Argentina; **MEFLG** - Museo Entomológico Francisco Luis Gallego, Universidad Nacional de Colombia, Medellín, Colombia; **MNHN** – Muséum National d’Histoire Naturelle, Paris, France; **MNRJ** - Museu Nacional da Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil; **MZSP** - Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil; **NHMUK** - The Natural History Museum, London, England; **ZSM** - Zoologische Staatsammlung München, München, Germany; **ZUEC** - Museu de Zoologia da Universidade Estadual de Campinas, Unicamp, Campinas, São Paulo, Brazil; **ZUEC-AVLF** - André V. L. Freitas Collection, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

2.2 Morphological procedures

Male genitalia dissections were made using standard techniques. Abdomens were soaked in hot 10 % KOH solution for 10 min before dissection, and dissected parts were stored in glycerol. Photographs of the male genitalia were taken using a Zeiss Discovery V20 Stereomicroscope.

2.3 Molecular procedures
Total genomic DNA was extracted from two legs of each specimen, by using the Invisorb Spin Tissue Mini Kit protocol (Stratec Molecular, Berlin, Germany), QIAGEN’s DNeasy® extraction kit and Illustra tissue and cells genomicPrep Mini Spin Kit (GE Healthcare Life Sciences), following the manufacturers’ instructions. We used three standard gene regions used in previous studies on nymphalid butterflies which have been shown to provide useful phylogenetic resolution at lower taxonomic levels (Kodandaramaiah & Wahlberg 2007, 2009; Leneveu et al. 2009; Aduse-Poku et al. 2009; Peña et al. 2010, 2011; Müller et al. 2010). The primer pairs used to amplify the mitochondrial gene COI (1498 base pairs (bp)) were LCO+HCO (Folmer et al. 1994) for the first half and Jerry+Paty for the second half (Simon et al. 1994) and for the nuclear genes the primer pairs were Frigga+Burre (Wahlberg & Wheat 2008) for GAPDH (691 bp) and rpS5degF+ rpS5degR (Wahlberg & Wheat 2008) for RpS5 (610 bp).

For the amplification of the first half of COI, PCR was carried out in a total volume of 25 µl using 2 µL of total DNA, 2.0 mM of MgCl₂, 40 µM of dNTPs, 0.5 µM of each primer, 1 U of GoTaq DNA Polymerase (Promega, Madison, WI, USA), and 10% of 1× Taq buffer. The amplification program included an initial denaturation step at 95°C for 5 min, followed by 34 cycles of denaturation at 94°C for 30 s, annealing at 42°C for 30 s, and polymerization at 72°C for 1 min, followed by an extension step at 72°C for 10 min (Silva-Brandão et al. 2005).

For the second half of COI and the two nuclear markers, PCR reactions were carried out in a total volume of 20 µl using 3 µL of total DNA, 1.5 mM of MgCl₂, 100 µM of dNTPs, 0.2 µM of each primer, 1 U of GoTaq DNA Polymerase (Promega, Madison, WI, USA), and 10% of 1× Taq buffer. The amplification program for the nuclear genes included an initial denaturation step at 95°C for 5 min, followed by 34 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 1 min, and polymerization at 72°C for 2 min, followed by an extension step at 72°C for 10 min. For the second half of COI, the amplification program was the same as the nuclear genes except by the annealing step, which was done at 50°C for 1 min. PCR products were purified of primers and deoxynucleotides with ExoSAP-IT (GE Healthcare, Buckinghamshire England), and then sequenced by ABI Prism BigDye Kit protocol in a 3500xL Genetic Analyzer (Applied Biosystems—Hitachi), with primers used for amplification.

Sequences were analyzed with the program FinchTV v. 1.4.0 (Geospiza Inc.). Those sequences were posteriorly aligned by eye in the Biological Sequence Alignment Editor Software (BioEdit) v. 7.2.4 (Tom Hall 2013, available at
http://www.mbio.ncsu.edu/bioedit/bioedit.html#downloads) with sequences obtained previously and available on GenBank.

2.4 Phylogenetic analysis

We conducted maximum parsimony (MP), Maximum likelihood (ML) and Bayesian inference (BI) analyses using combined-gene datasets. MP analyses as implemented in TNT v1.1 (Goloboff et al. 2003) were executed utilizing heuristic searches and the “New Technology Search” algorithms (Goloboff 1999; Nixon 1999) with 100 random additions. Clade support values were measured by using the resampling-based bootstrap approach (Felsenstein 1985) performing 1000 pseudo-replicates and 10 independent replicates and Bremer support. BI analyses were carried out in MrBayes v3.2 (Ronquist & Huelsenbeck 2003) and ML analyses were carried out in RaXML v7.3.1 (Stamatakis et al. 2008) on the CIPRES Science Gateway portal v3.1 (Miller et al. 2010). In all analyses we partitioned our datasets by gene sequences and also used the software TIGER (Cummins & McInerney 2011) to partition our combined dataset into 5 “bins” each containing a number of characters with similar relative rates: bin1 = 1670, bin2 = 471, bin3 = 330, bin4 = 178, bin5 = 150 which has been found to be informative for BI (Rota 2011).

For BI the calculations were run twice for 20 million generations each so that the final average standard deviation of split frequencies was below 0.01, sampling trees every 500 generations and using four simultaneous chains (one cold and three hot) in each run. The parameters and the model of evolution GTR + G were unlinked across character partitions. We discarded as burn-in the first 25% of the sampled trees that were not within the stationary distribution of log-likelihoods. Trees and posterior probabilities were summarized using the “50%-majority rule” consensus method.

For ML analysis 1000 rapid bootstrap replicates and a search for the maximum likelihood topology were selected and the data were modelled according to the GTR+CAT model for each partition independently.

2.5 Dating of divergences

Due to the lack of fossil records for the genus and no equally sampled outgroups, the phylogeny was reconstructed including only the described species of the clade composed by the genera Yphthimoides, Carminda, Pharneuptychia, Moneuptychia and the species Euptychoides castrensis, Stegosatyrus ocelloides and Graphita gripe and constrained it with
secondary calibration points from a fossil-calibrated Euptychiina phylogeny (Peña et al. 2010).

We used the Bayesian analysis software BEAST ver. 2.3.2 (Drummond & Rambaut 2007) on the CIPRES Science Gateway portal v3.1 (Miller et al. 2010) under a log-normal relaxed molecular clock. The DNA sequences were divided in five partitions using TIGER software (Cummins & McInerney 2011), with parameter values estimated separately for each partition. The combined dataset was analysed under the GTR+Γ model with a relaxed clock allowing branch lengths to vary following an uncorrelated log-normal distribution (Drummond et al. 2006). The analysis was run four independent times for 40 million generations (with pre-run burn-in of 400 000 generations) with sampled trees every 1000 generations and the results compiled using all runs.

We selected five terminals per Ypthimoides species and species from outgroups to maximize the gene coverage in the resulting dataset except for those species were there were less than five individuals per species. The selected calibration points were chosen from well-supported monophyletic groups from the Peña et al. (2010) Euptychiina phylogeny: the root of the tree to 16.8 ± 5 Ma, the crown age of Moneuptychia soter to 1.3 ± 4 Ma, the crown age of Carinda + Ypthimoides to 14.22 ± 5 Ma and the split age between Ypthimoides cipoensis and Ypthimoides angularis to 8.60 ± 4 Ma.

The tree priors were set either to the Birth-Death or the Calibrated Yule speciation processes as the tree prior in separate analyses to investigate and compare the impact of this parameter on the final age estimates. All other priors were left to the default values in BEAST.

Tracer v1.3 was used to analyze convergence, good mixing of MCMC and the Effective Sample Size of each estimated parameter to be higher than 200 and trees were summarized using TreeAnnotator v1.4.7 software, which are distributed with the BEAST package.

2.6 Biogeographic history reconstruction

With the objective of trying to reconstruct the biogeographical history of Ypthimoides genus in the Neotropical Realm based on ancestral areas of distribution and historical processes that helped to shape the current distribution of the group, biogeographic analysis was carried on the program Reconstruct Ancestral State in Phylogeny (RASP – Yu et al. 2011) using Statistical Dispersal-Vicariance Analysis (S-DIVA – Yu et al. 2010) which was based on the DIVA 1.1 software developed by Ronquist (1996). The analysis was run without restriction regarding the maximum number of ancestral areas in each node.
The Neotropical realm was subdivided into eight major geographical areas based on the current distribution of the extant species of *Ypthimoides*, as follows: Atlantic Forest South, Atlantic Forest North, Caatinga, Cerrado, Chaco, Amazon Forest, Andean region and Central America (Figure 5).

3. Results

*Ypthimoides* phylogeny

The Maximum Parsimony analysis produced 337 equally most parcimonious cladograms of length 12178 steps (CI = 0.184; RI = 0.799) and the strict consensus tree is shown in Figure 1. The Bayesian and the Maximum Likelihood analyses produced trees (Figures 2 and 3, respectively) that are largely congruent with the most parcimonious cladograms. The consistency in the patterns of relationships recovered regardless the method of analysis used implies a strong phylogenetic signal in the data. There were no differences in trees produced in Bayesian analyses regarding the type of data partitioning.

All the analyses recovered the genus *Ypthimoides* as non-monophyletic regarding the current taxonomic classification of the genus with high support values.

The true species of *Ypthimoides* appear in a well-supported (Bootstrap (bs = 97; posterior probability (pp = 1) major clade along with *Carminida, Graphita griphe, Stegosatyurs ocelloides* and the *Pharneuptychia* clade.

Of all the species from *Ypthimoides* used in the molecular analyses, three species appear nested along with some *Paryphthimoides* species, *Ypthimoides affinis, Y. maepius* and *Y. mimula*. *Ypthimoides mimula* appers to be sister to *Paryphthimoides grimon* or, given the genetic distance between these two *taxa*, they might even be subspecies from the same species. Another *Ypthimoides* species, *Y. punctata*, was proved to belong to a new genus along with *Hajjesia griseola*, with both been synonymized as a single species, *Sepona punctata* (Weymer, 1911), by Freitas et al. (2016).

*Ypthimoides eriphule* appears as an isolated branch which is sister to a clade composed by *Y. mimula, Y. maepius, Y. affinis, Paryphthimoides poltys, P. grimon, P. difficils*, *P. sylvina, Paryphthimoides* sp. nov2, *Paryphthimoides* sp., *Magneuptychia flavofascia* and some other species of *Magneuptychia, Cissia, Caeruleuptychia* and *Capronnieria (Paryphthimoides poltys* clade in Fig. 2) (with a relatively good supported (bs = 73; pp = 0.94).

The analysis of the male genitalia morphology has corroborated the removal of these species from *Ypthimoides* genus. Male specimens of *Ypthimoides* have a very short saccus
compared to the valva, which is in general robust and large (for comparison, see Figure 3). The species placed outside of *Yphthimoides* (*Y. maepius, Y. affinis* and *Y. mimula*) have a long saccus and a thin valva resembling male genitalia of *ParYphthimoides* species. *Y. eriphule* has a short saccus but the general shape of the male genitalia is quite distinct from the true *Yphthimoides*. Since the work of Peña et al. (2010) it is known that *Paryphthimoides* is not a natural group so the species identified as *Paryphthimoides* in our work (except *P. poltys*, the type species of the genus) will probably be placed in other genera.

Of all the current described species of *Yphthimoides* we were not able to collect individuals and extract DNA from *Y. mythra, Y. neomenas, Y. argyrospila* and *Y. austere*, but based on male genitalia comparison and wing pattern of the latter, these species do not belong to the genus *Yphthimoides* either. *Y. mythra* and *Y. neomenas* might be related to some *Paryphthimoides* species based on the somewhat long saccus and thin valva. The morphology of the male genitalia of *Y. argyrospila* is quite distinct with short saccus and thin valva. We were not able to analyze the male genitalia from *Y. austera* because the type is a female (as can be seen in Warren et al. 2013) and we have not collected any further specimen in the field and neither found any other specimen in butterfly collections but based on the wing color pattern, it is possible that *Y. austera* could actually be some aberration of a *Cissia* or *Magneuptychia* species. We could not analyze both the male genitalia or DNA samples of *Y. acmenis* either because no specimen was collected. The holotype is apparently lost and the only individual found was in the NHMUK collection with the abdomen missing. Therefore we cannot confirm that *Y. acmenis* is or is not part of the genus *Yphthimoides*.

All the remaining species of *Yphthimoides*, including the type species of the genus, *Yphthimoides yphthima*, used in the molecular analyses appear in a well-supported clade (bs = 76; pp = 0.93) and sister to *Carinda* genus (bs = 90; pp = 1). *Carinda* appears in the analysis as monophyletic with the relationship of its three species, *C. paeon, C. umuarama* and *C. griseldis* been well-supported (bs = 97; pp = 1) which is different from what was found by Peña et al. (2010) with *Carinda* (species identified as *Moneuptychia paeon* and *M. griseldis*) recovered as non-monophyletic.

Despite being well-supported the internal relationships of the clade *Yphthimoides* remain poorly understood using three genes. Small clades (for example the “renata clade”) and sibling species are well-supported but the relationships among these species inside these small clades have somewhat low support.

The well-supported relationships are the following: *Yphthimoides pacta* and *Y. patricia* are sister species (“pacta clade”) (bs = 100; pp = 1) and sister to all the remaining species in
the clade. In this clade the relationships with the highest bootstrap and posterior probability values are Yphthimoides yphthima and Y. straminea ("yphthima clade") which is also well-supported, Y. bella and Y. celmis which is also well-supported, Y. borasta sister which is also well-supported to Y. iserhardi and Y. cipoensis which is also well-supported.

The "yphthima clade" (bs = 100; pp = 1) is sister to a well-supported major clade (bs = 77; pp = 0.99) containing the remaining Yphthimoides species that are separated in two well-supported sister clades, the first one (bs = 85; pp = 1) composed by Y. gabiela, Y. bella + Y. celmis, Y. ochracea, Y. angularis and Y. borasta + (Y. iserhardi + Y. cipoensis), hereafter "celmis clade", and the second one (bs = 100; pp = 1) composed by Y. legualimai + Y. renata (from Colombia, Mexico and Costa Rica) (bs = 53; pp = 0.99), Y. manasses + Y. ordinaria (bs = 100; pp = 1), Y. nareta, Y. blanquita and Y. renata (from Brazil), hereafter “renata clade”.

Despite being part of the same major clade, the specimens identified as Yphthimoides renata were recovered as two different subgroups, one from Colombia and Central America and the other one from Brazil (see Barbosa et al. 2015, 2016). This clearly indicates that these specimens are part of a complex of cryptic species and that probably the individuals from Colombia, Mexico and Costa Rica must be a new species since the type species of Y. renata is from Suriname. The Brazilian state of Roraima, where one of the individuals from Brazil was collected, is very close to Suriname and both regions are located in the south part of the Guiana Shield which could be a natural barrier maintaining these species apart.

**Dating of divergences**

The BEAST analyses with either Birth-Death or Yule birth model recovered a tree whose topology is congruent with trees topologies recovered in the MP, ML and BI analyses (Figure 4). In the present work we are showing only the tree recovered with Yule birth model, since its likelihood value was higher than the Birth-Death model.

The age estimates from each node using the relaxed molecular clock produced wide credibility intervals, especially those near the root. According to Graur & Martin (2004) this should be expected when using secondary calibration points in the analyses.

Our estimated times indicate that the major clade composed by Yphthimoides, Carminda, Stegosatyrus ocelloides, Graphita gripe and Pharneuptychia clade appeared around 15.53 (± 4.52) Mya, during the beginning of the mid-Miocene and that the ancestral lineage split that give birth to the lineages of Carminda and Yphthimoides occurred around 11.86 (± 3.54) Mya, in the late Miocene, which is congruent with the dating results found by Peña et al. (2010) to these two genera in their work with Euptychiina biogeographic history.
The diversification of *Yphthimoides* started around 11.36 (± 3.38) Mya, at the end of the middle Miocene, when the ancestral lineage of “pacta clade” split from the ancestral lineage of the other species. The lineages of *Y. pacta* and *Y. patricia* split at approximately 6.99 (± 2.45) mya (late Miocene) and begun to diversify around 2.44 (± 1.40) Mya and 0.34 (± 0.25) Mya, respectively.

The ancestral lineage of the other species of *Yphthimoides* split into the ancestral lineage of “yphthima clade” and the ancestral lineage of “celmis clade” and “renata clade” around 10.43 (± 3.20) Mya. The “yphthima clade” had split into *Y. yphthima* and *Y. straminea* around 6.99 (± 2.45) Mya and the lineages begun their diversification around 1.83 (± 0.80) Mya and 1.25 (± 0.63) Mya, respectively.

The ancestral lineages of “celmis clade” and “renata clade” split around 9.72 (± 2.90) Mya and begun to diversify at approximately 8.51 (± 2.55) Mya and 6.50 (± 2.04) mya, respectively, given rise to the remaining extant species of the genus.

Taking into account the wide confidence intervals the origin of *Yphthimoides* and *Carminida* is somewhat between the middle and the end of the late Miocene. Some species have originated during the late Miocene, around 11 and 5 Mya, while other species have originated during the early Pliocene, around 5 and 3.6 Mya (Figure 4) and the major diversification of these species started through the early Pliocene to the late Pleistocene (3.8 to 0.31 Mya). In the *Taygetis* clade Matos-Maraví et al. (2013) have found that most species originated and diversified through the late Miocene to the middle to late Pliocene and only a few species originated during the Pleistocene.

**Biogeographic history**

According to the RASP optimal biogeographic hypothesis (Figure 6), to explain the actual distribution pattern of *Yphthimoides* species, 19 dispersal events, 12 vicariant events, a single extinction event and at least two duplication events (sympatric speciation) were needed. The duplication events were more noticeable mainly in the Cerrado area.

**4. Discussion**

4.1. **Systematics**

Murray & Prowell (2005) recovered *Yphthimoides* as non-monophyletic as well, but they used only two described species of *Yphthimoides*, *Y. renata* and *Y. erigone* (today synonymized with *Y. maepius*). Peña et al. (2010) recovered *Yphthimoides* as monophyletic using four described species, *Y. borasta*, *Y. leguialimai*, *Y. eipoensis* and *Y. angularis*. 
In both works (Murray & Prowell 2005; Peña et al. 2010) *Ypthimoides* was under sampled and the type species of the genus was not sampled as that was not the aim of both works so more in-depth phylogenetic comparisons will not be possible to be made.

Our inferred phylogenetic hypothesis based on three molecular markers is robust and shows that some taxonomic changes are in need. The genus *Ypthimoides* as currently assigned is not a natural group since four of the described species used in the molecular analyses were not recovered in the clade with the type-species in the tree.

Three of these species, namely *Ypthimoides mimula*, *Y. maepius* and *Y. affinis*, appear in a clade along with some putative species of *Paryphthimoides* and should be assigned to this genus in a first moment. According to the work of Peña et al. (2010) *Paryphthimoides* is not a monophyletic clade and probably some of its species will be reassigned to other genera, new or already described, but more deep phylogenetic and taxonomic studies are needed to correctly solve this issue.

*Ypthimoides eriphule* appear as an independent lineage sister to the clade composed by *Paryphthimoides*, *Y. mimula*, *Y. maepius* and *Y. affinis* and should be assigned to a new genus to be described.

Other species currently assigned to *Ypthimoides* and that have not been studied from the molecular perspective, namely *Y. mythra*, *Y. neomenas*, *Y. argyrospila* and *Y. austera*, should be reassigned to other genera based on the morphology of the male genitalia or wing color pattern, as is the case of *Y. austera*.

Species of the true *Ypthimoides* have a very distinctive male genitalia pattern although not autapomorphic. The saccus is very short, having in most species less than half of the valva size and never been longer than half of valva size. The valva is very robust and large and most species present well-developed projections ("teeth") at the internal margin of the posterior end of the valva (*cucullus*, sensu Klots 1956) (see Fig. Z).

The placement of *Y. eriphule*, *Y. mimula*, *Y. maepius* and *Y. affinis* out of the genus *Ypthimoides* is also corroborated by the male genitalia morphology. *Y. mimula*, *Y. maepius* and *Y. affinis* have long saccus compared to the size of valva, which is thin, delicate and constricted near the apex. Male genitalia of *Y. eriphule* is a slightly different of other species, with valve been robust and squared but tapering near the apex and the gnathos are very long when compared to the size of uncus. In the case of *Y. austera*, it was not possible to access male genitalia morphology but based on the wing color pattern is most likely that the holotype of this species is an aberrant form of some *Cissia* or *Magneuptychia* species.
The true *Yphthimoides* was recovered as sister to *Carinda* genus, also recovered in the present work as monophyletic. In the work of Peña *et al.* (2010) *Carinda* was recovered as non-monophyletic. *Carinda* has three described species and in our work *C. griseldis* appears as sister to *C. umuarama + C. paeon*, all with high support values.

Regarding *Yphthimoides*, the clade can be divided in four main subgroups, namely “pacta clade” (*Y. pacta + Y. patricia*), “yphthima clade” (*Y. yphthima + Y. straminea*), “celmis clade” (*Y. celmis, Y. bella, Y. gabriela, Y. ochracea, Y. angularis, Y. borasta, Y. cipoensis* and *Y. iserhardi*) and “renata clade” (*Y. renata, Y. legualimalai, Y. ordinaria, Y. manasses, Y. nareta* and *Y. blanquita*) with high support for each one of the subgroups.

The morphology of the male genitalia also corroborates these four subgroups (Fig. Z). In “renata clade” male genitalia possesses a more or less slender valva and a saccus length that has almost half size of valva. Also the internal margin of cucullus does not bear developed “teeth”, instead they are very small.

Species from “celmis clade” possesses a more squared valva and much shorter saccus when compared to the total length of the valva. Also the cucullus bears very developed “teeth” in its internal margin. In “yphthima clade” the valva is robust, long and somewhat oval shaped and in “pacta clade” the cucullus bears two sets of small “teeth” inwardly curved.

In “renata clade” all the species are very similiar, presenting a more or less uniform wing color pattern and some species of this clade appear to be part of complexes of cryptic species, as is the case of *Y. renata*. The phylogenetic analyses recovers this species as two separated groups, one containing the individuals from Central America and Andean region and the other one containing the individuals from Brazil.

The original locality of the holotype of *Y. renata* (Stoll, 1780) is Suriname, in the Guiana Shield and one of the individuals of *Y. renata* from Brazil that appears in the analyses was collected in the Brazilian state of Roraima, also localized in the Guiana Shield and near Suriname. So based on this information and on the molecular data, it is possible that the individuals from Brazil identified as *Y. renata* are indeed from the species *Y. renata* and those individuals from Central America and Andes might be a new species (Barbosa *et al.* 2016; Barbosa & Freitas in prep.). The Guiana Shield, especially the table-like mountains (*tepuis*) might have acted as a geographical barrier separating the *Yphthimoides* species from Andes and Central America and from Brazil.

4.2. *Diversification and biogeography*
According to Peña et al. (2010) early in Euptychiina evolution a dispersal event to central South America gave rise to a lineage that underwent through some dispersal events that produced at least two lineages. One of these lineages, of which Ypthimoides is a part of (Megisto clade), diversified in the south-eastern Brazil.

Almost all the species in the clade composed by Ypthimoides, Carinda, Graphita gripe, Stegosatyrus ocelloides and Pharneuptychia inhabit open habitats like grasslands, campos de altitude, campos rupestres and also forest edges. The exception being the sister-group of Ypthimoides, the genus Carinda, in which all species inhabit forested habitats.

The split between the ancestor of Carinda and the ancestor of Ypthimoides occurred at 11.86 Mya and probably the ancestor of Carinda changed from an open habitat to forested ones, the interior of forests. The ancestor of Ypthimoides remained in the open habitats and forest edges and started to diversify at more or less 11.36 Mya.

The ancestral lineage of Ypthimoides has probably originated in an area where today is the Cerrado biome. Some studies point out the origin of Cerrado between three to eight millions of years ago (Pinheiro & Monteiro 2010; Lehmann et al. 2014), from the late Miocene to early Pliocene with the oldest palynologic record of Cerrado flora dating back to 32,000 years ago (Ledru 2002). Since the origin of Ypthimoides dates back to approximately 11.86 Mya and giving the confident interval, the genus started to diversify almost at the same time of the origin of Cerrado biome.

Once settled in the Cerrado, some dispersal events occurred into the Atlantic Forest (both south and north portions), the Caatinga, the Amazon Forest and back into the Andes, where the species came to occupy the forest edges and forest canopy (as is the case of Y. borasta, Freitas A. V. L. pers. com.).

Some vicariant events occurred as well, splitting Y. borasta in the Atlantic Forest from Y. cipoensis and Y. iserhardi in the high altitudes of Cerrado (“Campos rupestres” or Rocky fields), splitting Y. cipoensis from Y. iserhardi in north and south portions of Espinhaço range, splitting the Colombian populations of Y. renata from the Central America populations of Y. renata (Y. renata is clearly a complex of cryptic species, Barbosa et al., in prep.) and splitting Y. manasses in the north portion of the Atlantic Forest from Y. ordinaria in south portion of Atlantic Forest and Chaco.

It is interesting to note that both Y. cipoensis and Y. iserhardi inhabits and are restricted to the Campos rupestres (Rocky fields) in Espinhaço range mountains that extends from Minas Gerais to Bahia states in Brazil (Rapini et al. 2002). These two species are the only Ypthimoides species in Brazilian biomes that are exclusive to high altitudes. Ypthimoides
*ochracea* can be found at higher altitudes (between 1800 and 2000 m.a.s.l.) in *Serra da Mantiqueira* in São Paulo state but it is not exclusive of these ambients of high altitude.

*Yphthimoides cipoensis* is now restricted to the south portion of Espinhaço, called Serra do Cipó, and *Y. iserhardi* is restricted to the north portion, called Chapada Diamantina. After their speciation event about 4.46 mya, somehow a geographical barrier has originated in the Espinhaço range keeping these two species spatially disjunct.

There is a topographical discontinuity between north and south portions of Espinhaço range which are known to have different flora (Bitencourt & Rapini 2013) with a few endemic species shared by both portions (Rapini *et al.* 2002, 2008) and according to Bitencourt & Rapini (2013) this gap of about 300km between the rock fields from Minas Gerais and Bahia is an important constraint preventing the dispersion of endemic plants. Apparently the geographical disjunction observed for *Y. cipoensis* and *Y. iserhardi* is due to this topographical discontinuity that split these two species apart.

The vicariant event splitting *Y. nareta* from *Y. blanquita* cannot be discussed further because the support of their relationship is very low. It is mostly likely that *Y. nareta* is closely related to *Y. renata* from Brazil and *Y. blanquita* is probably closely related to *Yphthimoides* species from Andes and Central America, although more molecular data (more genes) are needed to corroborate these assumptions.

Another interesting vicariant event led to the splitting of populations of *Y. renata* from Brazil in two groups: one in the Amazon Forest, near the Guiana Shield and other in the Atlantic Forest, *Cerrado* and *Caatinga*. The population of *Y. renata* in the Brazilian state of Roraima, in the Amazon Forest, is close to the type locality of the species located also in the Guiana Shield. The type-specimen is from Suriname.

This scenario is interesting because the population from Guiana Shield might be the true *Y. renata* species while the specimens identified as *Y. renata* from Central America, Andes and other Brazilian regions could be different species (EPB & AVLF, *in prep.*). Since we have only one individual from the Guiana Shield (YPH-0287), more individuals from the same location must be collected and the DNA extracted for further analysis in order to achieve a confident interpretation about this scenario.

Among *Yphthimoides* diversification two duplication events (sympatric speciation) occurred in *Cerrado* giving origin to the sister-species *Y. pacta* and *Y. patricia* and *Y. yphthima* and *Y. straminea*.

Regarding the the dispersal events that were responsible for the colonization of the Andean and Central America regions by *Yphthimoides* species, there were two of them which
have occurred probably when the Andes were already formed although its uplifting had accelerated during the Miocene-Pliocene. This accelerated uplifting could have contributed to isolate populations during that period (Elias et al. 2009 and references therein).

The first dispersal was made by the lineage that gave rise to *Y. leguialimai* and the specimens also identified as *Y. renata*. *Y. renata* from Andean region was then split in two lineages by a vicariant event. One lineage remained in the Andean region and the other one occupied the Central America region.

The second dispersal was made by the lineage of *Y. blanquita* about 4.74 mya. Once established the species to the low lands of the pacific side of the Andes, which has a physiognomy and climate conditions more similar to the Central America region than to the high altitudes of the mountains in the Andes. Although the analysis pointed out this dispersal event it is not very reliable due to the low support/stability of the relationship of *Y. blanquita* with *Y. nareta*. *Y. blanquita* is more probably related to the other species of the Andes and Central America and the colonization of these regions by *Yphthimoides* species most likely occurred just once, although more data and analysis are needed to certify this assumption.

4.3. Conclusions

The use of only three molecular markers has been showed to be sufficient to solve the phylogenetic relationships at the genus level and to recover which species belong or not to the genus been studied, although the three markers have not been sufficient to solve some of the internal relationships among species inside the genus.

*Yphthimoides* as currently recognized is not monophyletic, which is supported by molecular and morphological data and some taxonomic rearrangements must be done in order to make it a natural group. These rearrangements must take into account assigning the species that do not belong to the genus to other genera, new or described.

The true *Yphthimoides* species have originated about 11.86 mya when its ancestral lineage split from the ancestral lineage of the genus *Carinida* and then started to diversify and colonize several new habitats in both Brazil and Andes and Central America. A combination of dispersal and vicariant events was responsible to shape the current geographical distribution of *Yphthimoides* species in the Neotropical region.

The present studied is a contribution to improve the phylogenetic and biogeographical understanding of a particular and interesting genus of Euptychiina that has a somehow good number of species and also shows that more phylogenetic studies focused on the genus level
are needed to help solve all the non-natural groups that still exist in this subtribe and that are continuously being discovered.

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Table 1. Information of specimens used in the analysis with genbank accession numbers (in bold sequences obtained by the main author).

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Splendenspitychia furina CP02-39 Madre de Dios, Peru GU205868 GU205982 GU206043
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**Figure 1 (A-B-C).** Strict Consensus tree of 37 equally parsimonius trees inferred from the maximum parsimony analysis (length 12178; CI = 0.184; RI = 0.799) of the combined dataset. Numbers below and above branches are Bremer support and Absolute Bremer support (ABS) values. Branches are colored by species and clades of *Yphthimoides*.

**Figure 2.** Consensus tree from the Bayesian Inference analysis of the combined dataset, modeled with GTR+Γ. Numbers below branches are Posterior Probability values. Branches are colored by species and clades of *Yphthimoides*.

**Figure 3 (A-B-C).** Consensus tree from the Maximum Likelihood analysis. Numbers below branches are bootstrap values. Branches are colored by species and clades of *Yphthimoides*.

**Figure 4.** Estimated times of divergence derived from the BEAST analysis. Ultrametric tree scaled in Mya. Numbers in parenthesis and horizontal bars represent posterior probability values and 95% credibility intervals. Numbers without parenthesis represent estimated date of divergence for each node.

**Figure 5.** Map showing the biogeographic areas for *Yphthimoides* species.

**Figure 6.** Reconstruction of biogeographical hypothesis for *Yphthimoides* using *Statistical Dispersal-Vicariance* analysis with RASP. Geometrical figures represent historical events and are assigned by black arrows to the nodes were they probably occurred. Colored rectangles represent the ancestral areas of distribution at the nodes.
Figura 1A
Figura 1B
A - Mata Atlântica Sul
B - Mata Atlântica Norte
C - Caatinga
D - Cerrado
E - Chaco
F - Amazônia
G - Andes
H - América Central

Figura 5

Este capítulo é composto por um único artigo, a saber:


Resumo

Este capítulo é composto por um único artigo contendo a filogenia usando marcadores moleculares e as estimativas de datação da diversificação do clado *Pharneuptychia*, composto pelos gêneros *Moneuptychia* e *Pharneuptychia* e pela espécie *Euptychoides castrensis*.

Análises moleculares demonstraram que esses grupos não são monofiléticos como atualmente definidos. Os gêneros *Moneuptychia* e *Pharneuptychia* e a espécie *Euptychoides castrensis*, que se provou ser um complexo de espécies crípticas, fazem parte de um único clado, que divergiu de sua linhagem irmã à cerca de 15.53 milhões de anos atrás.

Com base nos resultados obtidos, as espécies do complexo “*Euptychoides castrensis*” devem ser realocadas no gênero *Moneuptychia*, grupo-irmão do gênero *Pharneuptychia*. 

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**Abstract.** The Satyrinae subfamily is among one of the lesser known groups of Nymphalide butterflies mainly due to its lack of bright wing color patterns and conspicuousness. Most species are entirely brown in their background wing color and because of this characteristic the species correct identification is very hard. In the past years several phylogenetic and taxonomic reviews had been published as a first attempt to tackle with the great amount of non-natural groups especially in the subtribe Euptychiina. Three of these groups are the genera *Pharneuptychia* and *Moneuptychia* and the species *Euptychoides castrensis*, which are distributed in open habitats of South America. Using molecular data the present work provides a phylogenetic hypothesis showing that neither *Pharneuptychia* nor *Moneuptychia* are monophyletic groups as currently recognized and that the species *Euptychoides castrensis* is in fact a complex of cryptic species. These three groups are part of the same clade and closely related to *Carminia*, *Yphthimoides*, *Stegosatyrtus ocelloides* and *Graphita gripe*. Based on the phylogenetic analyses, the genus *Pharneuptychia* is sister to the genus *Moneuptychia*, and *Euptychoides castrensis* proved to be a complex of cryptic species, therefore these species should be reassigned to *Moneuptychia*.

**Keywords.** Lepidoptera, brown butterflies, monophyley, polyphyletic, taxonomic rearrangement
Running title: Phylogeny of the *Pharneuptychia* clade
1. Introduction

The Lepidoptera family Nymphalidae is among the most admired and studied (Harvey 1991; Penz 1999; Brower 2000; Willmott 2003; Elias et al. 2009; Ortiz-Acevedo & Willmott 2013) group of butterflies around the world mainly due to their vast amount of morphological and wing color variation (Wahlberg et al. 2009).

In the past years its phylogenetic relationships within Lepidoptera (Mutanen et al. 2010; Regier et al. 2009, 2013; Kawahara & Breinholt 2014; Heikkilä et al. 2015) and among its subfamilies became very well-known and stable (Freitas & Brown 2004; Wahlberg et al. 2003, 2009).

But even before the subfamilies evolutionary relationships were at some point well established, and after that, most subfamilies internal phylogenetic relationships began to be scrutinized (Penz 1999; Penz & Peggie 2003; Wahlberg et al. 2005; Peña et al. 2006; Willmott & Freitas 2006; Zhang et al. 2007; Zhang et al. 2008; Marconato 2008; Kawahara 2009) seeking a better understanding of their evolutionary histories and patterns of diversification. Of these studied subfamilies, Satyrinae is perhaps the one with most taxonomic difficulties in need of resolution.

Since the phylogenetic studies with the nymphalid subfamily Satyrinae (Peña et al. 2006), the tribe Satyrini (Peña et al. 2011) and the subtribe Euptychiina (Murray & Prowell 2005; Peña et al. 2010), it became clear that the great majority of groups within these taxonomic units were not monophyletic and a huge effort would have to be undertaken in order to solve at least most of these mishmash (Marín et al. 2011).

Following this path several works dealing with phylogenetic relationships of lower taxonomic levels in the subfamily Satyrinae were published (Penz 2007; Penz et al. 2011; Penz et al. 2012; Penz et al. 2013; Kodandaramaiah & Wahlberg 2009; Kodandaramaiah et al. 2009; Kodandaramaiah et al. 2010; Price et al. 2011; Matos-Maraví et al. 2013; Seraphim et al. 2014; Peña et al. 2015).

Of these groups Euptychiina is certainly one of the most problematic (Murray & Prowell 2005; Peña et al. 2010) with most of its clades pervaded by non-natural genera.

Three phylogenetic studies dealing with some genera of Euptychiina and using molecular data were published so far. Matos-Maraví et al. (2013) studied the Taygetis clade and found that Taygetis and some other genera used in the study were not monophyletic and would need some taxonomic adjustments.

Seraphim et al. (2014) worked with the genus Hermeuptychia and the main results achieved was that all species of this genus were part of a complex of cryptic species and that
the so-called most common species of *Hermeuptychia, H. hermes*, was in fact not the most common one.

Barbosa *et al.* (*in prep.*), working with the genus *Ypthimoides*, found out that the genus as currently assigned was not a monophyletic group either and some of its species should be placed somewhere else.

Peña *et al.* (2010) also showed that a group close to *Ypthimoides* could also be non-monophyletic, the genus *Moneuptychia*. In this work species of *Moneuptychia* appeared in a clade along with *Euptychoides castrensis* and *Pharneuptychia* (two species of this work identified as *Moneuptychia, M. paeon* and *M. griseldis* belong in fact to the revalidated genus *Carminda* Dias, 1998 (Dias 2011), the sister group of *Ypthimoides*).

*Moneuptychia* was erected by Forster (1964) and comprises currently eight species, *M. soter* (Butler, 1877), *M. melchiades* (Butler, 1877), *M. itapeva* Freitas, 2007, *M. giffordi* Freitas, Emery & Mielke, 2010, *M. montana* Freitas, 2015, *M. pervagata* Freitas, Siewert & Mielke, 2015, *M. vitellina* Freitas & Barbosa, 2015 and *M. wahlbergi* Freitas, Barbosa, Siewert & Mielke, 2015 (Lamas 2004; Freitas 2007; Freitas *et al.* 2010, 2015), being well supported by at least one conspicuous morphological synapomorphy: the well-developed appendix angularis that project posteriorly in the male genitalia (for further information see Freitas 2007; Freitas *et al.* 2010; Freitas *et al.* 2015). One additional character pointed out by Freitas *et al.* (2015) is the callus present on the subcoastal vein supposedly related to sound production by males and that was described by Murillo-Hiller (2006) for *Euptychoides castrensis* (Schaus, 1902). This callus is also present in some species of *Pharneuptychia* Forster, 1964 (AVLF and EPB, unpublished).

*Pharneuptychia* was also erected by Forster (1964) to include two species, *P. phares* (Godart, [1824]) and *P. pharnaces* (Weymer, 1911). After the checklist of neotropical butterflies (Lamas 2004), the genus had included four more species, *P. boliviana* (Hayward, 1957), *P. innocentia* (C. Felder & R. Felder, 1867), *P. pharnabazos* (Bryk, 1953) and *P. romanina* (Bryk, 1953), comprising a total of six species.

In the phylogenetic study of Euptychiina Peña *et al.* (2010) had included two specimens of *Pharneuptychia, P. innocentia* and *Pharneuptychia* sp., and apparently the genus is non-monophyletic since the two specimens appeared in different clades. *Pharneuptychia* sp. in the “Megisto clade” and *P. innocentia* in the “Hermeuptychia clade”.

*Euptychoides castrensis* (Schaus, 1902) is currently assigned to the genus *Euptychoides* along with nine species (Lamas 2004). *Euptychoides castrensis* is the only species of the genus that do not occur in the Andes, being distributed in the open habitats of
Brazilian savanna and grass fields in high altitudes of Atlantic forest. The genus is likely polyphyletic (see Murray & Prowell 2005 and Peña et al. 2010) and one of its species, *Euptychoides griphet*, was shown to be a single lineage and reassigned to a new described genus, *Graphita* Nakahara, Marín & Barbosa, gen. nov. (Nakahara et al. 2016, *on-line first*).

In order to test the hypothesis of the monophyly of *Moneuptychia* and understand its relationships with *Pharneuptychia* and *Euptychoides castrensis* the present work uses molecular markers to infer the phylogeny and times of divergence of this clade.

2. Material and methods

2.1 Taxon sampling.

Our dataset was the same used by Barbosa et al. (*In prep.*) in the phylogenetic study of *Yphthimoides* and includes 461 terminal taxa representing 116 species of Satyridi from several localities in the Neotropical region and Southeast Asia (*Paleonympha opalina*). Most of them were used as outgroups since our preliminary hypothesis was that the genus *Moneuptychia* was not a natural group and would be part of a clade containing also the genus *Pharneuptychia* and the species *Euptychoides castrensis*. All sequences have been deposited in GenBank. The current classification of sampled species and the GenBank accession numbers can be found in Table 1 from Barbosa et al., *in prep*. Taxonomic nomenclature for genera and species follows Lamas (2004), with additions by Freitas (2004, 2007), Freitas & Peña (2006), Peña et al. (2006, 2010, 2011) and Wahlberg et al. (2009).

Specimens of *Moneuptychia*, *Pharneuptychia* and *Euptychoides castrensis* were collected in the field by several researchers and sent to us for molecular analyses and also examined in six public collections (see below). The Lamas collection of Neotropical butterfly type specimen photographs at the MUSM (also available online in Warren et al. 2013), representing most currently relevant names and recognized species of these groups (Lamas 2004).

The acronyms for the collections are: DZUP - Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil; MNHN – Muséum National d’Histoire Naturelle, Paris, France; MNRJ - Museu Nacional da Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil; MZSP - Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil; ZUEC - Museu de Zoologia da Universidade Estadual de Campinas, Unicamp, Campinas, São Paulo, Brazil; ZUEC-AVLF - André V.L. Freitas Collection, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.
2.2 Morphological procedures

Male genitalia dissections were made using standard techniques. Abdomens were soaked in hot 10 % KOH solution for 10 min before dissection, and dissected parts were stored in glycerol. Photographs of the male genitalia were taken using a Zeiss Discovery V20 Stereomicroscope.

2.3 Molecular procedures

Total genomic DNA was extracted from two legs of each specimen, by using the Invisorb Spin Tissue Mini Kit protocol (Stratec Molecular, Berlin, Germany), QIAGEN’s DNeasy® extraction kit and Illustra tissue and cells genomicPrep Mini Spin Kit (GE Healthcare Life Sciences), following the manufacturers’ instructions. We used three standard gene regions used in previous studies on nymphalid butterflies which have been shown to provide useful phylogenetic resolution at lower taxonomic levels (Kodandaramaiah & Wahlberg 2007, 2009; Leneveu et al. 2009; Adusc-Poku et al. 2009; Peña et al. 2010, 2011; Müller et al. 2010). The primer pairs used to amplify the mitochondrial gene COI (1498 base pairs (bp)) were LCO+HCO (Folmer et al. 1994) for the first half and Jerry+Paty for the second half (Simon et al. 1994) and for the nuclear genes the primer pairs were Frigga+Burre (Wahlberg & Wheat 2008) for GAPDH (691 bp) and rpS5degF+ rpS5degR (Wahlberg & Wheat 2008) for RpS5 (610 bp).

For the amplification of the first half of COI, PCR was carried out in a total volume of 25 μl using 2 μL of total DNA, 2.0 mM of MgCl₂, 40 μM of dNTPs, 0.5 μM of each primer, 1 U of GoTaq DNA Polymerase (Promega, Madison, WI, USA), and 10% of 1× Taq buffer. The amplification program included an initial denaturation step at 95°C for 5 min, followed by 34 cycles of denaturation at 94°C for 30 s, annealing at 42°C for 30 s, and polymerization at 72°C for 1 min, followed by an extension step at 72°C for 10 min (Silva-Brandão et al. 2005).

For the second half of COI and the two nuclear markers, PCR reactions were carried out in a total volume of 20 μl using 3 μL of total DNA, 1.5 mM of MgCl₂, 100 μM of dNTPs, 0.2 μM of each primer, 1 U of GoTaq DNA Polymerase (Promega, Madison, WI, USA), and 10% of 1× Taq buffer. The amplification program for the nuclear genes included an initial denaturation step at 95°C for 5 min, followed by 34 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 1 min, and polymerization at 72°C for 2 min, followed by an extension step at 72°C for 10 min. For the second half of COI, the amplification program was the same as the nuclear genes except by the annealing step, which was done at 50°C for 1 min. PCR products were purified of primers and deoxynucleotides with ExoSAP-IT (GE Healthcare,
Buckinghamshire England), and then sequenced by ABI Prism BigDye Kit protocol in a 3500xL Genetic Analyzer (Applied Biosystems—Hitachi), with primers used for amplification.

Sequences were analyzed with the program FinchTV v. 1.4.0 (Geospiza Inc.). Those sequences were posteriorly aligned by eye in the Biological Sequence Alignment Editor Software (BioEdit) v. 7.2.4 (Tom Hall 2013, available at http://www.mbio.ncsu.edu/bioedit/bioedit.html#downloads) with sequences obtained previously and available on GenBank.

2.4 Phylogenetic analysis

We conducted maximum parsimony (MP), Maximum likelihood (ML) and Bayesian inference (BI) analyses using combined-gene datasets. MP analyses as implemented in TNT v1.1 (Goloboff et al. 2003) were executed utilizing heuristic searches and the “New Technology Search” algorithms (Goloboff 1999; Nixon 1999) with 100 random additions. Clade support values were measured by using the resampling-based bootstrap approach (Felsenstein 1985) performing 1000 pseudo-replicates and 10 independent replicates and Bremer support. BI analyses were carried out in MrBayes v3.2 (Ronquist & Huelsenbeck 2003) and ML analyses were carried out in RaXML v7.3.1 (Stamatakis et al. 2008) on the CIPRES Science Gateway portal v3.1 (Miller et al. 2010). We partitioned our datasets by gene sequences in all analyses. We also used the software TIGER (Cummins & McInerney 2011) to subdivide our combined dataset into 5 “bins” each containing a number of characters with similar relative rates: bin1 = 1670, bin2 = 471, bin3 = 330, bin4 = 178, bin5 = 150 which has been found to be informative for BI (Rota 2011).

For BI the calculations were run twice for 20 million generations each so that the final average standard deviation of split frequencies was below 0.01, sampling trees every 500 generations and using four simultaneous chains (one cold and three hot) in each run. The parameters and the model of evolution GTR + G were unlinked across character partitions. We discarded as burn-in the first 25% of the sampled trees that were not within the stationary distribution of log-likelihoods. Trees and posterior probabilities were summarized using the “50%-majority rule” consensus method.

For ML analysis 1000 rapid bootstrap replicates and a search for the maximum likelihood topology were selected and the data were modelled according to the GTR+CAT model for each partition independently.
2.5 Dating of divergences

Due to the lack of fossil records for the genus and no equally sampled outgroups, the phylogeny was reconstructed including only the described species of the clade composed by the genera Moneuptchia, Pharneuptchia, Yphthimoides, Carminia and the species Euptchoides castrensis, Stegosatyrus ocelloides and Graphita gripha and constrained it with secondary calibration points from a fossil-calibrated Euptychiina phylogeny (Peña et al. 2010).

We used the Bayesian analysis software BEAST ver. 2.3.2 (Drummond & Rambaut 2007) on the CIPRES Science Gateway portal v3.1 (Miller et al. 2010) under a log-normal relaxed molecular clock. The DNA sequences were divided in five partitions, with parameter values estimated separately for each partition. The combined dataset was analysed under the GTR+I model with a relaxed clock allowing branch lengths to vary following an uncorrelated log-normal distribution (Drummond et al. 2006). The analysis was run four independent times for 40 million generations (with pre-run burn-in of 400 000 generations) with sampled trees every 1000 generations and the results compiled using all runs.

We selected five terminals per Yphthimoides species and species from outgroups to maximize the gene coverage in the resulting dataset except for those species were there were less than five individuals per species. The selected calibration points were chosen from well-supported monophyletic groups from the Peña et al. (2010) Euptychiina phylogeny: the root of the tree to 16.8 ± 5 Ma, the crown age of Moneuptchia soter to 1.3 ± 4 Ma, the crown age of Carminia + Yphthimoides to 14.22 ± 5 Ma and the split age between Yphthimoides cipoensis and Yphthimoides angularis to 8.60 ± 4 Ma.

The tree priors were set either to the Birth-Death or the Calibrated Yule speciation processes as the tree prior in separate analyses to investigate and compare the impact of this parameter on the final age estimates. All other priors were left to the default values in BEAST.

Tracer v1.3 was used to analyze convergence, good mixing of MCMC and the Effective Sample Size of each estimated parameter to be higher than 200 and trees were summarized using TreeAnnotator v1.4.7 software, which are distributed with the BEAST package.

3. Results

Pharneuptchia clade phylogeny

In our analyses we were able to sample almost all described Moneuptchia species (only M. melchiades was not sampled) and many specimens of Euptchoides castrensis from
several localities in Brazil. In our study *Pharneuptychia* is under sampled but our sampling was enough for our purposes.

The Maximum Parsimony analysis produced 337 equally most parcimonious cladograms of length 12178 steps (CI = 0.184; RI = 0.799) and the strict consensus tree is shown in Figure 1.

The Bayesian and the Maximum Likelihood analyses produced trees (Figures 2 and 3, respectively) that are largely congruent with the most parcimonious cladograms.

All the analyses recovered the genera *Moneuptychia* as non-monophyletic and *Euptychoides castrensis* as a complex of cryptic species mixed within *Moneuptychia* and sister to a monophyletic *Pharneuptychia*. All these three groups appeared as part of a major clade in which internal relationships are messy.

As has been shown by Peña et al. (2010), *Pharneuptychia innocentia* is not part of the genus *Pharneuptychia* and is more closely related to species of the clade *Iermeuptychia*. The three analyses recovered *P. innocentia* almost in the same position in the trees.

The major clade composed by the species of *Moneuptychia, Pharneuptychia* and *Euptychoides castrensis* has great support (ML bootstrap = 100; BI posterior probability = 1; MP Bremer = 10). In our analysis *Pharneuptychia* is composed by at least four subclades and appears as sister to *Moneuptychia + Euptychoides castrensis*. Of these four subclades only one, composed by YPH0465, YPH0570, YPH0571 and YPH0599, matches the wing pattern of a described taxon, *Pharneuptychia coeca* (Köhler, 1935), synonymized with *Pharneuptychia phares* by Lamas (2004). The other three subclades appear to be new taxa.

In BI and ML analyses the relationships of *Moneuptychia* species appear as follows: *M. wahlbergi* sister (bs = 70; pp = 0.87) to *M. itapeva + M. giffordi* (bs = 100; pp = 1); *M. soter* is sister (bs = 100; pp = 1) to *M. vitellina + Euptychoides castrensis* (bs = 100; pp = 1) from Ribeirão Grande in Brazilian state of São Paulo and *Moneuptychia pervagata + M. montana* (bs = 95; pp = 1) sister to *Moneuptychia sp. nov. 1 + Moneuptychia sp. nov. 2 + Moneuptychia sp. nov. 3 (bs = 64; pp = 0.90).

In MP analysis *Moneuptychia* species relationships are almost the same, *M. pervagata* sister *M. montana, M. soter* sister to *M. vitellina + Euptychoides castrensis* and *M. itapeva* sister to *M. giffordi*, all relationships of these pair of species are well supported (see Figure 1 for Bremer values) but the relationships among these pairs of species have low support in three analyses. With the exception of *Moneuptychia wahlbergi*, the phylogenetic relationships of all other species of *Moneuptychia* are stable and well-supported.
The specimens of *Euptychoides castrensis* are scattered throughout the tree in four small clades with no shared common ancestry in all analyses. In the first clade the relationships among specimens from Brazilian states of Pernambuco (PE), Alagoas (AL), São Paulo (SP, Atibaia and Serra do Japi), Bahia (BA, Santa Teresinha and Vitória da Conquista), Minas Gerais (MG, Vale do Rio Doce), Paraná (PR, Foz do Iguaçu) and Rio Grande do Sul (RS, Taquaruçu do Sul) are well-supported (bs = 99; pp = 1; Bremer = 10).

Second clade is composed by specimens from São Paulo (Taubaté), Minas Gerais (Alto Caparaó and Serra do Caraça) and Bahia (Chapada Diamantina) (bs = 99; pp = 1; Bremer = 8). In BI and ML this clade is sister to a clade composed by the third clade of *Euptychoides castrensis*, *Moneuptychia soter* and *Moneuptychia vitellina* (bs = 99; pp = 0.93). In MP analysis this clade appears in strict consensus tree as is sister to *M. soter* and *M. vitellina* + *Euptychoides castrensis* from Ribeirão Grande (SP), although with no support.

Third clade is well supported (bs = 99; pp = 1; Bremer = 10) and composed by specimens from São Paulo (Campos do Jordão), Paraná (Curitiba) and Rio Grande do Sul (São Francisco de Paula). In BI and ML this clade appears as is sister to a clade composed by *M. soter* and *M. vitellina* + *Euptychoides castrensis* from Ribeirão Grande (SP), with low support (bs = 49; pp = 0.57).

Finally fourth clade is composed by three specimens from Ribeirão Grande, São Paulo, Brazil and appear as sister to *M. vitellina*. But based on the wing color pattern, morphology of male genitalia and branch length it is safe to state that these three specimens are in fact individuals of *Moneuptychia vitellina* and were misidentified by the main author.

Based on the phylogenetic relationships of the sampled specimens of *Euptychoides castrensis* it is safe to say that this species is indeed a complex of cryptic species and at least five different species can be identified in the present phylogeny.

Murray and Prowell (2005) did not use any species of *Moneuptychia*, *Pharneuptychia* or *Euptychoides castrensis* in their analyses so further comparisons could not be made. In the work of Peña *et al.* (2010) *Euptychoides castrensis* was recovered as sister to *Moneuptychia itapeva*, *Pharneuptychia* sp. and *Moneuptychia soter*. The only comparison that can be made with the present work is that these species do belong to the same well-supported clade.

**Dating of divergences**

The BEAST analyses with either Birth-Death or Yule birth model recovered a tree whose topology is congruent with trees topologies recovered in the MP, ML and BI analyses
(Figure 4). In the present work we are showing only the tree recovered with Yule birth model, since its likelihood value was higher than the Birth-Death model.

The age estimates from each node using the relaxed molecular clock produced wide credibility intervals, especially those near the root, which this should be expected when using secondary calibration points in the analyses (Graur & Martin 2004).

Our estimated times indicate that the major clade composed by *Pharneuptychia* clade, the sister genera *Yphthimoides* and *Carinda* plus *Stegosatyrus ocelloidus* and *Graphita gripe* (in Figure 4 *S. ocelloidus* and *G. gripe* are subsumed in *Carinda + Yphthimoides* clade) appeared around 15.30 (± 4.52) Mya, during the beginning of the mid-Miocene when a splitting event had originated the ancestral lineages of *Pharneuptychia* clade and *Yphthimoides, Carinda, S. ocelloidus* and *G. gripe*. The *Pharneuptychia* clade lineage began to diversify around 11.35 (± 3.52) Mya, in the late Miocene.

All the species that are assigned to *Moneuptychia* nowadays apparently had their origins around the late Miocene and the early Pliocene (11.20 to 3.60 Mya). *Moneuptychia wahlbergi* began to diversify around 1.93 (± 0.86) Mya, during the lower Pleistocene and had split from *M. itapeva* and *M. giffordi* around 8.64 (± 2.75) Mya, but this relationship is not well-supported. *M. itapeva* and *M. giffordi* originated around 6.01 (± 2.20) Mya and began to diversify around 2.97 (± 1.24) Mya (late Pliocene) and 0.55 (± 0.58) Mya (middle to upper Pleistocene), respectively.

The lineages of *M. pervagata* and *M. montana* originated approximately around 6.88 (± 2.42) Mya and their diversification started around 0.69 (± 0.42) Mya (middle Pleistocene) and 0.33 (± 0.28) Mya (upper Pleistocene), respectively. The clade composed by *Moneuptychia* sp. nov. 1 + *Moneuptychia* sp. nov. 2 + *Moneuptychia* sp. nov. 3 begun to diversify around 2.39 (± 1.18) Mya (lower Pleistocene) with its origins around 9.02 (± 2.75) Mya in the late Miocene, but with low support.

*M. soter, M. vitellina* and *Euptychoides castrensis* (SP) had their origins around 4.74 (± 1.72) Mya, during the early Pliocene. *M. soter* began to diversify around 1.22 (± 0.59) Mya (lower Pleistocene) and the split between *M. vitellina* and *E. castrensis* (SP – fourth clade) had occurred around 1.63 (± 0.76) Mya, also in the lower Pleistocene, while their diversification started in the upper Pleistocene around 0.32 (± 0.18) Mya and 0.18 (± 0.18) Mya, respectively.

The lineage of *Pharneuptychia* began to diversify around 5.68 (± 2.00) Mya (late Miocene) with its origins around 11.35 (± 3.52) Mya, in the middle Miocene.
Regarding *Euptychoides castrensis* complex of cryptic species, the first clade (see the section *Pharneuptychia clade phylogeny*) had originated approximately around 9.48 (± 2.92) Mya, although with low support, and began to diversify around 7.03 (± 2.37) Mya, during the late Miocene.

Second clade also had its origins in the late Miocene (around 8.29 (± 2.59) Mya) and its diversification started around 6.39 (± 2.15) Mya. The third clade began to diversify more recently, around 3.51 (± 1.37) Mya, during the late Pliocene and its origins are rooted around 7.48 (± 2.41) Mya in the late Miocene, although with low support.

4. Discussion

In the phylogenetic analyses of the subtribe Euptychiina Peña et al. (2010) state that they could not test the monophyly of *Pharneuptychia* due to the inclusion of a single species in the dataset.

But in their work they have used one identified species of *Pharneuptychia*, *P. innocentia*, and one non-identified specimen of *Pharneuptychia*, *Pharneuptychia* sp., with *P. innocentia* recovered as part of the “*Hermeuptychia* clade”, along with some *Splendeuptychia*, *Amphidecta* and *Hermeuptychia* and *Pharneuptychia* sp. recovered as part of the “*Megisto* clade”, close to *Moneuptychia*, *Euptychoides castrensis*, *Carminda* and *Yphthimoides*.

In the present work the same patterns of relationships were recovered, with *Pharneuptychia* and *Moneuptychia* recovered as polyphyletic as currently recognized. *Pharneuptychia innocentia* appeared as part of a clade with *Hermeuptychia*, *Amphidecta*, *Splendeuptychia* and *Godartiana*, although with low support and the remaining specimens of *Pharneuptychia* recovered as closely related to species of *Moneuptychia* and *Euptychoides castrensis*, which was shown to be a complex of cryptic species.

Based on the present study the genera *Pharneuptychia* and *Moneuptychia* are sister groups and the species of *Euptychoides castrensis* should be reassigned to *Moneuptychia* with the addition of several new, undescribed species.

The *Moneuptychia* species and individuals identified as *Euptychoides castrensis* share a similar wing pattern, with the anal margin of the hindwing having a small concavity toward the tornus (see figure 3). This hindwing character is not present in *Pharneuptychia* species.

Although the sampling of *Pharneuptychia* species in our work is incomplete and the species have not been correctly identified to the level of species, it became clear that as currently assigned this genus is polyphyletic and therefore it needs a huge taxonomic revision study in order to correct the mistakes and place the species in the right genera.
As can be seen most of Euptychiina genera appear to be non-monophyletic and pervaded by complexes of cryptic species and the phylogenetic works published so far (Murray & Prowell 2005; Peña et al. 2010; Matos-Maravi et al. 2013; Seraphim et al. 2014) helped to shed some light into these taxonomic problems and started to solve them.

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References


Figure legends

**Figure 1.** Strict Consensus tree of 37 equally parimoniou trees inferred from the maximum parcimony analysis (length 12178; CI = 0.184; RI = 0.799) of the combined dataset. Numbers below and above branches are Bremer support and Absolute Bremer support (ABS) values. Branches are colored by species and clades of *Pharneuptychia, Moneuptychia* and *Euptychoides castrensis*.

**Figure 2.** Consensus tree from the Bayesian Inference analysis of the combined dataset, modeled with GTR+Γ. Numbers below branches are Posterior Probability values. Branches are colored by species and clades of *Pharneuptychia, Moneuptychia* and *Euptychoides castrensis*.

**Figure 3.** Consensus tree from the Maximum Likelihood analysis. Numbers below branches are bootstrap values. Branches are colored by species and clades of *Pharneuptychia, Moneuptychia* and *Euptychoides castrensis*.

**Figure 4.** Estimated times of divergence derived from the BEAST analysis. Ultrametric tree scaled in Mya. Numbers in parenthesis and horizontal bars represent posterior probability values and 95% credibility intervals. Numbers without parenthesis represent estimated date of divergence for each node.
Figura 1
Figura 4
Capítulo 3: Descrições e alterações taxonômicas de espécies do gênero *Ypthimoides* Forster, 1964

Este capítulo está dividido em cinco artigos, a saber:

3.1 - “A new species of *Ypthimoides* (Lepidoptera: Nymphalidae: Satyrinae) from the southern Atlantic forest region” – Este artigo trata da descrição de uma nova espécies do gênero *Ypthimoides* que passou despercebida pelos pesquisadores por muito tempo, apesar de ser extremamente comum em algumas regiões do Brasil, e foi publicado no periódico “Zootaxa” 3526: 31–44.


3.3 - “Species' from two different butterfly genera combined into one: description of a new genus of Euptychiina (Nymphalidae: Satyrinae) with unusually variable wing pattern” – Trabalho sinonimizando as espécies *Hartjesia griseola* e *Ypthimoides punctata* sob um mesmo e novo gênero, *Sepona*, e foi publicado no periódico “Revista Brasileira de Entomologia” 60: 157–165.

3.4 - “Description of two new species of the Neotropical genus *Ypthimoides* Forster, 1964 (Lepidoptera: Nymphalidae: Satyrinae) from the “renata clade””. Este trabalho descreve outras duas espécies novas de *Ypthimoides*, que foram reconhecidas como espécies novas principalmente por meio de dados moleculares, e foi publicado no periódico “Neotropical Biodiversity” 2(1): 87–98.

3.5 - “Redescription of *Ypthimoides patricia* (Hayward, 1957), with taxonomic notes on the name *Euptychia saltuensis* Hayward, 1962 and *Ypthimoides manasses* (C. Felder & R. Felder, 1867) (Nymphalidae: Satyrinae)” – Trabalho em fase final de preparação, será submetido ainda neste segundo semestre.

Resumo
Dados moleculares, usando a primeira parte (*Barcode*) do gene mitocondrial COI, conjuntamente com dados morfológicos, principalmente caracteres da genitália masculina e de imaturos, mostraram a existência de seis novas espécies de *Yphthimoides*.

A primeira foi descrita em 2012, em um trabalho publicado na *Zootaxa*. Em um segundo trabalho, três dessas novas espécies foram descritas, sendo duas delas com distribuição geográfica extremamente localizada (Barbosa et al. 2015). As duas espécies restantes foram descrita recentemente (Barbosa et al. 2016), uma com distribuição andina e a outra tendo sido coletada na região nordeste do Brasil, nos estados de Alagoas e Pernambuco.

Outros dois artigos tratam de alterações taxonômicas no *status* de outras duas espécies de *Yphthimoides*: o primeiro trabalho trata da sinonímização de *Y. punctata* com *Harjesia griseola* em um novo gênero descrito para essas espécies, *Sepona*; o segundo trabalho faz correções quanto à sinonímização de *Euptychia saltuensis*, passando de *Y. manasses* para *Y. patricia* e corroborando *Y. patricia* e *Y. manasses* como espécies distintas.
Article

A new species of Ypthimoides (Lepidoptera: Nymphalidae: Satyrinae) from the southern Atlantic forest region

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Abstract

This paper describes a new, abundant and widespread species of Ypthimoides Forster from the Atlantic forests of southern Brazil, Paraguay, and northern Argentina (Misiones) in open and secondary vegetation and forest edges. Adult and immature stage morphology is described, molecular data are provided, and the placement of the new species within the genus Ypthimoides is discussed.

Key words: Atlantic Forest, butterfly, Euptychiina, life history, molecular barcode, Poaceae

Introduction

The species-rich subfamily Satyrinae has been subject of intensive systematic studies especially in higher taxonomic levels in recent years as an attempt to clarify their poorly understood phylogeny and taxonomy (reviewed in Marín et al. 2011). These studies included broad phylogenetic studies focusing on higher classification (Murray & Prowell 2005; Peña et al. 2006, 2010), and definition of lower taxa within this subfamily, including descriptions of several new genera and species (Marín et al. 2011).

In southeastern Brazil, several new species have been discovered and described in recent years (e.g. Freitas 2004, 2007; Peña & Lamas 2005; Freitas et al. 2010, 2011), and several additional new taxa are awaiting description, especially in some large genera such as Ypthimoides Forster (Freitas 2004).

The genus Ypthimoides is a member of the diverse subtribe Euptychiina and was erected by Forster (1964) to include 24 species of medium sized brown butterflies, but no clear diagnosis to delimit the genus was given. Based on the original classification of Forster (1964), the genus Ypthimoides appeared to be an unnatural group of species (Freitas 2004), and Lamas (2004) made a major organization of the genus obtaining a list of 24 species (including two undescribed species from Peru). Since then, only one new species of Ypthimoides has been described (Freitas 2004).

Based on the current classification of Ypthimoides, most species are endemic to southeastern South America (Murray & Prowell 2005), with their maximum diversity observed in the Atlantic Forest and in the open cerrado areas of central Brazil. Accordingly, the discovery of several new species of Ypthimoides from this region is not unexpected with additional field and museum work.

The present paper describes a common and widespread species of Ypthimoides present in several forest and suburban habitats in southern Brazil, Paraguay, and northern Argentina. In addition, the morphology and natural history of the immature stages are described and the systematic position based on molecular data is briefly discussed.
Material and methods

**Study sites and rearing.** This species was studied in the field from four localities in the states of São Paulo and Rio Grande do Sul. 1) Santa Genebra Forest Reserve (Campinas, São Paulo, 620 m; 22°49’S, 47°06’W); 2) Ribeirão Cachoeira (Sousas, Campinas, São Paulo 700 m; 22°49’S. 46°55’W); 3) Observatório Astronômico (Morro dos Macacos, Valinhos, São Paulo, 860 m; 23°00’S. 46°57’W); 4) Campus of the Universidade Federal do Rio Grande do Sul (UFRGS) (Porto Alegre, Rio Grande do Sul, 75 m; 30°04’S, 51°07’W).

Fertile eggs were obtained from wild-captured females, which were confined in plastic bags along with leaves of several potential host-plants (species of Poaceae) and put under a source of heat (40W incandescent lamp). The intense heat triggers in most females the behavior of laying eggs. The eggs were laid on the leaves and/or scattered on the plastic bag. Larvae were reared in plastic containers cleaned daily, with fresh plant material provided every two or three days (following Freitas 2007). Observations and data were recorded on behavior and development times for all stages. Dry head capsules and pupal cases were retained in glass vials. Immature stages were fixed in Kahle solution when the number of specimens was sufficient. Voucher specimens of the immature stages were deposited in the Museu de Zoologia “Adão José Cardoso” (ZUEC), Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

**Morphology.** Adult dissections were made using standard techniques. Legs, palpi and abdomens were soaked in hot 10% KOH solution for 10 min before dissection, and dissected parts were stored in glycerol. Taxonomic nomenclature followed Lamas (2004), modified after Peña et al. (2006) and Wahlberg et al. (2009). Measurements were made and general aspects of early stages morphology were studied by using a Leica® MZ7.5 stereomicroscope equipped with a micrometric scale. Egg size was indicated by height and diameter. Head capsule width of larvae consisted of the distance between the most external stemmata; maximum total length for both larvae and pupae corresponded to distance from head to posterior margin of the tenth abdominal segment in dorsal view (as in Freitas 2007). Measurements were provided as minimum-maximum values. Scanning electron microscopy (SEM) was conducted using a JEOL® JSM-5800 microscope, and samples were prepared in accordance with the following protocol: Sample critical point dried using Bal-tec® - CPD030 equipment and attached with double stick tape to aluminum stubs; gold/palladium coated with a Bal-tec® - SCD050 sputter coater. Terminology for the early stages followed García-Barros & Martin (1995) for eggs and Stehr (1987) for larvae and pupae.

**Yphthimoides** specimens were examined in four public and private collections in Brazil (see below). The Lamas collection of neotropical butterfly type specimen photographs at the MUSM (also available online in Warren et al. 2012), representing most currently relevant names and recognized species of *Yphthimoides* (Lamas, 2004), except *Y. acmenis* (Hübner, 1823), *Y. patricia* (Hayward, 1957), *Y. punctata* (Weymer, 1911), and *Y. renata* (Stoll, 1780), was examined. The last four species are all clearly described in their original descriptions, and all but *Y. patricia* have pictures of adults. It is noteworthy, however, that the genitalia of *Y. patricia* is so distinct that there is no doubt it is not even like the one from the new species here described.

Specimens of *Y. renata*, a taxon very similar to the described species (including all taxa synonymized by Lamas, 2004) were studied from material coming from several different places in Brazil at the states of São Paulo, Paraná, Minas Gerais, Mato Grosso do Sul and Pernambuco, and also from Costa Rica (Guanacaste).

The acronyms for the collections are: **DD**, Coleção Diego Dolibaina, Curitiba, Paraná, Brazil; **DZUP**, Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil; **MZSP** - Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil; **ZUEC**, Museu de Zoologia da Universidade Estadual de Campinas, Unicamp, Campinas, São Paulo, Brazil.

**Genetic divergence and phylogenetic inference.** Genetic distance within and between species of *Yphthimoides*, and the phylogenetic relationship among them, were estimated to evaluate the molecular variability for those taxa. Genomic DNA was extracted from two legs of two adults of the *Yphthimoides n.* sp., one from Santa Bárbara, São Paulo (voucher code – Yph–sp) and another from Porto Mauá, Rio Grande do Sul (voucher code – Yph–612), and from three adults of *Y. renata* from Três Lagoas, MS (Table 1), by using the DNeasy Blood & Tissue Kit protocol (QIAGEN, Düsseldorf, Germany), DNA was stored in TE buffer at −20° C. The mitochondrial gene cytochrome c oxidase I (coxI, ca. 1,480 bp for specimens of *Yphthimoides* n. sp. and ca. 650 bp for adults of *Y. renata* from Brazil) was amplified by using the follow primer combinations: LCO + HCO (Folmer et al. 1994), and Jerry + PatII (Caterino et al. 2001). Reactions were done in a 25 μL final volume using 1 μL of total DNA, 2.0 mM
of MgCl₂, 40 μM of dNTPs, 0.2 μM of each primer, 1U of GoTaq DNA Polymerase (Promega, Madison, Wisconsin, USA), and 10% of 10X Taq buffer. The amplification program included an initial denaturation step at 95°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 47°C for 30 s, and polymerization at 72°C for 1.5 min, followed by an extension step at 72°C for 10 min (Silva-Brandão et al. 2005). PCR products were purified of primers and deoxynucleotides with ExoSAP-IT (GE Healthcare, Buckinghamshire England), and then sequenced by ABI Prism BigDye Kit protocol in an ABI 3700 automated sequencer, with primers used for amplification. Sequences were analyzed with the program FinchTV v. 1.4.0 (Geospiza Inc.). Those sequences were posteriorly aligned by eye with sequences obtained previously and available on GenBank (Peña et al. 2010) and with sequences of five individuals of Yphthimoides n. sp. from Argentina (provided by Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (MACN)) by using BioEdit v. 7.0.5.3 (Hall 1999). The final matrix comprised 21 sequences of 11 species of Yphthimoides (Table 1) and one species used as outgroup, Carminda paeon (Godart) (GQ864792) (named as Moneuptychia paeon in Peña et al., 2010, see also Dias 2011).

For comparison, the genetic distances among species of Yphthimoides, among individuals of the new species, and among sequences of Y. renata were determined by using the nucleotide substitution model Kimura-2-parameters (Kimura 1980) by using the program MEGA v. 5.0 (Tamura et al. 2011).

The phylogenetic relationships of the new species were estimated by using the Maximum Likelihood method. The program MEGA v.5.0 (Tamura et al. 2011) was used to determine the available substitution model which best fitted the coxl sequences and to run the analysis. The best fit model suggested was GTR+G (+G, parameter = 0.3801) (Nei & Kumar 2000), and the analysis was carried out under this model. Branch support was estimated by using the non-parametric bootstrapping procedure, with 1,000 replicates (Felsenstein 1985).

**TABLE 1. Data of evaluated specimens of Yphthimoides for the evolutionary history inference of Y. ordinaria.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Code</th>
<th>Source</th>
<th>GenBank accession</th>
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<tr>
<td>Y. angularis</td>
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<td>GU205876*</td>
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<td>JQ797609</td>
</tr>
</tbody>
</table>

*Murray & Prowell (2005); * Peña et al. (2010)

**Yphthimoides ordinaria** Freitas, Kaminski & Mielke, new species (Figs. 1–8)

*Yphthimoides ca. electra*; Brown 1992: 152, Fig. 31.
*Yphthimoides sp. ca. angularis*; Brown 1992: 152, Figs. 32, 33.
**Adult: Diagnosis.** Adults of both sexes are similar to *Yphthimoïdes renata* from southern Brazil (a common sympatric species, see Fig. 3 in Freitas 2004), but they can be easily distinguished from that species by the following wing pattern characters: 1) the ventral transverse lines crossing both wings are more wavy and thin in *Y. ordinaria* when compared to *Y. renata*, which have these lines always straight and broad; 2) the most basal transverse line on the ventral hindwing in *Y. ordinaria* is usually broken after crossing the discal cell, just before the stalk of CuA2, and continuous in *Y. renata*; 3) the ventral submarginal line in the hindwing is more wavy in *Y. ordinaria* when compared to *Y. renata*. The male genitalia of *Y. ordinaria* is similar to *Y. renata* (based on individuals from Brazil), but can be distinguished by the following characters: 1) in ventral view, the uncus of *Y. ordinaria* narrows toward the apex, ending in a point, while in *Y. renata* the uncus is enlarged in the middle portion, abruptly narrowing and ending in a fat tip; 2) the valve of *Y. ordinaria* in slender in all its extension, while in *Y. renata* the valve is enlarged at the middle, with a conspicuous dorsal concavity in the distal half; 3) the teeth in the valvae are much more conspicuous in *Y. ordinaria* than in *Y. renata*.

**FIGURE 1.** Adults of *Yphthimoïdes ordinaria*, ventral on the left, dorsal on the right. A, holotype male from Terra Rica, Paraná, Brazil; B, allotype female, same locality.

**Descriptions of adults: Male** (Fig. 1A). Forewing length 18–23 mm (average 20.8 mm, SD = 1.19, n = 17); hindwing length 14–20 mm (average 16.5 mm, SD = 1.55, n = 17). Eyes naked, entirely brown. Palpus length 2.0 times head height, beige with long brown hairs. The male palpus is shown in Fig. 2D. Antenna of males 9.0–10.0 mm in length, with 36 antennomeres extending to mid-costa; shaft rust brown, dorsally covered by dark brown scales, club with 9–10 antennomeres, not conspicuously developed. Hindwing outer margin slightly wavy. Male and female wing venation shown in Figs. 2A–B. Male foreleg (Fig. 2C) covered by long brown hairs and with two tarsomeres partially fused, the first two thirds the length of tibia, and the second extremely reduced; female foreleg with five tarsomeres (Fig. 2E). Wings with dorsal ground color dark brown with few markings, restricted to marginal and submarginal lines in both wings; hindwing with one or two ocelli in spaces CuA1–CuA2 and CuA2–2A (smaller or absent in some individuals); these are completely black. Ventral wings with a kind of mottled pattern, with darker spots on a slightly paler background; forewing crossed by two thin dark brown lines, the first more regular, extending from costa to 2A one-third distance from wing base to apex; the second line wavy, extending from costa to 2A at two-thirds from wing base to apex, delimiting a lighter distal area; a dark brown scalloped submarginal line and a brown regular marginal line extending from costa to 2A; one to four minute black ocelli with white pupil in cells M1–M2 (ocellus 1), M2–M3 (2), M3–CuA1 (3) and CuA1–CuA2 (4). Hindwing crossed by two thin dark brown lines from costa to anal margin, in similar position to those on forewing, the more base one more even; the more distal one wavy, delimiting a lighter distal area; a dark brown zigzag submarginal line and a brown regular marginal line extending from costa to 2A; a series of six black ocelli circled by orange scales and
with white pupil can be found in cells Rs–M1 (ocellus 1), M1–M2 (2), M2–M1 (3), M1–CuA1 (4), CuA1–CuA2 (5) and CuA3–2A (6); ocelli 1, 3, 4 and 6 usually small and reduced to few white scales circled by few black scales; ocelli 2 and 5 larger than the others, with double white pupil. No conspicuous androconial scales observed.

**FIGURE 2.** Morphological characters of *Yphthimoidea ordinaria*. A, male wing venation; B, female wing venation; C, male foreleg; D, male palp; E, female foreleg.

**Male genitalia** (Figs. 3A–C, 4). Saccus short and triangular in ventral view; tegumen rounded; gnathos long and pointed; uncus elongated and truncated distally (Fig. 4B); valvae elongated, trapezoidal, ending in a bump, internal margin with a series of small teeth (Figs. 4C–D); aedeagus straight; cornuti absent; juxta membranous.

**Female** (Figs. 1B, 5A). Forewing length 21–25 mm (average 23.4 mm; SD = 10.8, n = 14); hindwing length 17–22 mm (average 19.14 mm; SD = 1.17, n = 14). Antenna 9.0–11.0 mm in length, with 36 antennomeres extending to mid-costa. General color and pattern very similar to, but in general paler than that of males. Some females also show additional black ocelli in spaces M1–M2, and CuA1–CuA2, on ventral forewing (Fig. 1B). Female genitalia as in Figure 3D. Ductus bursae not sclerotized, as long as corpus bursae; corpus bursae ellipsoid and signa absent.
Remarks on color variation. Variation on the dorsal wing surfaces is practically absent and obvious seasonal variations have not been detected. The ventral surface of both wings shows weak variation in intensity of pigmentation and line shape, in the contrast of the paler distal area, and especially in the number (but not size) of the ocelli. No individuals lacking ocelli were observed, including those from dry season.

Description of immature stages. The morphological description and measurements of the immature stages below (with five larval instars) are based on material from Porto Alegre, Rio Grande do Sul. Additional data from Campinas region, São Paulo (with only four larval instars) are at the end of this section.

Figs. 5A–C. Male (A–C) and female (D) genitalia of *Yphthimoides ordinaria*. A, lateral view; B, ventral view; C, aedeagus (lateral view); D, ventral view. Abbreviations: ae, aedeagus; bu, corpus bursae; gn, gnathos; pa, papilla analis; sa, saccus; st, sterigma; te, tegument; va, valva; un, uncus.

Egg (Figs. 5B, 6). Spherical, cream, smooth, with a reticle of thin ridges forming a pattern of irregular pentagonal and hexagonal cells visible with SEM (Fig. 6). Height 1.2 mm; diameter 1.16 (n = 1). Duration 6 days (n = 10).

First instar (Figs. 5C, 7, 8). Head capsule width 0.66–0.74 mm; head scoli 0.08–0.10 mm (n = 10). Head capsule black, with enlarged chalazae, bearing a pair of short scoli on vertex, each with two long narrow setae, P1 and P2 respectively (Figs. 7A–B). Porp Pb between P1 and P2. Third stemma larger than the other stemmata. Body cream, smooth, with red longitudinal stripes; caudal filaments very short. Setae dark, elongated, several dorsal and subdorsal clubbed at the tip. Duration 9–10 days (n = 10). Head and body chaetotaxy are presented in the Figure 7.

Second instar (Fig. 5D). Head capsule width 0.92–1.00 mm; head scoli 0.14–0.20 mm (n = 10). Head dark brown with two diverging short scoli on vertex. Body beige, striped longitudinally with white and reddish; caudal filaments short. Duration 9–11 days (n = 10).
**Third instar.** Head capsule width 1.35–1.47 mm; head scoli 0.22–0.30 mm (n = 10). Head brown, with two diverging very short scoli on vertex. Body brown with several longitudinal dark brown stripes; caudal filaments short. Duration 10–12 days (n = 10).

**Fourth instar** (Fig. 5E). Head capsule width 1.77–1.92 mm; head scoli 0.30–0.38 mm (n = 10). Very similar to third instar. Duration 10–12 days (n = 10).

**Fifth (last) instar** (Fig. 5F). Head capsule width 2.43–2.82 mm; head scoli 0.44–0.50 mm (n = 9). Head brown, with two diverging short scoli on vertex. Body brown with many longitudinal dark stripes; mid-dorsal stripe conspicuously dark; ventral region dark brown; legs and prolegs light brown; caudal filaments short. Duration 10–11 days (n = 10).

**Pupa** (Fig. 5G). Short and smooth; mostly dark brown with white stripes bordering the wing caps; short pointed ocular caps with white ridges; cremaster dark in ventral portion; dorsal abdomen with a paired series of short subdorsal ridges bordered with white. Total length 10–12 mm (n = 5). Duration 15–17 days (n = 6).

**Additional rearing data.** Measurements of the immature stages from Campinas region (São Paulo, Brazil) with only four larval instars are presented below.

**Egg:** duration 8–14 days (n = 6), diameter 1.10–1.12, height 1.11–1.14 (n = 5); **first instar:** Duration 7–10 days, maximum length 6 mm (n = 5), head width 0.62–0.65, scoli 0.08–0.10 (n = 3); **second instar:** Duration 6–10 days, maximum length 11 mm (n = 5), head width 0.98–1.60, scoli 0.14–0.30 (n = 4); **third instar:** Duration 10–12 days, maximum length 20 mm (n = 5), head width 1.22–1.67, scoli 0.24–0.30 (n = 2); **fourth (last) instar:** Duration 8–23 days, maximum length 32 mm (n = 5), head width 2.40–2.64, scoli 0.37–0.50 (n = 2); pupa: duration 13–17 days, length 9–12 mm.

**Behavior and natural history.** Oviposition behavior was observed in Porto Alegre, Rio Grande do Sul. At 13:00 a female was observed flying slowly near the ground, apparently testing several substrates. After some time, the female was observed laying isolated eggs either in dead leaves and twigs or directly on the host plant. In this study site the host plant is the cultivated broadleaf carpetgrass *Axonopus compressus* (Sw.) P. Beauv. ("grama
missioneira”) (Poaceae). In the laboratory, larvae easily accepted this and two other common grasses: the African Guinea grass Panicum maximum Jacq. (“capim colonião”) and an unidentified species (all are common species of grasses in most known sites where Y. ordinaria occurs). Adults were observed only in open habitats, grasslands and forest edges, flying among grass patches and perching usually on the ground or in low vegetation. In south Brazil (from Porto Alegre population), all larvae presented five instars, except for larvae from two rearing lots from São Paulo state (from Campinas region) which passed through only four instars (see above). The difference in the number of instars can be related to different rearing conditions of larvae in São Paulo and South Brazil, but more studies are needed to clear such observations.

**FIGURE 5.** Life stages of Ypthimoides ordinaria. A, female perching near the ground just after oviposition (description of immatures based on eggs laid by this female); B, egg; C, first instar; D, second instar; E, fourth instar; F, fifth (last instar); G, pupa.

**Habitat.** Although more common on forest edges, Y. ordinaria is also found in several other different habitats, such as secondary forests, open pastures and even in urban areas (in abandoned overgrown grass lawns). Based on field records and museum specimens, the species appear to be associated with the semi-deciduous Atlantic Forest in the interior, in altitudes from 400 to 900 m. An exception is the coastal area of Rio Grande do Sul, where the species is present in the transitional mixed vegetation of the coastal plain. In Misiones (Argentina), this species is very common and flies throughout most of the province (E. N. Bustos, pers. com.).
FIGURE 6. Scanning electron microscopy of Yphthimoides ordinaria egg. A, lateral view; B, micropylar area (Mp); C, hexagonal cells of the exochorion with aeropyle in the rib intersections (arrows).

**Distribution.** Based on field observations, museum records and literature, the species is widespread in the Brazilian states of São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul, Paraguay, and is also present in Argentina, in Misiones province.

**Etymology.** The specific epithet is feminine and is the Latin adjective for “usual”, alluding to the commonness of this species in several different habitats and locations in southern South America.

**Types:**

- **Holotype.** Male (Fig. 1A) from Terra Rica, 600 m, Paraná, Brazil. Deposited in the collection of Departamento de Zoologia (DZUP), Universidade Federal do Paraná, Curitiba, Paraná, Brazil. Labels on the holotype (five labels separated by transverse bars): / Holotypus/ 09.X.2009 Brasil, Paraná, Terra Rica, Pq. [Parque] Mun. [sic] Três Morros, 600 m, Carneiro, Leite, Dias & Dolibaina leg./ Holotypus Yphthimoides ordinaria Freitas, Kaminski & Mielke det. 2012/ DZ 22.388/; DZUP

- **Allotype.** Female (Fig. 1B), from Terra Rica, Paraná, Brazil, also deposited in the DZUP. Labels on the allotype (five labels, separated by transverse bars): / Allotypus/ 17.XII.2009 Pq. [Parque] Mun. [sic] Três Morros, Terra Rica, Paraná, 600 m, [Brasil], Carneiro, Maia & Dolibaina leg./ Allotypus Yphthimoides ordinaria Freitas, Kaminski & Mielke det. 2012/ DZ 22.818/; DZUP.


**FIGURE 7.** Larval chaetotaxy of first instar of *Ypthimaoides ordinaria*. A, Head in frontal view; B, head in lateral view; C, body diagram in lateral view. For chaetotaxy abbreviations see Stehr (1987) and Murray (2001).


**Genetic distance.** The average genetic distance among *cox1* sequences from all sampled individuals of *Yphthimoides* was 0.069, ranging from zero among some individuals of *Y. ordinaria* from Argentina to 0.106.

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**FIGURE 8.** Scanning electron microscopy of first instar of *Yphthimoides ordinaria*. A, lateral view; B, head in frontal view; C, head and prothoracic shield in dorsal view; D, anal shield in dorsal view; E, detail of clubbed setae; F, detail of pointed setae.

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between *Yphthimoides celmis* (Godart) and *Yphthimoides renata* from Belize. The genetic distance between the two individuals of *Y. ordinaria* from Brazil was 0.001. The genetic distance among the three individuals of *Y. renata* from Brazil was 0.002. Genetic distance among all sequences of *Y. ordinaria* and sequences of the sister group *Y. leguialimai* + *Y. renata* was of 0.057.

**Phylogenetic relationships.** Based on sequences of the mitochondrial gene *cox1*, the monophyletic *Y. ordinaria* is sister to a clade composed of *Yphthimoides leguialimai* (Dyar) + the paraphyletic *Y. renata* (Fig. 9).

![Phylogenetic tree](image)

**FIGURE 9.** Phylogenetic relationships among 11 species of *Yphthimoides* based on DNA sequences of *cox1* and obtained by a Maximum Likelihood analysis. Values above branches are bootstrap branch support.

**Discussion**

In spite of recent efforts to clarify and organize the systematics of the subtribe Euphychina (*e.g.* Murray & Prowell 2005; Peña *et al.* 2006, 2010, 2011), most studies have focused on the higher classification of this tribe, while the limits of several genera remain poorly defined; in addition, there are many species within that subtribe awaiting description (Marín *et al.* 2011). This is the case for several species-rich genera, such as *Euphychia* Hühner, *Cissia* Doubleday, *Magneuphychia* Forster, *Splendeuphychia* Forster, *Taygetis* Hühner, and *Yphthimoides*. Additionally, because most of the above genera were erected without a clear diagnosis, the correct assignment of a new species in a valid genus is usually tentative, and for the moment is best done by comparison with the type species of each genus (*e.g.* Freitas 2004, 2007).

Based on the classification of Lamas (2004) for the genus *Yphthimoides*, at least three groups can be recognized based on characters of male genitalia (illustrated in Forster 1964), hereafter named: 1) the *yphthima*-group (containing the type species *Yphthimoides yphthima* (C. Felder & R. Felder), with oval valvae adorned with well-developed spines; 2) the *ordinaria*-group, with squared valve adorned with very small teeth; and 3) the *erigone*-group, with elongated valvae adorned with several irregular teeth. Since members of the first two groups
form a monophyletic lineage in Peña et al. (2010), and *Y. ordinaria* appeared as sister group to the clade *Y. renata* + *Y. leguajalmai*, the placement of this taxon in the genus *Yphthimoïdes* is a reasonable decision.

Genetic distance of all analyzed individuals of *Y. ordinaria* from three localities in Brazil and Argentina are virtually identical, with differences comparable to intraspecific differences observed within other butterflies, and well below 2% sequence divergence often found between closed-related species (Herbert et al. 2003a,b; Silva-Brandão et al. 2008, this study).

The present results showed that *Y. ordinaria* is a good species, well defined by molecular data and quite distinct from most other known *Yphthimoïdes* based on wing pattern. Given its commonness and broad geographic distribution, it is surprising that it has remained undescribed until now. A possibility is that *Y. ordinaria* has become more common in recent times, following the expansion of open disturbed habitats, in a process similar to that suggested to the ithomiines *Episcada doto canaria* (Brown & D’Almeida) and *Mechanitis polymnia casabranca* Haensch in southeastern Brazil (Brown & D’Almeida 1970). This likely process combined with the low attractiveness of small brown satyrines to most lepidopterists, has resulted in a common and widespread species that remained neglected and usually mistaken with other common described species (Peña et al. 2010).

No matter the reason that kept the species for so long without being described, it should be taken as a warning to taxonomists of the importance of continual field work, in both pristine as well as disturbed areas.

**Acknowledgements**

We would like to thank Keith S Brown Jr., Marcelo Duarte, Keith Willmott, Carlos Peña, Helena Romanowski, Diego Doldbaina, Cristiano Iserhard, Lidiane Fucolini, Poliana Araújo, Pavel Maravi and Niklas Wåhberg for helping in diverse phases of the development of the manuscript. We also thank Ezequiel O. Núñez-B. and the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN) for providing sequences from material from Argentina (which are part of their ongoing project to barcode the butterflies of Argentina). Sabrina Thiele, Fábio Luis dos Santos, Paula R. Furlanetti, Clara L. B. Sant’Anna and Danilo Ribeiro helped by providing additional specimens from several localities in Brazil, and Luiza Magaldi was responsible for sequencing specimens of *Y. renata* from Brazil. AVLF thanks FAPESP (grant 04/05269-9), and the Brazilian Research Council – CNPq (fellowship 302585/2011-7). KLSB and OHHM were supported by the Brazilian CNPq (480619/2008-5 and 302662/2009-0, respectively). LAK was supported by FAPESP (10/51340-8), and EPB thanks FAPESP (12/03750-8) for a fellowship. This publication is part of the RedeLep “Rede Nacional de Pesquisa e Conservação de Lepidópteros” SISBIOTA-Brasil/CNPq (563352/2010-7), and the BIOTA-FAPESP Program (2011/50225-3).

**Literature cited**


Lepidopterists' Society, 58, 7–12.
Uncovering the hidden diversity of the Neotropical butterfly genus *Ypthimoides* Forster (Nymphalidae: Satyrinae): description of three new species based on morphological and molecular data

Eduardo P. Barbosa · Ana K. Silva · Márlon Paluch · Ana Maria L. Azeredo-Espin · André V. L. Freitas

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**Abstract** Three new species belonging to the butterflies satyrine subtribe Euptychiina are described: (1) *Ypthimoides gabriela* n. sp. occurring in low to medium altitudes in the coastal forests between the states of Rio de Janeiro and Bahia, (2) *Ypthimoides bella* n. sp., from Brazilian Cerrado savannas, known from only two localities in the State of Goiás, and (3) *Ypthimoides iserhardi* n. sp., occurring in high altitudes in the rocky outcrops of Chapada Diamantina, in the interior of the Bahia state. Descriptions are based on wing shape, wing pattern, and morphology of male genitalia. Furthermore, molecular data from the “DNA barcode” (Cytochrome C Oxidase I, ca. 658 bp) was obtained and used to validate the three new species. Additionally, information about geographic distribution and habitat for the three new species is provided, and the systematic position of each of the three species is discussed based on a molecular analysis using another 11 additional species of *Ypthimoides*.

**Keywords** Atlantic forest · Cerrado · DNA barcode · Euptychiina · Species description · Integrative taxonomy

**Introduction**

In recent years, the subtribe Euptychiina (Lepidoptera: Nymphalidae: Satyrinae) has been subject of several systematic and taxonomic studies, including the description of several new taxa and the publication of phylogenetic hypotheses (see Murray and Prowell 2005; Marín et al. 2011 and references therein; Matos-Maraví et al. 2013; Seraphin et al. 2014). Nevertheless, the taxonomy of the group continues to be a challenge to researchers; the internal relationships in the subtribe are still not well established and several large genera are non-monophyletic (Freitas 2007; Marín et al. 2009, 2011).

*Ypthimoides* Forster, 1964 is exclusively Neotropical, occurring from Central America to Argentina, with highest species richness in southeastern Brazil (Murray and Prowell 2005). The genus comprises about 20 species of predominantly brown butterflies, and most species are associated with open habitats, such as forest edges and savannas, and consequently they became more abundant with forest disturbance (Freitas et al. 2012). In his original description of the genus, Forster (1964) did not provide a clear diagnosis, and the genus has subsequently been revealed to be clearly
polyphyletic (Freitas 2004). Lamas (2004) reorganized the genus to include 22 described and two undescribed species, but since then, although several undescribed species have been identified, only two additional species of *Ypthimoides* have been described (Freitas 2004; Freitas et al. 2012).

The present paper describes three new species of *Ypthimoides* from the Brazilian Atlantic Forest and Cerrado, based on an integrative taxonomic approach (e.g., Dayrat 2005; Yeates et al. 2011; Pante et al. 2015) using both morphological and molecular data.

**Material and methods**

*Ypthimoides* specimens were examined in four public and private collections (see below). The Lamas collection of Neotropical butterfly type specimen photographs at the MUSM (also available online in Warren et al. 2013), representing most currently relevant names and recognized species of *Ypthimoides* (Lamas, 2004), except *Ypthimoides acmenis* (Hübner, 1823), *Ypthimoides patricia* (Hayward, 1957), *Ypthimoides punctata* (Weymer, 1911), and *Ypthimoides renata* (Stoll, 1780), was examined. The last four species are all clearly described in their original descriptions, and all but *Y. patricia* have pictures of adults.

Data from museum specimens were obtained from four collections. The acronyms for the collections are as follows: DZUP—Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil; BMNH—British Museum of Natural History, London, England; ZUEC—Museu de Zoologia da Universidade Estadual de Campinas, Unicamp, Campinas, São Paulo, Brazil; and ZUEC-AVLF—André V. L. Freitas Collection, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

**Morphology study**

Adult dissections were made using standard techniques. Legs, palpi, and abdomens were soaked in hot 10% KOH solution for 10 min before dissection, and dissected parts were stored in glycerol. To examine the venation, wings were soaked in alcohol and NaClO solution (bleach) for few minutes until wings become transparent. Taxonomic nomenclature follows Lamas (2004), modified after Peña et al. (2006) and Wahlberg et al. (2009).

Drawings and measurements of wings, legs, and palpi were made using a Leica® MZ7.5 stereomicroscope equipped with a micrometric scale and a drawing tube. Photographs of the male genitalia were taken using a Zeiss Discovery V20 Stereomicroscope.

The following abbreviations are used: (FW) forewing, (HW) hind wing, (D) dorsal, and (V) ventral.

**Genetic distance and phylogenetic analysis**

The genetic distance between *Ypthimoides* species and the phylogenetic relationships among them were estimated to evaluate the validity of the placement of these new species in *Ypthimoides*. Genomic DNA was extracted from two legs, by using the Invistor SPIN Tissue Mini Kit protocol (Stratage Molecular, Berlin, Germany), from five adults from Santa Teresinha and Ubaíra, Bahia State, one adult from Pirenópolis, Goiás State, and five adults from Mucugê, Bahia state, Brazil. DNA was stored in TE buffer at -20 °C.

The mitochondrial gene Cytochrome C Oxidase I (COI, ca. 658 bp) was amplified by using the following primer combination: LCO+HCO (Folmer et al. 1994). Reactions were done in a 25-μL final volume using 2 μL of total DNA, 2.0 mM of MgCl2, 40 μM of dNTPs, 0.5 μM of each primer, 1 U of GoTaq DNA Polymerase (Promega, Madison, WI, USA), and 10% of 1× Taq buffer. The amplification program included an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 42 °C for 30 s, and polymerization at 72 °C for 1 min, followed by an extension step at 72 °C for 10 min (Silva-Brandão et al. 2005). PCR products were purified of primers and deoxynucleotides with ExoSAP-IT (GE Healthcare, Buckinghamshire England), and then sequenced by ABI Prism BigDye Kit protocol in a 3500XL Genetic Analyzer (Applied Biosystems—Hitachi), with primers used for amplification. Sequences were analyzed with the program FinchTV v. 1.4.0 (Geospiza Inc.). Those sequences were posteriorly aligned by eye in the Biological Sequence Alignment Editor Software (BioEdit) v. 7.2.4 (Tom Hall 2013, available at http://www.mbio.ncsu.edu/bioedit/bioedit.html#downloads) with sequences obtained previously and available on GenBank (Peña et al. 2010). The final matrix comprised 34 sequences of 14 species of *Ypthimoides* (Table 1) and one species used as outgroup, namely *Atlanteuptychia ernestina* (Weymer, 1911) (Freitas et al. 2013).

The genetic distances among species of *Ypthimoides* were determined by using the nucleotide substitution model Kimura-2-parameters (Kimura 1980) by using the program MEGA v. 6.0 (Tamura et al. 2013).

The phylogenetic relationships of the new species were estimated by using the maximum likelihood method. Maximum likelihood analyses were run with RAXML (Stamatakis et al. 2008) with 1000 rapid bootstrap replicates and a search for the maximum likelihood.
Table 1  Data of sequenced specimens of *Pphthalmoides* with code, sampling sites data, and GenBank accession numbers

<table>
<thead>
<tr>
<th>Species name</th>
<th>Code</th>
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<th>COI—GenBank accession</th>
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topology on the CIPRES portal (Miller et al. 2010). The
data were modeled according to the GTR+G model.

**Pthalmoides gabriela** Barbosa, Freitas and Paluch,
new species

Diagnosis. *Y. gabriela* resembles *Pthalmoides angulata* (Butler, 1867) in wing shape, but can be distinguished from the latter by the presence of more developed ocelli in both dorsal and ventral hindwing surfaces (absent or simplified in *Y. angulata*) and by the presence of a yellowish band crossing both wings in the ventral surface about two thirds from the base (absent in *Y. angulata*) (Figs. 1, 2, and 3).

**Description of adults**

Male (Figs. 1a, b and 2a–c). Forewing length 22–23 mm (average 22.4 mm, SD=0.51, n=8); hindwing length 19–21 mm (average 19.7 mm, SD=0.70, n=8). Eyes covered with sparse very short hairs, entirely brown. Palpus length approximately one third of antenna length, beige, with long dark brown and white hairs (male palpus in Fig. 2c). Antenna of males 10–12 mm
in length \((n=7)\), with 39–40 antennomeres extending to mid-costal shaft rust brown, with light cream scales in base of each antennomere until antenna mid-point; club with 10–11 antennomeres, with internal margin orange ochre, not conspicuously developed. Wings with dorsal ground color dark brown with few markings; a thin dark marginal line and a broader dark submarginal line following contours of wing margins; DHW two black ocelli with white pupils outlined by a faint yellow ring, first larger in CuA~1–CuA~2, second in CuA~2–
2A. HW outer margin markedly wavy. Ventral wings mostly light brown; VFW crossed by two dark brown lines extending from costa to CuA~2; first slightly irregular, one third distance from wing base to apex; second straight, at two thirds from wing base to apex, delimiting a broad light yellowish stripe; a dark brown zigzag submarginal line and a brown regular marginal line extending from costa to 2A; one to three tiny white ocelli outlined by a sooty ring of dark brown scales in M~1–M~2 (ocellus 1), M~2–M~3 (2), and CuA~1–CuA~2 (3). VHW crossed by two regular dark brown lines extending from costa to anal margin, first one third distance from wing base to apex; second at two thirds from wing base to apex, delimiting a broad light yellowish stripe; a thin dark brown zigzag submarginal line and a brown regular marginal line following wing contours extending from costa to 2A; a series of six black ocelli outlined by a sooty ring of dark brown scales and with white pupil can be found in Rs–M~1 (ocellus 1), M~1–M~2 (2), M~2–M~3 (3), M~3–
CuA~1 (4), CuA~1–CuA~2 (5), and CuA~2–2A (6); ocelli 1, 3, and 4 very small and reduced to few white scales circled by few dark brown scales; ocelli 2 and 5 larger than others, with large white pupil. No conspicuous androconial scales or patches observed. Male wing venation shown in Fig. 2a. Legs covered by dark and light brown scales; male foreleg and midleg are shown in Fig. 2d, e.

Male genitalia (Fig. 3). Saccus short (Fig. 3a) and triangular in dorsal view (Fig. 3b); tegumen rounded (Fig. 3a, b); gnathos long and pointed and half the size of valvae (Fig. 3b); uncus elongated, in dorsal view large with a constriction in the basal portion and truncate at apex (Fig. 3b, c); valvae elongated, trapezoidal, ending in a tapered apex in lateral view (Fig. 3a), internal margin with one series of small to medium-sized teeth (Fig. 3b–
d); aedeagus straight (Fig. 3e, f); cornuti absent; juxta membranous.

Female (Figs. 1c, d and 2b, f). Forewing length 24–26 mm (average 25.6 mm, SD=0.89, \(n=5\)); hindwing length 20–
23 mm (average 22.0 mm, SD=1.22, \(n=5\)). General color and pattern very similar to, but in general paler than, that of males. Palpus length approximately one third of antenna length. Antenna of females 10–12 mm in length \((n=5)\), extending to mid-costal. Female wing venation is shown in Fig. 2b. Female foreleg with five tarsomeres is shown in Fig. 2f.

**Remarks on color variation.** In the individuals examined, variation in the dorsal wing color pattern is virtually absent. Variation in the ventral wing color pattern is minimal, and restricted to the size and shape of the ocelli.
**Fig. 2** *Yphthimoides gabriela.* a Male wing venation. b Female wing venation. c Male palp. d Male foreleg. e Male midleg. f Female foreleg.

**Habitat.** Field records and museum specimens suggest that *Y. gabriela* is associated with the semi-deciduous Atlantic Forest, in altitudes ranging from 150 to 750 m.a.s.l., being found on forest edges, in secondary disturbed forests and grasslands.

**Fig. 3** *Yphthimoides gabriela,* male genitalia. a Lateral view. b Dorsal view. c Ventral view. d Edeagus (dorsal view). e Edeagus (lateral view)
**Distribution.** Based on field observations, museum records, and literature, the species is present in the Brazilian states of Bahia, Minas Gerais, Espírito Santo, and Rio de Janeiro (Fig. 4).

**Etymology.** The specific epithet refers to the female protagonist of the Brazilian novel “Gabriela, clave and cinnamon” (“Gabriela, cravo e canela,” in Portuguese). This romantic tale, written by Jorge Amado in 1958, is set in a small Brazilian town in south Bahia and is considered one of the author’s finest works.

**Type material**

Holotype. Male (Fig. 1a, b) with the following labels (five labels separated by transverse bars): / HOLOTYPE/Morro da Pioneria, Serra da Jibóia, Pedra Branca, Santa Teresinha, Bahia: Brazil, 12° 52’ 6” S 39° 28’ 14” W, 18.V.2013, 500–800 m, Ana K. Silva and M. Paluch leg/Holotypus—Y. Gabriela Freitas, Barbosa and Paluch det. 2014/DNA voucher—YPH0258 / ZUEC LEP 8574/ZUEC.

Allotype. Male (Fig. 1c, d) with the following labels (five labels separated by transverse bars): / ALLOTYPE/Morro da Pioneria, Serra da Jibóia, Pedra Branca, Santa Teresinha, Bahia: Brazil, 12° 52’ 6” S 39° 28’ 14” W, 18.V.2013, 500–800 m, Ana K. Silva and M. Paluch leg/Allotypus—Y. Gabriela Freitas, Barbosa and Paluch det. 2014/DNA voucher—YPH0248/ZUEC LEP 8575/ZUEC.

ZUEC LEP 8585, ZUEC LEP 8586; Ubáira, 1 female, same data (YPH-0051—genitalia prepared), ZUEC LEP 8587.

**Ypthimoides bella** Barbosa and Freitas, new species

Diagnosis. *Y. bella* has a ventral wing pattern that resembles *Ypthimoides yphithima* (C. Felder and R. Felder, 1867) and *Ypthimoides pacta* (Weymer, 1911), but it can be easily distinguished from these two species by the presence of conspicuous black ocellar marks on the DFW (absent in both *Y. yphithima* and *Y. pacta*). The male genitalia of *Y. bella* is also quite distinct from *Y. yphithima* and *Y. pacta*, being similar (although distinct) to *Ypthimoides celmis* (Godart, [1824]) (Figs. 5 and 6).

**Description of adults**

Male (Fig. 5a, b). Forewing length 21 mm; hind wing length 18 mm. Eyes naked, entirely brown surrounded by cream scales. Palpus length approximately one third of antenna length, covered with cream scales and long black and short cream hairs. Antenna of males 10 mm in length, with 41 antennomeres extending to mid-costa; shaft rust brown, with internal margin light orange ochre and with light cream scales at base of each antennomere; club with 12 antennomeres. Wings with dorsal ground color dark brown; a thin dark marginal line and a dark zigzag submarginal line following contours of wing margin; both wings present conspicuous large ocelli; DFW with four black ocelli with small white pupil in spaces M₁–M₂ (ocellus 1), M₂–M₃ (2), M₃–CuA₁ (3), and CuA₁–CuA₂ (4); ocellus 2 smaller than remaining; DFW with five black ocelli in spaces Rs–M₁ (ocellus 1), M₁–M₂ (2), M₂–CuA₁ (3), CuA₁–CuA₂ (4), and CuA₂–2A (5); ocelli 2 and 4 larger than remaining, ocelli 4 and 5 very small, all with white pupil (except ocellus 5). Ventral ground color light brown; VFW crossed by two dark brown lines extending from costa to CuA₂; first one third distance from wing base to apex; second irregular, at two thirds from wing base to apex; a dark brown zigzag submarginal line and a brown regular marginal line extending from costa to 2A; five black ocelli outlined by a ring of orange scales and white pupil present in spaces Rs–M₁ (ocellus 1), M₁–M₂ (2), M₂–M₃ (3), M₃–CuA₁ (4), and CuA₁–CuA₂ (5); ocellus 1 and 3 smaller than others. VHW crossed by two dark brown lines extending from costa to anal margin, first one third distance from wing base to apex; second wavy, at two thirds from wing base to apex, delimiting a lighter area; a thin dark brown zigzag submarginal line and a brown regular marginal line following wing contours extending from costa to 2A; a series of six black ocelli outlined by a ring of orange scales and with white pupil can be found in Rs–M₁ (ocellus 1), M₁–M₂ (2), M₂–M₃ (3), M₃–CuA₁ (4), CuA₁–CuA₂ (5), and CuA₂–2A (6); ocelli 2 and 5 larger than other; ocellus 3 small with orange ring fusioning with that of ocellus 2. All ocelli are surrounded by a continuous area of dark brown scales. No conspicuous androconial scales or patches observed.

**Fig. 5** *Ypthimoides bella*. a Holotype male, dorsal view. b The same, ventral view. c Allotype female, dorsal view. d The same, ventral view.
Male genitalia (Fig. 6). Saccus short (Fig. 6a) and triangular in dorsal view (Fig. 6b); tegumen rounded (Fig. 6a, b); gnathos long and pointed, half size of valva (Fig. 6a, b); uncus elongated, laterally enlarged in dorsal view, constricted near junction with tegumen and ending in a pair of short rounded bumps (Fig. 6a, b, c); valva short, trapezoidal, with a conspicuous costal expansion bearing small teeth, and ending in a pointed apex in lateral view (Fig. 6a), with internal margin bearing one series of medium to large-sized teeth (Fig. 6b, c); aedeagus straight in dorsal view (Fig. 6d) and slightly hunched in lateral view (Fig. 6e); cornuti absent; juxta membranous.

Female (Fig. 5c, d). Forewing length 22 mm; hindwing length 19 mm. General color and pattern very similar to, but in general paler than that of males. Due to limited number of specimens studied, differences in color and wing pattern observed in females cannot yet be reliably attributed to sexual dimorphism.

Remarks on color variation. Considering the very few known individuals of *Y. bella*, it is difficult to assess wing color pattern variation. In the individuals examined, variation in the dorsal and in ventral wing color pattern is restricted to the size of several ocelli and the shape of the dark lines.

Habitat. Based on field records and museum specimens, *Y. bella* appears to be associated with the open vegetation of rocky montane fields (known locally as campos rupestres).

Distribution. This species is known from only two localities in the state of Goiás in Central Brazil (Fig. 4).

Etymology. The specific epithet, *bella* (beautiful in Portuguese) refers to the distinctive, conspicuous ocelli observed on the ventral wings, a pattern contrasting with the generally inconspicuous pattern of ocelli observed in other species of *Yphthimoides*.

Type material

Holotype. Male (Fig. 5a, b) with the following labels (five labels separated by transverse bars): / HOLOTYPE/ Goiás (Goiás Velho), Caminho para as antenas, Goiás: Brazil. 600–680 m, 30.II.2013, 15° 55′ 37″ S 50° 07′ 47″ W, Junia Y. O. Carreira leg./Holotypus *Y. bella* Barbosa and Freitas det. 2014/DNA — YPH 0201/ZUEC LEP 9167/ZUEC.

Allotype. Female (without antennae) (Fig. 5c, d) with the following labels (five labels separated by transverse bars): / ALLOTYPE/Parque Estadual da Serra dos Pirineus, Pirenópolis, Goiás: Brazil, 1300–1330 m, 04.IV.2013, 15° 47′ 44″ S 48° 49′ 55″ W, Lucas A. Kaminski leg./Allotypus *Y. bella* Barbosa and Freitas det. 2014/DNA voucher—YPH 0337/ZUEC LEP 9168/ZUEC.


_Yphthimoides iserhardi* Freitas and Barbosa, new species

Diagnosis. *Y. iserhardi* is similar to _Yphthimoides cipoensis_ Freitas, 2004, but can be easily distinguished from the latter by the lighter tones in wing pattern, and by the ground color of the VFW, which varies from a rusty orange to a light yellowish ochre. The male genitalia resembles that of *Y. cipoensis* although the valvae of *Y. cipoensis* is a little bit squarer and the valvae of *Y. iserhardi* is more elongated, but the irregular teeth pattern is very similar (Figs. 7 and 8).
Description of adults

Male (Fig. 7a, b). Forewing length 20–21 m (average 20.66 mm, SD=0.51, n=6); hind wing length 16–19 mm (average 17.33 mm, SD=1.03, n=6). Eyes naked, entirely light brownish ochre surrounded by cream scales. Palpus length approximately one third of antenna length, covered with cream scales and long black and short light cream hairs. Antenna 10–11 mm in length (n=4), with 39–41 antennomeres extending to mid-costa; shaft rust brown, with internal margin bearing a thin orange ochre stripe; club with 10–12 antennomeres. Wings with dorsal ground color dark brown with few markings restricted to HW; a thin dark marginal line following contours of wing margin and a broader dark zigzag submarginal line, both obsolescent; DHW with three black ocelli outlined by a ring of orange scales, and with a white pupil in spaces Rs–M₁ (ocellus 1), Cu₁–Cu₂ (2), and Cu₂–2A (3), ocelli 1 and 3 reduced and usually absent.

Fig. 8 Ipthimoides izerhardi,
male genitalia. a Lateral view. b Dorsal view. c Ventral view. d Edeagus (dorsal view). e Edeagus (lateral view)
(condition observed in holotype, Fig. 7a); ocellus 2 larger and with double pupil in some individuals. DHW outer margin slightly wavy. VFW with dark brown edges and a rusty orange to yellowish ochre ground color in filling most of wing surface; crossed by a straight and slightly wavy dark brown line extending from costa to 2A two thirds from wing base to apex; this line faint from M3 to 2A; a dark brown zigzag submarginal line and a brown regular marginal line extending from costa to 2A; two minute black ocelli with white pupil in spaces M1–M2 (ocellus 1) and M3–CuA1 (2; absent in holotype). VHW crossed by two dark brown concave irregular lines extending from costa to anal margin, first one third distance from wing base to apex; second at two thirds from wing base to apex, delimiting a lighter area; a broad dark brown zigzag submarginal line and a brown regular marginal line following wing contours extending from costa to 2A; three black ocelli with white pupil in spaces M1–M2 (ocellus 1), CuA1–CuA2 (2), and CuA2–2A (3; absent in holotype); ocellus 3 smaller than other.

Male genitalia (Fig. 8). Saccus short (Fig. 8a) and triangular in dorsal view (Fig. 8b); tegumen rounded (Fig. 8a, b); gnathos long and pointed and half size of valva (Fig. 8b); uncus laterally enlarged in dorsal view, constricted near junction with tegumen and flattened at end (Fig. 8b, c); valva elongated, trapezoidal, and ending in a tapered apex in lateral view (Fig. 8a), internal margin of harpe with one series of irregular medium-sized teeth (Fig. 8b, c); acedeagus straight in both dorsal and lateral view (Fig. 8d, c); cornuti absent; without fulvita inferior.

Female (Fig. 7c, d). Forewing length 21–25 mm (average 22.5 mm, SD = 1.08, n = 4); hindwing length 19–23 mm (average 20.5 mm, SD = 1.09, n = 4). General color and pattern very similar to, but in general paler than that of males, with two ocelli in VFW in all known individuals. Palpus length approximately one third of antenna length.

Remarks on color variation. Variation on the dorsal wing surfaces is practically absent and obvious seasonal variations have not been observed. On the VFW, the predominant ground color can vary from rusty orange to a light yellowish ochre, and on the VHW, the intensity of the mottled pattern is also variable. The number of ocelli on the males’ ventral wings can also vary, with a minimum observed of one ocellus on the VHW and no ocelli on the VFW.

Habitat. Based on field records and museum specimens, Y. iserhardi appears to be associated with the open vegetation of rocky montane fields (known locally as campos rupestres), from 900 to 1600 m a.s.l.

Distribution. The species is endemic to the Chapada Diamantina region in the state of Bahia, and has been recorded from three localities: (1) Parque Municipal de Mucugê, Mucugê (12° 58’ 56” S, 41° 20’ 49” W, 1100 m a.s.l.), (2) Pico das Almas, Rio de Contas (13° 22’ 15” S, 41° 52’ 12” W, 1300 m a.s.l.), and (3) Morro da Mãe Inácia, Palmeiras (12° 27’ 00” S, 41° 28’ 00” W, 900 m a.s.l.) (Fig. 4).

Etymology. The specific epithet of this species is a tribute to our friend Cristiano Agna Iserhard, a young butterfly researcher who has been contributing in the past few years to our knowledge of butterflies through regional inventories, conservation, and community ecology studies in Brazil.

Type material
Holotype. Male (Fig. 7a, b) with the following labels (four labels separated by transverse bars); / HOLOTPUS/28-I-2005, Pico Das Almas, Rio De Contas, Bahia, [Brazil], 1400–1600 m, MIELKE and CASAGRANDE LEG./DZ 21.466 / Y. iserhardi, Freitas and Barbosa det. 2014/DZUP.
Allotype. Female (Fig. 7c, d) with the following labels (four labels separated by transverse bars); / ALLOTPUS/ 28-I-2005, Pico Das Almas, Rio De Contas, Bahia, [Brazil], 1400–1600 m, MIELKE and CASAGRANDE LEG./DZ 21.515/Y. iserhardi, Freitas and Barbosa det. 2014/DZUP.

Phylogenetic relationships
For Y. Gabriela and Y. iserhardi, intraspecific distances ranged from 0 to 0.7% (data not available for Y. bella, since only a single individual has been sequenced). Interspecific distances ranged from 4.2 to 10.4%, overlapping with intraspecific distances in the classes 5.8 and 7.0 %, both, respectively, between specimens of Y. renata (Stoll, 1780) from Brazil and Colombia and from Brazil and Costa Rica (Fig. 9), but a possible complex of cryptic species is involved in that case (EPB and AVLF in prep.). Based on sequences of the
mitochondrial gene Cox1, *Y. gabiela* and *Y. iserhardt* are monophyletic (Fig. 10), and well distinct from all other described species of *Yphthimoidea*. The analysis recovered *Y. gabiela* as sister to *Yphthimoidea ochracea* (Butler, 1867), *Y. bella* sister to *Y. celmis* and *Y. iserhardt* sister to *Y. cipoensis*.

Discussion

Whereas in the recent past few years most studies had focused in the higher classification of *Euptychiina* (Murray and Prowell 2005; Peña et al. 2006, 2010, 2011), the limits of several general remain poorly understood (Freitas et al. 2012) with many species awaiting description (Marín et al. 2011), although a few studies have started to uncover the limits of some genera (Matos-Maravi et al. 2013; Seraphin et al. 2014) and to describe some of these species (e.g., Freitas 2007; Pulido and Andrade 2008; Huertas et al. 2009; Zacca et al. 2013,

Fig. 10 Phylogenetic relationships among 14 species of *Yphthimoidea* based on DNA sequences of Cox1 and obtained by a maximum likelihood analysis. *Numbers below branches are bootstrap support.*
2014; Siewert et al. 2013). Here, we showed that Y. gabriela, Y. bella, and Y. iserhardi not only belong to the genus Ypthimoïdes, but that they are also species well supported by molecular data, wing pattern, and morphology of the male genitalia.

A clear result of the present study is that wing shape and pattern are not good predictors of relationship among species of Ypthimoïdes. For example, the wavy hindwing margin and wing pattern of Y. gabriela resembles that of Y. angularis, and the large and numerous ocelli on the VHW of Y. bella is very similar to the condition observed in Y. pacta and Y. yphthima. However, for both of the above examples, the closest species are not those suggested by wing shape and pattern. Instead, the ML analysis showed that Y. gabriela is closely related to Y. ochracea and Y. bella is sister to Y. celsis.

On the other hand, the male genitalia appear to be informative in terms of phylogenetic relationships: the male genitalia of all three new species are very similar to their putative sister species, with some small differences that are sufficient to diagnose them. For Y. bella and Y. celsis (its sister species), differences are in the pattern of the teeth in the apex of the valvae and in the size of the small teeth in the costal expansion (smaller in Y. celsis, see Forster 1964). For Y. gabriela and Y. ochracea, the differences in male genitalia are more marked (Barbosa et al. in prep.). Finally, for Y. iserhardi and Y. cipoensis, the differences are in the size of the teeth in the internal margin in the apex of the valvae, and by the absence of a conspicuous bump on the costal margin, which is present in Y. cipoensis (Freitas 2007).

The information provided by the first half of the Coxl barcode region was congruent with morphology. The intraspecific genetic distance within Y. gabriela and Y. iserhardi is very low and does not overlap with the interspecific distances. Conversely, the interspecific distances among the three new species and the other sequenced Ypthimoïdes are high enough to set these three species as valid species, corroborating the morphological evidence.

Based on museum records and field observations, the conservation status, at least for one of the three new species, needs special attention, namely Y. iserhardi. This species appears to be endemic to the rocky fields of Chapada Diamantina, occurring in fragile habitats with high anthropogenic pressure. This situation is very similar to that for its putative sister species, Y. cipoensis, a species occurring in similar habitats and also proposed as threatened by Freitas (2004).

In the past decade, five new species of Ypthimoïdes have been described (Freitas 2004; Freitas et al. 2012 and the present work). As more data are gathered, both molecular and morphological, additional undescribed species are expected to come to light, while others will certainly be removed from this genus. With this approach, hopefully the taxonomy of the genus Ypthimoïdes will reach a reasonable stability in the near future.

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Ethical standards The research contained within this manuscript is in accordance with Brazilian laws.

Conflicts of interest The authors declare that they have no conflict of interest.

References


Systematics, Morphology and Biogeography
‘Species’ from two different butterfly genera combined into one: description of a new genus of Euptychina (Nymphalidae: Satyrinae) with unusually variable wing pattern

André Victor Lucci Freitas, Eduardo Proença Barbosa, Keith Richard Willmott, Niklas Wahlberg, Gerardo Lamas

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A B S T R A C T
Sepona Freitas and Barbosa, gen. nov., is proposed for the Neotropical satyrine butterfly species Euptychia punctata Weymer, 1911 and its junior subjective synonym Euphytchia griseola Weymer, 1911 and Tagetis indecisio Ribeiro, 1931. The new genus has a distinctive wing pattern and shape of the valva in the male genitalia, the latter being a unique autapomorphy within the subtribe Euptychina. Based on molecular data, this genus is not sister to any other single euptychine genus, instead appearing as the sister to all remaining genera in the Tagetis clade. The present paper illustrates the complexity of the taxonomy of Euptychina, and the importance of using different sources of evidence in taxonomic studies.
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Introduction
In recent years, the highly diverse butterfly subfamily Satyrinae has been subject to several studies attempting to clarify its internal relationships and taxonomy (Murray and Prowell, 2005; Peña et al., 2006, 2010; Marin et al., 2011; Matos-Maravi et al., 2013; Siewert et al., 2013; Seraphim et al., 2014). These studies have revealed many non-monophyletic genera, and a number of complexes of cryptic species waiting to be disentangled, especially in the predominantly lowland, largely Neotropical subtribe Euptychina (e.g. Peña et al., 2010; Freitas et al., 2012a; Matos-Maravi et al., 2013; Zacca et al., 2013; Siewert et al., 2013; Seraphim et al., 2014).

Including 10 genera, the “Tagetis clade” is one of five major groups of Euptychina (Peña et al., 2010); a preliminary phylogeny for this clade (Matos-Maravi et al., 2013) showed that four genera, namely Harjesia Forster, 1964, Pseudodesis Forster, 1964, Forsterinaria R. Gray, 1973 and Tagetis Hübner [1819], are polyphyletic, requiring some revised generic combinations and the description of new genera. The genus Harjesia, as then conceived, included species placed in three different clades within the “Tagetis clade” (Matos-Maravi et al., 2013). In that phylogeny, Harjesia griseola (Weymer, 1911) appeared as sister to the entire “Tagetis clade” (Matos-Maravi et al., 2013), suggesting that it should be placed in a new genus.

Ongoing research into the phylogenetic relationships of another euptychine genus, Ypthimoïdes Forster, 1964, showed that this genus is clearly polyphyletic, with several species that should be reassigned to other genera (Freitas et al., 2012b; Barbosa et al., 2015, EPB and AVLF, in prep.). One species in particular, Ypthimoïdes punctata (Weymer, 1911), is quite distinct from all other described Ypthimoïdes, and further morphological studies revealed that Y. punctata and H. griseola are similar enough to be considered subjective synonyms.

This study presents evidence based on an integrative taxonomic approach (e.g., Dayrat, 2005; Yeates et al., 2011; Pante et al., 2015) using both morphological and molecular data for the synonymy of Y. punctata and H. griseola, and describes a new genus to harbor the resulting single species.
Material and methods

Adult specimens were studied in a number of American and European collections, and the following acronyms are used here: AWLV – Allan & Lesley Wolhuter collection, United Kingdom; DZUP – Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil; FMLMN – Florida Museum of Natural History, Gainesville, FL, USA; KWH – Keith R. Willmott & Jason P. W. Hall collection, Gainesville, FL, USA; LBCB: L. & C. Brévignon collection, French Guiana; MNHN – Muséum National d’Histoire Naturelle, Paris, France; MNRI – Museu Nacional do Rio de Janeiro, Rio de Janeiro, Brazil; MOBE: Mohamed Benneshah collection, France; MUSM – Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru; MZU – Museu Zoológico Universitário Jagielloniego, Krakow, Poland; NHMUK – The Natural History Museum, London, United Kingdom; YUGA – Yuvitka Garca collection, Santa Cruz, Bolivia; ZSM – Zoologische Staatssammlung München, München, Germany; ZUEC – Zoológico da Universidade Estadual de Campinas, Campinas, São Paulo, Brazil; ZUEC-AVLF – André V. L. Freitas Collection, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

Morphology

Dissections were made using standard techniques. Legs, palpi and abdomens were soaked in hot 10% potassium hydroxide for nearly 10 minutes before dissection, and dissected parts were stored in glycerol. In order to see the venation, wings were diaphanized by soaking them in alcohol and NaClO solution (bleach). Taxonomic nomenclature follows Lamas (2004a,b), modified by Peña et al. (2009) and Wahlberg et al. (2009). Drawings and measurements of wings, legs and palpi were made using a Leica® MZ7.5 stereomicroscope equipped with a micrometric scale and a drawing tube. Photographs of the male and female genitalia were taken using a Zeiss Discovery V20 Stereomicroscope. The following abbreviations are used: (FW) forewing, (HW) hind wing, (D) dorsal, (V) ventral.

Phylogenetic inference

Genomic DNA was extracted from two legs of adults by using the DNeasy Blood & Tissue Kit protocol (QiAGEN, Düsseldorf, Germany). DNA was stored in TE buffer at ~20 °C. The mitochondrial gene cytochrome c oxidase 1 (CO1), ca. 658 bp, corresponding to the ‘DNA barcode’ region for all specimens and the nuclear genes GAPDH for one specimen (YPH-0240) and Rp5S for the outgroups were amplified, purified and sequenced using standard techniques (see Silva-Brandão et al., 2005; Wahlberg and Wheat, 2008). The sequences of nuclear gene Rp5S for Harjesia grisola were obtained from GenBank.

All the sequences were aligned by eye with sequences obtained previously and available on GenBank by using BioEdit v. 7.2.4 (Hall, 2013, available at http://www.mbio.ncsu.edu/bioedit/bioedit.html#downloads). The final matrix comprised 32 specimens from species of 10 genera (including 11 specimens from the new genus Sepona) and three species used as outgroups, namely Hermeuptychia maineone (A. Butler, 1870). Paryphthimaoides grimon (Godart [1824]) and Splendepuphia doxes (Godart [1824]) (see Table 1 for the sequence codes).

The phylogenetic relationships of the new species were estimated using maximum likelihood. Analyses were run using RAXML (Stamatakis et al., 2008) with 1000 rapid bootstrap replicates and a search for the maximum likelihood topology on the CIPRES portal (Miller et al., 2010). The data were modeled according to the GTR + G model for each partition independently.

Table 1
Species of Euptychina with code, sampling site data, and GenBank accession numbers for sequenced genes.

<table>
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<th>Species name</th>
<th>Code</th>
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<th>COI</th>
<th>GAPDH</th>
<th>Rp5S</th>
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Sepona Freitas & Barbosa, gen. nov.

Type species: Euptychia punctata Weymer, 1911, here designated.

Diagnosis

Molecular data (Peña et al., 2010) place this genus within the “Tagetes clade” of the satyrine subtribe Euptychina. In terms of wing shape and pattern, adults are similar to those of some species of Harjesia, Pseudodehis and Tagetina Forster, 1964, which all have undulate rather than straight dark discal and postdiscal lines on the venal surface, but this genus can be distinguished from all other euptychines by the unique shape of the valvae in the male genitalia (Fig. 3A). The valvae bear a long, thin, inwardly curved projection arising abruptly from the otherwise rounded main body of the valva. See Table 2 for comparisons of additional morphological characters of Sepona punctata with representatives of other genera of the “Tagetes clade”. The relationships of Sepona to other euptychina genera, and justification for its recognition as a monotypic genus, are addressed further below under ‘Discussion’.

Etymology

Sepona is an arbitrary combination of letters, derived from the Latin transitive verb “sepono”, meaning to put aside, separate, or remove, in reference to the isolated position of the genus in comparison with other members of the “Tagetes clade”. It should be treated as a feminine noun.

Sepona punctata (Weymer, 1911) comb. nov.

Euptychia punctata Weymer, 20 April 1911: 205. Type Locality: Brazil, Minas Gerais. Syntype(s): not located.


Redescription

Male (Figs. 1, 2A-C-D-E, 4A-B-D): Eyes reddish brown, covered with sparse black hairs. Palpus 1.5 times as long as head, brown with light brown hairs (Fig. 2C): Antenna of males 9.0–10.0 mm in length with 36 antennomeres, extending to mid-coxa: shaft rust-brown dorsally, orange brown ventrally, sparsely scaled dorsally; club not conspicuously developed, including eleven segments, with apical portion (last five segments) dark brown. Forewing length 23–25 mm (n = 6); hindwing length 19–20 mm (n = 6). HW outer margin slightly undulate. Male wing venation shown in Fig. 2A. Wings with dorsal ground color dark brown with few markings, restricted to a suffused dark brown outer margin on DW, and to dark double marginal line, and a submarginal line following contours of marginal line on DHW. Ventral wings light brown; VFW crossed by two thin zigzag dark brown lines, extending from costa to 2A, first line one-third distance from wing base to

Table 2

Comparisons of Sepona punctata with exemplar species of related genera in “Tagetes clade” for adeagus and sacculus width, the value refers to length/h width ratio in the medial portion of each structure higher numbers are larger.

Fig. 1. Adult male of Sepona punctata – Jaru, Rondônia, Brazil. Dorsal above, ventral below.

apex; second line extending from costa to 2A at two-thirds distance from wing base to apex; a conspicuous lighter outer band is adjacent to second line, followed by a darker ocellar region (see next); a thin dark brown zigzag submarginal line with single black dots in vertices and a brown regular marginal line extending from costa to 2A; four dark ocelli in spaces R5–M1 (ocellus 1), M1–M2 (2), M2–M3 (3) and M3–Cua1 (4). VWI crossed by two thin dark brown lines from costa to anal margin, in similar position to those on forewing; a conspicuous lighter outer band is adjacent to second line, followed by a darker ocellar region (see next); a dark brown zigzag submarginal line with single black dots in vertices and a brown regular marginal line extending from costa to 2A; a series of five dark ocelli can be found in cells Rs–M1 (ocellus 1), M1–M2 (2), M2–M3 (3), M3–Cua1 (4) and Cua1–Cua2 (5). Details about ocelli size and shape discussed further below. No conspicuous parandrine scales observed.

Male genitalia (Fig. 3A–E). Saccus elongate; tegumen rounded and short; gnathos long and pointed, projecting upwards above uncus; uncus elongated, with lateral expansions in dorsal view, giving an arrowhead appearance; valvulae elongated, ending in a bump with a long thin pointed process; aedeagus curved; cornuti absent; juxta sclerotized, linking both valvae together.

Female (Fig. 2B, F, 4C–E). Forewing length 24–26 mm (n=2); hindwing length 20–22 mm (n=2). Antenna 11.0 mm in length, with 35 antennomeres, extending to mid-costal area. General color and pattern very similar to those of males. Female genitalia as in Fig. 3E, F. Ductus bursae partially sclerotized, corpus bursae rounded; a pair of conspicuous signa present.

Taxonomy and variation

Weymer (1911) described Euptychia punctata based on an unstated number of specimens from Minas Gerais, Brazil. Several pages later, he described Euptychia griseola based also on an unstated number of specimens from Mapiri, Bolivia. Later, Ribeiro (1931) described a third taxon as Togetis incedis. Based on one female from Brazil, Rio Jamari; this taxon was promptly synonymized with Euptychia griseola by May (1933). Descriptions of both E. punctata and E. griseola were also based on female specimens, and although this cannot be determined unambiguously from their original descriptions, the fact that the types are females in both cases suggests that males were unknown to the authors. No type specimen(s) of punctata has been found, but there is a single female in ZSM identified by Forster (1964) as the “Typus” of griseola, and we accept that it indeed represents a syntype (which we designate herein as lectotype), since this particular specimen (examined) matches precisely the illustration provided subsequently by Weymer (1911: pl. 47g, fig. 7). The female holotype (examined) of Togetis incedis Ribeiro is deposited in MNRI. Although appearing rather different in wing pattern, the names punctata and griseola apparently represent extremes of geographical variation within a single species. Variation on the dorsal wing surfaces is practically absent and obvious seasonal variations have not been detected. The ventral surface of both wings, however, shows much variation, especially in the number and size of the ocelli. Most individuals from central and southeastern Brazil and eastern Bolivia (“punctata” phenotype) have the ocelli reduced to small black dots, sometimes with a tiny white pupa on the VWI; they also present a more homogeneous ventral pattern (Fig. 4B, F). Conversely, individuals from western Amazonia and Guianas (the “griseola” phenotype) have more developed ocelli cirrled by yellowish cream scales and with a white pupa on both wings, and a conspicuous banded pattern on the ventral wings (Fig. 4A, B). Intermediate phenotypes between “punctata” and “griseola” are known from Acre and Rondônia in Brazil, and from Bolivia, and are usually more similar to the “griseola” phenotype (Fig. 4C, D). To our knowledge, no two of these three phenotypes (“punctata”, “griseola” and intermediate) have been recorded in sympatry. The two names were published several months apart, and we thus treat E. griseola as a junior subjective synonym of S. punctata n. syn.

Specimens examined (34, 27, 27; Bahia, 7; Rondônia, 5; Amazonas, 2, 2, 1; Acre, 3; Mato Grosso, 3; Mato Grosso do Sul, 1; Minas Gerais, 1; São Paulo, 1).
Fig. 2. Morphological characters of Sepona punctata. A, male wing venation – forewing above and hind wing below; B, female wing venation – forewing above and hind wing below; C, male palpus; D, male foreleg; E, male midleg; F, female foreleg.

Fig. 3. Male and female genitalia of Sepona punctata. A, male genitalia in lateral view; B, male genitalia in dorsal view; C, male genitalia in ventral view; D, male aedeagus (lateral view); E, female genitalia in ventral view; F, female genitalia: detail of the signa in corpus bursae. (sa) sacculus, (te) tegumen, (un) uncus, (va) valva, (bu) corpus bursae, (st) signum.


Other records: French Guiana. – Cayenne, Roura, Cacao [4°35'N,52'28"W] (Damico, R.), Feb 1996, 1♀ (LBCB) (Brévignion, 2008: 68, pl. 7, fig. 81, 82); Saint-Laurent-du-Maroni, Sault [3°37'N,53'12"W], 9 Oct 2011, 1♂ (MOBE) (Brévignion & Bencheshab, 2012: 46, pl. 3, fig. 11 [adult wings], 1♂ [male genitalia]).

Biology and distribution

Sepona punctata is known from eastern Ecuador to southeastern Brazil (Espírito Santo, Rio de Janeiro, Minas Gerais and São Paulo). There are also records from two sites in French Guiana (Brévignion, 2008; Brévignion and Bencheshab, 2012) (Fig. 6). In eastern Ecuador the species is extremely rare, and the few known localities are in the Andean foothills on the types of sandstone soils that frequently support stands of bamboo. Adults are usually scarce and rare in collections, although they were sometimes common in areas with large bamboo patches in the upper Jurú River, in Acre, Brazil (AVLF and K. S. Brown Jr., pers. obs.). The species is usually associated with forested habitats, but some populations in SE Brazil (the “punctata” phenotype) are known from riparian forests in the cerrado. The immature stages and hostplants are unknown.

Discussion

The position of Sepona punctata as a well-supported sister to the remaining genera in the “Toygetis clade”, and the polyphylectic
nature of Harjesia as illustrated by Matos-Maravi et al. (2013) and in the present paper (Fig. 5), clearly shows that this species is not part of Harjesia (which has as its type species Tagetes blanda Möschler, 1877). The reasons for erecting a new genus for this taxon are therefore clear: unless all species in the “Tagetes clade” are lumped into a single genus, an undesirable option given the morphological variation and taxonomic diversity within the clade, there is no way to circumscribe monophyletic genera in the clade without making this taxon a monobasic genus. In addition to its phylogenetic position, the male genitalia of S. punctata is quite distinct from all known species of Harjesia (Forster, 1984 and unpublished results of the authors), presenting several unique characters, including the extremely thin and curved aedeagus and the unique shape of the valvae (see Fig. 3A and Table 2).

The known wing patterns of S. punctata are highly variable, but although specimens from western Amazonia and Guianas are quite

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**Fig. 4.** Variation in wing pattern of Sepona punctata (all from Brazil). A. Abuna, Rio Madeira, Rondônia; B. Porto Velho, Rondônia; C. Estação Ecológica do Alto Acre, Acre; D. Porto Acre, PAD Humaitá, Acre; E. Concreção de Mato dentro, Minas Gerais; F. Parque Municipal do Trabjih, Pindamonhangaba, São Paulo (A, B, D – males; C, E, F – females).

**Fig. 5.** Relationships among Sepona punctata and selected species in the “Tagetes clade” and several outgroups inferred with maximum likelihood. Numbers near branch nodes are bootstrap branch support. Names in parentheses for Sepona punctata refer to the phenotype of the voucher specimens (see text).
divergent from those from southeastern Brazil, individuals with intermediate wing pattern are known from eastern Bolivia, and Acre and Rondônia, Brazil. In addition, these differences are not related to seasonal forms and, based on the few known individuals, there is low variation within populations, including in the sites where intermediate populations are known. These reasons were considered sufficient to not recognize subspecific taxa within this species.

The above-described variation in wing patterns throughout the distribution of *S. punctata* easily explains why this taxon was described as three different species, twice by the same author (Weymer, 1911), in three different genera (see the synonymic list above). This situation is a perfect example of how complex is the taxonomy of Euptychia, where most of the large genera are non-monophyletic, with species spread in two or more different clades (as is the case of *Splendueuptychia* Forster, 1964, Cissta Doubleday, 1848, and *Purpymphithmoïdes* Forster, 1964, see Peña et al., 2010).

Hopefully, forthcoming studies combining morphological and molecular data will help to disentangle the complex and species-rich clade which constitutes the subtribe Euptychina, providing a well resolved phylogeny that will serve as a framework for future studies focusing on diversification patterns of Neotropical butterflies.

**Conflicts of interest**

The authors declare no conflicts of interest.

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**References**


Description of two new species of the Neotropical genus *Yphthimoides* Forster, 1964 (Lepidoptera: Nymphalidae: Satyrinae) from the ‘renata clade’

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This paper describes two new species of Neotropical butterflies: *Yphthimoides blanquita* Barbosa, Marín and Freitas sp. nov., from the dry forest of northwestern Colombia, and *Yphthimoides narkata* Barbosa & Freitas sp. nov. from northeastern Brazil, based on morphological and molecular data. Adult morphology, including wing shape and pattern as well as male genitalia, is described in detail. Furthermore, analysis of the mitochondrial *Cox1* ‘barcode’ showed that both new species are quite distinct from all similar *Yphthimoides* species and additionally, the ‘renata clade’ is defined based on the presence of cornuti in the aedeagus.


Keywords: integrative taxonomy; Satyrini; butterfly; biodiversity; molecular data; dry forest

Introduction

In recent years, the study of biodiversity has become a recurrent and important theme.[1–5] This is because the accelerated habitat loss caused by human actions[6–9] has resulted in a drastic decrease in species richness, which represents a substantial part of biodiversity.[10,11] This has led to the extinction of many species before they are formally described, directly affecting biodiversity measures[6,12–14] and resulting in the loss of important information for biodiversity conservation and management.[15,16]

Exacerbating the problem of quantifying and reducing the loss of biodiversity is the issue that several undescribed taxa may be hidden within morphologically similar species complexes, as is the case of many butterflies in the subfamily Satyrinae, particularly in the subtribe Euptychina.[17,18] In addition, a number of Euptychina genera are non-monophyletic,[17,19–21] even though their species may be similar in external appearance. Such is the case of *Yphthimoides* Forster, 1964, a relatively speciose Euptychina genus whose species have been recently reorganized into at least three other Euptychina genera, and for which six new species have been described in the last decade.[22–26]

The present paper describes two additional cryptic species of *Yphthimoides*, previously identified as *Y. renata*, based on an integrative taxonomic approach[27–29] using morphological and molecular data.

Material and methods

Morphology

Adult dissections were made using standard techniques. Legs, palpi and abdomens were soaked in hot 10% KOH solution for 10 min before dissection, and dissected parts were stored in glycerol. Photographs of the male genitalia were taken using a Zeiss SteREO Discovery.V20 Stereomicroscope (Zeiss, Germany). Taxonomic nomenclature follows,[22] modified after[30] and[31]. The following abbreviations are used: (FW) forewing, (HW) hindwing, (D) dorsal and (V) ventral.

*Yphthimoides* specimens were examined in 10 public collections. The Lamas collection of Neotropical butterfly type specimen photographs at the MUSM (also available online in[32]) was examined, representing most of the currently relevant names and recognized species of *Yphthimoides* [22], except for *Y. acmenis* (Hübner, 1823), *Y. patricia* (Hayward, 1957), *Y. punctata* (Weymer, 1911) and *Y. renata* (Stoll, 1780). The last four species are all clearly illustrated (except for *Y. patricia*) and characterized in their original descriptions. It is noteworthy, however, that the genitalia of *Y. patricia* (EPB et al. in* prep*) is distinct such that there is no close relation with both new species described herein. Specimens of *Y. renata*, a taxon very similar to both new species described, including all taxa synonymized by Lamas[22], were studied from material obtained at several Brazilian localities in the states of Roraima, Maranhão, Mato Grosso do Sul,
Mato Grosso, Paraná, São Paulo, Minas Gerais, Bahia and Alagoas; as well as from Mexico, Colombia and Costa Rica (Guianaasce).


**Genetic distance and relationship analysis**

Genetic distances and the relationship between *Yphthimoidea* species were estimated to evaluate the validity of new species placement in the genus *Yphthimoidea*. Genomic DNA was extracted from two legs of each individual using the Invitrogen Spin Tissue Mini Kit protocol (Stratagene Molecular, Berlin, Germany) and stored in TE buffer at −20 °C.

The *barcode* region of the mitochondrial gene Cytochrome C Oxidase I (Cox1 = 568 bp) for all of examined specimens was amplified, purified and sequenced using standard techniques [33,34]. Sequences were analyzed with the program FinchTV v. 1.4.0 (Geospiza, PerkinElmer Inc., Waltham, MA), and posteriorly aligned using BioEdit v. 7.2.4 (Tom Hall 2013, available at http://www.mbio.ncsu.edu/bioedit/bioedit.html) with GenBank sequences (Yph_sp, CP08–88; CP12–03; MAL-04257; 07-SRNP-100018).[20] The final matrix comprised 44 sequences from 16 *Yphthimoidea* species (Table 1).

The placement of each species relative to others was obtained by constructing a neighbor-joining (NJ) phenogram (a ‘species identification phenogram’ sensu [35]) using the MEGA 6.0 program.[36] Genetic distances among *Yphthimoidea* species (Table 2) were determined using the program MEGA v. 6.0 [36] under Kimura-2-parameters (K2P) model of nucleotide substitution.[37]

**Taxonomy**

*Yphthimoidea blanquita* Barbosa, Marín and Freitas, sp. nov.

Diagnosis. *Yphthimoidea blanquita* sp. nov. resembles some individuals of *Y. renata* from Brazil, but they are easily distinguished from the latter by the rounder wing (they are more angulate in Brazilian *Y. renata*), by the size of VHW ocelli (smaller in *Y. renata*) and by the lighter ground color of V wings (darker in *Y. renata*). Male genitalia shows no consistent differences between *Y. blanquita* sp. nov. and other closely related *Yphthimoidea* species (namely *Y. renata*, *Y. manasses* (C. Felder & R. Felder, 1867), *Y. ordinaria* Freitas, Kaminski and Mielke, 2012 and *Y. legualaimai* (Dyar, 1913), see below).

**Description**

*Male* (Figure 1(A, B)). FW length: 22.0–23.8 mm (n = 2); HW length: 18.7–19.0 mm (n = 2). Eyes entirely naked and brown. Palpus length approximately 1.5 times the head width, beige, with long dark brown and short white hairs. Antenna 8.5–9.3 mm in length (n = 2) extending to mid-costa, with 39–40 antennomeres; shaft rust-brown; club with 10–11 antennomeres. Legs covered with short white hair and white cream scales. Ground color of D wings dark brown with few markings; a thin dark marginal line and a broader dark submarginal line following contours of wing margins. HW outer margin slightly wavy. DHW with two complete ocelli outlined by a yellow ring, with a middle black disk; first one larger with double white pupils in CuA1–CuA2, second one smaller with single white pupil in CuA2–2A. V wings mostly light brown. VFW with a dark brown zigzag submarginal line, and a brown regular marginal line extending from costa to 2A and crossed by two dark brown lines: first one slightly irregular, one-third from the wing base to the apex, extending from Sc to 2A; second one straight, at two-thirds from the wing base to the apex extending from R3 to 2A; one small complete ocellus in R5–M1. VHW with a thin dark brown zigzag submarginal line, and a brown slightly irregular marginal line following wing contours extending from costa to 2A and crossed by two regular dark brown lines: first one extending from costa to 2A, one-third from the wing base to the apex; second one at two-thirds from the wing base to the apex, extending from costa to anal margin and delimiting a broad light cream region that extends to the wing margin; a series of six complete ocelli outlined by a ring of yellow scales, black middle disk and with double white pupil in Rs–M1 (ocellus 1), M1–M2 (2), M2–M3 (3), M3–CuA1 (4), CuA1–CuA2 (5) and CuA2–2A (6); ocelli 1, 3 and 4 smaller than the others; ocelli 3 and 4 reduced to white pupils surrounded by yellow scales; ocelli 2 and 5 larger than others. No conspicuous androconial scales or patches observed.

*Male genitalia* (Figure 2(A–F)). Saccus short and triangular in D and V views (2B–C); tegumen rounded; gnathos long and pointed and half size of valva; uncus elongated and narrow in D view with a truncated apex;
Table 1. Sequenced specimens of *Yphthimoides* with voucher code, sampling sites data and GenBank accession numbers for *Cox*1 (~658 bp).

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<td>GU658811</td>
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<tr>
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<td>YPH-0508</td>
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valva elongated, trapezoidal and ending in a pointed apex in lateral view (2A); internal margin of valvae (cucullus, sensu [38]) with a series of very small teeth; aedeagus is straight in both D and lateral view (2E-F); comrui present (2D); fultura inferior absent.

**Female** (Figure 1C, D)). FW length: 21–25 mm (n = 5); HW length: 19–23 mm (n = 5). General color and pattern very similar to, but paler than that of males. Palpus length approximately one-third of antenna length.

**Female genitalia.** The few known females of *Y. blanquita* that are in the museum in Colombia are not available to be dissected. However, a forthcoming study about the ‘*Y. renata* species complex’ will further investigate female genitalia (Barbosa et al. in prep).

**Remarks on color variation.** In examined individuals (n = 15), variation on the D wing surfaces is practically absent, and obvious seasonal variation has not been observed. Observation in the V wing color pattern is minimal, and restricted to the size and shape of the ocelli.

**Habitat.** *Yphthimoides blanquita* sp. nov. is associated with dry and tropical forests at altitudes ranging from 200 to 1550 m, where it occurs at forest edges; in secondary disturbed forests and in fruit crop areas.
Table 2. Pairwise genetic distances of the COI gene among 16 *Yphthimoides* species.

<table>
<thead>
<tr>
<th>Species</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tr>
<td>1. <em>Y. borasta</em> (2)</td>
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<tr>
<td>2. <em>Y. angularis</em> (2)</td>
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<tr>
<td>3. <em>Y. cipoensis</em> (2)</td>
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<td>0.067</td>
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<td>4. <em>Y. iserhardi</em> (2)</td>
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<tr>
<td>5. <em>Y. yphthima</em> (2)</td>
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<td>0.065</td>
<td>0.064</td>
<td>0.071</td>
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<tr>
<td>6. <em>Y. straminea</em> (2)</td>
<td>0.067</td>
<td>0.057</td>
<td>0.064</td>
<td>0.066</td>
<td>0.058</td>
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<tr>
<td>7. <em>Y. renata</em> (BR) (7)</td>
<td>0.094</td>
<td>0.074</td>
<td>0.087</td>
<td>0.084</td>
<td>0.091</td>
<td>0.081</td>
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<tr>
<td>8. <em>Y. ochracea</em> (2)</td>
<td>0.073</td>
<td>0.057</td>
<td>0.055</td>
<td>0.056</td>
<td>0.063</td>
<td>0.054</td>
<td>0.084</td>
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<td>9. <em>Y. cenis</em> (2)</td>
<td>0.065</td>
<td>0.052</td>
<td>0.047</td>
<td>0.050</td>
<td>0.047</td>
<td>0.046</td>
<td>0.081</td>
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<td>10. <em>Y. bella</em> (1)</td>
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<td>0.057</td>
<td>0.076</td>
<td>0.070</td>
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<td>11. <em>Y. gabriela</em> (2)</td>
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<td>0.059</td>
<td>0.061</td>
<td>0.069</td>
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<tr>
<td>12. <em>Y. ordinaria</em> (2)</td>
<td>0.094</td>
<td>0.062</td>
<td>0.077</td>
<td>0.073</td>
<td>0.074</td>
<td>0.071</td>
<td>0.056</td>
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<td>0.069</td>
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<td>13. <em>Y. monastes</em> (2)</td>
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<td>0.069</td>
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<td>14. <em>Y. renata</em> (CA) (3)</td>
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<td>0.076</td>
<td>0.093</td>
<td>0.092</td>
<td>0.092</td>
<td>0.084</td>
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<td>0.084</td>
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<td>15. <em>Y. legualiamai</em> (3)</td>
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<td>0.076</td>
<td>0.078</td>
<td>0.080</td>
<td>0.075</td>
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<td>16. <em>Y. blanquita</em> (6)</td>
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<td>0.063</td>
<td>0.070</td>
<td>0.069</td>
<td>0.073</td>
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<td>0.054</td>
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<td>0.063</td>
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<tr>
<td>17. <em>Y. nareta</em> (2)</td>
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<td>0.072</td>
<td>0.080</td>
<td>0.083</td>
<td>0.081</td>
<td>0.074</td>
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<td>0.083</td>
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<td>0.060</td>
<td>0.066</td>
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</table>

Notes: Number in parenthesis is sample size. BR means ‘Brazil’ and CA means ‘Central America and Colombia’.
Distribution. Based on field observations and museum records, the species is present in northern Colombia in Andean foothills and in inter-Andean valleys and canyons (Figure 3), where it is sympatric with *Y. renata* from the Central American clade.

Etymology. The species name is dedicated to Dr Blanca Huertas, a prominent Colombian biologist and curator of the butterfly collection in the British Museum of Natural History (NHMUK), who has made a large contribution to the understanding of Satyridae systematics. ‘Blanquita’ is the diminutive of ‘blanca’, and can be used as an affectionate nickname in Colombia.

Conservation. *Yphthimoides blanquita* sp. nov. is present in several different habitats, including forest edges and secondary environments, suggesting that it can tolerate a degree of disturbance. However, natural habitats where *Y. blanquita* sp. nov occurs, including the dry forests of northwest South America, have experienced a noteworthy decline in recent years and are among the most threatened Neotropical biomes, remaining strongly underrepresented even in protected areas.[39,40] Thus, additional ecological data will be required for an adequate evaluation of the conservation status of this species.

Type material

**Holotype.** Male (Figure 1(A, B)) with the following labels (four labels separated by inverted transverse bars)


**Allotype.** (Figure 1(C, D)) with following labels (four labels separated by transverse bars): \`ALLOTYPUS\` ALLOTYPUS *Yphthimoides blanquita* Barbosa, Marín & Freitas det. 2015 DNA voucher – YPI10438 MEFLG \`Colombia, Antioquia, Santa Fe de Antioquia [Santa Fe de Antioquia], Cotove [Cotové], 6°32’06"N 75°49’52"W [6.5350; -75.8311], 519 msm. Jama 11/03/07 [11.III.2007], 11:00:00 Cultivo de Mango. Colector: M.A. Marín leg. gsm338-752\`Lepidoptera Nymphalidae Satyridae Yphthimoides sp, Identificado: M.A. Marín Fecha: 2009-06-09, Número de catalogo: 15692, 752 – gsm338.

**Paratypes** (all from Colombia). ZUEC – Antioquia: Amalfi, Picardia, 1 male, 12.X.2007, DNA voucher
Figure 2. Male genitalia of *Yphthimoides blanquita*. A = lateral view; B = dorsal view; C = ventral view; D = detail of cornuti in aedeagus (dorsal view); E = aedeagus (lateral view); F = aedeagus (dorsal view). Scale bar = 0.2 mm except in D, as assigned.


*Yphthimoides nareta* Barbosa and Freitas, sp. nov. Diagnosis. *Yphthimoides nareta* sp. nov. (Figure 4(A, B)) and *Y. renata* (Figure 4(C, D)) are very similar and almost indistinguishable based only on wing pattern. Two subtle differences are: (1) the mottled ground pattern on the VHW is yellowish in *Y. nareta* sp. nov. (it is grayish in *Y. renata*), and (2) a short dark line is present along part of the M2-M3 transverse vein in *Y. nareta* sp. nov. (this dark line is longer and more conspicuous, extending from M2–M3 to R sector, in *Y. renata*). As with *Y. blanquita* sp. nov., the male genitalia of *Y. nareta*
Figure 3. Map showing all recorded localities of *Ypthimoides blanquita* (based on all examined material).

Figure 4. Syntopic specimens of *Ypthimoides nareta* (Holotype), A - dorsal, B - ventral; and *Ypthimoides renata* (DNA voucher YPH0510). C - Dorsal, D - Ventral. Both from Alagoas, Brazil.
sp. nov. is apparently similar from that of the other closely related Yphthimoides species. The DNA barcode, however, was very effective to distinguish Y. nareta sp. nov. from Y. renata and from all other species of Yphthimoides.

**Description**

**Male** (Figure 4(A, B)). FW length: 22.0–22.5 mm (n = 2); HW length: 18.7–20.0 mm (n = 2). Eyes entirely naked and brown. Palpus length approximately 1.5 times the head width, beige, with long dark brown and short white hairs. Antenna of males 8.5–9.0 mm in length (n = 2) extending to mid-costae, with 39–40 antennomeres; shaft rast brown; club with 10–11 antennomeres. Legs covered by long dark and white hairs and creamy scales. Ground color of D wings dark brown with few markings; a thin dark marginal line and a broader dark submarginal line following contours of wing margins. HW outer margin slightly wavy. DHW with two complete ocelli outlined by a yellow ring, and black middle disk, first larger and with double with pupils in CuA1–CuA2, second smaller and with single pupil in CuA2–2A. V wings mostly light whitish brown; VFVW crossed by two dark brown lines: first one slightly irregular, one-third from the wing base to the apex and extending from Sc to 2A; second one straight, at two-thirds from the wing base to the apex extending from R3 to 2A; a short dark line is present in the M2–M3 transverse vein; a dark brown zigzag submarginal line and a brown regular marginal line extending from costa to 2A; two small complete ocelli in M1–M3 (ocellus 1) and CuA1–CuA2 (2). VIIVW crossed by two regular dark brown lines: first one extending from costa to 2A, one-third distance from the wing base to the apex; second one at two-thirds from the wing base to the apex, extending from costa to anal margin; a thin dark brown zigzag submarginal line and a brown slightly regular marginal line following wing contours extending from costa to 2A; a series of six complete ocelli outlined by a ring of yellow scales, with a black middle disk and with double white pupils (exception being the ocellus number 6, with a single pupil) can be found in Rs–M1 (ocellus 1), M1–M2 (2), M2–M3 (3), M3–CuA1 (4), CuA1–CuA2 (5) and CuA2–2A (6); ocelli 1, 2, 5, 6 and 6 larger than ocelli 3 and 4; ocelli 3 and 4 reduced to few white scales surrounded by some dark brown scales. No conspicuous androconial scales or patches observed.

**Male genitalia** (Figure 5(A–F)). Saccus short and triangular in D view (5B); tegumen rounded; gnathos long and pointed and half size of valva (5C); uncus elongated and narrow in D view with a slender constriction in the basal portion and a tapered apex; valva elongated, trapezoidal and ends in a pointed apex in lateral view (5A); internal margin of valvae ( cucullus, sensu [38]) with a series of very small teeth; aedeagus straight in both D and lateral view (5E, 5F); cornuti present (5D); fultura inferior absent.

**Female**. No females are known for this species, but based on other related species females should be similar in appearance to males.

**Remarks on color variation.** Based on the two known individuals of Yphthimoides nareta sp. nov., wing color variation cannot be assessed, but ocelli 3 and 4 are strongly reduced in the holotype while are complete in the paratype.

**Habitat.** The two known individuals of Y. nareta sp. nov. were collected at the edge of wet submontane Atlantic Forest in Alagoas, at altitudes ranging from 300 to 400 m.

**Distribution.** This species is known only from the Usina Serra Grande, in the State of Alagoas in northeast Brazil, where it is sympatric with Y. renata from the Brazilian elade.

**Etymology.** The epithet *nareta* is an anagram with the name *renata*, since this new species has a high resemblance in wing color pattern to *Yphthimoides renata* and can be easily misidentified with the latter.

**Conservation.** Yphthimoides nareta sp. nov. is known to be present only within remnants of the submontane Atlantic Forest in the Serra Grande region, Alagoas. Most of this region has been deforested, and the remaining habitats are under high anthropic pressure. However, without additional geographic data, no adequate evaluation of its conservation status can be made.

**Type material**

**Holotype.** Male (Figure 4(A–B)) with the following labels (five labels separated by inverted transverse bars):


**Molecular data**

**Genetic distances** (Table 2): For *Y. blanquita* sp. nov. and *Y. nareta* sp. nov., pairwise intraspecific distances ranged from 0.0 to 0.8%. Interspecific distances ranged from 4.3 to 9.4%, overlapping with intraspecific distances of the range 4.5–8.5% between *Y. renata* specimens (Stoll, 1780) from Brazil, Colombia and Central America. Regarding the specimens identified as *Y. renata*, a possible complex of cryptic species is
involved in that case (EPB and AVL in prep.) as can be seen by their relationships, forming two well distinguished clades (Figure 6).

Neighbor-joining relationships (Figure 6). Based on sequences of the barcode region of the mitochondrial gene Cox1, both Y. blanquita sp. nov. and Y. nareta sp. nov. are monophyletic entities, well distinct from all other Yphthimoidea species. The analysis recovered both species, with high bootstrap support, as belonging to the clade (hereafter ‘Y. renata species group’) that also includes Y. manasses, Y. ordinaria, Y. leguialaimai and Y. renata. The supposedly common and widespread Y. renata appeared divided in two not closely related but well supported clades: (1) the ‘Brazilian clade’, including the specimens from Brazil, and (2) the ‘Central American clade’, including specimens from Mexico, Costa Rica and Colombia. It is worth noting that Y. nareta sp. nov. was quite distant from the syntopic specimen of Y. renata sampled in the present study (YPII-0510 in Figure 6), showing that it is not simply an isolated divergent population of the latter.

Discussion
The recently described taxa of Euptychina can be roughly divided into two main groups: (1) butterflies that are quite distinct and easily recognized as new species or subspecies (e.g. [23,24,41–45]), and (2) cryptic species eventually distinguished by subtle external characters and/or morphology of genitalia (e.g. [20,25,46,47] this paper). The increasing use of molecular data such as the DNA barcodes has increased the identification of those in the second group (cryptic species), helping to uncover a high hidden biodiversity in Euptychina. In the present study, this approach revealed two new species in a complex of at least four species (including the two clades of Y. renata) previously assumed as phenotypic variation of the common and widespread Y. renata.
The ‘Ypthimoides renata species group’ is a well-supported clade by both molecular and morphological characters. All species in this clade have similar wing pattern (as previously discussed) and male genitalia, including the elongated valvae bearing very small teeth at the cucullus and the presence of cornuti in the aedeagus. The presence of cornuti in the aedeagus of all species in the ‘Ypthimoides renata species group’ is exclusive to this clade within the genus Ypthimoides (the reported lack of cornuti in Ypthimoides ordinaria...
by Freitas et al. [48] was a mistake, when in fact this species does have in conspicuous cornuti). Internal relationships within this clade, however, are still not resolved based on barcode data alone, but the presence of cornuti in this clade could be a synapomorphy based on its absence in the sister groups of *Yphthimosoides* (EPB and AVLF, in prep.).

The present work is a good example of how molecular data can help to taxonomic study of insects by revealing new potential undescribed species embedded in the variation of widespread common species.

**Author contributions**

EPB and MAM did the morphological studies; EPB obtained the molecular data and was responsible for manuscript preparation. EPB, MAM and AVLF organized the data and the final version of the manuscript. All authors contributed in the form of discussions/suggestions and approved the final manuscript.

**Acknowledgments**

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**References**


Redescription of *Yphthimoides patricia* (Hayward, 1957), with taxonomic notes on the name *Euptychia saltuensis* Hayward, 1962 and *Yphthimoides manasses* (C. Felder & R. Felder, 1867) (Nymphalidae: Satyrinae)

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**Abstract.** *Euptychia saltuensis* Hayward, 1962, currently regarded as a nomen dubium and possibly a subjective synonym of *Yphthimoides manasses* (C. Felder & R. Felder, 1867), is here treated as a junior subjective synonym of *Yphthimoides patricia* (Hayward, 1957) new synonym, based on morphological characters of the male genitalia and the DNA barcode. The taxonomic status of *Y. patricia* is re-examined, and a detailed redescription of the adult morphology, including the male genitalia, is presented. Information on the distribution, habitat and immature stages of *Y. patricia* is also provided. *Y. patricia* is clearly a distinct species from *Y. manasses* based on the molecular analysis of the animal barcode and the morphology of the male genitalia.

**Key words.** Integrative taxonomy, Euptychiina, Neotropical region, open grassland, species status.

**Introduction**

The butterfly subtribe Euptychiina ranges from Canada to southern South America (in addition to a single genus, *Palaeonympha* Butler, 1871, endemic to the Oriental
region), and is one of the most species-rich butterfly groups among the Nymphalidae (Peña et al. 2010, Marín et al. 2011). Notwithstanding increasingly intensive studies on this clade during the last decade, much work remains to be done, including systematic revisions at all taxonomic levels (Peña et al. 2006, 2010, Marín et al. 2011, Freitas et al. 2012, 2015).

This is certainly the case with Ypthimoides Forster, 1964, a genus erected to include 22 butterfly species that Forster characterized as being “medium-sized, mostly monochrome brown on the upper wing surface, with one or two almost always very small ocelli on the anal angle of the hindwings”. Later, Lamas (2004) reorganized the genus to include 24 species (22 described and 2 undescribed) and since then, five new species have been described (Freitas 2004; Freitas et al. 2012; Barbosa et al. 2015). Although Ypthimoides occurs broadly throughout the Neotropics, its diversity center is in the southeast, especially in southeastern Brazil. As with most other species-rich Euptychiina genera, Ypthimoides is possibly non-monophyletic with several undescribed species, including some that are cryptic. As a consequence, its current taxonomic composition should be carefully re-evaluated (Barbosa & Freitas in prep.).

The main objective of the present paper is to provide a detailed redescriptions of Ypthimoides patricia (Hayward, 1957), using the integrative taxonomic approach (e.g., Dayrat 2005; Yeates et al. 2011; Pante et al. 2015). Y. patricia is a widespread and common species that shows confusing phenotypic variation and can be very similar to other sympatric euptychiines.

Material and methods

Study sites and rearing. Ypthimoides patricia was studied in the field in more than 15 Brazilian localities in the Federal Units of São Paulo, Minas Gerais, Mato Grosso, Goiás and Distrito Federal. Fertile eggs were obtained from wild-captured females, which were confined in plastic bags along with leaves of several potential host-plants (Poaceae species) and put under a heat source (40W incandescent lamp). As for many Euptychiina, the intense heat triggers the behavior of laying eggs in most females. Larvae were reared in plastic containers cleaned daily, with fresh plant material provided every two or three days (following Freitas 2007), recording data on behavior and development times for all stages. Dry head capsules and pupal exuviae were preserved and stored in glass vials, and immature stages were fixed in Kahle-Dietrich solution (Triplehorn & Johnson 2005). All adults, preserved larvae, head capsules and
pupal cases have been deposited at the Museu de Zoologia “Adão José Cardoso” (ZUEC), Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

**Morphology.** Adult dissections were made using standard techniques. Legs, palpi and abdomens were soaked in hot 10% KOH solution for 10 min before dissection, and dissected parts were stored in glycerol. To assess the venation, wings were soaked in alcohol and NaClO solution (bleach) to dissolve the scales. Drawings and measurements of wings, legs and palpi were made using a Leica® MZ7.5 stereomicroscope equipped with a micrometric scale. Photographs of the male genitalia were taken using a Zeiss SteREO Discovery.V20 Stereomicroscope (Zeiss, Germany). Scanning electron microscopy (SEM) was conducted using a JEOL® JSM-5800 microscope (JEOL Ltd., Japan), and samples were prepared by critical point drying in a Bal-tec® – CPD030 (Leica Microsystems, Germany), attached with double-sided tape to aluminum stubs coated with gold/palladium with a Bal-tec® – SCD050 sputter coater (Leica Microsystems, Germany). Morphological nomenclature for genitalia largely followed Klots (1956). Terminology for the early stages followed Garcia-Barros & Martín (1995) for eggs and Stehr (1987) for larvae and pupae. The following abbreviations are used: FW – forewing; HW – hindwing; D – dorsal; V – ventral.

*Ypthimoides* specimens were examined in nine public and private collections in South America and Europe (see below). The Lamas collection of Neotropical butterfly type specimen photographs at the MUSM (also available online in Warren *et al.* 2013), representing most currently relevant names and recognized species of *Ypthimoides* (Lamas 2004), was examined. Nomenclature followed Lamas (2004), modified after Peña *et al.* (2006) and Wahlberg *et al.* (2009).

Acronyms for examined collections are: **DZUP** – Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil; **DZUP-OM** Olaf H. H. Mielke collection, Curitiba, Paraná, Brazil; **IML** – Instituto Miguel Lillo, Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina; **MACN** – Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina; **MUSM** – Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru; **NHMUK** – Natural History Museum, London, United Kingdom; **UFMG** – Universidade Federal de Minas Gerais taxonomic collection, Belo Horizonte, Minas Gerais, Brazil; **ZUEC** – Museu de Zoologia da Universidade Estadual de Campinas, Campinas, São Paulo, Brazil; **ZUEC-AVLF** - André V. L. Freitas Collection,
Universidade Estadual de Campinas, Campinas, São Paulo, Brazil. Examined specimens are listed in Table 1.

**Genetic distances.** Genetic distances within and between 14 *Ypthimoides* species were estimated to evaluate the genetic similarity of these taxa. Genomic DNA was extracted and the mitochondrial gene Cytochrome c Oxidase I (COI, ca. 658 bp, corresponding to the 'DNA barcode' – see Herbert *et al.* 2003) was amplified, purified and sequenced using standard techniques (see Silva-Brandão *et al.* 2005; Wahlberg & Wheat 2008). Sequences were examined with the program FinchTV v. 1.4.0 (Geospiza Inc.) and aligned by eye with sequences obtained previously and available on GenBank using BioEdit v. 7.2.4 (T. Hall 2013, available at http://www.mbio.ncsu.edu/bioedit/bioedit.html#downloads). The final matrix comprised 35 sequences of 14 *Ypthimoides* species (Table 1). Potential relationships were inferred by constructing a Neighbor Joining (NJ) phenogram (a ‘species identification phenogram’ sensu Janzen *et al.* 2005) using MEGA 6.0 (Tamura *et al.* 2013). Pairwise genetic distances between individuals were calculated using MEGA v. 6.0 (Tamura *et al.* 2013), with the Kimura two Parameter (K2P) model of nucleotide substitution (Kimura 1980). The frequency distribution of genetic divergence was plotted using pairwise values.

**Ypthimoides patricia** (Hayward, 1957)

*Euptychia patricia* Hayward, 1957: 115, 116, 119, fig. 6 (male genitalia). Holotype male, Chulumani, Sur Yungas, Bolivia, supposedly deposited in MACN (male genitalia preparation code MSS 11). Both the holotype and the male genitalia slide were not found in that collection, and appear to be lost.


*Ypthimoides manasses*: Freitas, 2004: 10, fig. 3 (male ventral); Silva *et al.*, 2012: 294, 295, tab. 1; Silva *et al.*, 2015: 6, tab. 1.

*Ypthimoides* sp. 1: Freire-Júnior & Diniz, 2015: 1209, 1210, tab. 3.

**Diagnosis.** *Ypthimoides patricia* (Fig. 1) differs from *Y. manasses* in the following characters: wings usually more rounded in *Y. manasses* than in *Y. patricia*; oceli in DHW cells CuA₁-CuA₂ and CuA₂-2A with single pupils in *Y. patricia*, but usually double (when present) in *Y. manasses*. Males of *Y. patricia* have a patch of dark
androconial scales on the middle of the DFW that is absent in _Y. manasses_. Valvae of the male genitalia (Figs. 2C, G) with two series of small ‘teeth’ on the posterior inside edge of the valva in _Y. patricia_, absent in _Y. manasses_, and in fact in all other _Yphthimoides_ species.

**Redescription of adults. Male (Figs. 1-6).** FW length: 16-20 mm (average 17.6 mm, SD = 1.17, n = 11); HW length: 12-15 mm (average 13.1 mm, SD = 0.88, n = 11). Eyes glabrous, entirely yellowish ochre (Fig. 3A). Male palpus (Fig. 3A, Fig. 4D) approximately twice the head height, covered with cream scales and black and cream hairs. Antenna of males 9 – 10 mm in length, with 43 – 45 antennomeres extending to mid-costa of FW; shaft rust-brown, with white scales at base of each antennomere (Fig. 3B), club not conspicuously developed, with 13 – 14 antennomeres, yellowish brown, with the four distal-most antennomeres darker (Fig. 3C). D ground color brown with a few markings; marginal and submarginal lines light brown and wavy, especially conspicuous in the IFW. DFW with a broad dark androconial area extending from the inner margin to Radial vein (Fig. 1A). This dark area is not easily viewed unless with direct light, especially in darker wet season forms. VFW crossed by two lines, submedian faint orange and wavier, across discal cell, extending from Sc to 2A, at one-third from the wing base; and postdiscal dark orange and straight extending from costa to 2A, at two-thirds from the wing base. VFW also with a light cream, broad postdiscal stripe extending from costa to 2A distad of dark orange postdiscal line. Two dark brown ocelli on DHW, outlined by a yellow ring and with silver pupil: one larger, between CuA$_1$ and CuA$_2$ and a smaller one between CuA$_2$ and 2A. VHW ground color light brown with several markings: in both wings marginal line straight and submarginal line wavy; VHW crossed by two orange lines extending from costa to anal margin, namely a submedian faint and slightly wavy line, at one-third from the wing base; and a postdiscal dark and straight line, at two-thirds from the wing base. A light cream postdiscal stripe, broader than that on the VFW, extending from costa to anal margin distad of dark orange postdiscal line. In wet season individuals, a series of six dark ocelli with white pupil and outlined by a ring of yellowish-creamy scales is present in VHW in wing cells Rs-M$_1$ (ocellus 1), M$_1$-M$_2$ (2), M$_2$-M$_3$ (3), M$_3$-CuA$_1$ (4), CuA$_1$-CuA$_2$ (5) and CuA$_2$-2A (6). Ocelli 2 and 5 larger than others. In some individuals ocelli 3 to 6 have double pupils. HW outer margin slightly wavy. Male wing venation shown in Fig. 4A. Legs covered by cream scales (Fig. 3D); male foreleg shown in Fig. 4C.
**Male genitalia (Figs. 2, 5).** Saccus short and triangular in dorsal view (Figs. 2A-B); tegumen rounded (Fig. 2B); gnathos long and pointed, almost half size of the valva length (Fig. 2B); uncus elongated with apex truncated distally (Figs. 2B-C; 5A); valvae elongated, trapezoidal, ending in a tapered apex in lateral view (Fig. 2A), internal margin with two series of small 'teeth', one series in apex and other series more internal (Figs. 2B-C; 5B); aedeagus straight (Fig. 2D); cornuti absent; fultura inferior membranous.

**Female (Figs. 1, 6G-L).** FW length: 19-22 mm (average 20.5 mm, SD = 1.22, n = 6); hind wing length: 16-20 mm (average 17.5 mm, SD = 1.52, n = 6). Eyes glabrous, entirely brown. Palpus length approximately twice the head height, beige with long brown hairs. General color and pattern very similar to, but in general paler than, that of males. Female wing venation shown in Fig. 4B. Female foreleg with five tarsomeres, as shown in Fig. 4E.

**Remarks on color variation (Figures 1, 6).** Two seasonal forms are known, affecting both sexes, with differences observed only on the underside. The wet season form is darker with larger and has more developed oceli, especially on the VHW; this form was reported as being dominant from November to April in Central Brazil (Figs. 1 E, 6 A, B, C, G, H). The dry season form is lighter, often yellowish, with reduced oceli on both wings, occasionally presenting a whitish postdiscal stripe distal of the postdiscal line crossing the wings ventrally; this form was reported as being dominant from May to September (Figs 1 A-D, F, 6 D, E, F, K, L). Intermediates (Figs. 6 I, J) were observed in transitions between seasons, from February to April and in October-November (AVLF pers. obs.).

**Description of immature stages.** The morphological description and measurements of the immature stages below are based on material reared from January to April 1999, obtained from three wild caught females from Itirapina, São Paulo, southeastern Brazil.

**Egg.** Barrel shaped, flattened at base, pinkish cream, with a reticle of 19-20 thin longitudinal ridges forming a pattern of irregular pentagonal and hexagonal cells visible under light microscope. Height and diameter 1.0 mm (n = 5). Duration 6 days (n = 15).
**First instar.** Head capsule width 0.62 – 0.64 mm; head scoli 0.08 – 0.10 mm (n = 5). Head capsule black, with enlarged chalazae, bearing a pair of short scoli on vertex, each with two long narrow setae, P1 and P2 respectively. Third stemma larger than other stemmata. Body cream, smooth, with red longitudinal stripes; caudal filaments very short. Setae dark, elongated, several dorsal and subdorsal clubbed at tip. Maximum length 6 mm. Duration 9–10 days (n = 15).

**Second instar** (Fig. 7A). Head capsule width 0.90 mm; head scoli 0.16–0.18 mm (n = 5). Head dark brown with two diverging short scoli on vertex. Body beige, with a broad dorsal dark stripe and several lateral longitudinal weakly marked stripes; caudal filaments short. Maximum length 9 mm. Duration 7–8 days (n = 10).

**Third instar** (Fig. 7B). Head capsule width 1.22–1.30 mm; head scoli 0.24–0.26 mm (n = 5). Head brown, with two diverging very short scoli on vertex. Body brown with a broad dorsal longitudinal stripe and several lateral longitudinal weakly marked intersecting brown stripes; caudal filaments short. Maximum length 13 mm. Duration 6–8 days (n = 5).

**Fourth instar** (Fig. 7C). Head capsule width 1.80–1.98 mm; head scoli 0.28–0.36 mm (n = 10). Very similar to third instar. Maximum length 21 mm. Duration 9–11 days (n = 5).

**Fifth (last) instar** (Fig. 7C,D). Head capsule width 3.08–3.23 mm; head scoli 0.55–0.58 mm (n = 4). Head brown, with two diverging short scoli on vertex. Body brown with a broad dark dorsal stripe and several lateral longitudinal weakly marked intersecting brown stripes; ventral region dark brown; legs and prolegs light brown; caudal filaments short. Maximum length 25 mm. Duration 17–19 days (n = 10).

**Pupa** (Fig. 7 E,F,G). Short and smooth; mostly dark brown with a mottled pattern of cream dots; short pointed ocular caps with white ridges; cremaster dark in ventral portion; dorsal abdomen with a pair of short bumps laterally in each segment. Total length 12 mm (n = 5). Duration 13-15 days (n = 6).

**Behavior and Natural History.** Oviposition behavior was not observed in the field and the natural host plant is unknown. In captivity, eggs were laid on provided host plant leaves and/or scattered on the inside of the rearing container (plastic bag). In the laboratory, larvae quickly accepted three common grasses: the African Guinea grass *Panicum maximum* Jaq. (“capim colonião”), the Molasses grass *Melinis minutiflora* P. Beauv. (“capim gordura”) and the Indian goosegrass *Eleusine indica* (L.) Gaertn
(“capim pé de galinha”). These three species are introduced in Brazil and no native grass species used by *Y. patricia* are known. Adults fly near the ground from early morning to afternoon. Both sexes were observed feeding on decaying fruits lying on the ground.

**Distribution and habitat.** Based on field observations, museum records and literature, the species is distributed in the Brazilian states of Pará, Maranhão, Bahia, Mato Grosso, Mato Grosso do Sul, Goiás, Minas Gerais, São Paulo and in the Distrito Federal; and in the Bolivian departments of La Paz and Santa Cruz (Fig. 8). This species is strongly associated with open habitats, including the Brazilian savannas (the cerrado biome, Figs. 9A, B), where it occurs in several environments, including the narrow gallery forests adjacent to the cerrado (Silva et al. 2015). It can be locally abundant, and is the dominant satyrine species in some cerrado sites (Silva et al. 2015, Freire-Júnior & Diniz, 2015, AVLF pers. obs.). It was also recorded in the rocky montane fields of Minas Gerais (known locally as “campos rupestres”) and in the Amazonian “canga” vegetation (iron-rich rock outcrops with associated vegetation that grows on thin soil) in the region of Carajás (south Pará State). The species has never been recorded in the Brazilian semiarid “caatingas”. In Bolivia, *Y. patricia* has been recorded in three localities in the region of Yungas near La Paz and in the region of Santa Cruz (Fig. 8).

**Genetic distances (Fig. 10).** Distances within species ranged from 0 % to 3.1 %, and interspecific distances ranged from 4.2 % to 11.0 % (mean genetic distances among 14 *Yphthimoides* species in Table 2), overlapping with intraspecific distances of 5.3 % and 6.9 %, respectively, between specimens of the *Y. renata* "complex" from Brazil and Colombia, and from Brazil and Costa Rica. Genetic distances among individuals of *Y. patricia* and *Y. manasses* ranged from 8.8 to 9.7 %. In conjunction with the morphological differences, these results and a species phenogram support *Y. patricia* and *Y. manasses* as distinct species (Fig. 11).

**Taxonomic comments.** Hayward (1957) described ten new taxa of Bolivian Satyrinae, assigning all of them to the genus *Euptychia* Hübner, 1818. The holotypes of six of those taxa (*E. susanna, E. pamela, E. patricia, E. phares boliviana, E. proxima* and *E. stella*) were stated to be deposited in the collection of the Museo del Instituto de Historia Natural "Sánchez Labrador" of San Miguel, Buenos Aires, Argentina. Decades
later, part of the Lepidoptera collection of the Instituto was transferred to the Museo Argentino de Ciencias Naturales (MACN), Buenos Aires, Argentina (A. Roig, pers. comm. to GL). GL examined that collection in 2005 and found the holotypes of *E. susanna* and *E. proxima*. For *E. patricia* and *E. phares boliviana*, only the labels were found. No trace could be discovered regarding the holotypes of *E. pamela* and *E. stella*. Presumably, the holotypes of these last four names are lost. Hayward described both *E. patricia* and *E. saltuensis* (Hayward 1962) based mostly on the wing color pattern, although drawings of male genitalia were also provided for both taxa. Despite the genitalia drawings being somewhat rough, they are detailed enough as to show the two series of teeth that make the valvae of *Y. patricia* so distinctive from all other species of Euptychiina, and very similar to those illustrated in the original description of *Euptychia saltuensis* (Hayward 1962). It is quite possible that variation in wing pattern explains why Hayward described two names for the same species. The holotype of *E. saltuensis* is very similar to wet season forms of *Y. patricia* (Fig. 1E), while the holotype of *Y. patricia* is more similar to dry season forms, as are the three individuals from Yungas, Bolivia deposited in the NHMUK (one of these, a female, is shown in Fig. 1 F). Lamas (2004) listed both *Y. patricia* and *E. saltuensis* in *Ypthimoides*, with *E. saltuensis* as a nomen dubium and possible subjective synonym of *Y. manasses*. By comparing Hayward's male genitalia drawings of *Y. patricia* and *E. saltuensis* with dissections of several *Ypthimoides* species, in conjunction with DNA barcode analysis to determine the range of intraspecific wing pattern variation within species, we treat *E. saltuensis* as a junior synonym of *Y. patricia* (new synonym).

**Remarks.** After GL examined the specimens in the collection of the MACN (Buenos Aires, Argentina) and found only the labels of *Euptychia patricia*, we concluded that the holotype is lost. Therefore, a neotype of *Euptychia patricia* is hereby designated to clarify the taxonomic status of the species and for nomenclatural stability.

**Neotype, male.**

**Examined material.**


Discussion

Despite ongoing efforts to increase the knowledge of the subtribe Euptychiina (see the introductory section), missing type specimens, including those of some common species, continue to create additional complications in the study of this group. This is the case with Ypthimoides patricia, addressed in the present study. Here, an integrative approach was used to tackle this difficult group within Ypthimoides: DNA barcodes helped to define species boundaries and pinpoint adult morphological characters (male genitalia in this case) that helped resolve the taxonomy of the species by allowing us to unequivocally identify missing or poorly preserved type specimens.

In the Euptychiina in particular, many genera are quite homogeneous in phenotype, and include species similar to those of other distantly related genera; in addition, some species can display seasonal forms (dry and wet season), and the unwary ecologist may easily assign incorrectly the same individual taxon to different species, or lump several separate species in a single one. In these cases, an integrative approach is particularly helpful to unravel taxonomic species complexes (Seraphim et al. 2014; Barbosa et al. 2015; Freitas et al. 2015). In addition, information on immature stages, habitat and behavior can add important information to taxonomic studies, highlighting
the importance of continuous field work for understanding the huge diversity of Neotropical butterflies.

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References


Figure legends

Figure 1. Ypthimoides patricia. A-B Male (dorsal, ventral), Brasília, DF, 20.VI.1972 (DZ 28.804); C-D, female (dorsal, ventral), Nova Xavantina, MT, 17-19.VIII.1997 (DZ 28.805). E – Male holotype of Euptychia saltuensis (dorsal left, ventral right); no locality data (IML). F. Female specimen of Y. patricia from Chairo, Bolivia, 11.VIII.1985 - BMNH(E) 1204804 (NHMUK). Scale bar = 1 cm. E. Photo by L. A. Kaminski.

Figure 2. Male genitalia of Ypthimoides patricia (A-D) and Ypthimoides manasses (E-H). Y. patricia: A – Lateral view; B – Dorsal view; C – Ventral view (Detail of valvae apex and uncus); D - Aedeagus (lateral view). Y. manasses: E – Lateral view; F – Dorsal view; G – Ventral view (Detail of valvae apex and uncus); H - Aedeagus (lateral view). Abbreviations: ae, aedeagus; gn, gnathos; sa, saccus; te, tegumen; va, valva; un, uncus.

Figure 3. Ypthimoides patricia: A. Eye and palpus details; B. detail of antennal shaft; C. Antennal club; D. Midleg. All from a male individual.

Figure 4. Ypthimoides patricia. A, male wing venation; B, female wing venation; C, male foreleg; D, male palpus; E, female foreleg.

Figure 5. Details of the male genitalia of Ypthimoides patricia in scanning electron microscopy. A. ventral-anterior view of uncus apex; B. detail of valva tip in ventral view showing the distinctive ‘teeth’.


Figure 7. Ypthimoides patricia. A. Egg, lateral; B. Egg, dorsal; C. First instar (fixed larva), lateral; D. second instar, dorsal; E. Third instar, dorsal; F. Fourth (paler) and Fifth (darker) instars, dorsal; G. Two fifth (last) instar larvae, dorsal; H, I. Pupae (two different individuals), lateral; J. Pupa, ventral.

Figure 8. Map showing localities for Ypthimoides patricia.
**Figure 9.** Habitat and live adults of *Yphthimoides patricia.* A. General view of the habitat in Brasília, Distrito Federal; B. Close view of the cerrado in Itapira, São Paulo; C, D Male and female, respectively, from Brasília, Distrito Federal.

**Figure 10.** Frequency distribution of pairwise individual genetic distances within (grey) and between (black) fourteen species of *Yphthimoides.*

**Figure 11.** Relationships among fourteen species of *Yphthimoides* based on DNA barcode sequences (ca. 658bp of *cox1*) obtained by a Neighbour-Joining analysis. Values above branches are bootstrap support.
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Table 2. Genetic distances among fourteen *Yphthimiodes* species.

| Species         | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  |
|-----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Y. borasii      |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Y. epivensis    | 0.055 |     |     |     |     |     |     |     |     |     |     |     |     |
| Y. iserhardi    | 0.054 | 0.037 |     |     |     |     |     |     |     |     |     |     |     |
| Y. yphthioida   | 0.075 | 0.064 | 0.067 |     |     |     |     |     |     |     |     |     |     |
| Y. colinii      | 0.062 | 0.048 | 0.044 | 0.048 |     |     |     |     |     |     |     |     |     |
| Y. angulicrus   | 0.072 | 0.067 | 0.064 | 0.069 | 0.054 |     |     |     |     |     |     |     |     |
| Y. straminea    | 0.068 | 0.064 | 0.063 | 0.057 | 0.044 | 0.058 |     |     |     |     |     |     |     |
| Y. marasser     | 0.089 | 0.083 | 0.076 | 0.090 | 0.070 | 0.070 | 0.070 | 0.083 |     |     |     |     |     |
| Y. renata       | 0.090 | 0.090 | 0.082 | 0.091 | 0.074 | 0.072 | 0.081 | 0.059 |     |     |     |     |     |
| Y. ordinaria    | 0.098 | 0.078 | 0.073 | 0.079 | 0.073 | 0.064 | 0.075 | 0.048 | 0.064 |     |     |     |     |
| Y. trilobata     | 0.090 | 0.077 | 0.075 | 0.081 | 0.068 | 0.068 | 0.080 | 0.053 | 0.053 | 0.056 |     |     |     |
| Y. ochracea     | 0.071 | 0.050 | 0.053 | 0.063 | 0.049 | 0.058 | 0.054 | 0.084 | 0.083 | 0.076 | 0.076 |     |     |
| Y. pecta        | 0.092 | 0.078 | 0.085 | 0.087 | 0.077 | 0.080 | 0.077 | 0.091 | 0.093 | 0.082 | 0.093 | 0.075 |     |
| Y. patricia     | 0.081 | 0.071 | 0.076 | 0.068 | 0.064 | 0.073 | 0.073 | 0.091 | 0.094 | 0.090 | 0.076 | 0.066 | 0.076 |
Barbosa et al. Figura 1
Barbosa et al. Figure 2
Barbosa et al. Figure 3
Barbosa et al. Figure 4
Barbosa et al. Figure 5
Barbosa et al. Figura 7
Barbosa et al. Figura 8
Barbosa et al. Figura 9
Barbosa et al. Figura 10
Barbosa et al. Figura 11
Capítulo 4: Descrições taxonômicas de novas espécies do gênero *Moneuptychia* Forster, 1964

Este capítulo é composto por um único artigo contendo a descrição das quatro novas espécies de *Moneuptychia* descobertas, cujo título é “*Four new species of Moneuptychia (Lepidoptera: Satyrinae: Euptychiina) from Brazil*”, e que foi publicado no periódico “*Zootaxa*” 3981(4): 521–541.

Dados moleculares, usando a primeira parte (*Barcode*) do gene mitocondrial COI, conjuntamente com dados morfológicos, principalmente caracteres da genitália masculina e de imaturos, mostraram a existência de quatro novas espécies de *Moneuptychia*.

Four new species of *Moneuptychia* (Lepidoptera: Satyrinae: Euptuchiina) from Brazil

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Abstract

This paper describes four new species of *Moneuptychia* as follows: *M. montana* Freitas, *M. vitellina* Freitas & Barbosa, *M. pervagata* Freitas, Siewert & Mielke and *M. wahlbergi* Freitas, Barbosa, Siewert & Mielke from south and southeastern Brazil. Details are presented on the morphology of adults of all species, and immature stages for two species, and we discuss the taxonomy and identification of the genus *Moneuptychia*. The mitochondrial *CoxI* “barcode” region was used for exploring the utility of this DNA marker to identify these species, giving strong support for all new species.

Key words: Atlantic Forest, Euptychoides castrensis, New Species, *Pharneuptychia*

Introduction

The subtribe Euptuchiina, a group with over 400 described species, is one of the most diverse groups of Satyrinae (Lepidoptera: Nymphalidae) (Lamas 2004; Peña et al. 2010). Euptuchiina are more speciose in lowland and premontane habitats, a pattern contrasting with the subtribe Pronophilina, another highly diverse satyrine clade whose diversity peaks in montane habitats (Adams 1986; Pyrcz 2009). However, in contrasting to the above generalities, the euptuchiine genus *Moneuptychia* Forster, 1964, is apparently more diverse in montane habitats (although not as rich as many Andean pronophilines), with few species reaching the lowlands.

The genus *Moneuptychia* was erected by Forster (1964) to include the single species *Euptychia soter* Butler, 1877, based on the absence of the gnathos (as subunci) in the male genitalia (Forster 1964: 92). Lamas (2004) listed five species in *Moneuptychia*, including three species previously assigned to the genus *Carminia* Ebert & Dias, 1998. Subsequently, Freitas (2007) and Freitas et al. (2010) showed that *Moneuptychia* presents at least one conspicuous synapomorphy: the well-developed appendices angulares that project posteriorly in the male genitalia, a character absent in the species of *Carminia*. Accordingly, excluding the species now placed in the genus *Carminia* Ebert & Dias 1998 (Ebert & Dias 1998; Dias 2011; Freitas 2007), the genus *Moneuptychia* was represented by only two species in the checklist of Lamas (2004): *Moneuptychia soter* (Butler, 1877) and *Moneuptychia melchiades* (Butler, 1877). Since then, two new species have been described (Freitas 2007; Freitas et al. 2010), and another six additional undescribed species at least have been identified as belonging to this genus.

The present paper describes four new species of *Moneuptychia* from southern Brazil, doubling the number of species in the genus from four to eight species.
Material and methods

Rearing methods. Fertile eggs were obtained by expressing the abdomen of fertile females, or by confining wild-captured females in plastic bags warmed by a 40W bulb, with leaves of several grasses potential host plant species. Larvae were reared in plastic containers cleaned daily, with fresh plant material provided every two or three days (following Freitas 2007). Data were recorded on behavior and development times for all stages, and dry head capsules and pupal castings were kept in glass vials. When there was sufficient material, immatures were fixed in Kahle solution. All samples from immatures (preserved eggs and larvae, head capsules and pupal castings) are deposited at the Museu de História Natural (Unicamp—ZUEC). All measurements were made using a stereomicroscope fitted with a calibrated micrometric ocular. Egg size is presented as height and diameter, and head capsule size is the distance between the most external ocelli (as in Freitas 2007).

Morphology. Adult dissections were made using standard techniques. Legs, palpi and abdomens were soaked in hot 10% KOH solution for 10 minutes before dissection, and dissected parts were stored in glycerol. Taxonomic nomenclature followed Lamas (2004), modified after Peña et al. (2006) and Wahlberg et al. (2009). Morphological terms for genitalia largely follow Klots (1956). Terminology for the early stages followed García-Barros & Martín (1995) for eggs and Stehr (1987) for larvae and pupae.

Specimens of *Moneuptychia* were examined in 10 public and private collections (see below). The Lamas collection of Neotropical butterfly type specimen photographs at the MUSM (also available online in Warren et al. 2013), representing most currently relevant names and recognized species of *Moneuptychia* and *Pharneuptychia* (Lamas, 2004) was examined.

The acronyms for the collections visited are: NHMUK—The Natural History Museum, London, England; DD—Coleção Diego Dolbaina, Curitiba, Paraná, Brazil; DZUP—Coleção Entomológica Padre Jesus Santiago Moure, Departamento de Zoológia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil; DZUP-OM—Coleção Olaf H. H. Mielke, Curitiba, Paraná, Brazil; IOC—Instituto Oswaldo Cruz, Rio de Janeiro, Rio de Janeiro, Brazil; MZUJ—Muzeum Zoologiczne Uniwersytetu Jagiellońskiego, Krakow, Poland; MZSP—Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil; USNM—National Museum of Natural History, Smithsonian Institution, Washington, DC, USA; ZUEC—Museu de Zoológia da Universidade Estadual de Campinas, Unicamp, Campinas, São Paulo, Brazil; ZUEC-AVLF—André V. L. Freitas Collection, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

Genetic divergence and phylogenetic inference. Genetic distance within and between species of *Moneuptychia*, were estimated to evaluate the molecular variability for those taxa. Genomic DNA was extracted from two legs of adults of *Moneuptychia* by using the DNeasy Blood & Tissue Kit protocol (QIAGEN, Düsseldorf, Germany). DNA was stored in TE buffer at −20° C. The mitochondrial gene cytochrome c oxidase I (*Coxl*, ca. 658 bp, corresponding to the ‘barcode’ region) was amplified by using the primer combination LCO + HCO (Folmer et al. 1994). Reactions were done in a 25 μL final volume using 1 μL of total DNA, 2.0 mM of MgCl2, 40 μM of dNTPs, 0.2 μM of each primer, 1U of GoTaq DNA Polymerase (Promega, Madison, Wisconsin, USA), and 10% of 10X Taq buffer. The amplification program included an initial denaturation step at 95°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 42°C for 30 s, and polymerization at 72°C for 1.5 min, followed by an extension step at 72°C for 10 min (Silva-Brandão et al. 2005). PCR products were purified of primers and deoxynucleotides with ExoSAP-IT (GE Healthcare, Buckinghamshire England), and then sequenced by ABI Prism BigDye Kit protocol in an ABI 3700 automated sequencer, with primers used for amplification.

Sequences were analyzed with the program FinchTV v. 1.4.0 (Geospiza Inc.).

Sequences were aligned by eye with sequences obtained previously and available on GenBank by using BioEdit v. 7.2.4 (Hall 2013, available at http://www.mbio.ncsu.edu/bioedit/bioedit.html#downloads). The final matrix comprised 43 sequences, including seven species of *Moneuptychia* (all four new species of *Moneuptychia* and three out of the four already described species, Table 1) and two species used as outgroups, namely *Euptychia mollina* (Hübner, [1813]) and *Iphthimoides borasta* (Schaus, 1902).

The phylogenetic relationships of the new species were estimated by using the Maximum Likelihood method. The program MEGA v. 6.0 (Tamura et al. 2013) was used to determine the available substitution model which best fitted the *Coxl* sequences and to run the analysis. The best fit model suggested was HKY + G (νG parameter = 0.18) (Hasegawa et al. 1985), and the analysis was carried out under this model. Branch support was estimated by using the non-parametric bootstrapping procedure, with 500 replicates (Felsenstein 1985).
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Pairwise genetic distances between individuals were calculated using MEGA v. 6.0 (Tamura et al. 2013), under Kimura two Parameter (K2P) model of nucleotide substitution (Kimura 1980), and the frequency distribution of genetic divergence was plotted using pairwise values.

**Moneuptychia montana Freitas, new species**
(Figs. 1, 2D, 3A,B, 4, 5)

**Adult: Diagnosis.** *Moneuptychia montana* n. sp. is distinguished from other *Moneuptychia* species by the distinctive ventral hind wing pattern, presenting a conspicuous post-discal white band in both sexes (absent in all other species of *Moneuptychia*) (Fig. 1, 2D).

![Image](image_url)

**FIGURE 1.** Holotype male (left) and allotype female (right) of *Moneuptychia montana* n. sp. Dorsal above, ventral below. Scale bar = 1 cm.

**Descriptions of adults: Male** (Figs. 1, 2D). Forewing length 16–18 mm (n = 10); hind wing length 13–14 mm (n = 10). Eyes naked, entirely brown. Palpus length 2.0 times head height, beige, with long dark brown and white hairs. Antenna of males 8 mm in length, with 32 antennomeres extending to mid-costa; shaft rust brown, dorsally covered by dark brown scales, club with 9 antennomeres, not conspicuously developed. Male foreleg covered by long beige hairs. Wings with dorsal ground color brown with few markings, restricted to marginal and submarginal lines in both wings; forewing with one small black ocellus in M₁–M₃; hind wing with two large black ocelli in M₁–M₃ and Cu₁₋₃, first completely black, second black outlined by an orange ring and with double white pupil. Forewing of males with a conspicuous callus on SC vein, close to distal end of swollen portion of vein (Figs. 3A–B). Ventral wings mostly brown; forewing crossed by one dark brown regular line extending from costa to Cu₁ at two-thirds from wing base to apex, delimiting a lighter distal area; a dark brown slightly scalloped submarginal line and a brown regular marginal line extending from costa to 2A; one to three small black ocelli outlined by an orange
ring with white pupil in R₅-M₁ (ocellus 1), M₁-M₂ (2) and M₃-CuA₁ (3). Hind wing crossed by two dark brown irregular lines from costa to anal margin, first thin and one-third distance from wing base, second broader and two-thirds distance from base and bordered distally by a broad whitish stripe; a dark brown zigzag submarginal line and a brown marginal line extending from costa to 2A; a series of six black ocelli outlined by an orange ring with white pupil can be found in Rs-M₁ (ocellus 1), M₁-M₂ (2), M₂-M₃ (3), M₃-CuA₁ (4), CuA₁-CuA₂ (5) and CuA₂-2A (6); ocelli 1, 3, 4 and 6 usually small and reduced to few white scales circled by few black scales; ocelli 2 and 5 larger than others, with double white pupil. Hind wing outer margin slightly wavy. No conspicuous androconial scales or patches observed.

**FIGURE 2.** Habitat and live adults of *Moneuptychia* spp. A. Open highland grassland (“campo de altitude”), Pico do Itapeva, Pindamonhangaba, São Paulo (habitat of *M. montana* n. sp., *M. pervagata* n. sp. and *M. itapeva*); B. Contact between grassland and altitude forest, Alto do Capivari, Campos do Jordão, São Paulo (habitat of *M. montana* n. sp., *M. itapeva*, *M. pervagata* n. sp. and *M. soter*); C. Trail inside altitude forest, Alto da Boa Vista, Campos do Jordão, São Paulo (habitat of *M. vitellina* n. sp. and *M. soter*); D. Male of *M. montana* n. sp. (Alto do Capivari, Campos do Jordão, São Paulo); E. Male of *M. vitellina* n. sp. (Alto da Boa Vista, Campos do Jordão, São Paulo); F. Female of *M. pervagata* n. sp. (Pico do Itapeva, Pindamonhangaba, São Paulo).
Male genitalia (Fig. 4). Saccus short and slender in ventral view; tegumen rounded; gnathos absent; appendix angularis extremely conspicuous, projecting posteriorly as a long process; uncus elongated, with a subtle median enlargement in dorsal view (Figs. 4B–C); valva elongated, narrowing towards end, with internal margin projecting internally in a structure with a series of small teeth (Figs. 4B–C); aedeagus slightly curved upwards; cornuti absent; juxta membranous.

Female (Fig. 1). Forewing length 18–19 mm (n = 5); hind wing length 14–16 mm (n = 5). Body entirely dark brown. General color and pattern very similar to that of males, with wings more rounded.

Remarks on color variation. Variation on the dorsal wing surface is small, limited to the number of dorsal ocelli (one or two). The ventral surface of both wings shows some variation in intensity of the whitish pigmentation, and size of the small ocelli (as described above in both sexes).
**Early stages.** Egg. Spherical, light cream, without conspicuous markings. The two expressed eggs hatched before being brought to laboratory to be photographed and measured.

**First instar** (Fig. 5A). Head capsule width 0.56–0.58 mm; scoli 0.06 mm (n = 2). Head dark brown, with enlarged chalazae, bearing a pair of short scoli on vertex, each with two long narrow setae ending in a fine point. Third stemmata larger than other stemmata. Body green, smooth, with red longitudinal stripes; caudal filaments very short. Legs, prolegs and caudal filaments light green. Setae dark elongated. Maximum length 4.2 mm. Duration 9 to 11 days (n = 2).

**Second instar** (Fig. 5B). Head capsule width 0.8 mm; scoli 0.10–0.12 mm (n = 2). Head dark brown with two diverging short scoli on vertex. Body green, striped longitudinally with white and reddish; caudal filaments short. Maximum length 6 mm. Duration 16 days (n = 1).

**Third instar** (Fig. 5C). Head capsule width 1.16 mm; scoli 0.24 mm (n = 1). Head brown, with two diverging very short scoli on vertex. Body brown with several longitudinal zigzag stripes; caudal filaments short. Maximum length 10 mm. Duration 14 days (n = 1).

**Fourth instar** (Fig. 5D). Head capsule width 1.54 mm; scoli 0.32 mm (n = 1). Head brown, with two diverging very short scoli on vertex. Body brown with several longitudinal zigzag stripes as in previous instar; caudal filaments short. Maximum length 15 mm. Duration 14 days (n = 1).

**Fifth (last) instar** (Figs. 5E–F). Head capsule width 2.26 mm; scoli 0.52 mm (n = 1). Head brown, with two diverging short scoli on vertex. Body brown with several zigzag longitudinal stripes; middorsal stripe conspicuously dark; ventral region dark brown; legs and prolegs light brown; caudal filaments short. Maximum length 22 mm. Duration 19 days (n = 1).

**Pupa** (Figs. 5G–I). Short and smooth; mostly dark brown, with short squared ocular caps; cremaster dark in ventral portion; dorsal abdomen with a paired series of short subdorsal white protuberances bordered with white. Total length 10.3 mm. Duration 12 days.

**Habitat, Behavior and Natural History.** *Moneuptychia montana* n. sp. occurs in open, natural grass fields, usually near forest edges (Figs. 1A–B), being rare or absent in disturbed places. Oviposition behavior was not observed in nature, and the host plant in the field is unknown. In the laboratory, larvae easily accepted *Bambusa gracilis* Hort. ex Rivière & C. Rivière (Poaceae), a common ornamental Chinese bamboo. Adults were observed only in open habitats and grasslands, flying among grass patches and perching usually on the ground. Males are apparently territorial, and were observed interacting with other males on sunny occasions, when it was possible to hear a clicking noise while two or more males flew together. Adults of both sexes were observed feeding on flowers of *Galantus brasiliensis* (Spreng.) E. L. Cabral & Bacigalupo (Rubiaceae). No courtship behavior was observed.
Distribution. The species is only known from the Serra da Mantiqueira, and has been recorded in a narrow region extending from Campos do Jordão (São Paulo) to Itatiaia (Minas Gerais and Rio de Janeiro), at altitudes from 1550 to 2300 m.

Etymology. The specific epithet is an adjective derived from the Latin (*montana* = of a mountain), and refers to the habitat of this species, which is known from the high mountains of the Mantiqueira mountain range.

Types. Holotype male. Deposited in the Museu de Zoologia da Universidade Estadual de Campinas (ZUEC), Campinas, São Paulo, Brazil. With the following labels separated by transverse bars: / Holotypus / Parque Nacional do Itatiaia, Itamonte, Minas Gerais: Brazil, 06.II.2014, 2100 m, 22°21’25”S 45°44’9”W, E. P. Barbosa & A. Tacioli leg. / Holotypus *Moneuptychia montana* Freitas det. 2014/ DNA voucher—YPH-0436 / ZUEC LEP 8897 /


Additional material. ZUEC-AVLF—São Paulo: Pico do Itapeva, Pindamonhangaba, 11-I-1990, 1 male (genitalia prepared); 09-III-2000, 1 male, 2 females; 10-I-2006, 1 male; AVLF leg.

**Moneuptychia vitellina** Freitas & Barbosa, new species
(Figs. 2E, 6, 7)

**Adult** Diagnosis. *Moneuptychia vitellina* n. sp. is similar to some species in the *Euptychoides castreensis* species complex. However, they are easily distinguished from the above species, and from all other known species of *Moneuptychia*, by the broad yellow outlining the ocelli on the ventral hind wing.

**Descriptions of adults** Male (Figs. 2E, 6). Forewing length 17–19 mm (n = 7); hind wing length 13–14 mm (n = 7). Eyes naked, entirely brown. Palpus length 2.0 times head height, beige, with long dark brown and white hairs. Antenna of males 8 mm in length, with 35 antennomeres extending to mid-costal; shaft rust brown, dorsally covered by dark brown scales, club with 12 antennomeres, not conspicuously developed. Male foreleg covered by long beige hairs. Wings with dorsal ground color brown with few markings, restricted to marginal and submarginal lines in both wings; forewing with no additional markings; hind wing with two large black ocelli outlined by an orange ring in Cu₄₄-Cu₄₃ and Cu₄₂-2A. Forewing of males presenting a conspicuous callus on SC vein, close to distal end of swollen portion of vein. Ventral wings mostly brown; forewing crossed by two dark brown lines, first indistinct, extending from discal cell to 2A one third from base; second extending from costa to 2A at two thirds from wing base; a dark brown irregular submarginal line and a brown regular marginal line extending from costa to 2A; ocelli absent. Hind wing crossed by two dark brown nearly straight lines from costa to anal margin, first one-third from wing base, second two-thirds from it; second crossing line delimiting a lighter marginal area; a brown irregular submarginal line and a brown regular marginal line extending from costa to 2A; a series of five black ocelli outlined by a broad yellow ring can be found in M₁-M₂ (ocellus 1), M₂-M₃ (2), M₃-Cu₅₁ (3), Cu₅₁-Cu₄₂ (4) and Cu₄₂-2A (5); ocelli 2 and 3 small; in some individuals ocellus 2 can be nearly absent, reduced to few yellow scales with one white scale in center; ocellus 2 usually double (when visible); ocelli 1, 4 and 5 larger than other; ocelli 1 and 4 with double white pupil. Hind wing outer margin slightly wavy. No conspicuous androconial scales or patches observed.

Male genitalia (Fig. 7). Saccus short and slender in ventral view; tegumen rounded; gnathos absent; appendix angularis extremely conspicuous projecting posteriorly as a long process; uncus elongated; valvae elongated, narrowing towards end, and with a single pointed process projecting internally (Figs. 7B–C); aedeagus slightly curved upwards, with a conspicuous triangular projection in left near tip (Fig. 7E); cornuti absent; juxta membranous.
FIGURE 6. Holotype male (left) and allotype female (right) of *Moneuptychia vitellina* n. sp. Dorsal above, ventral below. Scale bar = 1 cm.

FIGURE 7. Male genitalia of *M. vitellina* n. sp. A. lateral view; B. dorsal view; C. ventral view; D. aedeagus (lateral view); E. aedeagus (dorsal view). All morphological details following Fig. 4. Scale bar = 0.2 mm.
Female (Fig. 6). Forewing length 19–20 mm (n = 5); hind wing length 15–18 mm (n = 5). Body entirely dark brown. General color and pattern very similar to that of males, with wings more rounded.

Remarks on color variation. Variation on the dorsal wing surfaces is virtually absent. The ventral surface of both wings shows some variation in intensity of the yellow rings outlining the ocelli, and size of the small ocelli (as described above in both sexes).

Habitat, behavior and natural history. Moneuptychia vitellina n. sp. was observed in partially shaded areas, with open understory covered by grass (Fig. 2C). Adults of both sexes were observed flying near the edges of the shaded areas, where males perched and chased other males, but usually avoiding open areas with direct sunlight. Adults were observed perching on leaves from 10 to 50 cm above ground (Fig. 2E). Males are apparently territorial, and were observed interacting with other males, when it was possible to hear a clicking noise when two or more males flew together. No courtship behavior was observed.

Distribution. The species is known from the Serra da Mantiqueira, in a narrow region extending from Campos do Jordão (São Paulo) to Itatiaia (Minas Gerais and Rio de Janeiro), and also from the Serra da Bocaina, in altitudes from 1550 to 1850 m.

Etymology. The specific epithet is an adjective derived from the Latin (vitellus = egg yolk), and refers to the large amount of yellow outlining the ocelli on the ventral hind wing of both sexes.


Moneuptychia pervagata Freitas, Siewert & Mielke, new species
(Figs. 2F, 3C, 8–9)

Yphthimoides viviana; Iserhard et al. 2010: 312, Tab. 1.
Moneuptychia sp.; Santos et al. 2011: 255, Fig. 1.
Moneuptychia sp. n.; Dolibaina et al. 2011: 349, Tab. 1

Adult: Diagnosis. Moneuptychia pervagata n. sp. is similar to Moneuptychia itapeva Freitas, 2007. It can be distinguished from the latter by the following characters: the transverse dark brown bar crossing the ventral forewing two-thirds from wing base being much reduced or absent, and restricted to the discal cell (this line is more conspicuous, and extends from discal cell to 2A in M. itapeva); presence of marked ocelli in Rs–M₁, and CuA₁–2A on dorsal hind wing (these are absent in M. itapeva); ocelli in M₁–M₃ and CuA₁–CuA₃ on ventral hind
wing much larger compared to the remaining ocelli (they are more equivalent in size in *M. itapeva*). The male genitalia of *M. pervagata* n. sp. differ from that of *M. itapeva* by the shape of the gnathos being two rounded bumps below the uncus (conspicuous and forked in *M. itapeva*).

**Descriptions of adults: Male** (Fig. 8). Forewing length 14–17 mm (n = 15); hind wing length 11–14 mm (n = 15). Eyes naked, entirely brown. Palpus length 2.0 times head height, beige, with long dark brown and white hairs. Antenna of males 7–8 mm in length, with 35 antennomeres extending to mid-costa; shaft rust brown, dorsally covered by dark brown scales, club with 11 antennomeres, not conspicuously developed. Male foreleg covered by long beige hairs. Wings with dorsal ground color dark brown with few markings, restricted to marginal and submarginal lines in both wings; forewing with one minute black ocellus in M₁–M₂, hind wing with three black ocelli outlined by a light brown ring in Rs-M₁ (ocellus 1), Cu₁A₁–Cu₂A₂ (2) and Cu₂A₂–2A (3); ocelli 1 and 2 larger, ocellus 2 and 3 with white pupil (this double in ocellus 2). Forewing of males presenting a conspicuous callus on SC vein, close to distal end of swollen portion of vein (Fig. 3C). Ventral wings mostly brown; forewing crossed by two nearly straight dark brown lines, first very indistinct crossing discal cell one third from base; second extending from costa to 2A at two thirds from wing base; a dark brown zigzag submarginal line and a brown marginal line extending from costa to 2A; two black ocelli outlined by a yellowish ring in R₁–M₁ (ocellus 1) and M₁–M₂ (2); ocellus 2 large and with white pupil (both ocelli can be absent in some individuals). Hind wing crossed by two dark brown nearly straight lines from costa to anal margin, first one-third from wing base, second two-thirds from it; second crossing delineating a lighter medial area between line and ocellar region; a brown zigzag submarginal line and a brown regular marginal line extending from costa to 2A; a series of six black ocelli outlined by a yellowish ring can be found in Rs–M₁ (ocellus 1), M₁–M₂ (2), M₂–M₃ (3), M₃–Cu₁A₁ (4), Cu₁A₁–Cu₂A₂ (5) and Cu₂A₂–2A (6); ocellus 1 small and absent in some individuals, ocellus 2 completely black, ocelli 2 and 5 larger; ocellus 3 usually double (when visible); ocelli 3, 4, 5 and 6 with double white pupil. Hind wing outer margin rounded. No conspicuous androconial scales or patches observed.

![Figure 8](image_url)

**FIGURE 8.** Holotype male (left) and allotype female (right) of *Moneuptychia pervagata* n. sp. Dorsal above, ventral below. Scale bar = 1 cm.
Male genitalia (Fig. 9). Saccus short and slender in ventral view; tegumen rounded; gnathos as two rounded bumps below uncus; appendix angularis extremely conspicuous projecting posteriorly as a long process; uncus narrow and elongated, (Fig. 9B); valvae elongated, narrowing towards end, with internal margin projecting internally in a structure with a series of small teeth and with a single and small pointed process projecting internally (Figs. 9C–D); aedeagus slightly curved upwards, with a very small triangular projection in left near tip; cornuti absent; juxta membranous.

Female (Figs. 2F, 8). Forewing length 16–18 mm (n = 6); hind wing length 14–16 mm (n = 6). Body entirely dark brown. General color and pattern very similar to that of males, with wings more rounded.

Remarks on color variation. Variation on the dorsal wing surfaces is virtually absent. The ventral surface of both wings shows some variation in size and presence of ocelli (as described above in both sexes).

Early stages. Available pictures are of low quality and are not shown in the present paper, but in general appearance the immatures are very similar to those of *M. montana* and *M. itapeva* (Freitas 2007).

Egg. Spherical, light cream, without conspicuous markings. Height 0.80 – 0.84 mm mm; diameter 0.82–0.84 mm (n = 4). Duration 6 days (n = 1).

First instar. Head capsule width 0.58 mm; scoli 0.06 mm (n = 1). Head capsule dark brown, with enlarged chalazae, bearing a pair of short scoli on vertex, each with two long narrow setae ending in a fine point. Third stemmata larger than the other stemmata. Body green, smooth, with red longitudinal stripes; caudal filaments very short. Legs, prolegs and caudal filaments light green. Setae dark elongated. Maximum length 4.0 mm. Duration 8 days (n = 2).

Second instar. Head capsule width 0.8 mm; scoli 0.18 mm (n = 1). Head dark brown with two diverging short scoli on vertex. Body greenish beige with a conspicuous dorsal dark green stripe; caudal filaments short. Maximum length 7 mm. Duration 13 days (n = 1).

Third instar. Head capsule width 1.04 mm; scoli 0.24 mm (n = 1). Head brown, with two diverging very short scoli on vertex. Body beige with several longitudinal wavy stripes and a conspicuous dorsal dark stripe; caudal filaments short. Maximum length 8 mm. Duration 16 days (n=1).

Fourth instar. Head capsule width 1.4 mm; scoli 0.38 mm (n = 1). Head brown, with two diverging very short scoli on vertex. Body brown with several longitudinal wavy stripes and a conspicuous dorsal dark stripe; caudal filaments short. Maximum length 12 mm. Duration 14 days (n = 1).

Fifth instar. Head capsule width 1.84 mm; scoli 0.42 mm (n =1). Head brown, with two diverging short scoli on vertex. Very similar to previous instar. Maximum length 17 mm. Duration 15 days (n = 1).
Sixth (last) instar. Head capsule width 2.42 mm; scoli 0.52 mm (n = 1). Head brown, with two diverging short scoli on vertex. Body purplish brown with several zigzag longitudinal stripes; middorsal stripe conspicuously dark; ventral region dark brown; legs and prolegs light brown; caudal filaments short. Maximum length 25 mm. Duration 20 days (n = 1).

Pupa. Short and smooth; mostly dark brown, with short squared ocular caps; cremaster dark in ventral portion; dorsal abdomen with a paired series of short subdorsal white protuberances bordered with white. Total length 10.0 mm. Duration 15 days (n = 1).

Habitat, Behavior and Natural History. The species occurs in open, natural grass fields, usually near forest edges (Figs. 2A,B), being rare or absent in disturbed places. Oviposition behavior was not observed in nature, and the host plant in the field is unknown. Fertile eggs were obtained from a wild-caught female confined in a plastic bag along with leaves of several potential host-plants (species of Poaceae) and put under a source of heat (40W incandescent lamp). In the laboratory, larvae easily accepted the Bahia grass *Paspalum notatum* Flügge (Poaceae) (grama-batatais), a common species of grass. Adults were observed only in open habitats and grasslands, flying among grass patches and perching, usually on the ground. Males were observed interacting with other males on sunny occasions, when it was possible to hear a clicking noise when two or more males flew together. In the Serra da Mantiqueira, adults of both sexes were observed feeding on flowers of *Galianthe brasilienensis* (Rubiacceae) (Fig. 2F). No courtship behavior was observed.

Distribution. The species is known from the coastal mountains of south and southeastern Brazil, from Rio Grande do Sul to Rio de Janeiro, at altitudes from 800 to 1900 m.

Etymology. The specific epithet is an adjective derived from the Latin (*pervagata* = widespread), and refers to the wide distribution of this species.


Allotype female. Deposited in the Coleção Entomológica Padre Jesus Santiago Moure, Departamento de Zoologia, Universidade Federal do Paraná (DZUP), Curitiba, Paraná, Brazil. With the following labels separated by transverse bars: / ALLOTPYPUS / *Moneupychia pervagata* Freitas, Siwert & Mielle det. 2014 / Paraná, São José dos Pinhais, Pilão de Pedra, 1-II-2012. O. Mielle & Dobilaina leg. / DZ 30.646 /


*Moneuptychia wahlbergi* Freitas, Barbosa, Siewert & Mielke, new species

(Figs. 3D 10, 11)

**Adult: Diagnosis.** *Moneuptychia wahlbergi* n. sp. is similar to some species in the *Euptychoideae castrensis* species complex. However, both sexes are distinguished from the above species by the transverse dark reddish brown lines crossing the hind wing, which are bordered by yellowish scales in *M. wahlbergi* (absent in all other species of the *Euptychoideae castrensis* species complex).

**Descriptions of adults: Male** (Fig. 10). Forewing length 17–18 mm (n = 8); hind wing length 14–15 mm (n = 8). Eyes naked, entirely brown. Palpus length 2.0 times head height, beige, with long dark brown and white hairs. Antenna of males 9 mm in length, with 36 antennomeres extending to mid-costal; shaft rust brown, dorsally covered by dark brown scales, club with 12 antennomeres, not conspicuously developed. Male foreleg covered by long beige hairs. Wings with dorsal ground color brown with few markings, restricted to marginal and submarginal lines in both wings; forewing with one small black ocellus in M₁-M₂ (absent in some individuals); hind wing with three large black ocelli in M₂-M₃, CuA₁-CuA₂ and CuA₂-2A (last two ocelli outlined by an orange ring); in some individuals, one additional small ocellus is present in M₁-CuA₁. Forewing of males presenting a conspicuous callus on SC, close to distal end of swollen portion of vein (Fig. 3D). Ventral wings most brown; forewing crossed by two dark reddish brown lines, first extending from discal cell to 2A one third from base; second extending from costa to 2A at two thirds from wing base, curved basally in portion near costa; a dark brown zigzag submarginal line and a brown regular marginal line extending from costa to 2A; ocelli absent. Hind wing crossed by two dark reddish brown nearly straight lines from costa to anal margin, first bordered externally by yellowish scales, one-third from wing base, second bordered internally by yellowish scales, two-thirds from it; second crossing line delimiting a lighter marginal area; a brown irregular zigzag submarginal line and a brown regular marginal line extending from costa to 2A; a series of six black ocelli outlined by a pale yellow ring can be found in Rs-M₁ (occellus 1), M₁-M₂ (2), M₂-M₃ (3), M₃-CuA₁ (4), CuA₁-CuA₂ (5) and CuA₂-2A (6); ocelli 2 and 5 with double white pupil in some individuals; ocelli 1, 3 and 4 small and commonly absent or reduced to few yellow and black scales; ocelli 2, 5 and 6 larger than others. Hind wing outer margin slightly wavy. No conspicuous androconial scales or patches observed.
FIGURE 10. Holotype male (left) and allotype female (right) of *Moneuptychia wahlbergi* n. sp. Dorsal above, ventral below. Scale bar = 1 cm.

FIGURE 11. Male genitalia of *M. wahlbergi* n. sp. A. lateral view; B. dorsal view; C. ventral view; D. aedeagus (lateral view); E. aedeagus (dorsal view). All morphological details following Fig. 4. Scale bar = 0.2 mm.
Male genitalia (Fig. 11). Saccus short and slender in ventral view; tegumen rounded, conspicuous gnathos shaped as forked lateral structures with two pointed upright processes; appendix angularis extremely conspicuous projecting posteriorly as a long process; uncus elongated; valva elongated, narrowing towards end, with internal margin projecting internally in a structure with a series of small teeth (Figs. 11B–C); aedeagus slightly curved upwards; cornuti present as a membranous structure armed with several small teeth at tip; juxta membranous.

Female (Fig. 10). Forewing length 19–20 mm (n = 4); hind wing length 16–17 mm (n = 4). Body entirely dark brown. General color and pattern very similar to that of males, with wings more rounded.

Remarks on color variation. Variation on the dorsal wing surfaces is virtually absent. The ventral surface of both wings shows some variation, especially in the conspicuousness of the smaller ocelli (as described above in both sexes).

Habitat, behavior and natural history. The species was observed in open forests in regions of contact with cerrado savannas. No additional information is available on habitats, early stages, behavior and natural history.

Distribution. The species is known from low montane regions (700–1000 m) in Minas Gerais, Mato Grosso do Sul and the Federal District.

Etymology. The species is dedicated to Dr. Niklas Wahlberg, a prominent Finnish biologist who has made a large contribution to the understanding of Satyrinae evolution.


Allotype female. Deposited in the Coleção Entomológica Padre Jesus Santiago Moure, Departamento de Zoologia, Universidade Federal do Paraná (DZUP), Curitiba, Paraná, Brazil. With the following labels separated by transverse bars: / Allotypus / Minas Gerais, Cambuquira, 15-IX-1969, Ebert leg., ex-coll. Ebert / Holotypus Moneuptychia wahlerbergi Freitas, Barbosa, Siewert & Mielke det. 2014 / DZUP 26.530 /


Additional material. ZUEC-AVLF—Minas Gerais: Nova Lima, APE Manancial Mutuca, Parque Estadual da Serra do Rolo Moça, 20°01'01"S, 45°58'23"W, 06-X-2008, (DNA voucher PM 10-11), A. R. M. Silva leg. 1 male; Ibirité, Pq. Estadual da Serra do Rolo Moça, 29-IX-2013 (DNA voucher YPH-0414), 20°03'38.9"S, 44°03'03.1"W, 998 m, D. S. S. Lacerda leg., UFMG-IJE 1305193, 1 female.

Genetic divergence and phylogenetic inference. Distances within species ranged from 0 % to 3.5 %, and interspecific distances ranged from 4.1 % to 9.5 %, never overlapping with intraspecific distances (Fig. 12). The overall mean distance (variation) within each species was as follows: M. soter 0.3 %, M. itapeva 1.1 %, M. giffordii 0.0 %, M. pervagata 0.1 %, M. vitellina 0.0 %, M. montana 0.2 % and M. wahlerbergi 1.9 %. The genetic distances among the analysed species of Moneuptychia are shown in Table 2. Based on sequences of the mitochondrial gene CoxI, all new species here described are monophyletic (Fig. 13), and well distinct from all other described species of Moneuptychia. Also, the analysed species of Moneuptychia appeared separated in two main clades, but confirmation as to whether these represent actual species groups is dependent on a broader study with more genes (Barbosa et al. in prep.).
FIGURE 12. Frequency distribution of pairwise individual genetic distances within (grey) and between (black) the seven species of *Moneuptychia*.

**TABLE 2.** Genetic distances among all *Moneuptychia* species (exception of *M. melchiades*).

<table>
<thead>
<tr>
<th>Species</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>M. soter</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. <em>M. giffordi</em></td>
<td>0.065</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. <em>M. itapeva</em></td>
<td>0.055</td>
<td>0.059</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. <em>M. pervagata</em></td>
<td>0.077</td>
<td>0.078</td>
<td>0.074</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. <em>M. vitellina</em></td>
<td>0.043</td>
<td>0.078</td>
<td>0.070</td>
<td>0.082</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. <em>M. montana</em></td>
<td>0.071</td>
<td>0.077</td>
<td>0.055</td>
<td>0.068</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>7. <em>M. wahlbergi</em></td>
<td>0.074</td>
<td>0.071</td>
<td>0.067</td>
<td>0.090</td>
<td>0.071</td>
<td>0.077</td>
</tr>
</tbody>
</table>

**Discussion**

Recent studies in the genus *Moneuptychia* showed that this genus is supported by at least one conspicuous synapomorphy: the well-developed appendix angularis that project posteriorly in the male genitalia (see Freitas 2007; Freitas et al. 2010), present in the four new species herein described. One interesting additional character of wing venation is also shared by all four new species: in male forewings, a conspicuous callus is present on the vein...
FIGURE 13. Phylogenetic relationships among seven species of *Moneuptychia* based on DNA sequences of *Cox1* and obtained by a Maximum Likelihood analysis. Values above branches are bootstrap branch support.
SC, close to the distal end of the swollen portion of the vein (Fig. 3). This callus was first described by Murillo-Hiller (2006), and is supposedly related to sound production by males. This structure was overlooked by Freitas (2007) and Freitas et al. (2010); re-examination of additional Moneuphytia species revealed that it is present in Moneuphytia soter (Butler, 1877), the type species of the genus (Fig. 3F), in Moneuphytia itapeva Freitas, 2007 (Fig. 3E), and in Moneuphytia giffordi Freitas, Emery & Mielke, 2010 (very subtle; not figured here) (Moneuphytia melchiades (Butler, 1877), has not been examined). This structure has also been observed in some species of Pharneuphytia Forster, 1964 (AVLF and EPB, unpublished), and in Euptychoides castreensis (Schaus, 1902) (the species for which the structure was first described by Murillo-Hiller (2006)). In the phylogenetic analysis of Peña et al. (2010), using molecular data, Moneuphytia (except those species now belonging to Carminda Dias, 1998), Pharneuphytia (with exception of Pharneuphytia innocentia (C. Felder & R. Felder, 1867), and E. castreensis form a well-supported clade, suggesting that the presence of this callus might be a useful synapomorphy for this group. Further investigation of additional morphological characters in species belonging to the above clade could help to better understand the relationships among species of these three genera.

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Considerações finais

A inclusão de dados moleculares em pesquisas na área de taxonomia e sistemática proporcionou um aumento na resolução de problemas filogenéticos e taxonômicos mais desafiadores, como complexos de espécies cripticas e processos de diversificação de espécies muito recentes e isso se reflete positivamente para um melhor entendimento e, consequentemente, uma melhor conservação da diversidade de espécies.

Entretanto, o uso de dados moleculares não veio para substituir a morfologia nos trabalhos sistemáticos e sim para complementar e auxiliar quando a morfologia sozinha não é suficiente para resolver algumas questões que requerem uma combinação de esforços de diferentes áreas.

A combinação de diferentes fontes de dados tem sido de grande valor do ponto de vista taxonômico, pois tem permitido além da identificação de espécies alocadas em gêneros aos quais não pertencem de fato, o reconhecimento e a descrição de inúmeras espécies novas, o que é extremamente importante para a ciência. A descoberta e descrição das espécies novas permitem um melhor entendimento da biodiversidade e uma inferência mais acurada das relações evolutivas entre os grupos estudados.

No caso de estudos com borboletas em particular, e lepidópteros em geral, a combinação de morfologia e DNA tem sido uma ferramenta muito poderosa para se resolver questões filogenéticas e taxonômicas de alguns grupos que são mais difíceis de identificar corretamente ao nível de espécie, como é o caso das borboletas, em sua maioria pequenas e marrons, da subfamília Satyrinae (Nymphalidae).

Por serem muito similares entre si, torna-se extremamente complicado encontrar caracteres únicos para que se possa definir e identificar os limites entre cada espécie dessa subfamília, especialmente algumas subtribos como Pronophilina e Euptychiina. Por isso, no passado, muitos dos gêneros e espécies desse grupo foram descritos pelos pesquisadores da época com base em caracteres simplesiomórficos, ou seja, caracteres ancestrais compartilhados e que não são úteis para a separação dos grupos, como é o caso do trabalho de Forster (1964) com os satiríneos da Bolívia.

Com o advento de estudos filogenéticos usando dados moleculares e em alguns casos uma combinação de dados moleculares e morfológicos, o que se percebeu é que essas subtribos e gêneros são na verdade uma miríade de agrupamentos não naturais e que, portanto, muitas das identificações de espécies eram erradas e essas deveriam ser realocadas.
Como ainda não se conhece exatamente a amplitude desse problema taxonômico nesses grupos de satiríneos, os trabalhos de sistemática filogenética, principalmente ao nível genérico e que fazem uso de dados moleculares, se tornam extremamente importantes para auxiliar na descoberta e resolução desses problemas.

Quatro desses grupos de satiríneos foram objeto de estudo no presente trabalho, os gêneros *Yphthimoïdes*, *Moneuptychia* e *Pharneuptychia* e o complexo de espécies sob o nome “*Euptychoïdes castrensis*”.

Com base em trabalhos filogenéticos anteriores e análises preliminares de morfologia de genitália masculina, no caso das espécies de *Yphthimoïdes*, existiam dúvidas sobre o monofilétismo desses grupos e o uso de marcadores moleculares para o estudo desses grupos ao nível genérico pareceu muito promissor.

O resultado final dos trabalhos corroborou a hipótese do não monofilétismo desses quatro grupos. *Yphthimoïdes* como atualmente definido conta com pelo menos quatro espécies que não compartilham um ancestral em comum com o restante do gênero e sim com um clado do qual fazem parte algumas espécies de *Paryphthimoïdes*, relativamente distantes das espécies verdadeiras de *Yphthimoïdes*.

Já as espécies dos gêneros *Moneuptychia* e *Pharneuptychia* e a espécie *Euptychoïdes castrensis* compartilham um ancestral em comum, fazendo parte de um único clado no qual aparecem relativamente “misturadas” umas às outras. Uma das espécies de *Pharneuptychia*, *P. innocentia*, faz parte de outro clado, próximo das espécies de *Hermeuptychia* e algumas espécies de *Splendeuptychia*.

Já *E. castrensis* se mostrou sendo na realidade um complexo de espécies crípticas, não compartilhando um ancestral em comum único dentro do clado *Pharneuptychia*.

Com base nas análises efetuadas e nos resultados obtidos, *Moneuptychia*, *Pharneuptychia* e *Euptychoïdes castrensis* como atualmente reconhecidos são grupos polifiléticos. O complexo *Euptychoïdes castrensis* deveria ser realocado para o gênero *Moneuptychia* e a espécie *Pharneuptychia innocentia* deveria ser retirada do gênero *Pharneuptychia* e realocada em um novo gênero.

O que se pode concluir com os resultados alcançados nesse trabalho é que o uso de três marcadores moleculares, especificamente COI, GAPDII e RpS5, para estudos filogenéticos ao nível genérico é suficiente para que se consiga definir os limites desses gêneros com um bom suporte e estabilidade. Porém as relações evolutivas internas não são eficientemente resolvidas em alguns casos e nesses casos, a inclusão de ao menos mais um marcador molecular se torne necessária para que as relações filogenéticas entre as espécies
sejam mais bem resolvidas, além de uma amostragem de espécies e indivíduos suficientemente grande e até mesmo completa dos grupos sob estudo.

Espera-se que num futuro próximo os estudos de sistemática filogenética consigam, por meio da combinação de diferentes fontes de dados, proporcionar uma melhor resolução e compreensão das relações de ancestralidade comum entre as espécies e dos processos por trás da enorme biodiversidade existente no planeta, antes que boa parte dessa biodiversidade seja perdida.
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Em observância ao §5º do Artigo 1º da Informação CCPG-UNICAMP/001/15, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "Relações filogenéticas e padrões de distribuição biogeográfica dos clados Yphthimoides Forster, 1964 e Phaeneuptychia Forster, 1964 (Nymphalidae: Satyrinae)", desenvolvida no Programa de Pós-Graduação em Biociências e Tecnologia de Produtos Bioativos do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

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