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MARIA CLARA FERREIRA DE AMORIM

Revisão taxonômica de *Erioscelis emarginata* (Mannerheim, 1829) (Coleoptera: Scarabaeidae, Dynastinae)

Recife
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Dissertação apresentada ao Programa de Pós-Graduação em Biologia Animal da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de mestre(a) em Biologia Animal. Área de concentração: Taxonomia

Orientador (a): Luciana Iannuzzi

Coorientador (a): Paschoal Coelho Grossi

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Acho que eu nunca tive tanta coisa para ser grata por durante toda minha vida do que neste momento. Fazer essa dissertação foi... tudo. Tantas coisas novas pra descobrir e aprender. Tantas pessoas novas pra conhecer. Tantas formas novas de sofrer (haha). Mas sobretudo, tanta coisa nova pra amar. Estudar biologia foi algo que caiu no meu colo... eu descobri sem querer, e por isso demorou muito pra eu descobrir que eu tinha acertado em cheio. E não deveria vir como surpresa que entomologia veio da mesma forma. Caiu no meu colo, foi absolutamente sem querer. Mas eu aprendi que eu amo de tal forma que eu me arrependo do dia que eu desisti de testar essa área durante a graduação.

E por que eu estou falando disso? Bem, porque se caiu no meu colo foi porque teve muita gente que fez cair; mas eu devo essa em especial a duas pessoas muito queridas pra mim. Milena, minha querida, não acho que você tem noção do que assistir sua defesa de TCC despertou em mim na época. Se não fosse por você insistir tanto em me trazer de volta pra área acadêmica, em dar uma chance ao azar, acho que eu nunca teria mexido os pauzinhos tão rápido. E, ainda mais, nada poderia ter ido pra frente se a Professora Luciana Iannuzzi não tivesse me dado uma chance. Eu não poderia ter tido mais sorte, e ainda não acredito até hoje que ela aceitou me orientar: eu, a doida que não tinha experiência nenhuma no que estava me propondo a estudar, só muita vontade e garra. E, no entanto, ela me aceitou de braços abertos e fez de tudo pra tirarmos um projeto do chão mesmo com o pouco tempo e experiência. Professora, eu espero não ter te feito subir nas paredes demais, mas eu espero também que saiba o quanto essa oportunidade me permitiu aprender e abriu tantas novas portas que eu jamais esperava ser capaz. Eu devo tanto a você por isso e espero um dia poder retribuir tudo que significou pra mim!

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de laboratório: João! Tem poucas pessoas que eu conheci na vida que se dispõem tanto a ajudar quanto João, mesmo que ele jure de pé junto que odeia fazer isso. Às vezes eu fico até mal pensando no quanto ele me ajudou a me situar nesse novo mundo e quão pouco eu pude fazer pra ele em retorno. João, sua amizade significou muito pra mim, de coração, e eu espero que tenha ficado claro. Eu também espero que você saiba que pode contar comigo pra o que der e vier, espero um dia poder ajudar tanto quanto você me ajudou.

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Trabalhar com o que se gosta é bom, e conhecer pessoas que também gostam do mesmo é fenomenal... Mas sem suporte do lado de fora acho que eu não teria sobrevivido. E tudo começa com a família. Não tem nenhuma conquista minha que não seja diretamente uma conquista dos meus pais. Pai, suas cartas sempre foram mais eloquentes que as minhas, mas eu espero que pelo menos dessa vez eu faça jus a tudo que você já fez por mim. Assim como pra mamãe, que por ela eu falaria mil vezes mais de tudo que me aflige, espero que dessa vez eu esteja falando o bastante pra você saber o que ambos significaram pra mim. Por todas as noites que eu liguei do nada chorando porque eu tava cansada. Por todas as vezes que eu precisei de ajuda pra fazer uma escolha. Por todas as vezes que eu queria que a gente pudesse estar junto de novo, mas os quilômetros de distância dificultaram um pouquinho. Se

não fosse por vocês, eu não estava aqui, em tantos sentidos diferentes. Obrigada por tudo. E que felicidade de ter vocês comigo mais uma vez.

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I'm past patiently waitin' I'm passionately mashin' every expectation
Every action's an act of creation
I'm laughin' in the face of casualties and sorrow
For the first time, I'm thinkin' past tomorrow
And I am not throwin' away my shot

(MIRANDA L.M., Hamilton, 2015).

RESUMO

A tribo Cyclocephalini é atualmente conhecida como um dos grupos mais diversos de besouros, com conhecida importância econômica devido a espécies praga e polinizadores. Dentre as espécies da tribo, *Erioscelis emarginata* se destaca por atuar na polinização de diversas espécies de Araceae, algumas das quais são popularmente conhecidas como copo-de-leite. Recentemente, evidências tem surgido sobre uma grande variação entre as populações desses besouros, principalmente na forma e coloração do corpo, sendo levantada a hipótese que se trataria de um complexo de espécies. Portanto, esse estudo se propôs a revisar a taxonomia de *E. emarginata*, bem como propor uma abordagem inovadora para a análise e descrição de cor em besouros. A partir da análise da morfologia externa desses besouros, foi feita a redescrição de *E. emarginata* e a descrição de uma nova espécie que ocorre em simpatria com a localidade tipo de *E. emarginata*, Serra do Cipó (Minas Gerais). Caracteres anteriormente descritos puderam ser confirmados em grande parte, mas algumas divergências também foram encontradas, e alguns caracteres novos foram adicionados, em especial relacionados ao dimorfismo sexual e a caracteres de peças bucais. Também produzimos um mapa de distribuição geográfica e de potencial de nicho, bem como a adaptação de uma chave de identificação taxonômica para o gênero. Para o estudo da cor no tegumento dos insetos, esse estudo propôs o uso de agrupamento k-means para a obtenção de cores dominantes a partir de fotos. A partir de um estudo de caso com as populações simpátricas de *E. emarginata*, foi possível demonstrar a utilidade dessa metodologia para análises entre populações, bem como descrições de grupos de animais, trazendo uma importante discussão sobre todos os fatores que podem levar a alterações nas cores observadas. Com esse estudo, trazemos uma melhor compreensão dos limites taxonômicos de *E. emarginata* e com avanços nos estudos da cor em insetos, contribuindo com futuros estudos de ecologia e taxonomia destes grupos.

Palavras-chave: Taxonomia; Cyclocephalini; Polinizador de Araceae; Colorimetria; Agrupamento K-means.

ABSTRACT

The Cyclocephalini tribe is currently known as one of the most diverse groups of beetles, with recognized economic importance due to pest and pollinator species. Among the species in the tribe, *Erioscelis emarginata* stands out for its role in pollinating various species of Araceae, some of which are popularly known as calla lilies. Recently, evidence has emerged of significant variation between populations of these beetles, particularly in body shape and coloration, leading to the hypothesis that this could be a species complex. Therefore, this study aimed to review the taxonomy of *E. emarginata*, as well as to propose an innovative approach for the analysis and description of color in beetles. Based on the analysis of the external morphology of these beetles, *E. emarginata* was redescribed, and a new species that occurs in sympatry with the type locality of *E. emarginata* (Serra do Cipó, Minas Gerais) was described. Previously described characters were largely confirmed, but some discrepancies were found, and new characters were added, particularly regarding sexual dimorphism and mouthpart characters. We also produced a geographic distribution and niche potential map, as well as an adapted taxonomic identification key for the genus. For the study of color in the insect integument, this study proposed the use of k-means clustering to obtain dominant colors from photographs. Through a case study of the sympatric populations of *E. emarginata*, we were able to demonstrate the usefulness of this methodology for analyses between populations and for describing animal groups, contributing to an important discussion on all factors that may lead to changes in observed colors. With this study, we provide a better understanding of the taxonomic boundaries of *E. emarginata*, along with advances in color studies in insects, contributing to future ecological and taxonomic studies of these groups.

Keywords: Taxonomy; Cyclocephalini; Araceae Pollinator; Colorimetry; K-means Clustering.

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Introdução Geral

Revision of *Erioscelis emarginata* (Mannerheim, 1829) (Scarabaeidae: Dynastinae: Cyclocephalini)

Color Clustering Method: A Novel Quantitative Approach on the Study of Insect Colors from Photos with an Emphasis on Beetles

SUMÁRIO

1. INTRODUÇÃO GERAL

Os besouros da tribo da Cyclocephalini (Scarabaeidae, Dynastinae), são atualmente reconhecidos como um dos grupos mais ricos da ordem Coleoptera, com mais de 550 espécies descritas (MOORE; CAVE; BRANHAM, 2018a). Esses besouros têm recebido cada vez mais atenção, devido ao seu grande potencial econômico, especialmente ao se considerar as pragas agrícolas que advém de larvas de algumas espécies da tribo (MOORE; CAVE; BRANHAM, 2018a). Além disso, estima-se que cerca de 900 espécies de plantas neotropicais dependam dos serviços ecossistêmicos de polinização de besouros Cyclocephalini, em especial das famílias Annonaceae, Araceae, Arecaceae, Cyclanthaceae, Magnoliaceae e Nymphaeaceae (MOORE; JAMESON, 2013; SCHATZ, 1990). No entanto, quando se trata do cenário taxonômico da tribo, muito ainda se encontra incerto, o que tem gerado uma atenção maior de estudos sistemáticos ao grupo (MOORE; CAVE; BRANHAM, 2018a).

Em termos sistemáticos, apesar de Cyclocephalini ser uma tribo bastante relevante e bem estudada, com mais de 550 espécies em 14 gêneros, há questionamentos sobre a monofilia da tribo e pouco se sabe sobre a sua filogenia (MOORE; CAVE; BRANHAM, 2018a). Há alguns gêneros taxonomicamente instáveis na tribo, como é o caso do gênero asiático *Peltonotus* Burmeister, 1847, que já foi transferido para a subfamília Rutelinae devido ao labrum e que tornou a ser considerado um Dynastinae posteriormente (MOORE; CAVE; BRANHAM, 2018a), mas que estudos moleculares dão força à hipótese anterior (MCKENNA et al., 2015). Também há gêneros que apresentam instabilidade interna, como é o caso de *Cyclocephala* Dejean, 1821, o gênero mais especioso da tribo, com cerca de 360 espécies alocadas em diversos subgêneros, e *Erioscelis* Burmeister, 1847, que, mesmo com apenas cinco espécies, vem sendo questionada quanto a seu status taxonômico e monofilia (MOORE; CAVE; BRANHAM, 2018a, 2018b).

Erioscelis é um gênero de besouros conhecidos pelo fato de algumas de suas espécies serem polinizadores de Araceae (MOORE; JAMESON, 2013), plantas que apresentam inflorescência do tipo espádice, popularmente conhecidas como “copo-de-leite”. Normalmente esses besouros são de fácil identificação entre os Cyclocephalini por apresentarem protarsômeros, incluindo as garras, não alargados em ambos os sexos. Em machos dos demais gêneros, estes são grossos e encurtados.

O gênero reúne cinco espécies, incluindo *Erioscelis emarginata* (Mannerheim, 1829) como espécie tipo e mais quatro congêneres: *E. columbica* Endrödi, 1966, *E. proba* Sharp, 1877, *E. sobrina* Höhne, 1921 e *E. peruana* Saylor, 1946 (ENDRŐDI, 1985). *Erioscelis* foi revisado por Saylor (1946) e posteriormente foi incluído na revisão da subfamília Dynastinae de Endrödi (1966; 1985). E, no entanto, pouco foi discutido das características que distinguem este gênero de outros Cyclocephalini além dos protarsos dos machos, possivelmente em função da grande divergência entre os caracteres encontrados na espécie tipo e as outras espécies do gênero (ENDRÖDI, 1966; ENDRŐDI, 1985; MOORE; CAVE; BRANHAM, 2018a; SAYLOR, 1946).

Originalmente, *E. emarginata* foi descrita em *Apogonia* Kirby, 1818 e posteriormente transferida para *Erioscelis*, como espécie tipo por monotipia, com a adição de congêneres ocorrendo em trabalhos subsequentes. O nome da espécie provavelmente se deve à profunda emarginação do clípeo, a característica que mais facilmente a diferencia das demais espécies do gênero (ENDRÖDI, 1985). Essa espécie também difere morfologicamente das demais congêneres por apresentar dois dentes protibiais (as demais espécies apresentam três), pelo tamanho ligeiramente maior e pelo tegumento consideravelmente menos esculpido (ENDRÖDI, 1966; ENDRÖDI, 1985). A área de ocorrência também facilita sua identificação, sendo atualmente reconhecido a presença para *E. emarginata* principalmente no centro-sul brasileiro, desde Goiás até o Rio Grande do Sul, sendo também encontrado em Missiones (Argentina) e Concepción (Paraguai), as quatro demais espécies de *Erioscelis* se distribuem principalmente na Amazônia (Fig. 1; BARROS et al., 2020; DUPUIS; PERRIN, 2020; MOORE; CAVE; BRANHAM, 2018b).

Evidências têm surgido que indicam que a espécie *E. emarginata* apresenta uma variação maior do que o esperado a nível de espécie, entre suas populações. Barros et al. (2020), ao utilizar análises de morfometria geométrica, encontraram uma significativa variação na forma do élitro e do pronoto entre quatro populações encontradas em território brasileiro (Distrito Federal, Minas Gerais, São Paulo e Rio de Janeiro). Avaliando estas e outras populações, detectou-se também uma aparente diferença da morfologia externa de cada grupo (L. Iannuzzi e P. Grossi, comunicação pessoal) (Fig. 2). No entanto, as diferenças entre as populações não se restringem a variações regionais. Duas populações simpátricas na localidade tipo apresentam um contraste notável, especialmente em suas cores, bem como outras características

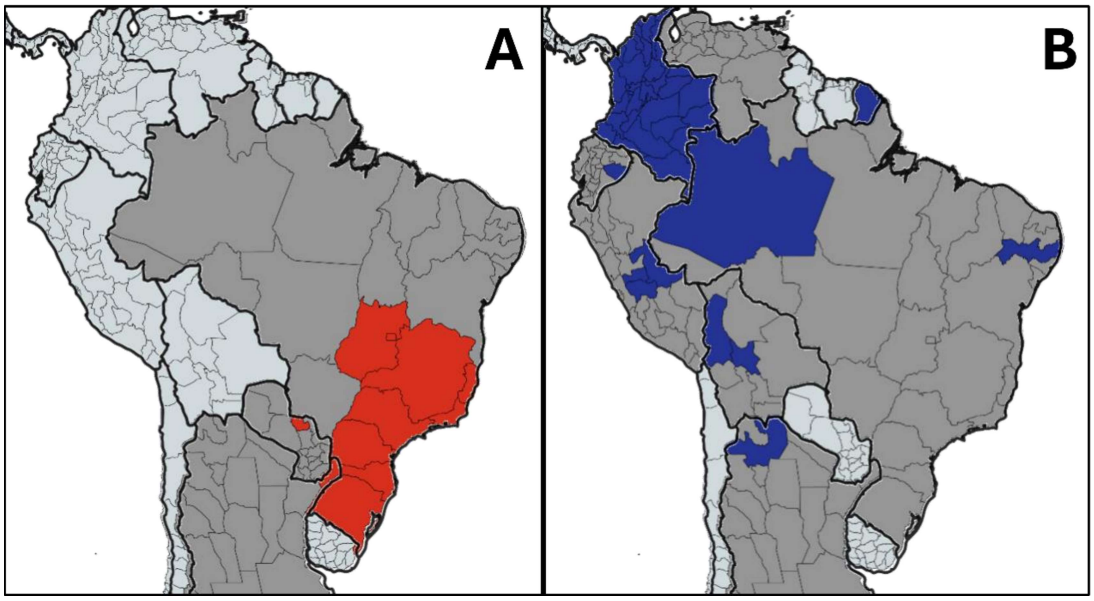

Figura 1 – Mapa simplificado da distribuição geográfica de *Erioscelis emarginata* (A, vermelho) e seus congêneres (B, azul) baseado na literatura vigente (Fonte: BARROS et al., 2020; DUPUIS; PERRIN, 2020; MOORE; CAVE; BRANHAM, 2018b).

como tamanho (observações pessoais). Neste cenário, umas das principais variáveis que poderia estar causando essa diferença é a polinização feita por estes besouros.

As diversas plantas que *E. emarginata* poliniza apresentam distribuições bastante diversas ao longo da área de ocorrência deste besouro (BARROS et al., 2020; PEREIRA et al., 2014), incluindo variações na fauna na localidade tipo, onde essas populações são encontradas. No que se refere aos dados de associação com as plantas, *E. emarginata* apresenta registro de polinização em *Thaumatophyllum* form *selloum* (GOTTSBERGER; JR., 1984), *T. adamantinum* e *T. uliginosum* (PEREIRA et al., 2014), *T. mello-barretoanum* (SANTOS, 2017), *T. lundii* (MAYO, 1991), *T. bipinnatifidum* e *Philodendron melinonii* (MALDONADO et al., 2015), *P. cipoense* (observações pessoais de P. Grossi), *Xanthosoma striatipes* (SCHROTTKY, 1910). A distribuição dessas aráceas no território de seu polinizador apresenta grandes variações (Tabela 1), com espécies de endemismo local, como *T. adamantinum* e *P. cipoense* que apenas são encontradas na Cadeia do Espinhaço (MG). Além disso, apenas *T. bipinnatifidum* pode ser encontrada em toda a extensão da distribuição de *E. emarginata*, devido a seu constante uso ornamental (DEWIR et al., 2023; Tabela 1). Diferentes habitats também são comuns entre essas plantas, como é o caso de *T. adamantinum* e *T. uliginosum*, que ocorrem em ambientes de

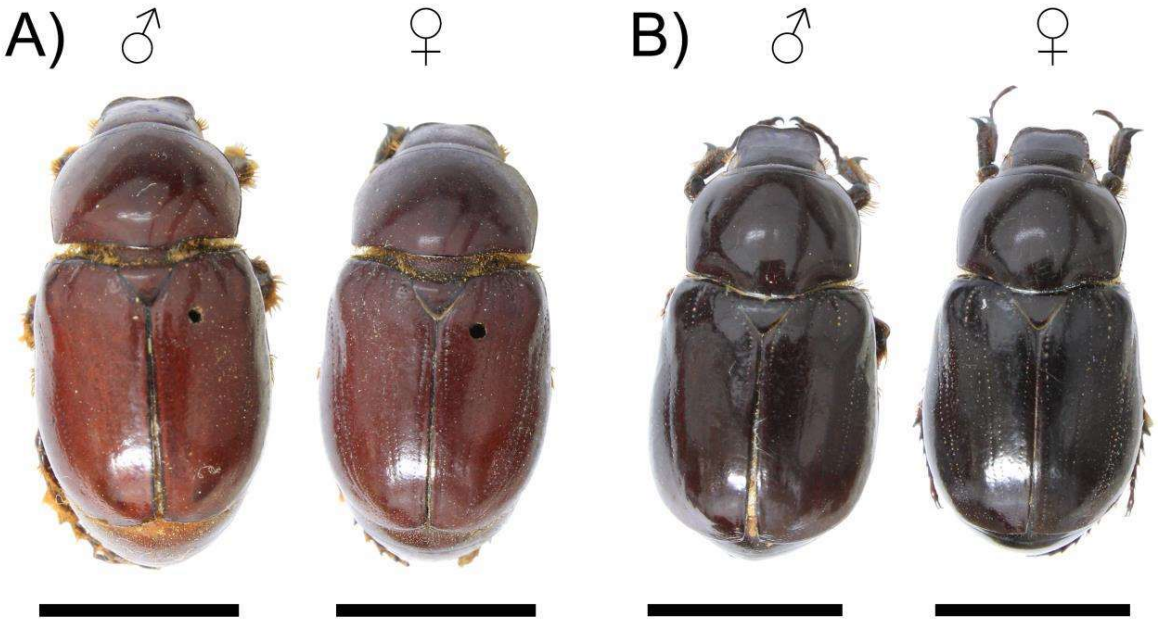

Figura 2 - Habitus de espécimes *Erioscelis emarginata* (Mannerheim, 1829), coletados em Jaboticatubas/Minas Gerais (A); Brasília/Distrito Federal (B). Barra = 1 cm. Fonte: BARROS (2017).

terreno rochoso ou aquático, respectivamente, mas na mesma região (PEREIRA et al., 2014). A ocorrência de larvas de *E. emarginata* em diferentes habitats pode ser um fator que desencadeie a divergência das populações, considerando que características do solo podem influenciar a sobrevivência de larvas de besouro (BARNETT; JOHNSON, 2013).

Além disso, mesmo entre os diversos polinizadores que podem ser encontrados entre os Cyclocephalini, *Erioscelis* se destaca por apresentar uma polinização bastante peculiar, que vem sendo extensamente estudada nos últimos anos, especialmente quando se trata da espécie tipo do gênero, *E. emarginata* (Fig. 3; MOORE; CAVE; BRANHAM, 2018a; PEREIRA et al., 2014). O processo costuma ocorrer durante cerca de um dia inteiro, tendo início por volta das 18:30, e sendo dividido principalmente em duas etapas: a fase feminina (Fig. 3A, B), onde os besouros são atraídos à inflorescência por odores e oferta de benefícios ao polinizador (alimento, termogênese e abrigo); e a fase masculina (Fig. 3C), na qual há a dispersão do besouro, recoberto de pólen, induzida pela parada na produção de odores e benefícios (GOTTSBERGER; SILBERBAUER-GOTTSBERGER, 1991; MALDONADO et al., 2015; MOORE; CAVE; BRANHAM, 2018a; PEREIRA et al.,

Tabela 1. Distribuição de plantas polinizadas por *Erioscelis emarginata* de acordo o território em que esses besouros podem ser encontrados.

Espécie	Distribuição dentro do território de *E. emarginata*	Referências
Philodendron cipoense	Minas Gerais (Cadeia do Espinhaço)	(SAKURAGUI, 2001)
Philodendron melinonii	Rio de Janeiro	(MALDONADO et al., 2015)
Thaumatophyllum adamantinum	Minas Gerais (Cadeia do Espinhaço)	(BARROS et al., 2020; PEREIRA et al., 2014)
Thaumatophyllum bipinnatifidum	Todos estados do Brasil; Argentina; Paraguai	(COELHO et al., 2015; GBIF.ORG, 2024)
Thaumatophyllum form *selloum*	São Paulo; Minas Gerais; Paraguai	(GOTTSBERGER; JR., 1984; MAYO, 1991)
Thaumatophyllum lundii	Distrito Federal; Minas Gerais	(CHACON; MARTINS; AMARAL, 2014; MAYO, 1991)
Thaumatophyllum mello-barretoanum	Distrito Federal; Minas Gerais; Rio de Janeiro	(GOTTSBERGER; SILBERBAUER-GOTTSBERGER; DÖTTERL, 2013; MAIA et al., 2019; RIZZO, 2009)
Thaumatophyllum uliginosum	Distrito Federal; Minas Gerais	(MAYO, 1991)
Xanthosoma striatipes	Distrito Federal; Minas Gerais; São Paulo; Paraguai	(GOTTSBERGER; SILBERBAUER-GOTTSBERGER; DÖTTERL, 2020; MADISON, 1981)

2014). A peculiaridade dessa relação se encontra justamente na termogênese das inflorescências, como um dos diversos mecanismos para manter os besouros dentro da espádice (Fig. 3A, B). A geração de calor, que pode chegar em média a 42°C em seu pico de produção, parece servir dois principais propósitos: volatilizar os odores

florais, com o pico de produção de calor na fase feminina, e oferecer calor aos insetos, com a manutenção de uma temperatura 10°C maior que o ambiente até próximo ao final da fase masculina (PEREIRA et al., 2014). Além do calor, as inflorescências também dispõem de estames inférteis, que são preferidos para alimentação por parte dos besouros (MALDONADO et al., 2015); um abrigo seguro e quente onde passar a noite, na forma da câmera polinizadora (MOORE; CAVE; BRANHAM, 2018a; SILBERBAUER-GOTTSBERGER et al., 2001); e a exsudação de resina, durante a fase masculina, fundamental para que o pólen seja capaz de aderir à cutícula lisa do besouro (PEREIRA et al., 2014).

Diante da especificidade desse sistema de polinização, não é uma surpresa que seja comum que diversos besouros apenas sejam adaptados para polinizar uma ou poucas espécies de plantas (SILBERBAUER-GOTTSBERGER et al., 2001). Um dos principais fatores que geram especificidade entre plantas e polinizador, envolve os diversos odores exalados pelas inflorescências para atrair os besouros (GOTTSBERGER, 1990; Fig. 3A), resultando em preferência e especiação por

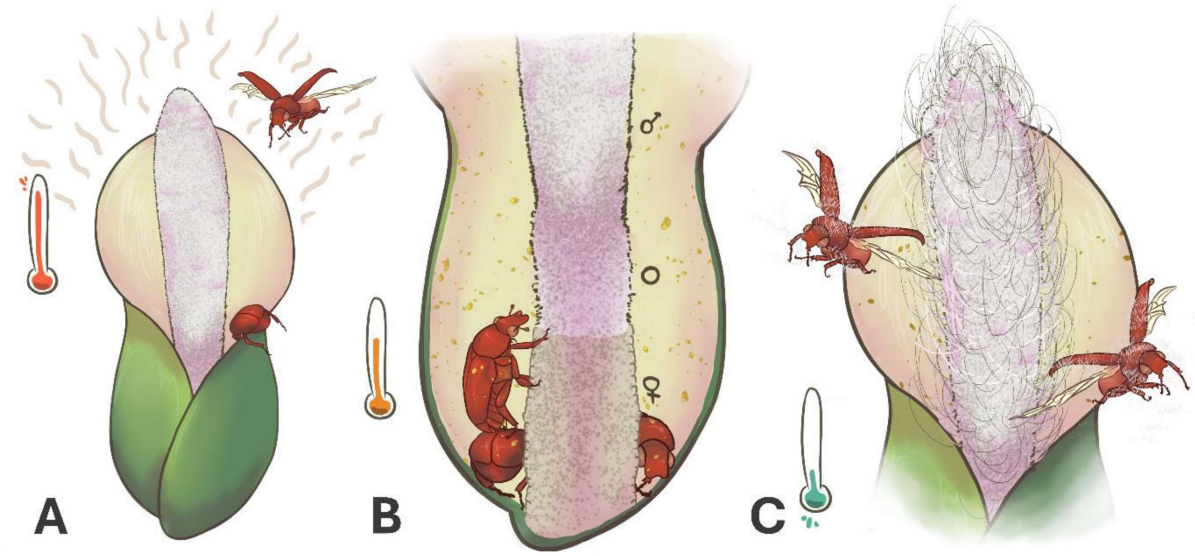

Figura 3 – Esquema representando as etapas de polinização realizadas pelo besouro *Erioscelis emarginata* em inflorescências espádice de diversas espécies de *Philodendron*. Em (A), o besouro é atraído às inflorescências a partir de odores que são volatilizados pela planta após o aumento da temperatura da espádice a cerca de 42°C, por volta das 18h. Durante a noite e o dia seguinte (B), a inflorescência se mantem em uma temperatura maior do que o ambiente, cerca de 10°C a mais, e começa a produzir resina enquanto os besouros se alimentam de estames inférteis. Na noite seguinte, por volta de 18h, a produção de calor cessa e os besouros caminham para fora da inflorescência, tendo o pólen mais facilmente grudado a sua superfície lisa devido à resina ((Baseado em: GOTTSBERGER; SILBERBAUER-GOTTSBERGER, 1991; MALDONADO et al., 2015; MOORE; CAVE; BRANHAM, 2018a; PEREIRA et al., 2014).

isolamento reprodutivo. Isso se deve especialmente devido aos grandes agrupamentos de besouros que podem ser encontrados nas câmaras polinizadoras (MOORE; CAVE; BRANHAM, 2018a), facilitando o cruzamento de indivíduos com as mesmas preferências. No entanto, *E. emarginata* não apenas é conhecida por visitar uma grande gama de aráceas diferentes, mas também por ser atraída por variados odores exalados por essas plantas (BARROS et al., 2020; GOTTSBERGER; JR., 1984; GOTTSBERGER; SILBERBAUER-GOTTSBERGER; DÖTTERL, 2013, 2020; MALDONADO et al., 2015; MAYO, 1991; PEREIRA et al., 2014).

Da mesma forma, outro fator de grande importância na variabilidade das populações de *E. emarginata*, se refere à coloração do tegumento dos indivíduos (Fig. 2). O padrão descrito para essa espécie é em tom castanho avermelhado (ENDRÖDI, 1985; SAYLOR, 1946), mas uma grande variação na coloração tem sido observada entre os indivíduos, desde castanho escuro a preto. Isso se dá especialmente, ainda que não apenas, entre as populações simpátricas encontradas na localidade tipo da espécie (observação pessoal). Apesar de variações no padrão de coloração serem esperadas, consistências nas divergências encontradas em cada populações podem ser um indicativo importante de que a espécie poderia na verdade se tratar de um complexo, visto que cor tem um papel importante nas pressões evolutivas (CHAPMAN, 1998). A cor pode influenciar na sobrevivência de insetos, seja através de proteção por camuflagem e mimetismo (CHAPMAN, 1998), ou da influência na fisiologia dos insetos, sendo fundamental para a devida termorregulação, já que os insetos são pequenos animais poiquilotermos. Ainda, há também o papel da produção de melanina para o sistema imunológico (GOURGOULIANNI et al., 2023).

Apesar da importância, estudos colorimétricos em populações de *E. emarginata* tem sido inviável em virtude da escassez de metodologias facilmente aplicáveis para comparar cores em insetos de uma forma quantitativa. A espectrofotometria e estudos moleculares são técnicas costumeiramente utilizadas em estudos em Lepidoptera, devido à importância das cores para esse grupo (BELDADE; MCMILLAN; PAPANICOLAOU, 2008). No entanto, o acesso a essas tecnologias depende de um grande aporte financiamento e demanda a destruição de amostras, inviabilizando o uso de material de coleção (KAFLE, 2019; SIUZDAK, 1996). Recentemente, outras metodologias de mais fácil acesso vêm sendo aplicadas, como as análises de melanização e via ferramentas de edição de foto (BADEJO et al., 2020; ELLERS; BOGGS, 2003). Apesar da facilidade de aplicação, essas técnicas

apresentam alguns impedimentos, pois às vezes não permitem a inteira compreensão do espectro de cor ou demandam alguma seleção aleatória de pixels. Em contraste, há uma metodologia já aplicada em plantas que é capaz de extrair as cores dominantes presentes em uma foto, no espectro RGB de cores (vermelho, verde e azul), baseada no uso de agrupamento K-means (GIBERT et al., 2022).

A aplicação de agrupamento K-means para o estudo de cores em insetos pode contribuir extensamente em estudos taxonômicos a partir de avaliações quantitativas e descrições precisas. Besouros da família Chrysomelidae são conhecidos por sua coloração bastante variada, sendo esse fator fundamental para sua sobrevivência e evolução (STRICKLAND et al., 2019; TAN et al., 2017). O mesmo acontece em Coccinellidae, que é uma das famílias mais conhecidas por apresentar diversos padrões de cor e diversos estudos quanto a influência da cor sobre a evolução do grupo (ANDO; NIIMI, 2019). Estudos de colorimetria em larvas de besouro tem sido importante na taxonomia, mesmo com as descrições qualitativas atuais (WIZEN; GASITH, 2011), mas métodos mais acurados são providenciais, visto que variações de outros caracteres larvais podem ser de mais difícil distinção.

Muitos fatores podem afetar a coloração de insetos, sendo de suma importância a sua investigação (CHAPMAN, 1998). Apesar do valor em utilizar a técnica de agrupamento K-means para o estudo da colorimetria, só existem registros em plantas até então (GIBERT et al., 2022). Nesse sentido, é de fundamental importância um estudo aprofundado sobre todas aplicações e limitações do uso de agrupamento K-means para o estudo da colorimetria desses animais.

Por fim, todas essas evidências nos levam a supor que *E. emarginata* se trata de um complexo de espécies e que estudo colorimétricos e morfológicos servirão para melhor entender o estado de diversificação no grupo. Dada a importância ecológica e a imprecisão na identificação taxonômica de *E. emarginata* diante da variação morfológica populacional, esse estudo se propôs a verificar se a segregação das populações resultou em modificações sugestivas a diversificação de linhagens distintas. Além disso, esse estudo também se propôs a analisar minuciosamente a possibilidade de utilização de agrupamento K-means como ferramenta para melhor analisar e descrever as cores dos insetos. Para tanto, foi lançado mão de vasto referencial teórico e alguns estudos de casos, com um enfoque especial nos besouros *E. emarginata*.

2. OBJETIVOS

2.1. OBJETIVO GERAL

Realizar uma revisão taxonômica de *Erioscelis emarginata* (Mannerheim, 1829), testando a hipótese de que o táxon se trata de um complexo de espécies e explorando suas variações morfológicas e de cor por meio de métodos inovadores de análise de imagem.

2.2. OBJETIVOS ESPECÍFICOS

1. Revisar a taxonomia de *E. emarginata* através de análises de sua morfologia externa
2. Verificar se as variações morfológicas encontradas entre as populações são características específicas ou intraespecíficas;
3. Redescrever a espécie e as suas supostas variações morfológicas;
4. Gerar mapas de ocorrência e projeções de nicho de *E. emarginata* e suas supostas variedades;
5. Determinar o potencial e os limites do uso de agrupamento K-means para obtenção das cores dominantes do tegumento de um inseto através de fotos;
6. Aplicar o método de estudo de cores dominantes em populações de *E. emarginata* e compreender se seriam passíveis de distinção por sua cor.

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4. ARTIGO 1 – Revision of *Erioscelis emarginata* (Mannerheim, 1829) (Scarabaeidae: Dynastinae: Cyclocephalini)

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Abstract

Erioscelis emarginata (Mannerheim, 1829), an Araceae pollinator scarab beetle from Brazil, was revised and a new species rom Serra do Cipó (Brazil), *Erioscelis sp1* **sp. n.,** is described. The new species was included in the key for the genus; the distribution of the *emarginata* complex was updated; both species were illustrated and compared; and we discussed the niche distribution for *E. emarginata* in Brazilian biomes.

Key-words: Coleoptera; Beetle; Taxonomy; Neotropical Region.

Introduction

Erioscelis Burmeister, 1847 is a small genus of neotropical Cyclocephalini beetles previously comprising five species, with its type species, *E. emarginata* (Mannerheim, 1829) (Endrödi, 1966, 1985; Saylor, 1946). All species are known to inhabit the Neotropics, with four of them being found mainly, but not exclusively, in the Amazon Forest, and *E. emarginata* being found at the Atlantic Forest and the Cerrado phytogeographical domains (Moore et al., 2018a; Ratcliffe & Cave, 2002). Originally, *Erioscelis* was described by monotypy (Burmeister, 1847), and has since been revised twice, once by Saylor (1946) and once by Endrödi (1966), in his Cyclocephalini monography. Besides its type species, four other species were also described in the genus: *E. proba* Sharp, 1877, *E. sobrina* Höhne, 1921, *E. peruana* Saylor, 1946 and *E. columbica* Endrödi, 1966.

After 39 years since the last taxonomic study of the genus, a geometric morphometry study has unveiled variation in *E. emarginata* between the shape of elytra and

pronotum from populations of different Brazilian regions (R. P. Barros et al., 2020). In that study, it is mentioned the possibility of such variations being due to the mosaic distribution of the many aroid species that *E. emarginata* pollinates, with scent compositions proven to differ greatly from one to the other (Gottsberger et al., 2020; Maldonado et al., 2015; Mayo, 1991; Pereira et al., 2014; Pohl, 1932; G. K. N. Santos, 2017). While beetles can be generalist pollinators, Cyclocephalini species are usually expected to be specialists on one plant and attracted by a specific scent (A. C. D. Maia, pers. comm. 2023), with scent preference being an important factor in plant-pollinator coevolution (Friberg et al., 2014). This tendency to specificity could be causing reproductive isolating events between the populations of *E. emarginata*. Hence, this study sets out to determine whether the populations of this species are in fact isolated groups that represent a species complex.

In this paper, we provide a redescription of *E. emarginata*, after the holotype examination, encompassing all its known variation, as well as a description of the new species, with known occurrence maps and a niche projection models whenever possible. Finally, this study also updated the key to the genus from Endrödi (1985), as to include the new species and encompass the observed variations.

Material and Methods

Material examined. For this study, 429 adult specimens (242♂ 183♀) deposited in the following institutions were examined (curators in parenthesis):

CEIOC – Coleção Entomológica do Instituto Oswaldo Cruz, Rio de Janeiro, Rio de Janeiro, Brazil (Márcio Felix);

CERPE – Coleção Entomológica da Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil (Paschoal Coelho Grossi);

CEUFPE – Coleção Entomológica da Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil (Luciana Iannuzzi);

DZUP – Coleção Entomológica Pe. J.S. Moure, Universidade Federal do Paraná, Curitiba, Paraná, Brazil (Lúcia Massutti Almeida);

EPGC – Everardo and Paschoal Grossi Collection, Nova Friburgo, Rio de Janeiro, Brazil;

MZUSP - Museu de Zoologia, Universidade de São Paulo, São Paulo, São Paulo, Brazil (Sônia A. Casari);

ZIN – Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia (Andrey Frolov).

Morphological Analysis. Characters of the external morphology of 429 specimens were analyzed with a Leica MZ6 stereomicroscope, including the male genitalia and mouthparts. For the dissection, specimens were pre-soaked in a solution of water and liquid detergent to soften the muscles and avoid damage. The dissected pieces (mouthparts and male genitalia) were mounted on cardboard with white glue and pinned beneath the specimen.

Images were taken with a HITACH TM4000Plus II Benchtop Scanning Electron Microscope and an Axiocam 105 attached to the Zeiss V20 stereomicroscope. Body size was measured by length, from clypeus apex to elytral apex, and width, from the wider portion of the pronotum. Description and redescription were based on the holotypes, with intraspecific differences being indicated separately, following the terminology of Endrödi (1985); Lawrence et al. (2011); Saylor (1946); species description and redescription followed Ratcliffe (2013). The diagnosis only included characters that differentiate between the two species discussed in this study.

For examined material, type specimens' labels were transcribed between quotation marks each (“”), using slash marks to differentiate each line (/). For other examined material, the information of the labels was recorded as follows: country of sampling in upper case, name of state or province in bold lower case, municipality, other information, sex, date, collector, collection the material is deposited in as well as voucher number.

Map. From the labels of the studied specimens, coordinates were extracted, using the centroid of the described location whenever coordinates were not provided, estimated from Google maps coordinates. The data was then inserted in the R program v 4.2.2 (R Core Team, 2022) to produce occurrence maps for both species.

Environmental Niche Modeling. To project the occurrence area of *E. emarginata*, the coordinates were also used to make an environmental niche distribution modeling. For environmental data, only bioclimatic layers were analyzed, as the addition of biotic data from the pollinated plant species was not viable due to some species being known as ornamental plants (Dewir et al., 2023), rendering their known distribution untrustworthy

as background. Furthermore, accounting for possible correlation of variables and their importance for the beetle biology, we used a variance inflation factor (VIF) test to select five out of 19 bioclimatic layers (size 30") available at Worldclim: Isothermality (3), Highest temperature of warmest month (5), Lowest temperature of coldest month (6), Precipitation of Wettest Month (13), Precipitation of Driest Month (14).

The environmental niche modeling was then run in the R program v 2.2.2 (R Core Team, 2022) using the Maxent algorithm (Phillips et al., 2006), with 10000 background points, replication by subsample and reserving 25% of the data for testing the acquired models. From the ten resulting models, the five that presented a true skill statistic (TSS) value above average were selected to make a consensus model, from each a niche distribution map was made.

Finally, due to the low amount of sampling locations, it was not possible to project a niche distribution for *Erioscelis sp1* **n. sp.**, which is only known from the type locality.

Key to genus. To update the key to the *Erioscelis* genus originally written by Endrödi (1985), all descriptions were taken into account as well as the specimens listed in this study and deposited material from other congeneric species housed in CERPE, *E. peruana* and *E. columbica*. Photos *E. peruana* were also requested from California Academy of Sciences, but *E. sobrina* specimens could not be located.

TAXONOMY

Erioscelis emarginata (Mannerheim, 1829) (Figs. 1A, B; 2; 3A; 4A, B, C; 5; 7A, B, E, F, G; 8A; 9; 10)

Cyclocephala emarginata Dejean, 1821: 57 [*nomen nudum*];

Apogonia emarginata Mannerheim, 1829: 54-55 (original description);

Erioscelis emarginata: Burmeister, 1847: 73 (new combination); Gemminger and Harold, 1869: 1244; Schrottky, 1908: 23; Schrottky, 1910: 67-68; Luederwaldt, 1915: 309; Luederwaldt, 1916: 294; Gesellschaft, 1921: 109; Pohl, 1932: 522, 528; Arrow, 1937: 19; Blackwelder, 1944: 254; Saylor, 1946: 61-64, 66 (genus redescription); Endrödi, 1966: 409-411 (tribe redescription); Gruner, 1971: 846; Gottsberger and Amaral, 1984: 391, 399-405; Endrödi, 1985: 173-174 (identification key to the genus); Gottsberger, 1986: 41-43; Gottsberger, 1987: 133; Gottsberger, 1990: 364; Mayo, 1991: 617-618; Gottsberger and Silberbauer-Gottsberger, 1991: 23-28; Silberbauer-

Gottsberger et al., 2001: ; Vaz-de-Mello et al., 2001: 5; Riehs, 2005: 63; Weber, 2008: 523; Dötterl et al., 2012: 1539-1543; Breeschoten et al., 2013: 105; Gottsberger et al., 2013: 793-809; Moore and Jameson, 2013: 1-43; Maia et al., 2014: 6-7; Pereira et al., 2014: 679, 685-690; Maldonado et al., 2015: 290-300; Gottsberger, 2015: 296-297, 326; Milet-Pinheiro et al., 2017: 8-9; Gonçalves-Souza et al., 2017: 534, 541; Gonçalves-Souza et al., 2018: 2-3, 8; Moore et al., 2018b: 26, 37, 59-61 (taxonomical synopsis to the tribe); Moore et al., 2018a: 275-276 (catalog of the tribe); Barbosa et al., 2019: 3, 5; Barros et al., 2020: 97-102; Gottsberger et al., 2020): 587.

Type material. *Apogonia emarginata* **Lectotype** (designated by P. G. Grossi; Barros et. al, 2020) BRAZIL: ♂ green label "Brasilia / 6024 - 1", yellow label "Zoological Institute / Russian Academy / of Sciences / St. Perterburg", red label "LECTOTYPE / *Apogonia emarginata* / Mannerheim, 1929 / P.C. Grossi des. 2019" (ZIN). **Paralectotype**. BRAZIL: ♀ green label "Brasilia / 6024 - 1", yellow label "Zoological Institute / Russian Academy / of Sciences / St. Perterburg", yellow label "PARALECTOTYPE / *Apogonia emarginata* / Mannerheim, 1929 / P.C. Grossi des. 2019" (ZIN).

Non-type material. BRAZIL. **Distrito Federal.** Taguatinga. Campus UCB: 5♂ (CEUFPE – 003930; 003931; 003932; 003933; 003934), 29.XI.2005, A. Maia, leg; Brasília. 1♂1♀ (CEUFPE); 2♂ (CERPE), 1.I.1990, E. & P. Grossi leg; 13♂15♀ (CEUFPE); Campus UNB. Setor da Biologia: 4♂2♀ (CERPE), 21.I.2018; Guará. Reserva Ecológica do Guará: 8♂3♀ (CEUFPE - 005215; 005213; 005207; 003937; 004029; 004031; 005208; 003938; 005212; 004032; 004030), 1993, E. G. Gonçalves leg; 1♀ (CEUFPE - 005210); **Minas Gerais.** Serra do Cipó. Campo Rupestre: 4♀ (MZUSP - 52539; 52542; 52543; 52540), 3.XI.1972, M. Jazima & J. Semik leg; MG010, km 108. Em flor de Araceae. 1♀ (CERPE), XI.2009, G. W. Fernandes leg; Jaboticatubas. Serra do Cipó ICMBio: 1♂ (CERPE), 27.XI.2015, Pinheiro-Costa leg; Serra do Cipó: 5♂2♀ (CEUFPE - 004033; 005206; 004034; 005211; 005209; 005214; 004035), 9.IX.1999, E. G. Gonçalves, leg; Santana do Riacho. Parada do Juquinha. Em *Philodendron cipoensis*: 3♂ (CERPE), 12.x.2023; Cordisburgo. Lagoa Bonita: 1♀ (CERPE), I.2011, F. Z. Vaz-de-Mello leg; São Gonçalo do Rio Preto. PEPreto: 1♂ (CEUFPE), 3.XII.2014, Schlindwein leg; Estrada. PEPreto: 2♂1♀ (CEUFPE), 16.XII.2011, J. A. P. Silva leg; 2♂ (CEUFPE), 7.XI.2013, Schlindwein et al. leg; Córrego. PEPreto: 2♀ (CEUFPE), 8.XII.2011, J. A. P. Silva leg.; Heliporto PERPreto:

1♂ (CERPE), 16.XII.2011, J. A. P. Silva leg; Juquinha. Serra do Cipó: 1♀ (CEUFPE); 31.X.2011, Carvalho leg; Teófilo Otoni: 1♂ (CEUFLA), 11.X.2008, M. F. S. Oliveira leg; Ijací: 1♂ (CEUFLA), 24.10.2002; *Caxambu*. Praça central (BB): 1♀ (CEUFLA), 23.XI.2005, A. Maia leg; Lavras: 1♀ (CEUFLA), 12.X.1986, L. F. G. Tavares leg; 1♀ (CEUFLA), 23.V.1998, G. Ferrari leg; 1♀ (CEUFLA), 28.IX.1998, Daniel B. Junior leg; 1♂ (CEUFLA), 10.01.1999, Paulo Pêgas leg; 1♂ (CEUFLA) 14.VI.1999, L. M. Carvalho leg; 1♂ (CEUFLA), 23.X.1999, P. Peres leg; 1♀ (CEUFLA), 09.XI.1999, Karina de Almeida leg; 1♂ (CEUFLA), 25.VI.2000, Diego Caraffini leg; 1♀ (CEUFLA), 5.VII.2000, T. B. Rodrigues leg; 1♂ (1 CEUFLA), 5.XI.2000, Gustavo Coelho leg; 1♀ (CEUFLA), 10.XI.2000, Claudio T. leg; 1♀ (CEUFLA), 11.XII.2000, L. S. Guimarães leg; 1♀ (CEUFLA), 2.XII.2000, A. M. Ribeiro leg; 1♀ (CEUFLA), 10.XII.2000, A. B. Azevedo leg; 1♀ (CEUFLA), 17.IV.2001, Oliveira leg; 1♂ (CEUFLA), 20.III.2002, L. O. Ribeiro leg; 1♂ (CEUFLA), 6.VII.2002, M. C. Nery leg; 1♀ (CEUFLA), 14.VII.2002, Rafael Carneiro leg; 1♂ (CEUFLA), 19.IX.2002, L. E. J. Malut leg; 1♂ (CEUFLA), 28.XII.2002, Vladmir leg; 1♂ (CEUFLA), 10.X.2003, R. V. Almeida leg; 1♀ (CEUFLA), 20.V.2004, D. C. R. Viela leg; 1♂ (CEUFLA), 17.XI.2004, E. D. Oliveira leg; 1♀ (CEUFLA), 01.XII.2004, Alano Xavier de S. Filho leg; 1♀ (CEUFLA), 04.IV.2005, P. P. Botrel leg; 1♀ (CEUFLA), 10.V.2005, R. Bianconi leg; 1♀ (CEUFLA), 01.IX.2006, L. M. Oliveira leg; 1♀ (CEUFLA), 12.IV.2008, G. B. Pinto leg; 1♂ (CEUFLA), 10.VIII.2008, J. F. B. Assis leg; 1♂ (CEUFLA), 22.IX.2008, G. C. Rocha leg; 1♂ (CEUFLA), 9.X.2008, Luis Felipe leg; 1♂ (CEUFLA), 12.X.2008, I. C. L. Proença leg; 1♂ (CEUFLA), 20.IX.2008, R. A. L. Silva leg; 1♂ (CEUFLA), 2.XI.2008, R. G. A. Mesquita leg; 62♂27♀ (CEUFPE), X.2016, R. P. Barros leg; UFLA. 920m. -21.227°S -44.979°W 9♂5♀ (CERPE), 16.21.X.2016, Grossi, Vaz-de-Mello, Duarte & Carvalho leg; Campus Universitário da UFLA. 21°13'40"S 44°57'50"W: 1♀ (CERPE), 19.X.2009, Korasaki leg; Parque Ecológico Quedas do Rio Bonito. Manual: 1♀ (CERPE), 22.X2016, P. R. M. Duarte leg; São Lourenço. 875 mts 2♂ (CERPE), 11.X.1991, Fe. & F. Soares leg; Prados. Serra São José. Parque das Libélulas. Manual: 1♂ (CERPE), 21.X.2016, P. R. M. Duarte leg; **Espírito Santo.** 11040: 1♂1♀ (MZUSP – 52528; 52533); **Rio de Janeiro.** Itatiaia. 700m. Estação Biológica: 1♂ (CEIOC - 66085), 18.X.1933; 1♀ (CEIOC - 66086), 23.X.1939, J. F. Zikan leg; 1♂ (CEIOC - 66088), 6.X.1940, J. F. Zikan leg; 1♀ (CEIOC - 66087), 23.X.1940, J. F. Zikan leg; PARNA Itatiaia: 1♂1♀ (CERPE), 24.X.2011, L. S. B. Calazans leg; Parque N. do Itatiaia. Campo Belo. 1♂1♀ (CERPE), 12.X.1957, J. Becker leg; *Nova Friburgo*. Sans Souci. 1000m: 2♂4♀ (CERPE),

6.XI.2005, E. & P. Grossi leg; 3♂5♀ (8 CERPE), 4.I.1995, E. & P. Grossi leg; **São Paulo.** Santana da Parnaíba: 1♂ (CEUFLA), 12.X.2007, A. R. Lohmann leg; Ip. 18647: 1♀ (MZUSP – 525337), IX; Ipiranga. 18596: 1♂ (MZUSP – 52541); Campinas: 1♀ (CEUFLA), 24.X.2001, V. Domin leg; Nova Odessa: 4♂ (CEUFPE), XI.2014, A. Maia leg; 1♀ (CEUFPE); 26♂24♀ (50 CEUFPE), XI.2014, A. C. D. Maia leg; *Cotia*. 23°37'S 46°53'W. 813m. Monstera flower. 8♂3♀ (CERPE), 18.IX.2015, F. F. Curcio; Itú. Fazenda Pau D'Alho: 1♀ (CERPE), 13.XI.1960, U. Martins leg; São Paulo. Santo Amaro: 1♂ (CERPE), X.1960, J. Lane leg; **Paraná.** Curitiba: 1♂ (MSUZP – 52538); Palmeiras. 865m. Rodovia Regis Bittencourt. Posto de Gasolina: 1♂ (CERPE), 2.III.2005, E. J. & P. Grossi leg; Cel. Domingos Soares: 1♀ (CERPE), 26.X.2014, E. M. Taques leg; Campo Mourão. Parque Estadual do Lago Azul. 600m: 3♂1♀ (CERPE), 10.X.2010, Dolibaina, Carneiro, Mielke & Dias leg; 2♂ (CERPE), D. Dolibaina leg; Antonina. Res. Natural Morro da Mina. 25°22'904"S 48°47'050"W 1♀ (CERPE), 18.VII.2008, M. L. P. Guedes leg; Ortigueira: 1♂ (CERPE), X.1945, nufe. graimbê; Tibagi. Parque Estadual do Guartelá. Em inflorescência de "costela de adão": 10♂20♀ (CERPE), 15.XII.2011. FWT Leivas, M. Caterino & A. Tshechin leg. **Santa Catarina.** Nova Teutônia: 1♂1♀ (MZUSP – 52527; 52525), XII.1936, B. Pohl; 1♀ (MZUSP – 52535), XI.1936, B. Pohl; 27°11'S. 52°23'L: 2♂2♀ (CEIOC – 66083; 66083; 66083; 66084), 2.XI.1939, Fritz Plaumann leg; 1♀ (MZUSP – 52536), VIII.1948, Dirings leg; 1♂ (DZUP – 541487), 19.X.1950, F. Pleuman leg; 1♀ (DZUP – 541488), 31.X.1951, F. Pleuman leg; 1♂ (DZUP – 541489), 4.XI.1951, F. Pleuman leg; 27°11'S. 52°23'L: 3♂7♀ (DZUP – 541490; 541491; 541492; 541493; 541494; 541495; 541485; CERPE), XII.1974, Fritz Plaumann leg; 27°11'S. 52°23'L. 300-500m: 1♂ (CERPE), X.1975, Fritz Plaumann leg; Hamonia. 16226: 1♂1♀ (MZUSP – 52524; 52530), X; 16226: 1♂1♀ (MZUSP – 52526; 52532), XI; Rio Vermelho: 1♂ (MZUSP – 52531), VIII.1948, Dirings leg; Corupá. 1♂ (DZUP – 521486), X.1937, A. Maller leg; Est: 1♀ (MZUSP – 52534), X.1956, Dirings leg; D 46. 60m: 1♀ (CERPE), X.1960; D 46. 60m: 1♀ (CERPE), IX.1964; Garopaba. Praia do Rosa: 1♂ (CERPE), 12.XI.2005, E. C. Santos leg; Tubarão. S 28°28'00" W 49°00'25": 2♂2♀ (CERPE), 9.XI.2010, J. S. Prophiro leg; 1♀ (CERPE), XI.1911, Luedini leg; Pinhal. D 46. 700m: 1♂ (CERPE), XII.58; ARGENTINA. **Misiones.** Loreto. Exp. St: 2♂ (MZUSP – 52523; 52529), Dr. A. Ogloblin leg. **No Data.** 1♀ (CEIOC), 3.X.1928, J. F. Zikan leg; (Ipiranga?):1♀ (CEIOC), 10.X1933, Hbd-Duiro leg; 1♀ (CEIOC), 12.XI.1924, J. F. Zikan leg; 1♀ (CEIOC),

6.X.1926, J. F. Zikan leg; 1♂ (CEIOC), 3.IX.1926, J. F. Zikan leg; 1♂ (CEUFLA); 1♂ (ZIN); no. 415: 1♂ (CERPE); 713 Sul. 1♀ (CERPE), 6.XI.1997, M. C. Silva leg.

Type locality. "Serra da Lapa", nowadays the municipality of Santana do Riacho (Serra do Cipó), Minas Gerais, Brazil.

Diagnosis. Color reddish brown (Fig. 1); length: 20-24mm, width 7.5-10mm. Mouthparts with extremities slightly sharp (Fig. 4A, C). Elytral interstriae II with rows of coarse punctation (Fig. 5C); apex of the elytra sub-oblong (Fig. 5B, C). Disc of the sternite VI with fine punctation accompanied by setigerous coarse punctures (Fig. 7A); lateral portion of the tergites with coarser and denser punctation (Fig. 7B). Aedeagus with paramera broad, inner margin straight or very slightly curved (Fig. 9A, E); parameres' basal margin usually V-shaped (Fig. 9A, E); parameres moderately angled downward in relation to the phallobase, forming a C-shape (Fig. 9I).

Redescription. Length: 24mm, width: 9.6mm. Body brown with a reddish undertone (Fig. 1A, B); smooth dorsal surface; covered in very sparse micropunctures all over the body, all other punctures ocellated; **Head.** Mostly covered in fine punctures, dense in the clypeus, sparser and larger in the frons, fading in the posterior portion of the frons but becoming denser towards the ends of the posterior portion and the ocular canthus area (Fig 2A); clypeus square, apex strongly emarginate, margin narrow but well-defined (Fig. 2A); fronto-clypeal suture marked, black in color; globular large eyes; ocular canthus setigerous (Fig. 3C); antennae with ten antennomers, lamellae with three antennomers (Fig. 3C); **Mouthparts (Fig. 3, 4A, C).** Labrum short and densely setigerous only laterally, not visible in dorsal view (Fig. 3D); mandibles not visible dorsally, dorsal and ventral surface covered in setae; mandibular apex not divided and triangular shaped, outer lateral angle slightly obtuse and sharp (Fig. 4A); prostheca well developed and heavily fringed with setae; mandibular mola square shaped, apical portion (up to the middle) plicate and with very fine punctures between the ridges, basal portion (beyond the middle) covered in fine and very dense punctures; mesal corner of mandible base with short pubescent process (Fig. 3G); maxilla densely setigerous, galea pentadentate with sharp teeth, lacinia and stipe clearly separated by a suture and almost twice as long as wide, cardo also separated by a suture but very short (Fig. 3F); mentum oval-shaped, lateral with long fine setae slightly densely distributed and

base sparsely and finely punctate (Fig. 3E); prementum and ligular lobes fused; ligular lobes emarginate at the apex (Fig. 3E); labial and maxillary palpi 3-segmented, with the middle segment being the shortest and the apical segment being slightly larger than the rest (Fig. 3E, F); **Pronotum (Fig. 2A).** Wider than long, base bisinuate, lateral and posterior margin narrow and well-defined; posterior margin arcuate and anterior margin straight; apical corners forming well-defined obtuse angles; lateral widened in the middle, rounded; fine and sparse punctation, fading into micropunctures towards the disc; big spots of darker color in the lateral portion; prosternal process short, with the apex covered in long fine setae; **Mesosternum.** Covered in long setae medially, lateral portion with coarse punctures, dense anteriorly and fading towards the middle; mesepisternum and metepisternum setigerous; **Metasternum.** Covered in long setae all throughout, except for an ellipsis in the very middle; **Elytra (Fig. 5A, B, C).** Twice as long as wide; micropigmented with reddish undertone all throughout the surface (Fig. 5A), color darker near the sutural stria, humeral and apical callus as well as the twin striae (Fig. 5A, B); scutellum triangular; humeral and apical callus well defined; elytra covering up to the middle of the propygidium (5th abdominal sternite); elytral suture coarsely punctate at the base and fading along the length of the elytra (disappearing in the median region) (Fig. 5C); twin striae punctate starting from under the humeral callus area ending at the apical callus (Fig. 5C); striae I display finer puncture, fading towards the apical callus area; striae II with larger punctures closer to the humeral callus, fading towards the median area; striae III with larger punctures and are the best marked striae, not fading; striae IV are barely marked, displaying finer punctures that fade very close to the humeral callus; most of interstriae finely and sparsely punctate, interstriae I and II with sparse coarser punctures (Fig. 5C), distributed in a row in the latter; apex of the elytra sub-oblong and densely punctate (Fig. 5B, C); **Legs (Fig. 2B, 6).** Profemur with two setigerous carinae in the ventral face crossing the length of the middle portion and the anterior portion, with dense setigerous punctation covering the posterior portion, dorsal face with two setigerous carinae not visible ventrally; meso and metafemur with three dorsal setigerous carina, one across the middle length of the femur and two at the edges of the ventral face, with these presenting much sparser setae in the metafemur; dorsal face with only one setigerous carinae across the middle length; mesofemur with setigerous punctures at the lateral portion of the dorsal surface, while the metafemur displays little to no punctures at this area; protibiae bidentate (Fig 2B♂), displaying three setigerous

carinae ventrally: one across the middle length with longer setae, another closer to the inner edge fading only near the spur, and a short transverse carinae near the edge close to the tarsi; inner margin of protibiae covered with fine setae, longer near the protibial spur, outer margin with coarse setigerous punctures; ventral face of the protibiae with two rows of very dense setae across its length, near both sides; meso and metatibiae with two setigerous carinae in an inverted “V” shape (Fig. 6), with the vertex at the outer face and the rows of setae continuing at the ventral and dorsal face, the outer V shaped carinae is separated into two carinae in the mesotibiae; outer face of metatibiae with a short row of seta in the middle near the apex (Fig. 6); meso and metatibiae’s dorsal face with dense and setigerous punctures from the middle of the disc until near the apex, inner margin with a setigerous carinae (Fig. 6); row of spines at the apex of the meso and metatibiae continuous; five tarsomeres with setae, meso and metatarsomeres I and II with one or two spines, metatarsomeres II and III with a row of setae laterally, all tarsomeres IV shorter (Fig. 6B); **Abdomen.** Tergites covered in punctures in the lateral, very dense in tergite I through III, slightly sparser at IV and sparse at V (Fig. 7B); sternites with a continuous row of long setae medially, longer at the disc (Fig. 7A); antero-lateral portion of sternites II and III presenting a row of coarse puncture, while this portion of IV and V is impunctate; V sternite 1.5 times longer than the others; sternite VI rugose from the base to the middle, fading into fine punctures (Fig. 7A); disc of sternite VI with fine punctation and two groups of coarse setigerous punctures, one in each side of the sternite (Fig. 7A, female); sternite VI’s posterior margin narrow and biemarginate (Fig. 8B), with longer setae laterally and shorter medially; **Propygidium (Fig. 8A).** Densely punctate in the whole disc, with coarse setigerous punctation and fine punctures, fading laterally; **Pygidium (Fig. 8A).** Wider than long; base rugose close to the edge, fading into a short band of non-setigerous dense punctation; disc finely and sparsely punctate; apex with few sparse and coarse punctures with long setae, margin of the apex covered in long fine setae; **Aedeagus.** In dorsal view (Fig. 9A, E): parameres symmetrical, broad and elongated, almost entirely straight, with the apical 1/3 arrow shaped, apex rounded and slightly hook-shaped; outer edge of the parameres nearly parallel, with a pair of teeth on the lateral at around the apical 1/3; lateral dilation slightly protruding from the medial portion of each side; inner margins straight; base truncate and broader than apical and medial regions, basal margin V-shaped; phallobase slightly longer than the parameres and smooth, except on the postero-lateral portion that has sparse fine punctures, apical

portion with deep V-shaped depression extending to the medial portion; apodeme oval shaped and as long as phallobase, with shallow vertical sulcus at the disc; in lateral view (Fig. 9I): parameres moderately angled downward in relation to the phallobase, forming a C-shape; parameres' middle portion densely setose ventrally.

Sexual dimorphism. Females differ from males by: protibiae tridentate, with a female-only basal short tooth (Fig 2B♀); protibiae with coarse puncture typically lacking setae on the ventral face near the outer edge; metafemur's lateral portion usually with more numerous coarse setigerous punctures; mesotibiae's outer V shaped carinae never fused, with outer face always lacking the setigerous carinae; hind tarsi about as long as the tibiae (hind tarsi in males 1/3 longer); sternite's row of setae interrupted in the disc (at times, barely so); V sternite usually with additional setae beyond the usual setae in a row (males tend to only have setae in a row, none outside of it); sternite VI disc's coarse punctures presented in only one disc group (separated in two lateral groups on the male), posterior margin not emarginated and with setae longer medially (Fig. 7A); pygidium lacking rugosity band on the base; apex of pygidium with very few punctures (less than in males).

Intraspecific variation. Length: 21-25mm, width: 8-12mm, color can vary from brown to dark brown, with reddish undertones. **Head.** Clypeus square to trapezoid. **Pronotum.** Punctation can vary in size and density, from micro to medium punctation, and from very to mostly sparse. **Elytra.** Elytral sutural striae can vary in size of punctation and may fade closer to the beginning (becoming barely apparent), fade in the median region or continue until the apex of the elytra. Interstriae punctures can also vary in size, from very fine to coarse, with some being barely visible, interstria II's row of punctures may be barely noticeable, very short or long and marked. The apical punctures are fine or very fine, dense all throughout the apex or only closer to the sutural striae. **Legs.** Metafemur's setigerous puncturing from the lateral portion of the dorsal surface may vary in number, denser closer to the lateral margin; protibiae's outer margin covered in coarse punctures with or without fine setae in the males; mesotibiae and metatibiae's outer V-shaped carina may be fused ventrally or separated into two rows of setae (more commonly separated in the mesotibiae and fused in the metatibiae) (Fig. 6C, D), outer face may present or not a short row of setae across the middle near the apex (more commonly absent in the mesotibiae and present in the

metatibiae) (Fig. 5F, G); row of spines at the apex of the meso and metatibiae continuous or presenting a gap before the last 3 spines, these becoming more inclined than the rest; metatarsomeres I to IV may present a row of setae laterally, meso and metatarsomeres I and II have one to three spines, III may present one spine; **Abdomen.** Lateral punctation of tergites III and IV may vary in density (from very dense to slightly sparse); V sternite's may present some setigerous punctures outside of the row of setae; sternite VI rugosity can be short, barely visible near the apex, or continue until the middle of the sternite, fading into a fine punctation that may itself fade medially or not at all; **Propygidium.** Coarse punctation may be lacking in the disc area and fade towards the apex; fine punctation may present as dense, slightly dense, sparse or lacking; **Aedeagus.** in any location (Fig. 9A, E, F): parameres may be almost entirely straight or depressed medially; parameres' lateral teeth can be hardly visible or more pronounced; lateral dilation may be barely visible dorsally or more pronounced; inner margin may be slightly curved, making a small slit at the center of the parameres; parameres' base broader or as broad as the apical and medial regions; in some southern individuals (Paraná and Santa Catarina – Brazil) (Fig. 9B, G) parameres are very broad, apical 1/3 may be slightly enlarged, more rounded; inner margin's mostly straight slightly curved near the postero-medial portion, making it slightly bulbous; basal margin round.

Comparative notes. *E. emarginata* can be easily distinguished from most other Cyclocephalini by the protarsomeres and inner protarsal claw not enlarged. From all congeneric species of *Erioscelis* (characters in parenthesis) this species is easily differentiated by deeply emarginated clypeus (non-emarginate or slightly emarginate clypeus), protibiae bidentate in males (protibiae tridentate in both sexes), surface less sculptured (surface more strongly sculptured) and larger size, ranging from 20-24mm (16-20mm); from *E. proba* and *E. columbica* (characters in parenthesis) by the reddish dark brown (black colored body). *Erioscelis emarginata* is most similar to *E. peruana*, especially similar in color and angle of the third teeth of the protibiae, however these species can still be easily distinguished by (*E. peruana* in parenthesis) the clypeus deeply emarginate (slightly emarginate) and surface moderately and finely to coarsely punctate (densely and coarsely to very coarsely punctate), specially on the elytra and pygidium.

Distribution (Fig. 10). This species occurs in most of the central-south of Brazil (Distrito Federal, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, Rio Grande do Sul), Argentina (Misiones) and Paraguay (Concepción).

Remarks. As it can be seen in the intraspecific variation section, there are many characters in *E. emarginata* that vary widely from one individual to the next. As some of these variations could only be seen in one population from Serra do Cipó, it was possible to separate them into a new species, *Erioscelis sp1* **sp. n.**. This study was also able to identify three major paramera morphotypes, with morph A (Fig. 9E) and morph B (Fig. 9F) being found all throughout the distribution of *E. emarginata*, while morph C (Fig. 9G) was only found in some of the specimens observed from Paraná and Santa Catarina, Southern Brazil (CEIOC – 66083; MZUSP – 52531; CERPE). However, these specimens could only be separated by aedeagus characters; no other external characteristics could differentiate the morphs observed. Though male genitalia differences can be fundamental for speciation, due to the key-and-lock mechanism of mating (Simmons, 2014), there is not enough evidence to separate these populations.

Some characters diverted slightly or greatly from their original description and subsequent redescriptions. One such character was the color description of *E. emarginata*. Though the species has been known to present as reddish-brown (Endrödi, 1966; Saylor, 1946), the original description used the term *piceo-castaneus* (in latin) to describe it, implying a rather darker hue of brown. However, later redescriptions used the term “reddish brown”, that is closer the actual color seen on the lectotype and many other specimens, also helping to differentiate from the new species; hence here we also adopt the later terminology.

Besides color, another important character that was revised were the teeth of the protibiae. Originally, this species had been the only one in the genera to have only two teeth, making it an important diagnostic character to differentiate species. However, after close inspection, a short third tooth could be found in all the females examined. It is most surprising that this is the first time this character has been described, as both Saylor (1946) and Endrödi (1966) have been able to examine specimens of both sexes for their redescription of *E. emarginata*. However, two factors may have impacted their analysis: 1) it is much smaller than the other two, nonetheless still similar to other

species of Dynastinae (Ratcliffe & Cave, 2002); 2) the protibiae teeth were usually found to be very worn, making them even smaller than usual, losing the pointy edge that can still be found some better-preserved specimens. According to the size of the structures found in all the females, here we describe them as a short third tooth from *E. emarginata* females, together with other sexually dimorphic characters, some new and some that has already been described.

There is also much to be said about how worn the specimens studied are. Most of the beetles studied presented plentiful of scratches on its surface, as well as very weary protibiae, especially on the length of the teeth, but also including the outer margin. Some of the scratches, especially in the pronotum and elytra, may also come from the period these beetles spend on the aroid inflorescence during pollination, as there has been said these inflorescences can become so crowded with beetles it overflows (Gottsberger & Jr., 1984). However, these would hardly be enough to explain the great weariness of the protibiae. This weariness, however, could be a great indicative of and edaphic lifestyle, as proposed for *Pseudogeniates* Ohaus, 1910, a Rutelinae genus (Jameson & Ocampo, 2012). Although other Cyclocephalini has been known to be edaphic, this habit has previously only been inferred as probable for Erioscelis, as little is known about its biology (Moore et al., 2018b).

Besides characters and ecology, some changes were also found on the limits of the distribution of *E. emarginata*. Although Moore et al. (2018b) indicated that the species could also be found in French Guiana, Ecuador and a northern state of Brazil (Pará), we have taken these locations out of the list after careful consideration due to the lack of evidence, especially since it was not recorded in recent fauna inventory efforts from French Guiana (Dupuis & Perrin, 2020) and no specimen was found in collections from the Pará State or in other contacted collections with corresponding locations. Besides the removal of these locations, we were also able to add the state of Espírito Santo from Brazil, previously not indicated in other studies.

These changes in the distribution of E. emarginata are in accordance with the environmental niche model obtained for the species (Fig. 10; AUC = 0.96), with a potential lack of suitable niche around the northern region, but very suitable values in Espírito Santo. The consensus model was mainly influenced by the locality's isothermality and maximum temperature, that are factors mainly affected by seasonality, showing the beetle's main predisposition to occupy areas with tropical and subtropical climate by the Köppen-Geiger classification (Peel et al., 2007). In addition

to the currently known distribution range of *E. emarginata*, six other Brazilian states were flagged with a high probability (>60%) of having the estimated suitable niche for *E. emarginata*: Ceará, Paraíba, Pernambuco, Bahia, Mato Grosso. All these states are included in the known distribution of at least one of the plants pollinated by these beetles, especially *Thaumatophyllum bipinnatifidum* for its widely spread ornamental uses (Dewir et al., 2023). Future efforts of beetle sampling in these areas should take into account the possibility of finding this species.

With this study, we expand the current knowledge about *E. emarginata* on the known range of its populations, new sexually dimorphic characters, description of the mouthparts and ventral surface, as well as the whole variation observed in the specimens.

***Erioscelis sp1* sp. n. Amorim, Iannuzzi and Grossi (Figs. 1C, D; 3B; 4D, E, F; 6; 7C, D, H; 8B; 11)**

Type material. *Erioscelis sp1* **sp. n. Holotype.** BRAZIL: ♂ “Santana do Riacho / Em *Philodendron cipoense* / 29.x.2023 Parada do Juquinha/ *Erioscelis emarginata* (3) / Erioscelis sp. nov. (1)”; “Holótipo”. Genitalia mounted. (CERPE) **Paratypes.** BRAZIL: ♀ “Jaboticatubas MG / Serra do Cipó ICMBio / Brasil 27.11.2015 / Pinheiro-Costa leg” “L 250 / P1599/ Philodendron uliginosum” (CEUFPE); ♀ “Jaboticatubas MG / Serra do Cipó ICMBio / Brasil 27.11.2015 / Pinheiro-Costa leg” “L 250 / P1599/ Philodendron uliginosum” (CEUFPE); ♂ “Jaboticatubas MG / Serra do Cipó ICMBio / Brasil 27/11/2015 / Pinheiro-Costa leg”, “96373”, Genitalia Mounted (CERPE); ♂ “Jaboticatubas MG / Serra do Cipó ICMBio / Brasil 06/12/2015 / Pinheiro-Costa leg”, “L 250 / *Philodendron cipoense*” (CERPE); ♂ “Jaboticatubas MG / Serra do Cipó ICMBio / Brasil 26/11/2015 / Pinheiro-Costa leg”, “L 250 / *Philodendron cipoense*” (CERPE); ♂ “Jaboticatubas MG / Serra do Cipó ICMBio / Brasil 27/11/2015 / Pinheiro-Costa leg”, “L 250 P1599 / *Philodendron uliginosum*” (CERPE); ♂ “Jaboticatubas MG / Serra do Cipó ICMBio / Brasil 06/12/2015 / Pinheiro-Costa leg”, “L 250 / *Philodendron cipoense*” (CERPE); ♂ “Jaboticatubas MG / Serra do Cipó ICMBio / Brasil 27/11/2015 / Pinheiro-Costa leg”, “L 250 P1599 / *Philodendron uliginosum*”, “96371” (CERPE); ♀ “Jaboticatubas MG / Serra do Cipó ICMBio / Brasil 27/11/2015 / Pinheiro-Costa leg”, “L 250 P1599 / *Philodendron uliginosum*”, “96372” (CERPE); ♂ “Jaboticatubas MG /

Serra do Cipó ICMBio / Brasil 27/11/2015 / Pinheiro-Costa leg", "L 250 P1599 / *Philodendron uliginosum*" (CERPE).
Holotype of the new species was deposited in Coleção Entomológica da Universidade Federal Rural de Pernambuco (CERPE) and the paratypes were deposited in in the same collection and in Coleção Entomológica da Universidade Federal de Pernambuco (CEUFPE).

Type locality. BRAZIL, Minas Gerais, Santana do Riacho, Serra do Cipó.

Diagnosis. Black body with a deep red undertone (Fig. 1); length: 19-22mm; width: 9-11mm. Mouthparts with extremities slightly round (Fig. 4B, D). Elytral interstriae II lacking rows of coarse punctation (Fig. 5F); apex of the elytra oblong (Fig. 5E, F). Disc of the sternite VI with fine punctation but lacking setigerous coarse punctures (Fig. 7C); lateral portion of the tergites with finer and sparser punctation (Fig. 7D). Aedeagus with paramera broad, inner margin curved, more so near the postero-medial portion that appears slightly bulbous (Fig. 9C, H); parameres' basal margin rounded; parameres strongly angled downward in relation to the phallobase, forming a C-shape (Fig. 9J).

Description. Length: 21mm; width: 11mm. Black with a deep red undertone (Fig. 1); smooth dorsal surface; covered in very sparse micropunctures all over the body, all other punctures present as ocellated; **Head.** Mostly covered in fine punctures, dense in the clypeus, sparser and larger in the frons, fading in the posterior portion of the frons but becoming denser towards the ends of the posterior portion and the ocular canthus area (Fig. 2A); clypeus nearly square, apex strongly emarginate (Fig. 2A), margin narrow but well-defined; fronto-clypeal suture marked; globular large eyes; ocular canthus setigerous (Fig. 3C); antennae with ten antennomers, lamellae with three antennomers (Fig. 3C); **Mouthparts (Fig. 3, 4B, D).** Labrum short and densely setigerous only laterally, not visible in dorsal view (Fig. 3D); mandibles not visible dorsally, dorsal and ventral surface covered in setae; mandibular apex not divided and truncated, outer lateral angle slightly obtuse and rounded (Fig. 4B); prostheca well developed and heavily fringed with setae; mandibular mola square shaped, apical portion (up to the middle) plicate and with very fine punctures between the ridges, basal portion (beyond the middle) covered in fine and very dense punctures; mesal corner of mandible base with short pubescent process (Fig. 3G); maxilla densely setigerous,

galea pentadentate with slightly rounded teeth, lacinia and stipe clearly separated by a suture and almost twice as long as wide, cardo also separated by a suture but very short (Fig. 3F); mentum oval-shaped, lateral with long fine setae slightly densely distributed and base sparsely and finely punctate (Fig. 3E); prementum and ligular lobes fused; ligular lobes emarginate at the apex (Fig. 3E); labial and maxillary palpi 3-segmented, with the middle segment being the shortest and the apical segment being slightly larger than the rest (Fig. 3E, F); **Pronotum (Fig. 2A).** Wider than long, base bisinuate; lateral and posterior margin narrow and well-defined, posterior margin arcuate and anterior margin straight; apical corners forming well-defined obtuse angles; lateral widened in the middle, rounded; fine and sparse punctation, fading into micropunctures towards the disc; big spots of darker color hardly seen in the lateral portion; prosternal process short, with the apex covered in long fine setae; **Mesosternum.** Covered in long setae medially, lateral portion with coarse punctures, dense anteriorly and fading towards the middle; mesepisternum and metepisternum setigerous; **Metasternum.** Covered in long setae all throughout, except for an ellipsis in the very middle; **Elytra (Fig. 5D, E, F).** Twice as long as wide; micropigmented with deep red undertone all throughout the surface (Fig. 5D), color lighter and redder near the suture (Fig. 5E, F); scutellum triangular; humeral and apical callus well defined; elytra covering up to the middle of the propygidium (5th abdominal sternite); elytral suture coarsely punctate at the base and fading along the length of the elytron (disappearing in the median region) (Fig. 5F); twin striae punctate starting from under the humeral callus area ending at the apical callus (Fig. 5F); striae I barely visible, fading at the very beginning; striae II with larger punctures closer to the humeral callus, fading towards the median area; striae III with larger punctures and are the best marked striae, not fading; striae IV are barely marked, displaying very fine punctures that fade very close to the humeral callus; most of interstriae very finely and sparsely punctate, interstria I with sparse coarser punctures (Fig. 5F); apex of the elytra oblong and densely punctate (Fig. 5E, F); **Legs (Fig. 2B, 6).** Profemur with two setigerous carinae in the ventral face crossing the length of the middle portion and the anterior portion, with dense setigerous punctation covering the posterior portion, dorsal face with two setigerous carinae not visible ventrally; meso and metafemur with three dorsal setigerous carina, one across the middle length of the femur and two at the edges of the ventral face, with these presenting much sparser setae in the metafemur; dorsal face with only one setigerous carinae across the middle length; mesofemur with

setigerous punctures at the lateral portion of the dorsal surface, while the metafemur displays little to no punctures at this area; protibiae bidentate (Fig 2B♂), displaying three setigerous carinae dorsally: one across the middle length with longer setae, another closer to the inner edge fading only near the spur, and a short transverse carinae near the edge close to the tarsi; inner margin of protibiae covered with fine setae, longer near the protibial spur, outer margin with coarse setigerous punctures; ventral face of the protibiae with two rows of very dense setae across its length, near both sides; meso and metatibiae with two setigerous carinae in an inverted "V" shape (Fig. 6), with the vertex at the outer face and the rows of setae continuing at the ventral and dorsal face, the outer V shaped carinae is separated into two carinae in the mesotibiae; outer face of metatibiae with a short row of seta in the middle near the apex (Fig. 6); meso and metatibiae's dorsal face with dense and setigerous punctures from the middle of the disc until near the apex, inner margin with a setigerous carinae (Fig. 6); row of spines at the apex of the meso and metatibiae continuous; five tarsomeres with setae, meso and metatarsomeres I and II with up to two spines, metatarsomeres I through IV with a row of setae laterally, all tarsomeres IV shorter (Fig. 6B); **Abdomen.** Tergites covered in finer punctures in the lateral (Fig. 7B), dense in tergite I and II, slightly sparser at III and sparse at IV and V (Fig. 7D); sternites with a continuous row of long setae medially, longer at the disc; antero-lateral portion of sternites II through V presenting a row of coarse puncture; V sternite 1.5 times longer than the others; sternite VI rugose near the base, disc glabrous and with very fine and few punctures (Fig. 7C); sternite VI's posterior margin narrow and biemarginate (Fig. 8D), with longer setae laterally and shorter medially; **Propygidium (Fig. 8C).** Densely punctate in the whole disc, with coarse setigerous punctation and fine punctures, fading laterally; **Pygidium (Fig. 8C).** Wider than long; base rugose close to the edge, fading into a short band of non-setigerous dense punctation; disc finely and sparsely punctate; apex with few sparse and coarse punctures with long setae, margin of the apex covered in long fine setae; **Aedeagus.** In dorsal view (Fig. 9C, D, H): parameres symmetrical, broad and elongated, slightly depressed in the medial portion, with apical 1/3 arrow shaped, apex rounded and slightly hook-shaped; outer edge of the parameres nearly parallel, with a pair of teeth on the lateral at around the apical 1/3; lateral dilation protruding from the medial portion of each side; inner margin curved, more so near the postero-medial portion that appears slightly bulbous; base truncate and as broad as the apical and medial regions, basal margin rounded; phallobase

slightly longer than the parameres and smooth, except on the postero-lateral portion that has sparse fine punctures, apical portion with deep V-shaped depression extending to the medial portion; apodeme oval shaped and as long as phallobase, with shallow vertical sulcus at the disc; in lateral view (Fig. 9J): parameres strongly angled downward in relation to the phallobase, forming a C-shape; parameres' middle portion densely setose ventrally.

Sexual dimorphism. Females differ from males by: protibiae tridentate, with a female-only basal short tooth (Fig 2B♀); protibiae's outer margin with coarse puncture typically lacking setae; metafemur's lateral portion usually with more numerous coarse setigerous punctures; mesotibiae's outer V shaped carinae never fused, with outer face always lacking the setigerous carinae; hind tarsi about as long as the tibiae (hind tarsi in males 1/3 longer); sternite's row of setae interrupted in the disc (at times, barely so); V sternite usually with additional setae beyond the usual setae in a row (males tend to only have setae in a row, none outside of it); VI's sternite's posterior margin not emarginated and with setae longer medially (Fig. 7C); pygidium's base lacking rugosity band; apex of pygidium with very few punctures (less than in males).

Intraspecific variation. Length: 19-22mm; width: 9-11mm; color can vary from very dark brown to black, with deep red undertones that may be more or less prevalent (Fig. 5D). **Head.** Clypeus square to trapezoid. **Pronotum.** Punctation can vary in size and density, from micro to medium punctation, and from very to mostly sparse. **Elytra.** Elytral sutural striae can vary in size of punctation and may fade closer to the beginning (becoming barely apparent), fade in the median region or continue until the apex of the elytra. Interstriae punctures can also vary in size, from very fine to coarse, with some being barely visible. The apical punctures are fine or very fine, dense all throughout the apex or only closer to the sutural striae. **Legs.** Metafemur's setigerous puncture from the lateral portion of the dorsal surface may vary in number, denser closer to the lateral margin; protibiae's outer margin covered in coarse punctures with or without fine setae in the males; mesotibiae and metatibiae's outer V-shaped carina may be fused ventrally or separated into two rows of setae (always separated in the mesotibiae and fused in the metatibiae) (Fig. 6C, D), outer face may present or not a short row of setae across the middle near the apex (always absent in the mesotibiae and present in the metatibiae) (Fig. 6F, G); row of spines at the apex of the meso and metatibiae

continuous or presenting a gap before the last 3 spines, these becoming more inclined than the rest; meso and metatarsomeres I and II have up to two spines, III may present one spine; **Abdomen.** Lateral punctation of tergites III and IV may vary in density (from slightly dense to sparse); sternite VI punctation may fade medially or not at all, fine or very fine, sparse or slightly dense; **Propygidium.** Coarse punctation may be lacking in the disc area and fade towards the apex; **Aedeagus.** Parameres' lateral teeth can be hardly visible or more pronounced; lateral dilation may be barely visible dorsally or more pronounced; base slightly broader or as broad as the apical and medial regions.

Comparative notes. From most other Cyclocephalini, *Erioscelis sp1* **sp. n.** can be easily distinguished by its simple protarsomeres and inner protarsal claw not enlarged. From *Erioscelis*, this species is easily differentiated by the characters in parentheses: from *E. proba*, *E. columbica*, *E. sobrina* and *E. peruana* by deeply emarginated clypeus (non-emarginate or slightly emarginate clypeus), protibiae bidentate in males (protibiae tridentate in both sexes), surface less sculptured (surface more strongly sculptured) and larger size, ranging from 20-22mm (16-20mm); from *E. sobrina*, *E. peruana* and *E. emarginata* by the very dark brown to black colored body (reddish dark brown color).

Distribution (Fig. 11). This species has only been collected from October to December in the Serra do Cipó region, from Minas Gerais (Brazil) up to this point in time.

Remarks. *Erioscelis sp1* **sp. n.** is very close in appearance to *E. emarginata* and occurs in sympatry with this species, with both sharing the same type locality (Fig. 11). Although it has been identified as the later until this revision, this study confirms morphological characters that clearly separates this population from *E. emarginata*.
In spite of the color of both species being similar, both dark brown, no specimens as dark as *Erioscelis sp1* **sp. n.** has been identified as *E. emarginata*. There are two factors that could have caused a darker color in this case: storage method and melanism. Such variation could be due to storage method differences, since how a specimen is stored may slightly change their color from its original presentation (Novák et al., 2023). On the other hand, there are specimens of both species that have been exposed to the same storage methods and the color has remained within the expected limits for each species. When it comes to pigmentation, however, it is possible that the

colors have darkened due to environmental factors rather than just genetic variation, as seen in many other insects (True, 2003), which could indicate that these species could diverge further in niche than initially assumed. Further studies could help elucidate the mechanisms involved in these color differences and help better understand the biology of these species.

On this species biology, all the specimens of *Erioscelis sp1* **sp. n.** sampled were found in inflorescences of *Thaumatophyllum uliginosum* and *Philodendron cipoense*. On the other hand, as *E. emarginata*, as defined in this study, has also been recorded on these plant hosts before (Pereira et al. 2014; personal observation of Grossi, 2023), this beetle should not be the sole pollinator of these species. Similar to *E. emarginata*, the specimens examined presented great weariness on the protibiae and many scratches on its dorsal surface, indicating the same habit of life as previously discussed.

On regional variation and plant hosts

As previously stated, although there were many characters that varied intraspecifically in *E. emarginata*, most variations could be found rather consistently in all sampling locations, only appearing inconsistent when differentiating the new species. However, during specimen examination, some characters were more easily found in some places rather than others.

Specimens found around Distrito Federal often lacked the row of setae found in the metatarsomere I, while the beetles from other locations typically present this row; the same tarsomere was also occasionally found with only up to one spine in them. Beetles from Southeastern (São Paulo, Rio de Janeiro, Minas Gerais, Espírito Santo) and Southern Brazil (Paraná, Santa Catarina) more frequently presented tarsomere I with up to 3 spines in much higher prevalence (though still rare); tarsomere II with up to 2 spines rather than 1 more often than not; sternite II with setae on the latero-anterior row of punctures more often than not (rare in others). Also, specimens from Southeastern Brazil usually were found with the V sternite presenting setae in addition to the usual ones found in a row more often than not (only occasionally in others); and specimens from Southern Brazil usually had pygidium with overall punctures rather coarser (rarely or occasionally found to be so in others). And, finally, there were the three southern specimens found with parameres with morphotype C (Fig. 9G), broader

and wider than the typical parameres found in *E. emarginata* and with a small circle near the base of the central orifice.

When all is considered, the variation observed seems excessive considering the biogeographic variations these animals are subjected to (IBGE, 2019; Köppen, 1936), as well as the ensemble of host plants in its occurrence area (R. P. Barros et al., 2020). As the new species was more easily found in *P. cipoense*, an endemic species of Espinhaço Range in Minas Gerais, it seems indeed plausible that populations may be in process of differentiation according to different host plant species, as previously postulated.

With such a specific pollination system (Pereira et al., 2014), differences on the plant assemblage may be creating preferences for species for some populations and hindering the genetic flux between them. For one, distribution could be a big factor, with some host plants having been known to occur only in some parts of the beetle's distribution, such as *T. adamantinum* (endemic to the Espinhaço Range in Minas Gerais) (Gonçalves-Souza et al., 2017), *T. lundii* and *T. uliginosum* (DF and MG) (R. P. Barros et al., 2020; Gottsberger et al., 2013; Maia et al., 2019; Mayo, 1991; Sakuragui et al., 2018), *T. mello-barretoanum* (DF, MG, RJ) (Gottsberger et al., 2013; Maia et al., 2019; Rizzo, 2009). There can also be differences in the habitat of these plants, as *T. adamantinum* occurs in rocky terrain while *T. uliginosum* is known to be aquatic, remaining apart even while being in the same location (Pereira et al., 2014). Another factor to consider is the ornamental value of these aroids, as can be seen with *T. melinonii*, that has a known range mostly restricted to Amazonian Forest (Zona & Christenhusz, 2015) but could be collected in Rio de Janeiro (Maldonado et al., 2015); as well as *T. bipinnatifidum*, that has been heavily used as an ornamental plant and may therefore be found all throughout Brazil (Dewir et al., 2023).

The differentiation of *Erioscelis sp1* **sp. n.** from *E. emarginata* is a good start in understanding how these beetles respond to such isolating pressures, as it does seem like the new species is attracted by only a small part of the plants *E. emarginata* can pollinate. However, as data on plant is hardly ever available in labels from specimens found in collection, much of the data about these dynamics is lost in the process. Future studies on the ecology of these beetles' pollination and how they have evolved may also help further the taxonomic knowledge of the genus.

Key to *Erioscelis* species (adapted from Endrödi 1985)

1. Apex of clypeus deeply emarginated. Sides of clypeus nearly parallel or slightly convergent, anterior angles broadly rounded. Anterior tibiae bidentate in males or with a third short tooth in females. Head, pronotum and pygidium nearly smooth. Pronotum surface smooth and with a few very fine punctures only on sides. Punctation of elytra fine to coarse, elytral striae distinct. Aedeagus with lateral tooth rather removed from apex. 2
- Apex of clypeus truncate or shallowly emarginated. Sides of clypeus usually rather convergent, anterior angles slightly rounded. Anterior tibiae tridentate in both sexes. Head, pronotum and pygidium strongly sculptured, that of pygidium mostly very strongly so. Pronotum surface moderately to strongly punctate, especially on the sides. Punctation of elytra coarse to very coarse, elytral striae from very to slightly distinct. Aedeagus with lateral tooth usually closer to the apex 3
2. Reddish brown, shining; mouthparts with extremities slightly sharp. Disc of the sternite VI with fine punctation and setigerous coarse punctures. Interstria II with coarse rows of punctation; apex of the elytra sub-oblong; lateral portion of the tergites with coarse and dense punctation; Aedeagus with paramera's inner margin straight or very slightly curved; parameres' basal margin usually V-shaped; parameres moderately angled downward in relation to the phallobase, forming a C-shape. 21-25mm – Brazil (Distrito Federal, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, Rio Grande do Sul), Argentina (Misiones), Paraguay (Concepción) ..... *E. emarginata* (Mannerheim, 1829)
- Black in color with a deep red undertone; mouthparts with extremities slightly round. Disc of the sternite VI with fine punctation but lacking coarse punctures. Interstria II lacking coarse rows of punctation; apex of the elytra oblong; lateral portion of the tergites with fine and moderately dense punctation; Aedeagus with paramera's inner margin curved and slightly bulbous near the postero-medial portion; parameres' basal margin rounded; parameres strongly angled downward in relation to the phallobase, forming a C-shape. 19-22mm – Brazil (Serra do Cipó) ..... *Erioscelis sp1* **sp. n.**
3. Black colored. Last abdominal segment of male simple or only slightly biemarginate at the apex. Pygidium regularly convex, less so in females. *4*
- Redish colored. Last abdominal segment of male biemarginate at the apex. Pygidium regularly convex in both sexes 5

4. Head regularly finely and rather sparsely punctate. Clypeus sides strongly contracted to apex. Pronotum finely and densely punctate in the sides, disc smooth and with little to no punctures, punctation in female stronger. Elytra very coarsely and slightly densely punctate, nearly rugose. Pygidium densely punctate, apical part much more sparsely punctate. Aedeagus; paramera with a small dilation before lateral tooth, basal portion lacking dilation. 16-20 mm. — Colombia, Ecuador, Peru, Brazil (Amazonas), Bolivia (*Cyclocephala curta* Kirsch, 1873, *nomen nudum*; *Erioscelis obtusa* Prell, 1914) ..... *E. proba* Sharp, 1877
- Head often strongly and densely punctate, stronger on the frons, finer and sparser on vertex. Clypeus sides slightly contracted to apex. Pronotum very distinctly coarsely and slightly densely punctate in the sides, fading into a fine and sparse puctation in the middle of the disc, punctation similar in both sexes. Elytra coarsely and very densely punctate, not rugose or only barely so. Pygidium uniformly densely punctures coarse. Aedeagus: paramera without dilation before lateral tooth, basal portion distinctly dilated. 17 mm. — Colombia ..... *E. columbica* Endrödi, 1966
5. Sides of pronotum slightly emarginated before basis, therefore hind angles distinct, surface everywhere distinctly coarsely punctate, on disc rather sparsely, on the sides very densely so. Last abdominal segment of male biemarginated. Sides of clypeus considerably converging to à truncated apex, anterior angles broadly rounded, surface wrinkled and punctate. Punctures of elytra forming dense rows, carinated, elytral striae hard to distinct. Pygidium coarsely and densely punctate, except in apical part which is very finely and sparsely punctate. Aedeagus; paramera very broad, lateral dilation rather long, apices acuminated. 17 — 20 mm. — Venezuela, Brazil (Pernambuco) ..... *E. sobrina* Hóhne, 1921
- Sides of pronotum rounded in the middle, almost straightly convergent both apically and basally, punctation on disc fine and sparse, on the sides much coarser, slightly dense. Apex of last abdominal segment in male simply curved. Apex of clypeus slightly emarginated, anterior angles slightly marked, shortly rounded, surface densely and coarsely punctate. Punctures of elytra forming very dense rows, intervals narrow, carinated, elytral striae mostly distinct. Pygidium with very coarse and moderately dense punctures. Aedeagus; paramera similar to those of preceding species, but slender. Female unknown. 19 mm. — Peru ..... *E. peruana* Saylor, 1946

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Figure Captions

Figure 1. Habitus of male and female *Erioscelis emarginata* (Mannerheim, 1829) (2 ZIN), as well as *Erioscelis sp1* **sp. n.** (1 CERPE; 1 CEUFPE), including the labels of each type. Scale bar = 5 mm.

Figure 2. Diagnosing characters of *Erioscelis emarginata*. A. Head and clypeus (MZUSP – 52531); B and C. Protibiae of female and male (2 CEUFPE) respectively, with arrow indicating a short third tooth. Scale bars = 1 mm.

Figure 3. Head of *E. emarginata*, detailing the ventral region of the head with setae (A) and without (B), as well as the eye (C), labrum (D), mentum (E), maxilla (F) and mandibula (G).

Figure 4. Details of the mouthparts of *Erioscelis emarginata* (A, C) and *Erioscelis sp1* **sp. n.** (C, D). Their mandibulae (A, B) and maxillae (C, D) can be differentiated by sharp or round extremities.

Figure 5. Elytra of types of *Erioscelis emarginata* ♂ (A, B, C; ZIN) and *Erioscelis sp1* **sp. n.** ♂ (D, E, F; CERPE), with detailed drawing of puncture pattern found in the elytra, with the twin striae being numbered from I-IV and an arrow indicating rows of puncture in the interstria (C, F). Scale bar = 5 mm.

Figure 6. Metaleg of *Erioscelis emarginata*: metatibia (A) (♂ UFLA) and metatarsus (B) (♂ CERPE). Metatibiae details are highlighted by drawings in dorsal view (C-D), ventral (E) and lateral (F-G); arrows indicate fused (C) and not fused (D) dorsal rows of setae, as well rows ventral (E) and lateral (G) rows of setae. Scale bar = 1 mm.

Figure 7. Sternite VI of *Erioscelis emarginata* (♀ CEUFLA and ♀ CEUFPE) (A, B) and *Erioscelis sp1* **sp. n.** (♀ CEUFPE) (C, D), with arrow indicating the coarse puncture found in the disc (A, C); and lateral of the tergites V, IV and III from left to right (B, D). Scale bars = 1 mm.

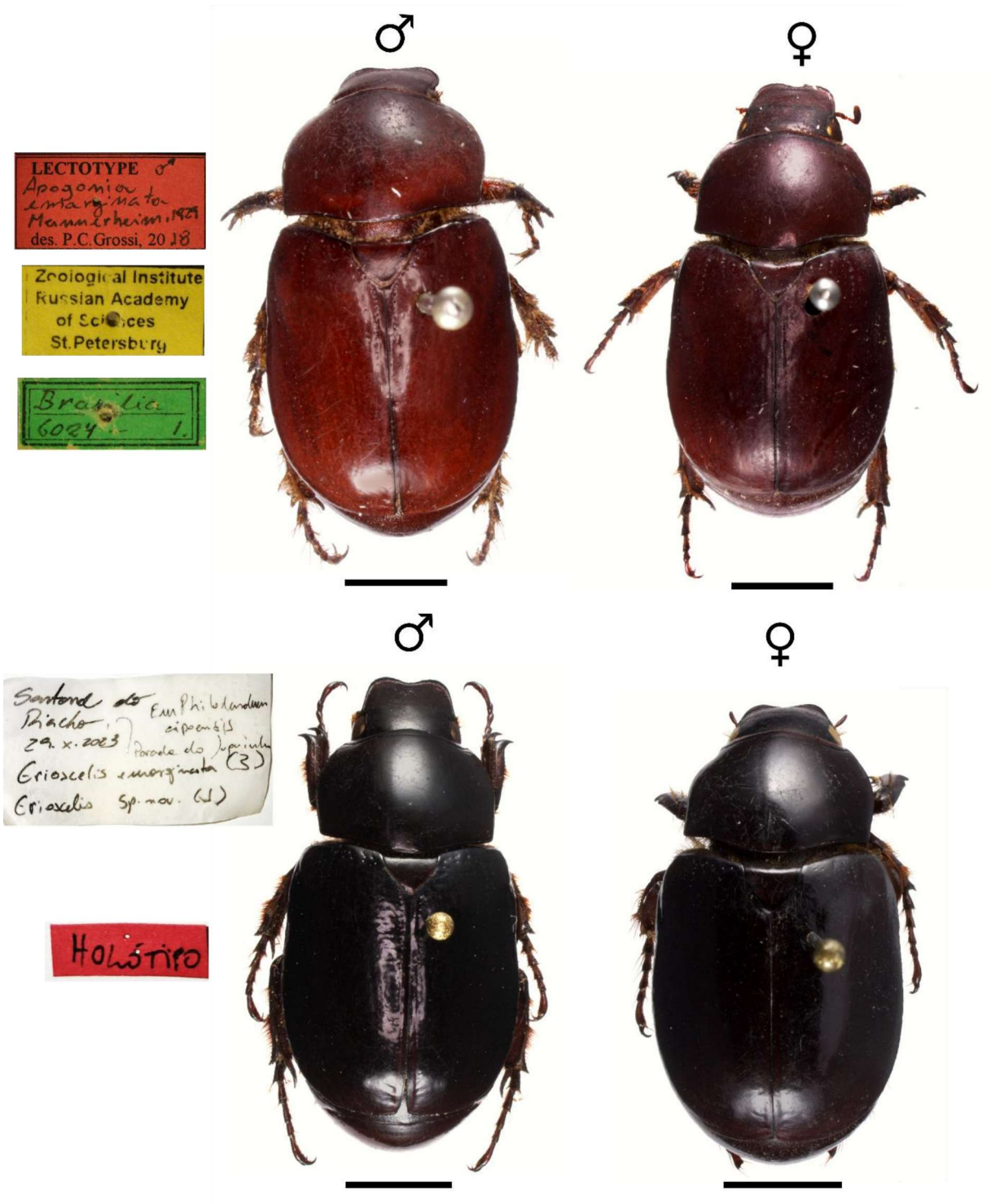
Figure 8. Abdomen of *Erioscelis emarginata* ♂ (A, B) (lectotype; ZIN) and *Erioscelis sp1* **sp. n.** ♂ (C, D) (holotype; CERPE), in dorsal view, focusing on the pygidium (A, C) and ventral view, focusing on the VI Sternite (B, D). Scale bar = 1 mm.

Figure 9. Dorsal view of *E. emarginata*'s parameres (typical morphotypes in A, E, F and southern morphotype in B, G) and *Erioscelis sp1* **sp. n.** parameres (C, H) and phallobase (D) (1 CERPE). Aedeagus, in lateral view, of *Erioscelis emarginata* (I; MZUSP – 52524) and *Erioscelis sp1* **sp. n.** (J; CERPE). Scale bar = 0.5 mm.

Figure 10. Environmental niche modeling for *Erioscelis emarginata* in Brazil (AUC = 0.96), probability of niche suitability is represented by color, as shown in the legend. Yellow dots represent occurrence data.

Figure 11. Occurrence map of *Erioscelis emarginata* (in red) and *Erioscelis sp1* **sp. n.** (in blue). Locations marked with a question mark (?) have no certain coordinates but are part of the beetle's distribution. Scale bar is in kilometers.

Figure 1.


♂
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emarginata
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Zoological Institute
Russian Academy
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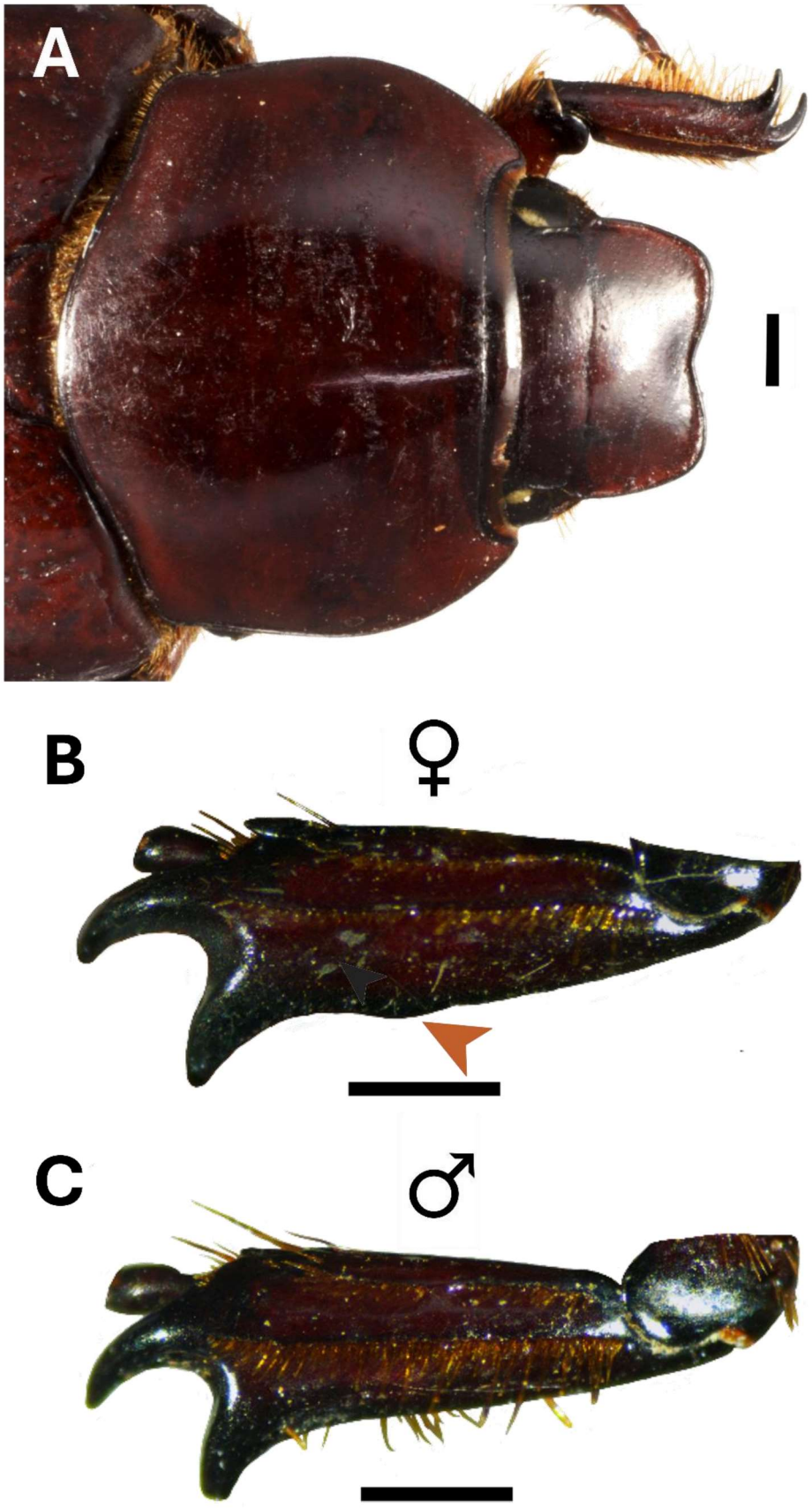
Figure 2.



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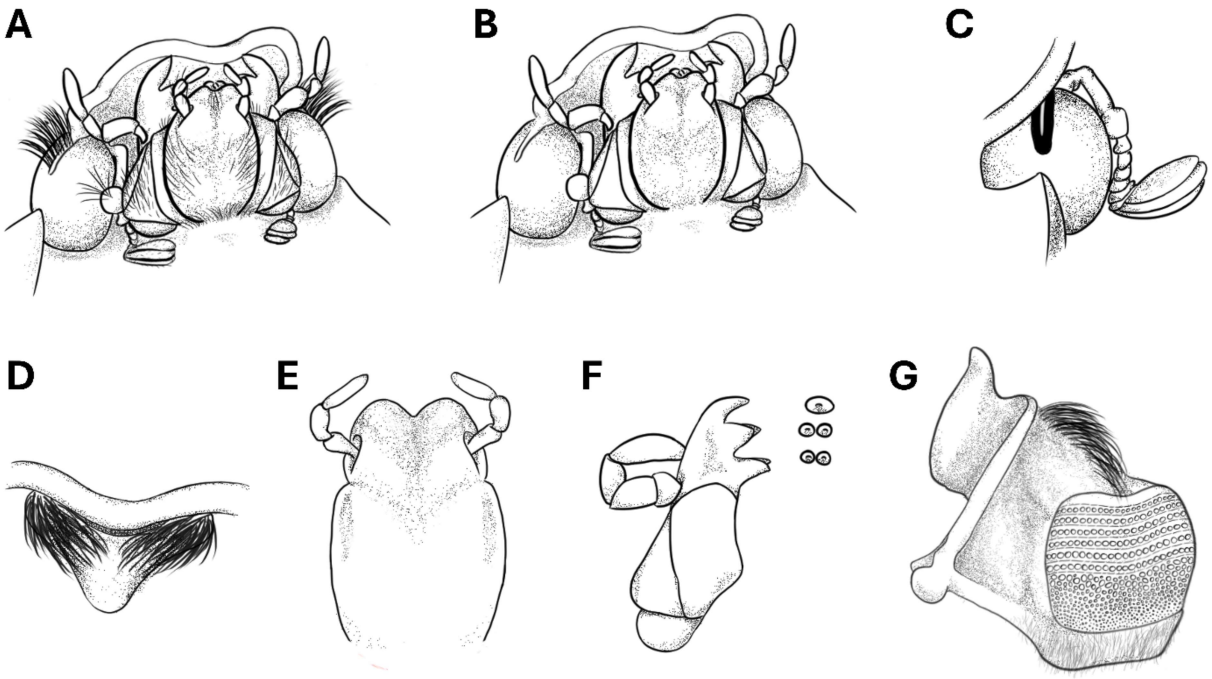

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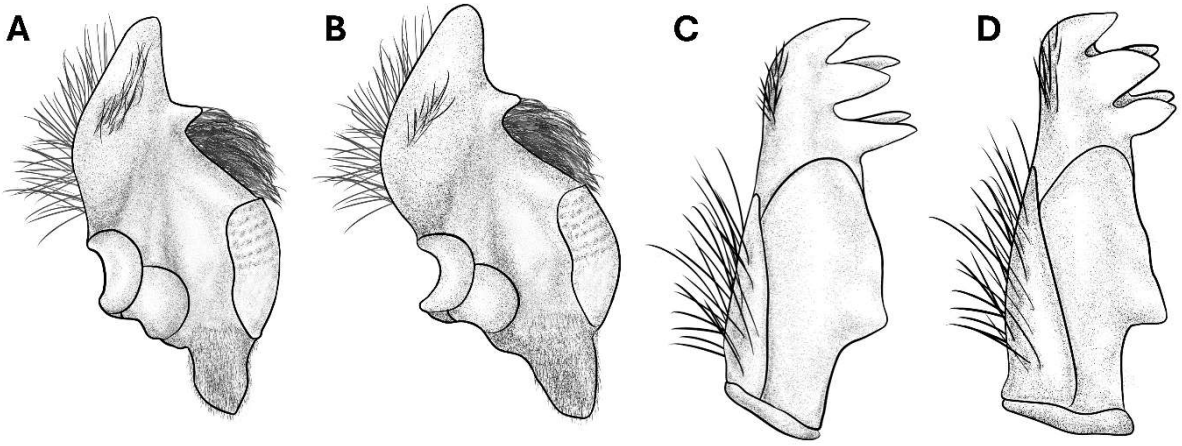

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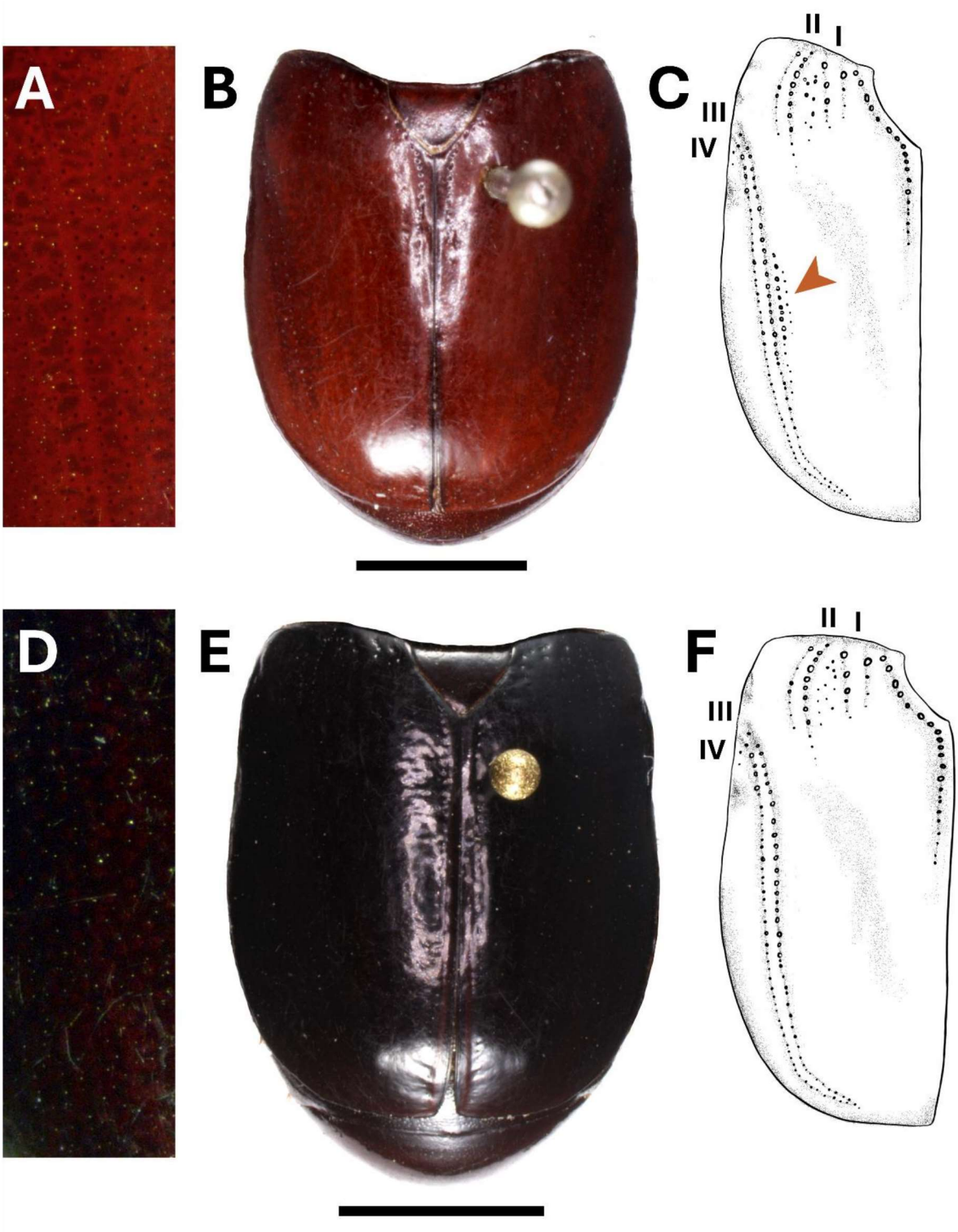

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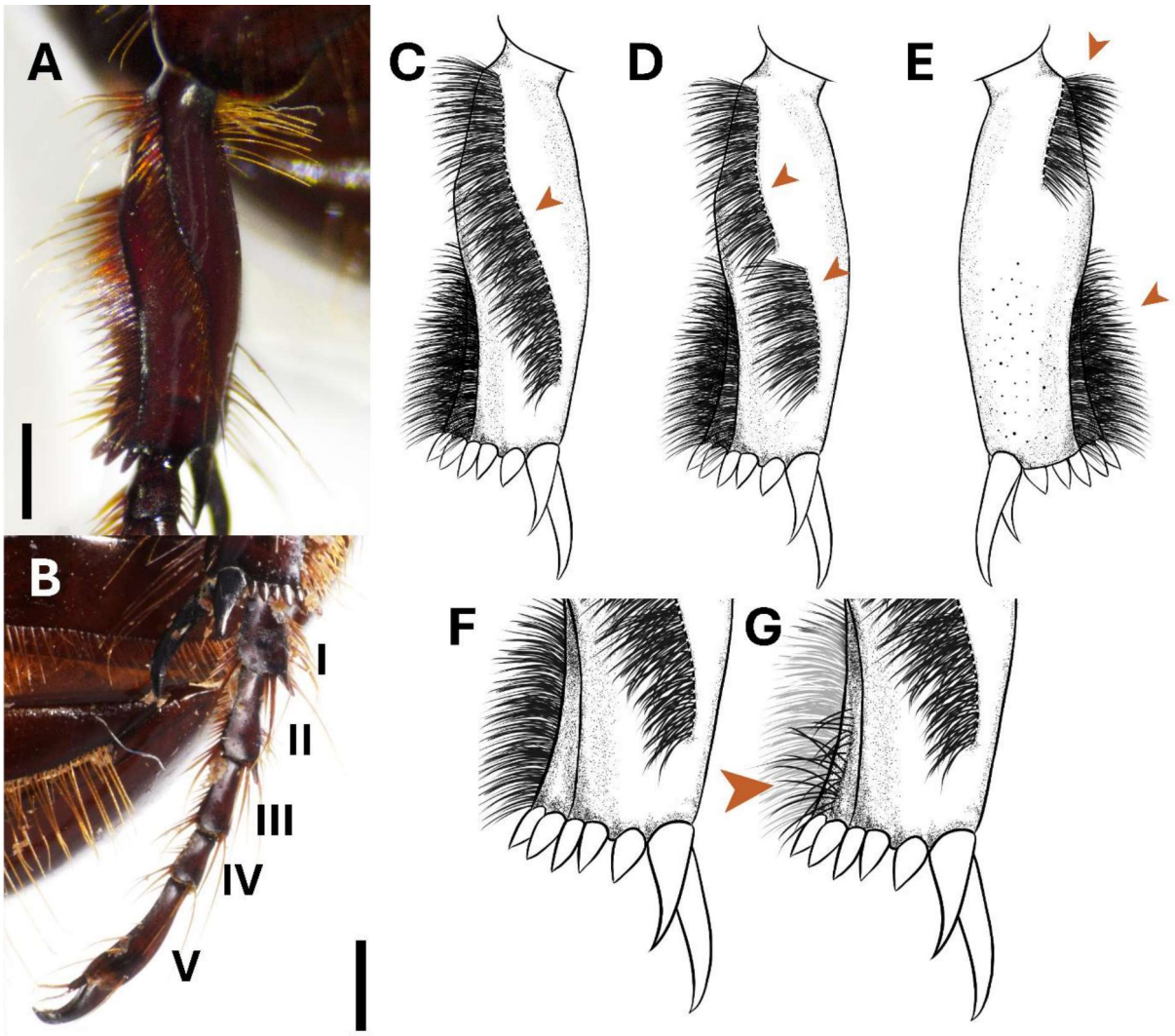


Figure 7.

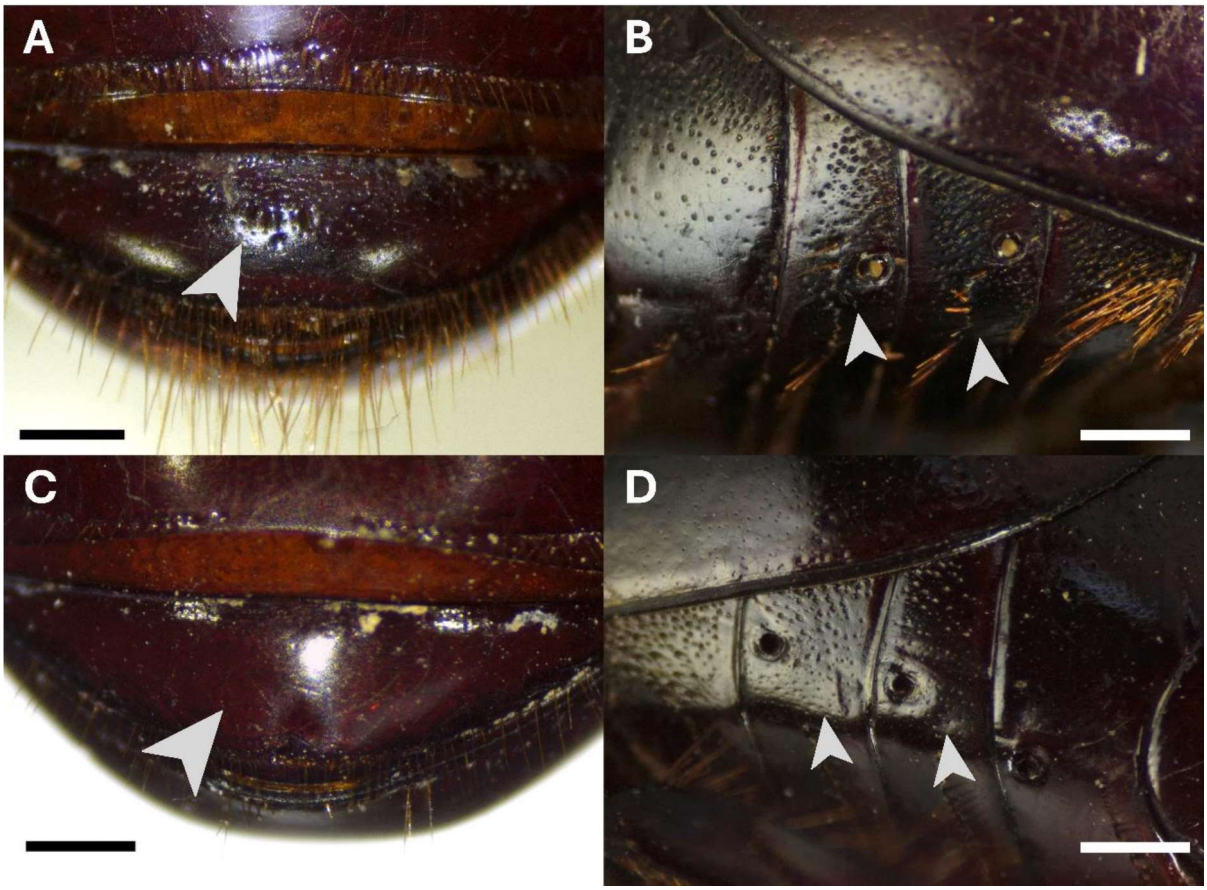
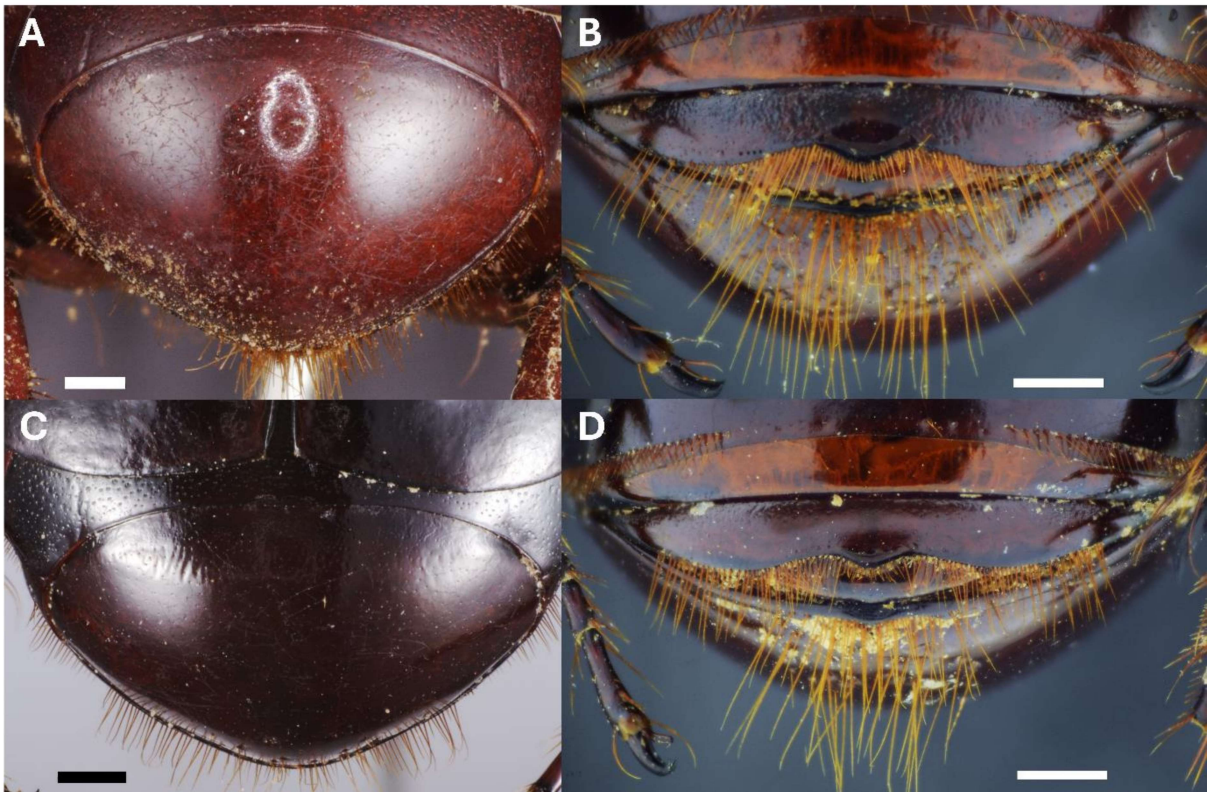


Figure 8.



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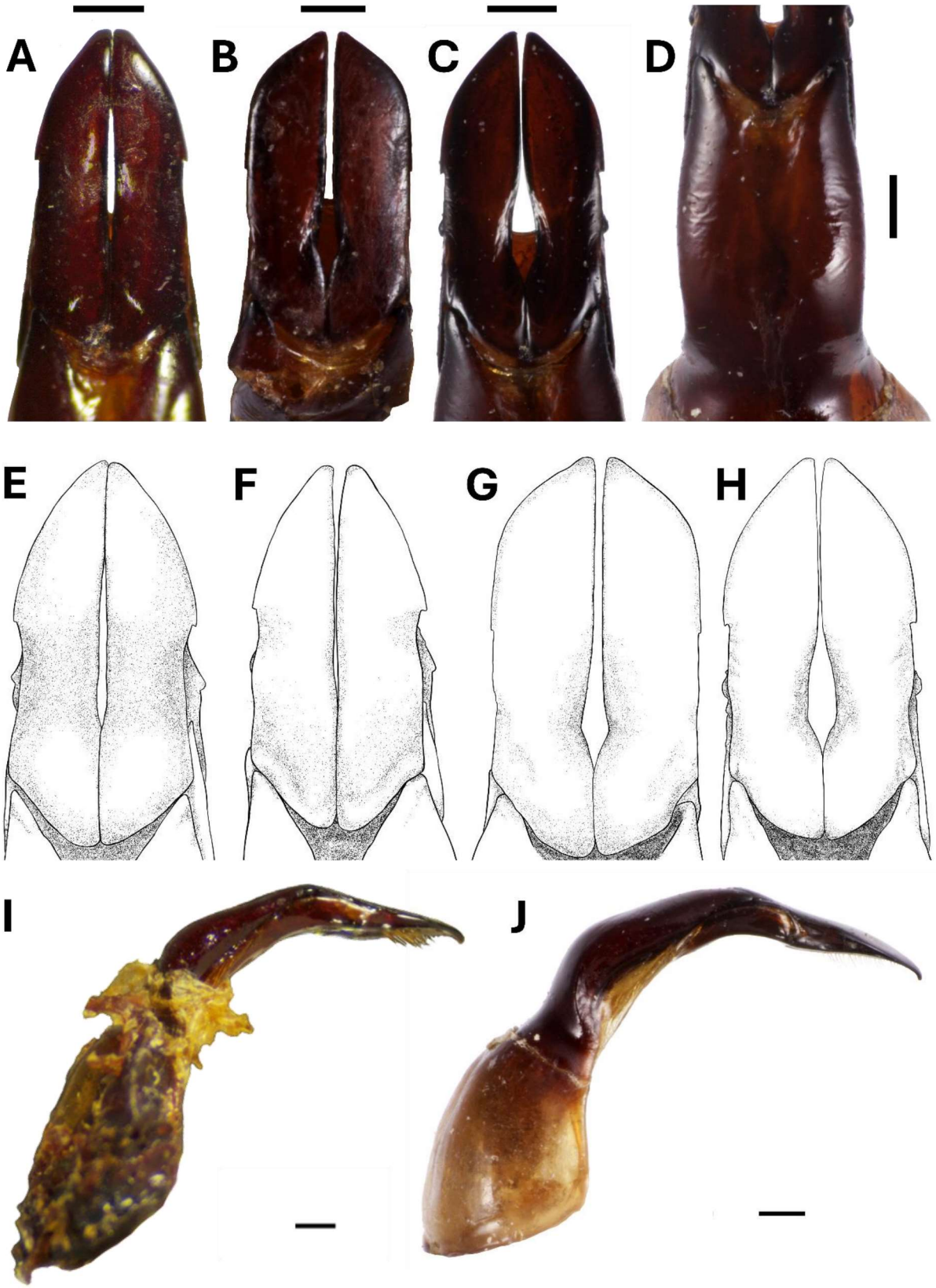


Figure 10.

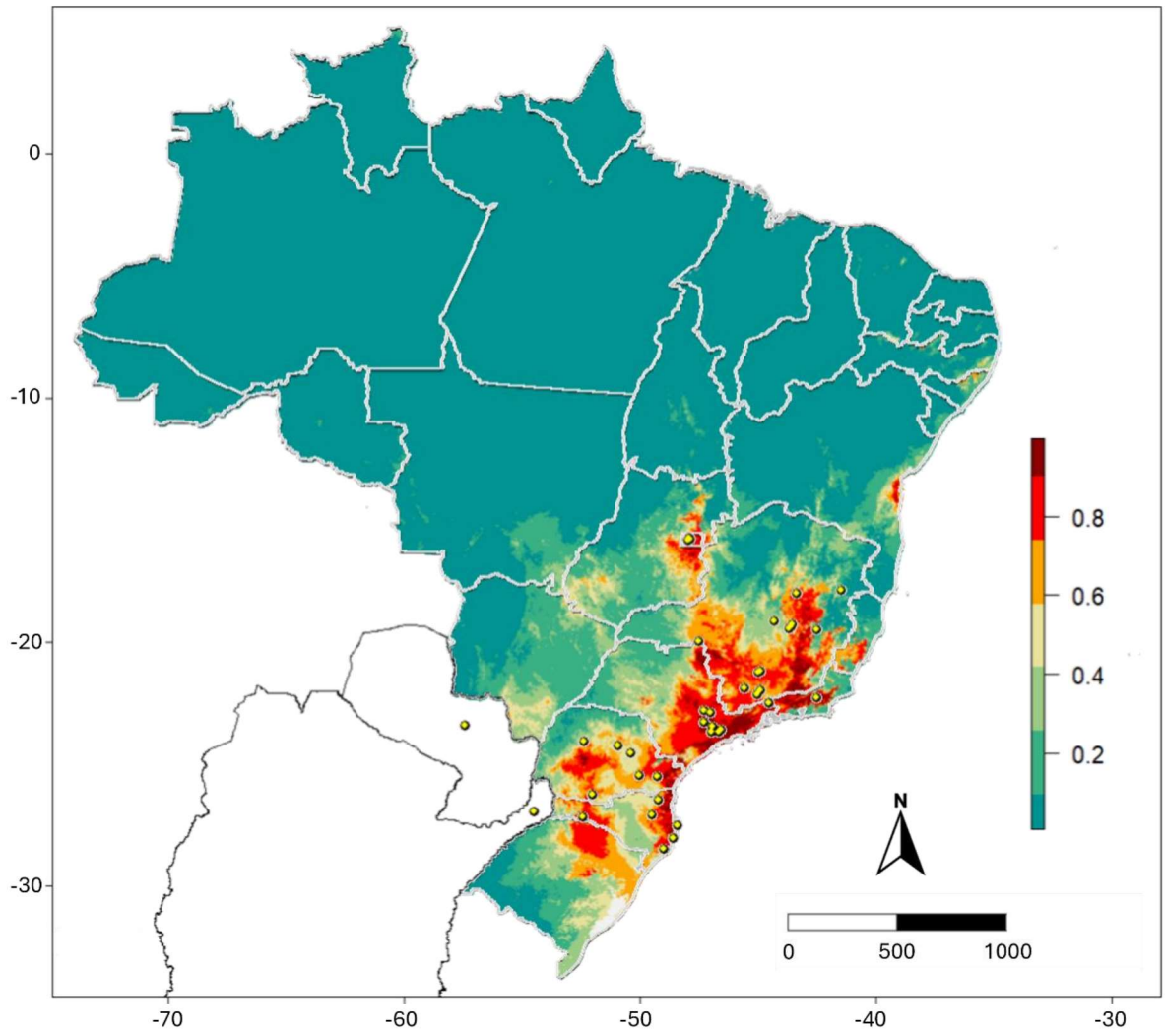
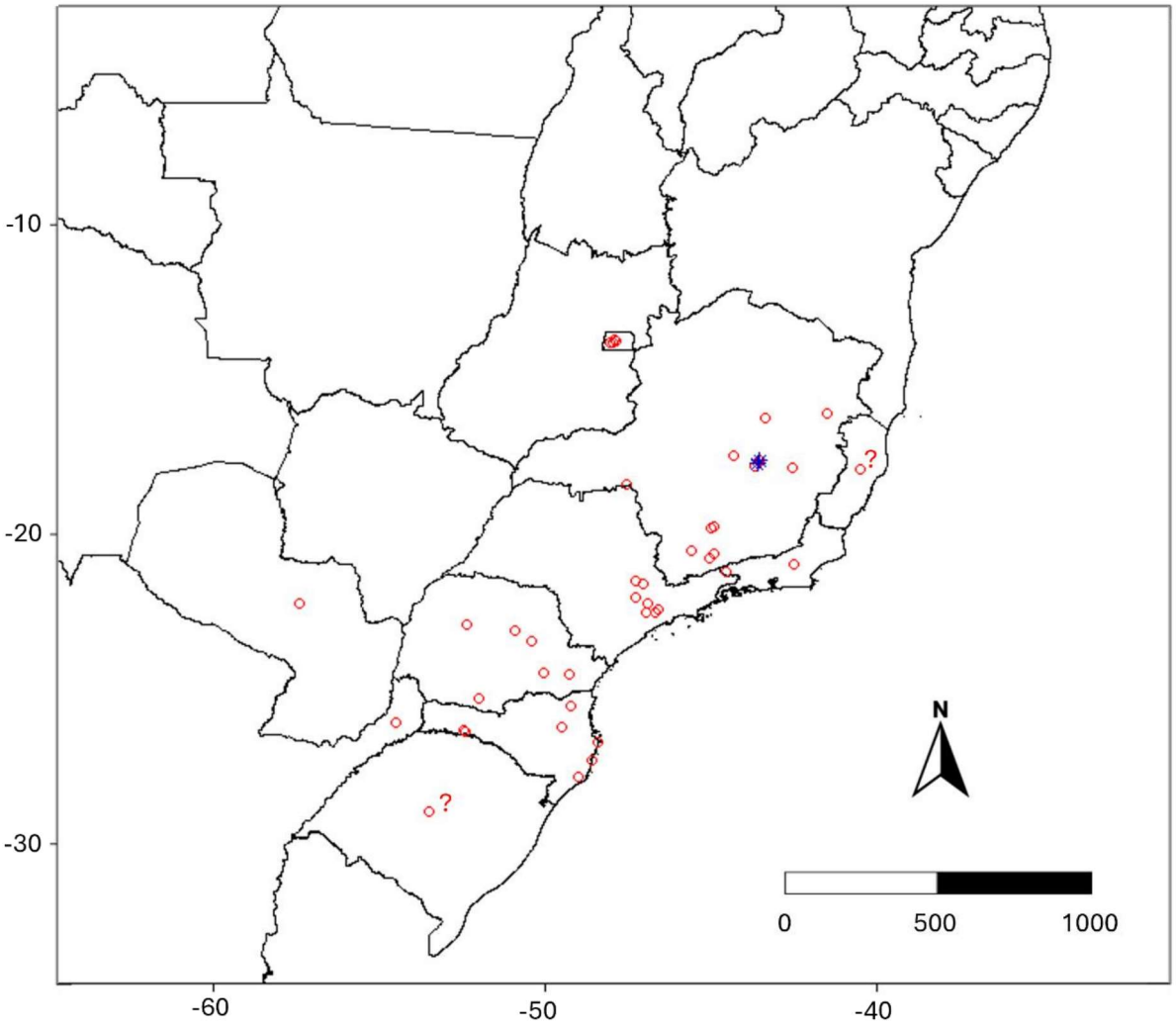


Figure 11.



5. ARTIGO 2 – Color Clustering Method: A Novel Quantitative Approach on the Study of Insect Colors from Images with an Emphasis on Scarab Beetles

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Abstract

Color is a particularly important factor in the survival and reproduction of many insects. However, the methods used to study color patterns have been relying heavily on technologies that are too sophisticated to be widely used, such as mass spectrometry and spectrophotometry. Recent studies have been able to evaluate melanization through photos of insects, but accurate quantification of all its colors has only been studied in plants using pixel clustering. The k-means clustering method can provide color codes for a *k* number of dominant colors, being very helpful for taxonomic, evolutionary and ecological studies. Here we explain how dominant color clustering can be applied, as well as the limitations for its uses in insects, exploring a case study of two sympatric populations of *Erioscelis emarginata* (Scarabaeidae: Dynastinae). Based on color clustering and PERMANOVA, we were able to confirm the subtle differences in colors between the populations, and to produce a color table that should facilitate future identification of each group. Despite the many factors that can affect the colors captured by a camera, such as the equipment, specimen preservation methods or even environmental factors, we believe that this study can greatly contribute to further the knowledge of insect coloration patterns and its role in their evolution and ecology.

Key-words: Colorimetry; K-mean clustering; Taxonomy; Color description; Comparative analysis.

Introduction

Colorimetry, the science that studies and measures color, has been the origin of many mysteries in biology because of its large variety of presentation and importance for insects (Hoffmann, 1984). There are many facets to the influence of color in survival, with benefits summarized in three categories: signaling, physiology and body protection (Badejo et al., 2020). Following this logic, signaling is the most direct aspect, encompassing not only mimicry and camouflage that directly affects the chance of an insect to survive, but also the role of color on mating rituals, commonly used to recognize mates of the same species, as well as a means to sexual selection, particularly common in iridescence and other colors on butterfly wings (Córdoba-Aguilar et al., 2018; Ellers & Boggs, 2003). Physiology is very linked to color as well, as insects are small and poikilothermal animals, highly benefiting from absorbing or reflecting heat through pigments, for example (Córdoba-Aguilar et al., 2018). And, finally, color has also been linked to body protection, since some molecules responsible for color also play a big role in the immune system, such as phenoloxidase (Gourgoulianni et al., 2023). Each and every one of these aspects can play a big part in evolution in general, as they affect the fitness of an individual and therefore can directly affect speciation.

Nonetheless, while study areas that analyze form went through a recent revolution, mainly due to geometrical morphometrics (Slice, 2007), most of the methods employed to evaluate color are rather established in studies on butterflies, with a few recent additions from eusocial insects (Badejo et al., 2020). And yet, not only are some of these methods hard to apply to groups other than Lepidoptera, but most are also too expensive to be widely employed. The use of genetical analysis is a fitting example of this scenario. Not only are there evidence that very few genes are responsible for color markings in butterflies, but there have also been many studies involving their color genetics (Parchem et al., 2007), resulting in a large genetic library that can be consulted for new studies and therefore enabling large scale analysis of color evolution (Beldade et al., 2008). Though other insects, such as hymenopterans (Badejo et al., 2020; Miyazaki et al., 2014), have been building up new date on the genes that affect color, the lack of data base already available can make it harder and more expensive to use these factors to analyze color.

Other common methods include mass spectrometry, which analyzes the substances a sample is composed of by measuring the mass of each molecule after

ionization (Siuzdak, 1996), and spectrophotometry, which analyzes how much light a substance can absorb at different wave lengths (Kafle, 2019). Spectrometry has been vastly used in butterfly studies due to its capacity to properly identifying the molecules responsible for the color patterns (Thuy et al., 2021), and though there is a need to have data banks on the molecules being observed, even knew discoveries can be compared since mass numbers do not change. This method has limitations, however, discerning only the molecules and not expressly the colors being displayed; other setbacks include the need to destroy samples, complicated when dealing with museum specimens, and being rather expensive (though chromatography is a viable substitute, results may not be as clearly read) (Siuzdak, 1996). Spectrophotometry, on the other hand, is able to perfectly obtain the colors displayed by the sample, and recent advances has made it possible to avoid the need to destroy the samples, enabling recent studies on butterflies (Tuomaala et al., 2012). However, such machines can be even more expensive and hard to use, also not necessarily obtaining as clear information about what molecules are responsible for the colors.

There are also more simple and cost-effective methods that rely on taking photos and analyzing the color variation in them (Badejo et al., 2020). The analysis of color through these methods can be a bit rudimentary at times, with hymenopteran studies making use of random pixels in photo editing software (Miyazaki et al., 2014) and the proportion of the pigmented area (de Souza et al., 2014; Tannure-Nascimento et al., 2008). However, the method of determining melanization levels has been very established, widely used in butterflies and recently also applied in hymenopteran studies (Badejo et al., 2018; Ellers & Boggs, 2003). For this technique, photos are converted into black and white and a software such as ImageJ is used to infer the percentage of melanization observed (Ellers & Boggs, 2003). While it is very helpful for comparing colors of different groups of insects, it highly depends on one of the groups being darker than the other and does not take into account other more subtle color differences, such as whether there is more of a blue hue or red hue.

There is, however, a method vastly employed by designers when dealing with photos and pictures in order to extract a detailed palette of the dominant colors that can be found in an image, based on clustering pixels that have similar colors (Chang & Mukai, 2022). Since this method is usually based on the three dimensions of color deemed red, green and blue (RGB) (Chang & Mukai, 2022), it can extract detailed variation on what colors are being more or less dominant in a picture besides just their

melanization. Although, to the best of our knowledge, this method has yet to be used in zoology studies, it has already been used in to study the color of plants (Gibert et al., 2022) and its use could fill the void of these methodologies when it comes to insect colors. Such an easy-to-apply methodology would not only benefit studies on population and community dynamics, but also have an impact on how color is treated in taxonomy.

From all the fields in biology that come in contact with color, taxonomy has seen the least amount of evolution on how to approach it. Even though systematic studies have found ways to include color in phylogenies, such as through molecular variables (OTAKI et al., 2006; Sagegami-Oba et al., 2007), the clades' description in entomology has mostly clung to subjective popular names of the observed colors, depending on how the author perceives the color. However, color is an especially subjective character, as each person may perceive it differently, either calling it by different names or not even perceiving enough variation when there could be many (Nassau, 2001; Winawer et al., 2007). This ambiguity can both lead to the need of corrections of a described color, but also impair the ability to correctly identify a species according to color. *Erioscelis emarginata* (Mannerheim, 1829) beetles are a notable example of this problem. This species has originally been described as "body pisceo-castaneus", a Latin term that implies mainly a darker brown (Mannerheim, 1829); however, later redescriptions have gone back on this color and described it as "reddish brown" after examining the type material (Endrödi, 1966; Endrödi, 1985; Saylor, 1946). This distinction is particularly important when considering that there is a morphologically different population of this beetle, sympatric to the type location, that has colors that are so dark it can be considered black, a color closer to its congenerics (Endrödi, 1985). Understanding how to deal with color variations that are hard to discern just by looking can facilitate studies with many other beetles, especially ones that can greatly vary in color such as Chrysomelidae and Coccinellidae (Ando & Niimi, 2019; Strickland et al., 2019). It would also be helpful to identify larvae stage, since color has already been shown to be an effective discerning character, but variations on larvae are often subtle (Wizen & Gasith, 2011).

Therefore, in order to facilitate the study of color in insects and its description, this paper introduces the use of dominant color extraction by k-means clustering as a method of studying color variation in the insect cuticle. Though this is not the first time this method is being used to study color differences in biology, this is the first known

instance in which it is suggested for insects and, therefore, here we evaluate all the factors that may influence its use when considering the particularities of this group, including factors that may play a part in color variation. We also explore how to apply the method, with a special focus on taxonomy, by using two sympatric populations of *Erioscelis emarginata* as a case study on colorimetric analysis.

Dominant color method

The proposed methodology analyzes the dominant colors present in a photo based on K-mean clustering, according to functions known from the R program v. 4.2.2 (R Core Team, 2022). Here we focus our efforts in the function "extract_colours" of the "rPlotter" package (Jacquemoud & Purdon, 2006), that uses the "kmeans" function in the "stats" package (R Core Team, 2019). We explain how K-mean works for clustering colors and how to build a script for this package in order to properly use it.

All photos taken to illustrate this study were taken with two equipments: Canon EOS Rebel T100 with an EF-S 18-55mm f/4-5.6 IS STM lens; Nikon D5300 attached to Zeiss Stemi 506 stereo microscope.

Dominant colors extraction – Calculation

In order to extract the dominant colors present in a picture, the "extract_colours" function can be surmised in four steps: I) loading and resizing the pictures (maintaining the aspect ratio), important to reduce time of processing and noise that could cause overfitting of the clustering (Ying, 2019); II) melting the image into a long-format data frame of RGB values and then reshaping it into a wide-format data frame (suitable for clustering); III) color clustering through the use of "kmeans"; IV) translation of the dominant colors of the clusters into the Hexadecimal code (HEX) and plotting the dominant colors.

To apply the K-means clustering to gather dominant colors, the act of partitioning the data into k clusters $\{C_1, C_2, \ldots, C_k\}$ and defining its centroids $\{\mu_1, \mu_2, \ldots, \mu_k\}$ is random in the beginning, but will need to be repeated until the total sum of squares from the points in all the cluster is at its minimum (R Core Team, 2023), calculated by the following formula:

$$J = \sum_{i=1}^{k} \sum_{x \in C_i} \|x - \mu_i\|^2$$

Where *x* is a point in a *i* cluster (C_i), with μ_i as is its centroid. Each centroid is calculated based on the medians of the RGB values of *n* points present in the cluster (R_j, G_j, B_j):

$$R_{\mu_i} = \frac{1}{n}\sum_{j=1}^{n} R_j \qquad G_{\mu_i} = \frac{1}{n}\sum_{j=1}^{n} G_j \qquad B_{\mu_i} = \frac{1}{n}\sum_{j=1}^{n} B_j$$

Finally, with the centroids with the minimal differences correctly picked, the function translates the RGB values displayed by these points to the HEX color code. Though not explicitly stated, this is probably done due to the nature of using color in coding, being easier to deal with HEX codes than RGB values. This translation, thankfully, can easily done and easily reversed due to the nature of both codes: RGB describes color according to values from 0 to 255 on each of the three dimensions, with 256 possible variations in each (Duckett, 2011); HEX, on the other hand, uses alphanumerical coding, consisting of numbers from 0 to 9 and letters from A to F (representing 10 to 15), one pair for each color dimension, which leads to 256 possibilities for each dimension as well (Robbins, 2012). As an example, pure red would be displayed as (255, 0, 0) in RGB, but #FF0000 in HEX (FF = 16 * 16 = 256).

How to script the extract_colours function

Before using the “extract_colours” function, some preparations of the materials are needed. Besides downloading and reading the necessary package libraries, one should add the file path where the R program can find the images as well as build a table to later receive the color codes to be acquired. If the images are not to be read in their entirety, be sure to crop them properly before running the code. Below is an example on how to achieve such a result:

```
#file path
files <- list.files(path = "C:/Users/mcamo/Downloads/Colorimetria t1/cores",
                    pattern = "\\.png$", full.names = TRUE)
#creating table to extract 5 dominant colors found in the photos
color_table <- data.frame(File = character(), HEX1 = character(),HEX2 = character(),
```

```
                HEX3 = character(), HEX4 = character(), HEX5 = character(),
                stringsAsFactors = FALSE)
```

Each image will need to have their colors extracted and inserted in the table, so making the table run in iterations is the best solution. In the iterations, the colors extracted can be separated into columns for easier use later on.

```
for (i in 1:70) {
  #which file to use (by iteration)
  file = files[i]
# Function to extract colors from an image, adding each color to a column of the table
colors <- extract_colours(url_img = file, num_col = 5, rsize = 200)
a <- gsub("#", "", colors[1])
b <- gsub("#", "", colors[2])
c <- gsub("#", "", colors[3])
d <- gsub("#", "", colors[4])
e <- gsub("#", "", colors[5])}
```

This function can be altered to cluster any number of dominant colors by changing the number inserted in “num_col”. The other parameter, “rsize”, is used in order to standardize the size of the pictures, avoiding too much data to be compared and overfitting during clustering (Ying, 2019). As a result, *n* color codes will be shown in HEX color code (in the example above, these codes are being fed to the table and therefore can be assessed latter on).

On account of the number of dominant colors that should be extracted, it will mainly depend on how many colors appear to be displayed on the color pattern of the chosen structure, as well as the level of “error colors” that are captured, derived by debris and imperfections. Insects usually present an array of dirtiness on their surface, be it dirt or pollen and other such debris, and some can be found with a waxy surface, adipocere, or even fungi and algae (Higley et al., 2016; Schneeberg et al., 2017). Though cleaning the specimens, be it chemically or mechanically, may help avoid errors, other factors such as physical imperfections need to be accounted for, such as scratching. Avoiding these imperfections by discarding the most affected individuals is required, but it is not always possible to find enough specimens in perfect conditions, particularly when working with insect collections. Therefore, the total sum of colors acquired should take into account the number of colors needed to display all the observed pattern and it’s variations, as well as enough “error colors” to later be

discarded. As a rule of thumb, five colors should suffice for unicolored species: one color as the lighter variation, one as the mid tone, one as the darker tone, two colors to be discarded. If the specimens are in very good condition, four colors might suffice. For multicolored animals, check the data before running the analysis to make sure the colors are being correctly compared with each other; this clustering method ranks each color by the proportion of pixels displaying it. Although it should be rare, a pattern may vary enough in a group that a color may shift ranks in some specimens, so checking that all colors are correctly compared to each other can avoid errors in the analysis.

Though HEX coding is the best color format for coding and graphing, non-qualitative statistical analysis demands numeric values to function, making HEX not suitable for such analysis due to its alphanumerical nature (Robbins, 2012). However, since translating HEX and RGB is very straightforward and does not loose information, as stated before, translating back the HEX code to RGB is the easiest way to acquire entirely numeric data. Though possible to be done in in any step, the translation is more easily executed while extracting the table. Following the example established, one could simply add columns to the table created before starting the clustering of colors and add the following lines to the iteration that extracts the colors (making sure to repeat the code for each color as to easily add them to the table):

```
rgb <- paste(as.vector(col2rgb(colors[1])), collapse = " ")
rgbs <- strsplit(rbg, " ")[[1]]
r <- rgbs[1]
g <- rbgs[2]
b <- rgbs[3]
```

With these numbers separated in columns, statistical analysis dependent on values can be run by considering each color dimension (e.g. R from dominant color 1) as a variable. Although reading the analysis results will depend on the statistics used, it will also indicate what dimension of color is affected by the group differences: red, green or blue; not just whether the colors are different or darker.

Photos – shooting and treating

Another very important step to consider when applying this technique is the act of taking the photos. When using a camera, the light that hits the sensor is typically recorded in RGB code, applying a number from 0 to 255 to each of the three-color dimensions (Freeman, 2013). Due to that, all factors affecting how light hits the sensor

and how the sensor computes the color can induce methodological errors that need to be considered while doing a study on color with photos, including uniformizing all the equipment chosen to take photos. Most of the changes, however, can be attributed to lighting, as exposure has a big effect on what is acquired (Freeman, 2013). Although the structure needs to be well illuminated, hard lighting can induce a shiny surface and heighten the contrast of a photo, risking to loose details and information on the color naturally observable (Freeman, 2013). To avoid this problem, diffusing the light might be necessary, which can be easily achieved by using parchment paper (MasterClass, 2021) either in the light source or covering the specimen. Another great strategy is to bounce the light off other areas that are not being analyzed, but this must be done carefully to illuminate all specimens in the same manner and angles.

It is also important to note that in addition to lighting, the exposure of a photograph is entirely dependent on three other factors: the lens aperture, which regulates the amount of light that has room to pass through the lens; the shutter speed, which regulates the amount of light that has time to pass through the lens; and the ISO settings, which regulates the sensitivity of the camera sensor to light (Freeman, 2013). For this reason, it would be advisable to include the settings used on the camera when disclosing the materials and methods of a study.

Finally, proper post-photo editing can help standardize the lighting errors that may have occurred during the shootings, but editing should be restricted to correcting the lighting values to bring the photos closer to the natural appearance of the specimens. Although photo editing can be done in softwares such as Photoshop and Lightroom (Freeman, 2013), batch editing would produce a better end result in this case, as it could better unify the output. The best software currently available to the author's knowledge is PhotoScape X v 3.7 (Mootools, 2014), able to batch edit with filters that automatically correct contrast and backlighting. Any filters used should be described in the methodology.

Structural coloring

Finally, one should also consider that the colors displayed by insects can be derived from pigment or cuticle structure, though usually both can be responsible for it (Badejo et al., 2020; Hoffmann, 1984). Capturing light from pigment with a camera is a straightforward process, as pigment can only absorb some wavelengths of light and reflects others (Vigneron & Simonis, 2010) that will be captured by the camera, which

will define the color displayed. When it comes to structural colors, however, what wavelength is reflected depends on the structure where the light hits, able to change depending on the angle of the light source (Hoffmann, 1984; Vigneron & Simonis, 2010).

Iridescence is one of the easiest to recognize structural colors, as the change in colors is visible when the light source shifts the angle and is usually derived from thin-film or multilayer interference, as well as grating (diffraction) and photonic crystals (Kinoshita et al., 2008; Vigneron & Simonis, 2010). Many insects display iridescence in their cuticle, such as the *Morpho* Fabricius, 1807 butterflies, Jewel beetles and *Chrysina* Kirby, 1828 scarabs (Kinoshita et al., 2008). Since iridescence implies a change in color depending on the angle of the source of light, it is possible to capture both the non-iridescent hue, as well as all the colors it can change into depending on how the insect is lighted during the photoshoot, and therefore each color can be properly analyzed with the clustering method (Fig. 1). The technique can be a bit trickier to master, but many sources can be helpful on for photographing insects (Parent, 2023).

Another well-known structural color that insects can display is ultraviolet (UV) reflection, more frequently studied in butterflies (Kemp, 2008; Obara et al., 2008), but the use of the clustering method is not as easy in this case. Although most camera sensors are able to capture UV light, cameras usually come with filters and are therefore incapable of capturing UV photos unless modified; and even then, the camera will probably need UV transmitting filters and if lens to work (Davies, 2017). All that considered, the use of this method for UV markings may demand more funding and expertise than for other colors, which may not be the best solution considering there are already well stablished solutions for taking photos (similar to melanization) and many methods of in situ and ex situ experimentations customarily used in butterflies that can be taken as inspiration (Kemp, 2008; Obara et al., 2008).

Case study

In order to understand how to evaluate differences in color with the clustering method, here we exemplify its use by evaluating the dominant colors in the elytra of two sympatric populations of the beetle *E. emarginata*, discernible through external morphological characters and also known to display different color hues (Fig. 3; personal observation). For this study, 26 specimens were assessed from five

entomological collections (curator in parenthesis): CERPE – Coleção Entomológica da Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil (Paschoal Coelho Grossi); CEUFPE - Coleção Entomológica da Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil (Luciana Iannuzzi); MZSP - Museu de Zoologia, Universidade de São Paulo, São Paulo, São Paulo, Brazil (Sônia Aparecida Casari); ZIN – Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia (Andrey Frolov). From these, 23 specimens with less scratched surface were selected to have their left elytron photographed by an Axiocam color 105 coupled to a Leixa MZ6 stereomicroscope.

The photos were then treated in the software PhotoScape v3.7 (Mootools, 2014) in order to uniformize color variation (filters used: autocontrast and backlight correction), and finally cropped into 1mm transversal strips of the widest part of the left elytra, avoiding areas where it was too shiny due to the lustrous surface of the beetle (Fig. 2). These strips were then fed to the R program v4.2.2 (R Core Team, 2022), using the "extract_colours" function in order to obtain the five dominant colors in the photos (k = 5). Only the three first colors were translated into RGB coding and analyzed through PERMANOVA (post-hoc pairwise) and NMDS between population A (n =14) and B (n=9), with the last two colors being considered as "error colors", especially considering the scratching of the surface and some fat that could not be properly removed. Each of the color dimensions was considered a different variable.

As a result, there was a clear difference in colors displayed by populations A and B ($F_{1,21}$ = 31,24; P < 0,001; Fig. 2). Population A presented a warmer color scheme, redder in tone, while population B displayed darker, cooler colors, closer to black and with higher values of blue and green than population A. In case this difference was to be discussed in a taxonomic paper, displaying the colors such as can be seen in Table 1 can help better visualize the whole of the variations of the colors that can be expected while dealing with each group. For this table, the maximum, minimum and mean values of each dimension of color (RGB) was taken from each dominant color, so that the table can more easily surmise the raw data obtained from each specimen (Fig. 2).

Applications and limitations

As it can be seen with *E. emarginata*, the use of dominant colors extraction by k-means clustering is very simple to put into practice, less demanding than other

methods and can be used for both population comparisons and taxonomical descriptions. The importance of a readily available possibility to ascertain slight nuances in color is especially clear in these beetles. Although it may seem like there is a clear difference in the coloration of the elytra between the two populations, some of the differences can be barely distinguishable to the eye. With this detailed analysis, we can lay the data on a table (Table 1) and confirm that the colors are in fact barely overlapping, with an almost clearcut distinction of what population display what color (Fig. 2).

Considering these results, there is a pressing issue in the descriptions of this species coloration. When considering the darker hues on population B, it would be a better fit with the original description of *E. emarginata*, that mentions a darker brown rather than a reddish brown (Mannerheim, 1829), and also be rather similar to the black of other congeneric species such as *E. columbica* Endrödi, 1966 and *E. proba* Sharp, 1877 (Endrödi, 1985). However, not only does the holotype of *E. emarginata* show a lighter reddish brown, in accordance with later descriptions (Endrödi, 1966; Endrödi, 1985; Saylor, 1946), but so does most other populations of this species from locations other than its type (personal observation; Fig. 3). Inconsistency on the labels used for coloration can cause confusing and impair the ability to properly identify species. Since the clustering method can extract exact colors, such variations can be avoided and tables including most of the observable variation can help avoid the subjectivity of color describing as it stands today (Table 1), being therefore able to complement the descriptive methodology currently in place in favor of a wider comprehension of what can be observed.

Even though this method can be very helpful when used in taxonomical and ecological studies, caution is needed when treating color as a character. Color patterns displayed can depend on many factors and vary both inter and intraspecifically (Chapman, 1998; Hoffmann, 1984). Though color can be used to compare clades, it is fundamental to support changes observed with other variables, such as morphological characters related to form, molecular variation or niche divergence, and to always consider all factors that can be causing the variations observed. Here we examine some of the most common factors that could cause such differences and how they could have affected the populations of *E. emarginata*.

Material and color preservation

The preservation method used to store insects in collections is one of the most important factors to be considered when selecting specimens to run colorimetric analysis, as it can alter the color pattern of the cuticle. Novák et al. (2024) confirmed on beetle larvae that both the fixative substance used to kill the specimens and the storage substance has been shown to affect the colors displayed by the cuticle. Though the study focused on how the color can dull and loose brightness, it can also be seen in some photos how the hues displayed can turn more yellowish (Fig. 4). To avoid discoloration and other problems that may come with these substances, such as damaging the wings of butterflies, other methods that are customarily employed include freezing under -20°C and killing jars with a cotton ball infused with a killing substance (Santos & Fernandes, 2020), as these methods avoid the material entering in direct contact with the cuticle.

Though initially it may seem like this would be a huge impairment on studies made with animals from collection, the case study with E. emarginata here exemplified shows how even then the differences between groups can be analyzed. With the whole sample being derived from collections, no data could be acquired on the materials used for killing and storing the specimens, but we can assume not all specimens were subjected to the same treatments. Nonetheless, the variation that the preservation methods may have caused in the samples were similar enough in each population that comparation was still able to show clear differences in color between the groups (Fig. 2). Further studies on the amount of variation that can be expected due to preservation methods could enable considering the variation that can be expected in future studies that use collection specimens for color comparison, considering the importance of these animals.

Studying the color of collection specimens is also particularly interesting in order to facilitate taxonomical identification, at times greatly dependent on color (Rafael et al., 2024), but still being subjected to hue changes due to preservation. Therefore, describing all the colors found in specimens, including variations found in situ or in preserved animals, can help future researchers more easily differentiate clades.

Environmental variables

Namely, environmental factors are one of the most important to consider in color variation studies since it is known to induce phenotypic plasticity, with different factors inducing different morphotypes even with the same genome (Pigliucci et al., 2006).

Though sexual dimorphism is a widely known example and can hinder identification for many species (Chapman, 1998; Rafael et al., 2024), juvenile hormones are fundamental to dictate morphotype, but are also highly affected by many external factors such as photoperiod, nutrition, temperature, humidity and even population density (Nijhout & Wheeler, 1982). With that in mind, the ability to easily compare colors could facilitate the use of color as a variable to better understand environmental factors and how they affect ecology and evolution.

For factors that affect phenotype through hormones, the combination of molecular analysis with color variables could enlighten what is varying. And yet, some factors are also capable of directly influencing the colors displayed. As a great example, higher temperature is known to be able of enhancing the activity of phenoloxidase enzyme during the larval stage, a fundamental molecule for both melanization and the immune system of insects (Gourgoulianni et al., 2023). As the production of pheomelanin is highly dependent on the presence of thiols, but eumelanin can be produced independent of its presence, a higher activity of phenoloxidase during the larval stage could induce a higher production of eumelanin and, therefore, darken the color of the specimen (Gourgoulianni et al., 2023; Sugumaran & Barek, 2016). A similar effect could be seem changing the diet of the larvae, ingesting more thiol rich substances would lead to a lighter population (Sugumaran & Barek, 2016).

These possibilities are especially interesting when considering the case study of *E. emarginata*, that have similar coloration and undertone, but a different level of melanization. As both can be found in the same locality, the same type locality, it can be assumed that such changes are not directly related to local weather conditions. However, larvae live in microenvironments that do not depend exclusively on the macro-scale factors but are highly susceptible to change in temperature and moisture (Johnson et al., 2010; Lepage et al., 2012). Soil type, vegetation and altitude can influence the temperature of the soil, as well as availability of dietary resources (Oliver et al., 1987; Shreve, 1924). If the color change that was observed did not in fact derive from genetic differences but environmental influences, the niche of these populations may diverge further during its developmental stage than initially assumed. Further studies on the genetic profile of these populations could help elucidate not only what is causing these color changes, but also new ecological knowledge indirectly.

Similarly to temperature, humidity can also affect the displayed color not only by activating dormant parts of the DNA, due to phenotypic plasticity (Hoffmann, 1984), but it can also affect the color pattern in an even more direct manner than temperature. One of the best-known cases is *Dynastes hercules* (Linnaeus, 1758), known to change color when exposed to different levels of humidity, greenish under normal conditions and black under very high humidity (Rassart et al., 2008). This phenomenon occurs due to the nature of light and structure of insect's cuticle. The green color normally observed in this beetle is actually due to a 3D structure in its cuticle, not pigment, and when water inflates this structure, it changes the refractive index and therefore changes the perceived color to black (Rassart et al., 2008). Though not a common phenomenon, other insects are known to show such effects as well, such as some *Plusiotis* Burmeister, 1844 (Scarabaeidae) and *Aspidomorpha* Agassiz, 1848 (Chrysomelidae), metallic beetles that may even influence water influx according to pH levels (Neville, 1977).

When it comes to nutrition, much of its influence is indeed direct, since the productions of many molecules depend directly on what is or not ingested, and not only on what is decoded from the genotype (Futahashi & Osanai-Futahashi, 2021). In addition to the importance of the intake of thiols for the melanization cycle, as discussed for the *E. emarginata* beetles (Sugumaran & Barek, 2016), carotenoid pigments, responsible for some red, orange and yellow colors, are also entirely dependent on what is eaten, and their presence can even indicate the capacity of a lineage to properly digest and metabolize certain substances (Chapman, 1998; Hoffmann, 1984). Flavonoids also fall into this category since they are plant pigments that can be found in plant-eating insects (Chapman, 1998). A dietary imbalance could also lead to differences in resources allocation (Chapman, 1998), which could result in increased or decreased production of pigments and other cuticle components.

Pollution is a very interesting factor in color changes, especially considering that many insects are already used as bioindicators due to variation caused by environmental stress (Rafael et al., 2024). Both melanin and carotenoid pigmentation have been well known to be directly affected by the presence of pollution such as heavy metals and pesticides, in vertebrates (Lifshitz & St Clair, 2016). Many insects are also known to change color or exhibit pigmentation defects, especially in orders already widely used as bioindicators such as Odonata, Hymenoptera and Lepidoptera (Bybee et al., 2016; Kozminov et al., 2021; Skaldina & Sorvari, 2017). The change in the

environment itself is also known to cause interference in selective pressures, which can cause, for example, the over melanization of some moth species due to industrialization (Riley, 2013).

All these environmental factors, and the many others particular to each group, bring forth and array of questions that could be analyzed with the help of colorimetric analysis. At the community level, color can be used as a biomonitoring tool (Bybee et al., 2016), but it is also an important variable that has been largely unaccounted for in large trends of communities. Do insects occupying similar niches show similar colors? How different are the colors of insects found in different climates? Has the recent increase in temperature affected the overall color of a community? Can social castes be distinguished by slight nuances in color? How much does a species change in color according to the food it is fed during its larval stages? How does pollution affect color?

Using color as an explanatory variable in statistical analysis on facilitated by the k-mean clustering would be especially enlightening by providing a nuanced understanding of color variation that can be quantified (Chang & Mukai, 2022). The color dimensions extracted can be individually evaluated by many statistical tests such as PERMANOVA, GLM and GLMM, but test such as ANOVA, that require normality and homogeneity of the data, should be avoided (Silva et al., 2022); non-parametric tests can also be considered if the independent variable is continuous, including Spearman's rank correlation coefficient. For graphing, PCA and CVA are great alternatives to show how different variables affect each study group.

Conclusion

The use of k-means clustering to obtain the code of dominant colors in a photo was indeed able to help differentiate two populations that are not readily discernible by the naked eye. On the other hand, its use in insects in special should be considered with caution, as many studies with insects depend on preserved animals, subject to the direct influence of the fixative and killing substances applied to them (Novák et al., 2024), and other than such alterations, environmental factors need to be heavily considered while comparing groups of insects according to their color patterns (Hoffmann, 1984). However. these obstacles can be seen as double-edged sword, as anything that can change color can be studied by the changes it makes. In the end, while there is a need to consider all factors that may influence future studies on color, we hope that this study can bring to light the uses and limitations of clustering

methodology for the study of color through pixels, a method that can also be integrated to many other techniques already used, facilitating how we deal with color in all areas of entomology.

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Figure Captions

Figure 1. Iridescence of the pygidium of *Pelidnota sumptuosa* (Vigors, 1825) (habitus on A); the colors captured on the pygidium shifts depending on the view: green in dorsal and view (B) and an array from yellow to red in posterior view (C). Scale bar = 0.5mm (A) and 0.2mm (B, C).

Figure 2. Scheme (A) and results (B) of the analysis of the dominant colors found in two sympatric populations of *Erioscelis emarginata* (Mannerheim, 1829) found in their type location, Serra do Cipó (Minas Gerais, Brazil). In (A), a scheme of the extraction of 1mm transversal strips of the widest part of the left elytra and the data analysis on de R program (R Core Team, 2022). In (B), an NMDS represents the difference between the two populations confirmed by a PERMANOVA analysis.

Figure 3. Habitus of an *Erioscelis emarginata* (Mannerheim, 1829) beetle from Population B, from Serra do Cipó, Minas Gerais (A), in comparison with other groups of beetles: four populations from *E. emarginata*, including a specimen from Population A, from Serra do Cipó, Minas Gerais (B); one from Lavras, Minas Gerais (D); one from Federal District (E); and one from Santa Catarina (C); as well as two congeneric species, *E. proba* (F) and *E. columbica* (G). Scale bar = 0.5mm.

Figure 4. Color variation of the elytra of *Retrachydes thoracicus* (Olivier, 1790) (Cerambycidae) subjected to preserving substances. In A, the photo was shot *in situ* (Vale do Catimbau, IX.2023), with the beetle alive; and in B is the same beetle after 6 months being preserved in 70% alcohol. Both photos were taken with the same equipment (Canon T100, 18-55mm lens).

Figure 1.

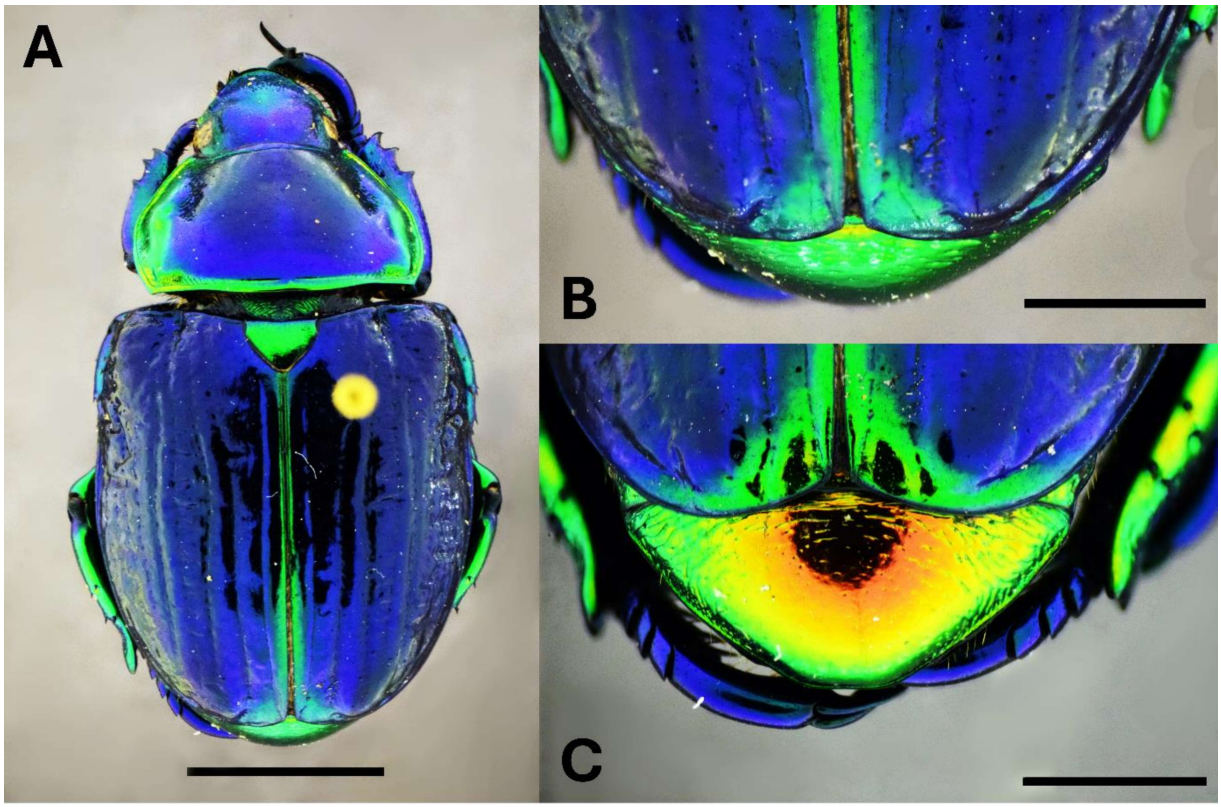

Figure 2.

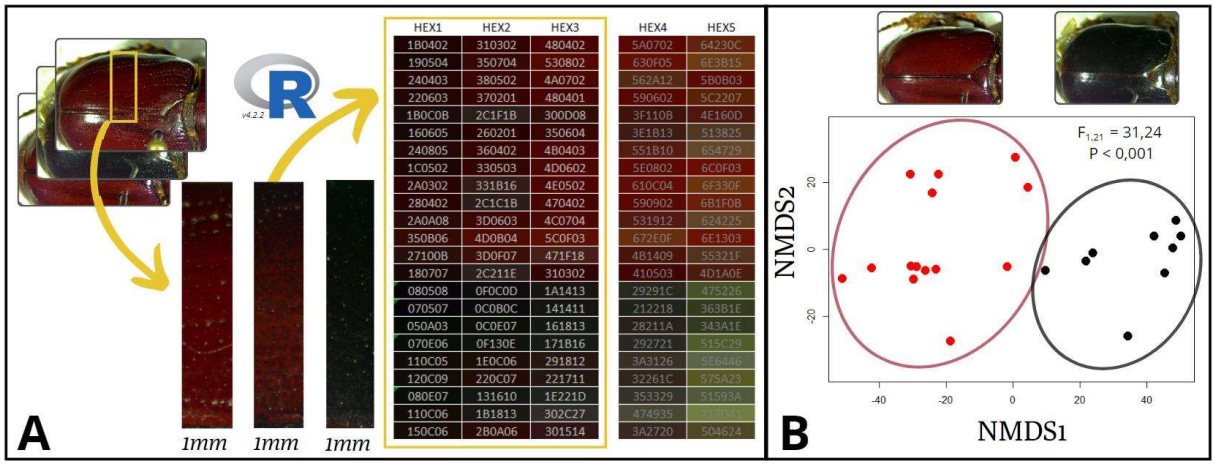

Figure 3.

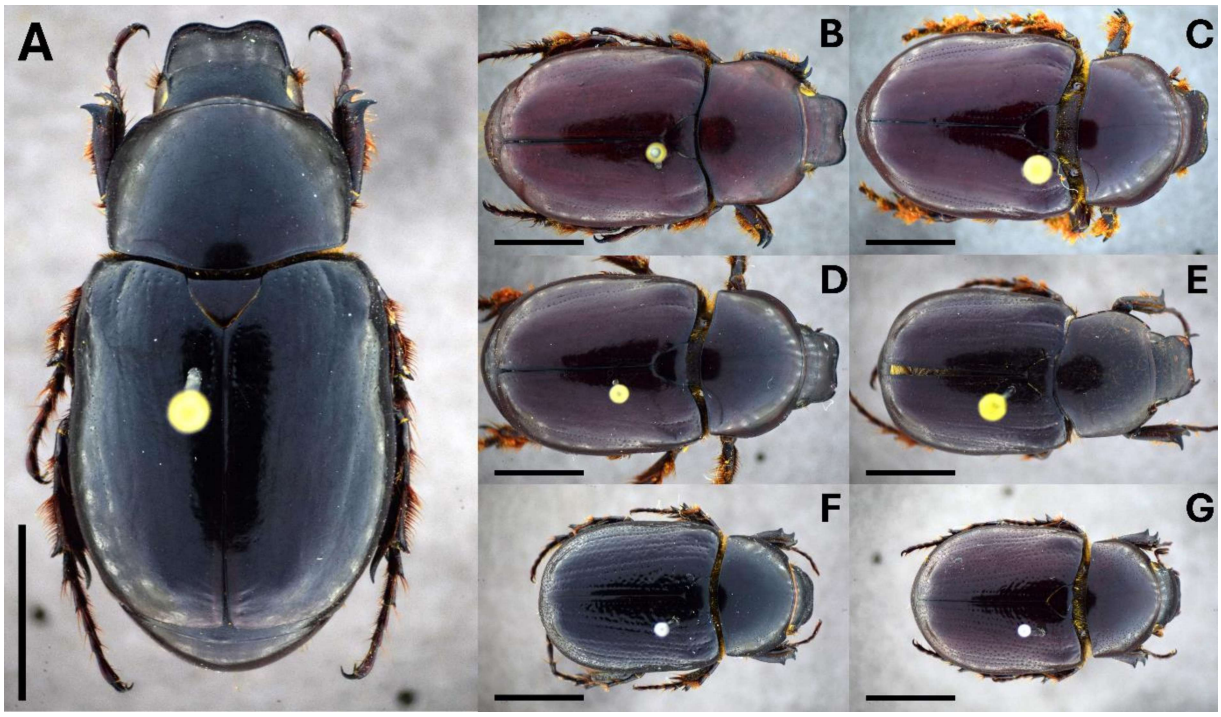

Figure 4.

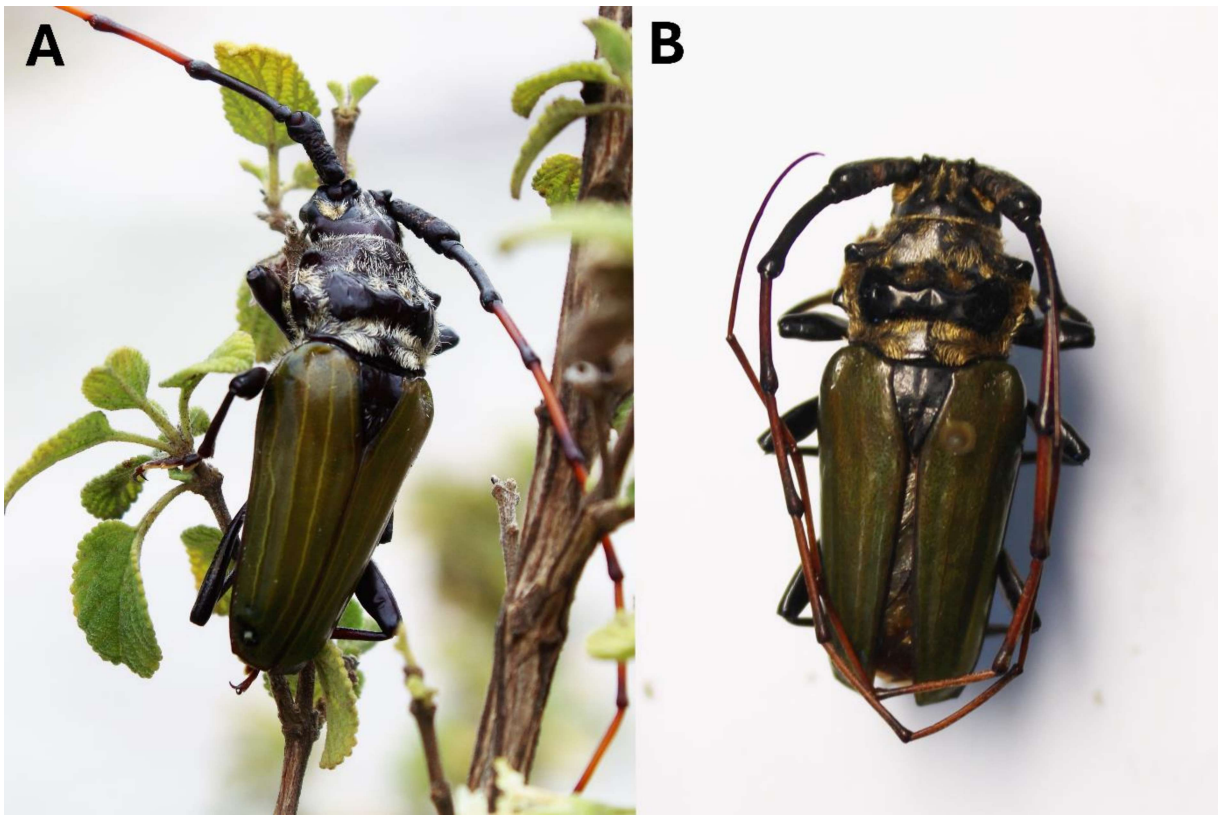

Table 1 – Description of the array of possible variations of three main dominant colors extracted from the left elytra of two sympatric populations of *Erioscelis emarginata*. The colors are described according to hexadecimal color coding. Colors of the cells represent the color code.

		1st Color	2nd Color	3rd Color
Population A	Minimum	160302	260201	300301
	Mean	230605	340704	490602
	Maximum	35100B	4D211E	5C1F18
Population B	Minimum	050503	0C0A06	141411
	Mean	080C06	130C0C	1E1813
	Maximum	150E09	2B1813	302C27

6. CONSIDERAÇÕES FINAIS

A partir da análise morfológica das populações de *Erioscelis emarginata*, foi possível aprimorar a delimitação taxonômica da espécie, por meio da sua redescrição e a descrição de uma nova espécie, que ocorre em simpatria com a localidade tipo de *E. emarginata*.

A partir de análises dos espécimes tipo e não tipo, também foi possível descrever novos caracteres encontrados nesses besouros, contribuindo especialmente com a primeira descrição do aparelho bucal em *Erioscelis*, assim como a inclusão de diversos caracteres sexualmente dimórficos previamente desconhecidos. Alguns dos caracteres previamente descritos para *E. emarginata* não foram observados entre os espécimes analisados nesse estudo e a terminologia de certos termos foi atualizada. Detalhes da história de vida da espécie também foram avaliados através da distribuição de nicho potencial de *E. emarginata* e das distribuições das plantas que estes besouros visitam e contribuem para a polinização.

Esse estudo também contribuiu com uma inovadora metodologia para a análise quantitativa de cor no tegumento dos insetos, facilitando estudos comparativos e descritivos de padrões de cores, demonstrando seu potencial através da diferenciação das populações de *E. emarginata* e da nova espécie de *Erioscelis*. Esperamos que o uso de agrupamento K-means para a obtenção das cores dominantes encontradas em fotos de insetos possa contribuir especialmente com descrições taxonômicas, facilitando a identificação de espécies que apresentam nuances em seus padrões de coloração.

Diante do que foi apresentado, acreditamos haver necessidade de continuação nos estudos taxonômicos do gênero *Erioscelis*, com um enfoque especial nas variações morfológicas e no histórico biogeográfico do gênero. A inclusão de análises de colorimetria também seriam interessantes para a compreensão do grupo, diante da possibilidade de contribuir com fatores da história de vida pouco conhecida e evolução de *Erioscelis*. Análises e descrições colorimétricas quantitativas nos demais grupos de insetos também serão fundamentais para solucionar questões taxonômicas e ecológicas, sendo interessante focar de início em avaliar a evolução das cores e contribuir com a descrição mais informativa deste caráter.