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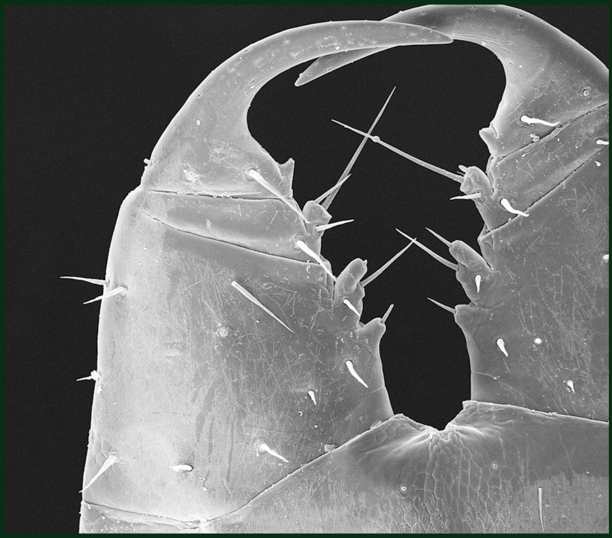

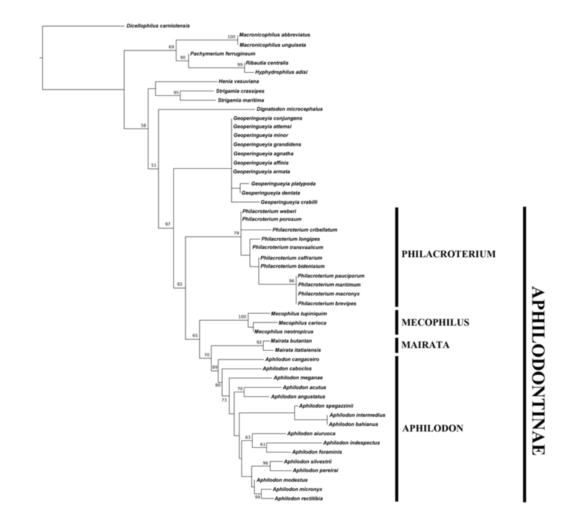

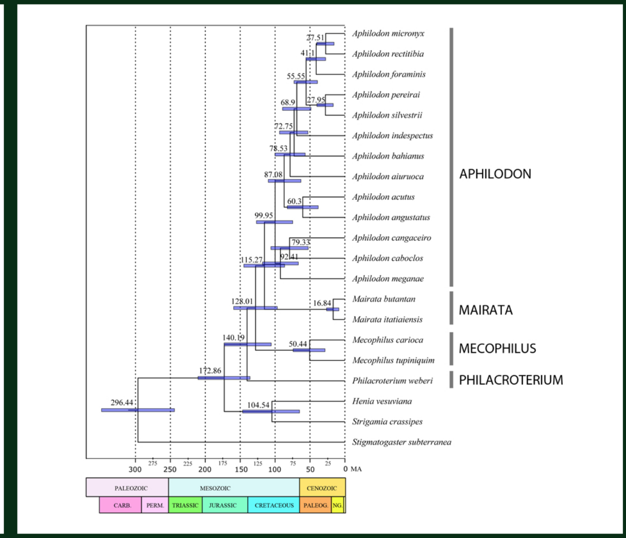

Instituto de Biociências

Universidade de São Paulo

2021

Capa: esquerda acima: *Aphilodon cangaceiro* **n. sp.**
direita acima: forcípula de *Aphilodon acutus* **n. sp.**
esquerda abaixo: filogenia de evidência total de Aphilodontinae
direita abaixo: cronograma evolutivo de Aphilodontinae
por Victor C. Calvanese

Victor de Carvalho Calvanese

Sistemática e biogeografia de Aphilodontinae Silvestri, 1909, com a primeira abordagem molecular para a subfamília (Geophilomorpha: Geophilidae)

Systematics and biogeography of Aphilodontinae Silvestri, 1909, with the first molecular approach to the subfamily (Geophilomorpha: Geophilidae)

Tese apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Doutor em Ciências Biológicas, na Área de Zoologia.

Orientador: Dr Antonio Domingo Brescovit

São Paulo
2021

Calvanese, Victor de Carvalho

Sistemática e biogeografia de Aphilodontinae Silvestri, 1909, com a primeira abordagem molecular para a subfamília (Geophilomorpha: Geophilidae) / Victor de Carvalho Calvanese; orientador Antonio Brescovit -- São Paulo, 2021.

147 p.

Tese (Doutorado) -- Instituto de Biociências da Universidade de São Paulo. Programa de Pós-Graduação em Zoologia.

1. Sistemática. 2. Biogeografia. 3. Aphilodontinae. I. Brescovit, Antonio, orient. II. Título.

Comissão Julgadora:

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Prof. Dr. Antonio Domingos Brescovit

“*There are only two ways to live your life. One is as though nothing is a miracle. The other is as though everything is a miracle.*”

Albert Einstein

A todos aqueles que contribuem
com a preservação da natureza

Agradecimentos

Suporte financeiro

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pela concessão da bolsa de estudos de doutorado, processo número 169641/2017–1.

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pelo apoio vinculado ao Programa de Excelência Acadêmica (PROEX), editais I-2018 e I-2019.

Laboratórios envolvidos

Laboratório de Coleções Zoológicas (LCZ), Instituto Butantan

Laboratório de Biologia Celular, Instituto Butantan

Laboratório de Evolução Molecular (LEM), Departamento de Zoologia, IB-USP

Agradecimentos especiais

A bióloga Adele M. M. S. Silva, por participar ativamente de todas as coletas de campo, contribuindo de forma fundamental para a obtenção de grande parte do material aqui analisado. Também pelas discussões e revisão do trabalho.

Prof. Dr. Antonio Domingos Brescovit, por todos os valorosos ensinamentos. Também pelo entusiasmo e seriedade que demonstrou ao longo de todos esses anos.

Dr. Luis Alberto Pereira e Dr. Lucio Bonato, por estarem sempre dispostos em ajudar, por contribuir com minha formação e colaborar com a construção deste trabalho.

Manuel Antunes Júnior e Beatriz Vieira Freire, pela dedicação no treinamento técnico para extração de DNA e sequenciamento molecular.

Beatriz Mauricio, pelo auxílio na obtenção de imagens através de microscopia eletrônica de varredura.

Ao corpo docente do IB, agradeço pelo respaldo teórico e técnico.

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Capítulo 1. Introdução geral

1- Do subfilo Myriapoda a subfamília Aphilodontinae

Myriapoda

Myriapoda é um subfilo de Arthropoda cujos integrantes podem ser facilmente reconhecidos por sua tagmose típica, onde o corpo é dividido entre cabeça e tronco multissegmentado (Ruppert *et al.*, 2005). Análises filogenéticas baseadas tanto em morfologia como em dados moleculares corroboram com o monofiletismo do grupo, que foi dividido em quatro classes, sendo Chilopoda grupo irmão de Progoneata, clado que abarca Symphyla, Diplopoda e Pauropoda (Paulus, 2000; Giribet *et al.*, 2005). Entre as chamadas "grandes classes", Diplopoda conta com cerca de 12 mil espécies (Sierwald & Bond, 2007) e Chilopoda com cerca de 3.200 espécies (Bonato *et al.*, 2011). Já Symphyla apresenta cerca de 200 espécies (Szucsich & Scheller, 2011) e Pauropoda pouco mais de 800 (Scheller, 2011). Os miriápodes são todos terrestres, de hábitos crípticos e com preferência por ambientes úmidos, contudo, acabam por atuar sobre diferentes nichos ecológicos (Voigtlander, 2011). Assim, enquanto integrantes de Symphyla, Diplopoda e Pauropoda são principalmente fitófagos ou detritívoros, Chilopoda é composto apenas por espécies com estilo de vida predador (Ruppert *et al.*, 2005; Voigtlander, 2011).

Chilopoda

Quilópodes são também conhecidos como lacraias, centípedes ou centopéias, e ocorrem em todos os continentes exceto Antártica (Bonato & Zapparoli, 2011). O grupo se destaca dentro de Myriapoda por apresentar morfologia bastante peculiar em relação às outras classes, sendo distinguido por englobar espécies com um segmento forcipular em parte anterior e órgãos sexuais localizados em parte posterior do corpo (Linnaeus, 1758; Newport, 1844; Attems, 1926; Lewis, 1981). Edgecombe (2011) se baseou em filogenia e discutiu que a posição dos órgãos sexuais em parte posterior do corpo é uma plesiomorfia compartilhada com outros artrópodes. Contudo, recuperou três novidades evolutivas para Chilopoda: segmento forcipular, presença de um dente na segunda maxila em fase embrionária e núcleo do esperma portando um cume em espiral.

Atualmente Chilopoda é dividido entre cinco ordens viventes, incluindo Scutigeromorpha, Lithobiomorpha, Craterostigmomorpha, Scolopendromorpha e Geophilomorpha, mais a extinta Devonobiomorpha. Baseado em análise filogenética sobre dados morfológicos e moleculares, Edgecombe & Giribet (2004) estabeleceram as relações entre as linhagens viventes de centípedes. Nesta hipótese Scutigeromorpha é mais basal, seguida de Lithobiomorpha e Phylactometria, um clado representado por Craterostigmomorpha, Scolopendromorpha e Geophilomorpha (Figura 1A).

Contudo, logo surgiram outros dados moleculares que indicaram uma relação diferente entre as ordens. Mallatt & Giribet (2006) analisaram os marcadores nucleares 28S e 18S rRNAs com o objetivo de esclarecer as relação filogenética de Ecdysozoa, e recuperaram Scutigeromorpha e Craterostigmomorpha como mais basais a Amalpighiata, um clado com Lithobiomorpha, Scolopendromorpha e Geophilomorpha. Esta hipótese foi posteriormente corroborada por Fernández *et al.* (2014) em filogenia baseada em transcriptoma (Figura 1B).

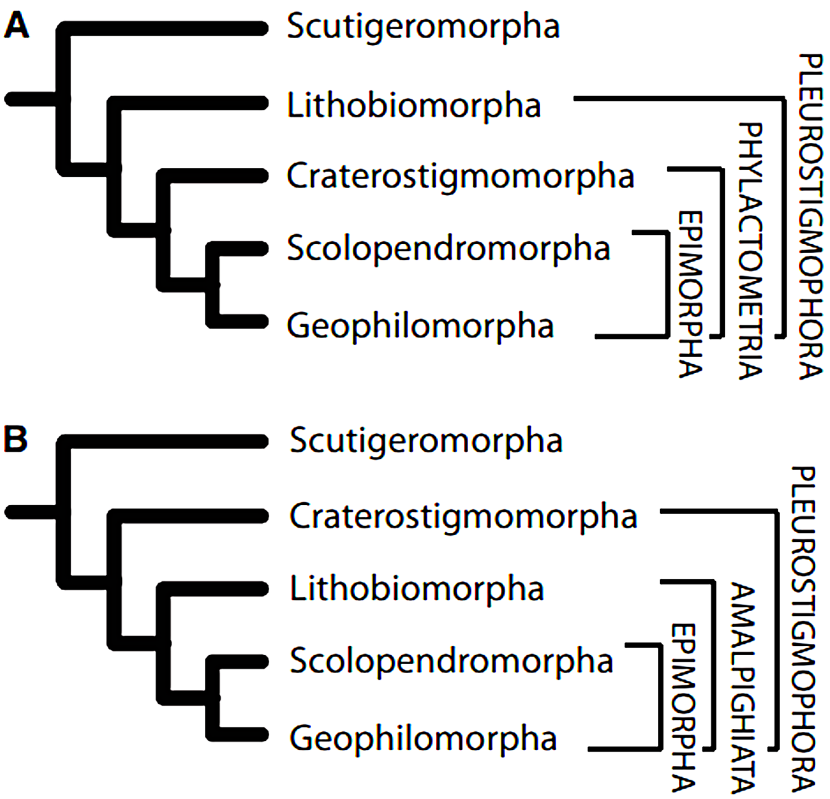

Figura 1. A–B, Relações filogenéticas entre as ordens de Chilopoda: **A**, hipótese com Phylactometria, baseada em dados morfológicos e dados morfológicos e moleculares combinados; **B**, hipótese com Amalpighiata, baseada em dados moleculares. Retirado de Fernández *et al.* (2014).

Geophilomorpha

O nome Geophilomorpha deriva do grego e significa *geo*, “terra”, *philo*, “que gosta” e *morpha*, “forma”, fazendo referência ao plano corpóreo observado no grupo, bem adaptado ao ambiente subterrâneo. De fato, diversas características morfológicas indicam a aptidão dos geofilomorfos para a vida dentro de galerias, que também podem ser observadas como convergentes em outros animais, tais como perda de pigmentação, ausência de olhos ou ocelos e alongamento do corpo (Ribera *et al.*, 2010). A ocupação de galerias subterrâneas a partir do ambiente de serapilheira, a mais de 320 milhões de anos atrás (Fernández *et al.*, 2014), rendeu a Geophilomorpha uma alta taxa de especiação, sendo esta ordem a mais diversa, contando atualmente com mais de 1.200 espécies (Bonato *et al.*, 2014; Figura 2).

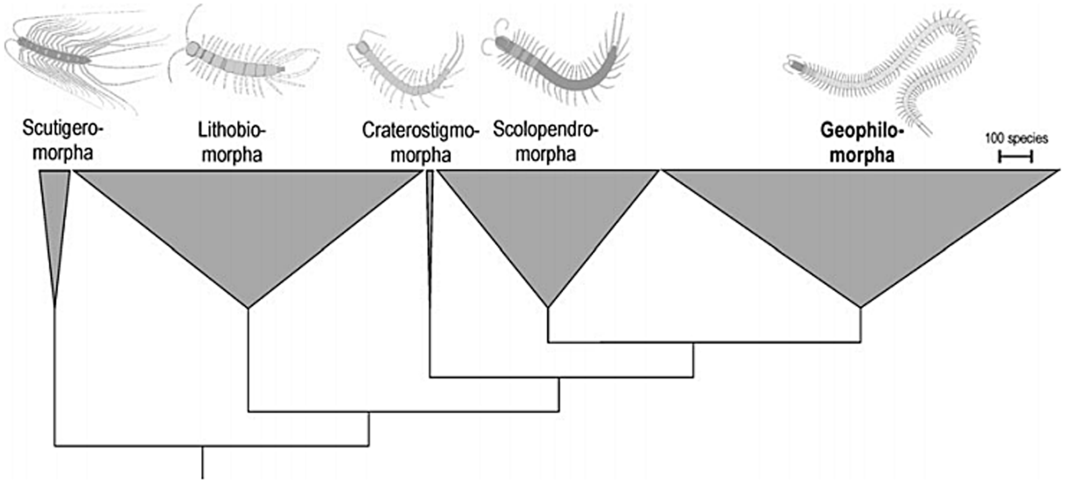

Figura 2. Relações filogenéticas e riqueza taxonômica das ordens existentes de Chilopoda (de Bonato *et al.*, 2014). A largura dos ramos terminais são proporcionais ao número de espécies conhecidas para cada uma das ordens.

Historicamente aceita como um grupo natural, Geophilomorpha foi constantemente suportada em análises filogenéticas, sendo que Edgecombe (2011) reportou para a ordem dez sinapomorfias morfológicas não ambíguas: 1) perda dos ocelos e supressão de alguns nervos óticos; 2) número fixo de 14 artículos antenais; 3) duas áreas laterais portando sensilas base-cônicas no último artículo antenal; 4) mandíbula reduzida, e sem mobilidade dos músculos tentoriais anteriores; 5) tronco composto de no mínimo 27 segmentos portadores de pernas; 6) tronco homogêneo, sem alternância de tergitos curtos e longos; 7) tergito dos segmentos portadores de pernas divididos entre pretergito e metatergito; 8) espiráculos presentes ao longo de todo corpo, com exceção do primeiro e último segmentos; 9) chiasmata traqueal separada por tricomas espongiformes; 10) perda da capacidade de regeneração na antena e pernas.

Embora os primeiros integrantes do grupo tenham sido descritos por Linnaeus (1758), a relação entre as espécies de Geophilomorpha passaram a ser melhor compreendidas quando Pocock (1895) delimitou o grupo como ordem e Cook (1896) estabeleceu a primeira classificação em famílias, baseando-se na morfologia dos apêndices bucais. Neste trabalho, nove famílias foram incluídas em Geophilomorpha: Gonibregmatidae Cook 1896, Oryidae Cook, 1896, Himantariidae Bollman, 1893; Disargidae Cook, 1896, Ballophilidae Cook, 1896, Schendylidae Cook, 1896, Dignathodontidae Cook, 1896, Geophilidae Leach, 1815 e Dicellophilidae Cook, 1896.

Nos anos seguintes, Attems (1903), Verhoeff (1908) e Brölemann (1909) propuseram novas classificações, e com destaque, Attems (1929) publicou uma notável monografia sobre a sistemática de Geophilomorpha. Neste trabalho considerou dez famílias, incluindo Azygethidae Chamberlin, 1920, Gonibregmatidae (sinônimo sênior de Disargidae), Himantariidae, Mecistocephalidae Bollman, 1893 (sinônimo sênior de Dicellophilidae), Neogeophilidae Silvestri, 1918, Oryidae, Schendylidae (incluindo Schendylinae e Ballophilinae), Sogonidae Chamberlin, 1912, Soniphilidae Chamberlin, 1912 e Geophilidae (incluindo Geophilinae, Dignathodontinae, Aphilodontinae Silvestri, 1909, Chilenophilinae Attems, 1909 e Pachymerinae Attems, 1926).

A classificação proposta por Attems (1929) foi modificada por Crabill (1961, 1970), e posteriormente por Lewis (1981), onde o último passou a reconhecer 11 famílias em Geophilomorpha: Himantariidae, Schendylidae, Oryidae (sinônimo sênior de Azygethidae), Mecistocephalidae, Geophilidae (sinônimo sênior de Sogonidae e Soniphilidae), Chilenophilidae (incluindo Chilenophilinae e Pachymeriinae), Eriphantidae Crabill, 1970, Dignathodontidae, Aphilodontidae, Gonibregmatidae e Neogeophilidae.

Novas modificações foram implementadas por Edgecombe & Giribet (2007), e corroboradas em revisão por Bonato *et al.* (2011), onde Geophilomorpha passou a ser dividida em 13 famílias: Aphilodontidae, Ballophilidae, Dignathodontidae, Eriphantidae, Geophilidae, Gonibregmatidae, Himantariidae, Linotaeniidae Cook, 1899, Macronicophilidae Verhoeff, 1925, Mecistocephalidae, Neogeophilidae, Oryidae e Schendylidae.

Pouco depois, em análise filogenética de Geophilomorpha proposta por Bonato *et al.* (2014) sobre dados morfológicos e moleculares, apenas sete famílias foram reconhecidas: Mecistocephalidae, Gonibregmatidae, Oryidae, Schendylidae (incluindo Schendylinae e Ballophilinae), Geophilidae (incluindo Aphilodontinae, Dignathodontinae, Geophilinae, Linotaeniinae, Macronicophilinae e Ribautiinae Brölemann, 1909), Himantariidae e Zelanophilidae Bonato, Drago & Murienne, 2014.

Sistemática e distribuição de Geophilidae

Leach (1814) foi o primeiro a estabelecer um táxon supra específico para agrupar os geofilomorfos ao propor o gênero *Geophilus* Leach, 1814. Logo depois o mesmo autor (Leach, 1815) propôs Geophiloidea a fim de abarcar a grande variedade de novas espécies que vinham sendo conhecidas. Por cerca de 80 anos Geophiloidea abrigou todas as espécies conhecidas de geofilomorfos, até que Pocock (1895) estabeleceu o grupo como ordem Geophilomorpha e Cook (1896) considerou o grupo dividido em nove famílias. Neste esquema *Geophilus* pertence a Geophilidae, uma família limitada para agrupar espécies com a mandíbula portando apenas uma lamela pectinada, labrum com três peças distintas e telopoditos da segunda maxila com três artículos e garra terminal sem projeções (Cook, 1896).

Historicamente, a composição de Geophilidae se mostrou instável, somando uma grande quantidade de hipóteses de relação distintas (Attems, 1929; Crabill, 1961, 1970; Lewis, 1981; Edgecombe & Giribet, 2007). No entanto, como discutido por Bonato *et al.* (2014), para concordar com a naturalidade dos grupos, Geophilidae pode englobar seis principais linhagens, consideradas como subfamílias: Geophilinae, Aphilodontinae, Dignathodontinae, Linotaeniinae, Macronicophilinae e Ribautiinae. Neste cenário, a família representa mais que a metade de toda diversidade conhecida para Geophilomorpha, contando com mais de 110 gêneros e cerca de 650 espécies.

Sobre esta classificação, duas subfamílias apresentam distribuição majoritariamente holártica: Dignathodontinae, com cerca de 20 espécies em quatro gêneros restritos a região mediterrânea e Europa central, e Linotaeniinae com cerca de 50 espécies em sete gêneros principalmente distribuídos entre América do Norte e Eurásia, e que também conta com alguns representantes nos Andes e arquipélago japonês (Bonato *et al.*, 2011).

Geophilinae e Ribautiinae apresentam ampla distribuição, com um grande número de gêneros e espécies registradas para todos os continentes exceto Antártica (Bonato *et al.*, 2011). Contudo, contrastam em distribuição, uma vez que Geophilinae concentra a maior parte de sua diversidade no sudeste dos Estados Unidos e centro-oeste da Europa, já Ribautiinae tem maior diversidade em parte Neotropical, África e Oceania (Attems, 1929; Lewis, 1981).

Considerada a subfamília menos diversa, Macronicophilinae conta com apenas quatro espécies em um gênero restrito ao bioma Amazônico, em parte subequatorial da América do Sul (Foddai *et al.*, 2000).

Aphilodontinae segue um padrão de distribuição Gonduânica, até então com 14 espécies divididas em três gêneros que ocorrem sobre parte centro-sul do continente sul-americano, e mais 11 espécies em um gênero sul-africano (Calvanese *et al.*, 2019).

Aphilodontinae

Representantes da subfamília Aphilodontinae são facilmente identificados por serem os únicos geofilomorfos a apresentar forcípula com apenas três artículos, caracterizada pela fusão do trochanteroprefemur com o fêmur (Silvestri, 1909; Calvanese *et al.*, 2019). Outras características também são peculiares no grupo, como a redução de alguns apêndices bucais, como as peças do labrum e telopodito da segunda maxila, e posição da glândula de veneno, translocada da forcípula para alguns segmentos do tronco (Verhoeff, 1937; Calvanese *et al.*, 2019).

Ao longo do tempo poucos pesquisadores se dedicaram em estudar as espécies de Aphilodontinae, assim, o desenvolvimento da sistemática do grupo pode ser sintetizado basicamente em quatro momentos: 1) delimitação do táxon e descrição das primeiras espécies Neotropicais, conduzidos por Filippo Silvestri e Henry Wilfred Brölemann no final do século XIX e início do século XX. 2) Descrição de um novo gênero com novas espécies sul-africanas, desenvolvido por Carl August Graf Attems na primeira metade do século XX. 3) Revisão das espécies sul-africanas e descrição de novas espécies para esta região, por Karl Wilhelm Verhoeff e Reginald Frederick Lawrence, no meio do século XX. 4) Revisão das espécies Neotropicais e sul-africanas, descrição de novas espécies Neotropicais e primeiros estudos sobre a evolução do grupo, do início do século XXI até os tempos atuais, por Luis Alberto Pereira, Lucio Bonato, Antonio Domingos Brescovit e Victor de Carvalho Calvanese.

A primeira espécie de Aphilodontinae, *Aphilodon spegazzinii* Silvestri, 1898 foi registrada para La Plata, Argentina, como espécie tipo de *Aphilodon* Silvestri, 1898, um gênero recém proposto em Geophilidae. Logo depois foi somado ao grupo *Aphilodon micronyx* Brölemann, 1902, que no entanto foi subitamente transferido, e utilizado por Brölemann como tipo do monotípico *Mecistauchenus* Brölemann, 1907, também de

Geophilidae. Em seguida Silvestri (1909) descreveu *A. modestus* Silvestri, 1909 para Misiones, Argentina, *A. intermedius* Silvestri, 1909 para Tacuru-Pucu, Paraguai, e *A. angustatus* Silvestri, 1909 para Misiones, Argentina, Paraguarí no Paraguai e Mato Grosso, Brasil (Silvestri, 1909). Também descreveu *Aphilodon weberi* Silvestri, 1909, a primeira espécie do grupo registrada na África do Sul. O autor propôs ainda *Mecophilus* Silvestri, 1909, com a espécie tipo *M. neotropicus* Silvestri, 1909 para Foz do Iguaçu, Brasil. Neste mesmo trabalho Silvestri desvinculou o grupo de Geophilidae e criou Aphilodontidae Silvestri, 1909, uma nova família de Geophilomorpha que passou a abarcar *Aphilodon*, *Mecophilus* e *Mecistauchenus*.

Embora Silvestri (1909) tenha proposto *A. weberi* com ocorrência para África do Sul, o conhecimento do grupo para esta região experimentou maior progresso apenas após os trabalhos de Attems (1926, 1929). Este autor considerou Aphilodontinae membro de Geophilidae e propôs *Philacroterium* Attems, 1926, com designação subsequente de *Philacroterium cribellatum* Attems, 1928 como espécie-tipo e descrição de *P. pauperum* Attems, 1928, ambas registradas para Cape Province. Attems (1926, 1928, 1929) contudo não discutiu o posicionamento de *A. weberi*, fora de *Philacroterium,* mesmo que este compartilhe com outros representantes sul-africanos importantes características na segunda maxila e labrum.

Verhoeff (1937) revisou o status das espécies de Aphilodontinae, e estabeleceu que *Philacroterium* é sinônimo júnior de *Aphilodon*. O autor também considerou *P. pauperum* como sinônimo júnior de *A. weberi* e descreveu os sul-africanos *Aphilodon porosus* Verhoeff, 1937 para Cape Province e *A. caffrarius* Verhoeff, 1937 para Hoedspruit. Logo depois o mesmo autor descreveu *Aphilodon brevipes* Verhoeff, 1938 para KwaZulu Natal, também na África do Sul.

Attems (1947) revalidou tanto *Philacroterium* como *P. pauperum* e apresentou as espécies sul-africanas de *Aphilodon* dentro de *Philacroterium*. Neste trabalho o autor também traz uma chave de identificação para as quatro espécies consideradas no gênero, incluindo *P. cribellatum*, *P. pauperum*, *P. caffrarius* e *P. porosus*.

Lawrence (1955) revisou as espécies sul-africanas propostas por Attems (1928) e Verhoeff (1937, 1938) e concordou com a proposta taxonômica de Verhoeff (1937), onde Aphilodontinae é dividida apenas entre *Aphilodon*, *Mecistauchenus* e *Mecophilus*, sendo *Philacroterium* considerado inválido. Neste trabalho Lawrence descreveu os sul-africanos *Aphilodon bidentatus* Lawrence, 1955 para Kokstad, *A. longipes* Lawrence, 1955 para KwaZulu Natal e *A. macronyx* Lawrence, 1955 para Richmond.

Mais tarde Lawrence (1963) descreveu mais três espécies sul-africanas, *Aphilodon maritimus* Lawrence, 1963 e *A. pauciporus* Lawrence, 1963, ambas para KwaZulu Natal, e *A. transvaalicus* Lawrence, 1963 para Rustenburg.

Posteriormente, as espécies Neotropicais voltam a ser acessadas por Pereira *et al.* (2007). Neste trabalho os autores apresentaram uma nova descrição para *Aphilodon angustatus,* baseado em um material recém obtido no centro-oeste do Brasil, e compararam as principais características morfológicas conhecidas para as espécies Neotropicais de *Aphilodon*, incluindo *A. spegazzinii*, *A. angustatus*, *A. modestus* e *A. intermedius*. Neste trabalho as espécies de *Aphilodon* foram incluídas em "Aphilodontidae", considerada pelos autores como uma família.

Em revisão sobre a taxonomia dos geofilomorfos, Bonato *et al.* (2011) consideraram Aphilodontidae distinta de Geophilidae e com três gêneros válidos, *Aphilodon* com cerca de 15 espécies (Neotropicais e sul-africanas, incluindo a espécie tipo de *Philacroterium* referida como "*A. cribellatus*") e os monotípicos *Mecistauchenus* e *Mecophilus*.

Pouco depois, Bonato *et al.* (2014) publicaram a primeira análise filogenética para Geophilomorpha baseada em evidência total (Figura 3). Neste trabalho duas espécies de Aphilodontinae, *A. angustatus* e *A. weberi* foram recuperadas juntas em Geophilidae. Nesta filogenia também se evidenciou a relação de proximidade entre as espécies de Aphilodontinae e espécies de *Geoperingueyia* Attems, 1926, um gênero historicamente alocado em Geophilinae (Attems, 1929; Lawrence, 1955, 1963).

Bonato *et al.* (2016) apresentaram em catálogo Aphilodontinae como subfamília de Geophilidae e com quatro gêneros válidos. Nesta classificação, *Aphilodon* contou com 14 espécies Neotropicais e sul-africanas, *Mecistauchenus* incluiu *M. micronyx*, e *Mecophilus* incluiu *M. neotropicus*. No mesmo catálogo, *Philacroterium* foi considerado válido e representado apenas pelo tipo *P. cribellatum*, sendo a sinonímia proposta por Verhoeff (1937) entre *A. weberi* e *P. pauperum* considerada válida.

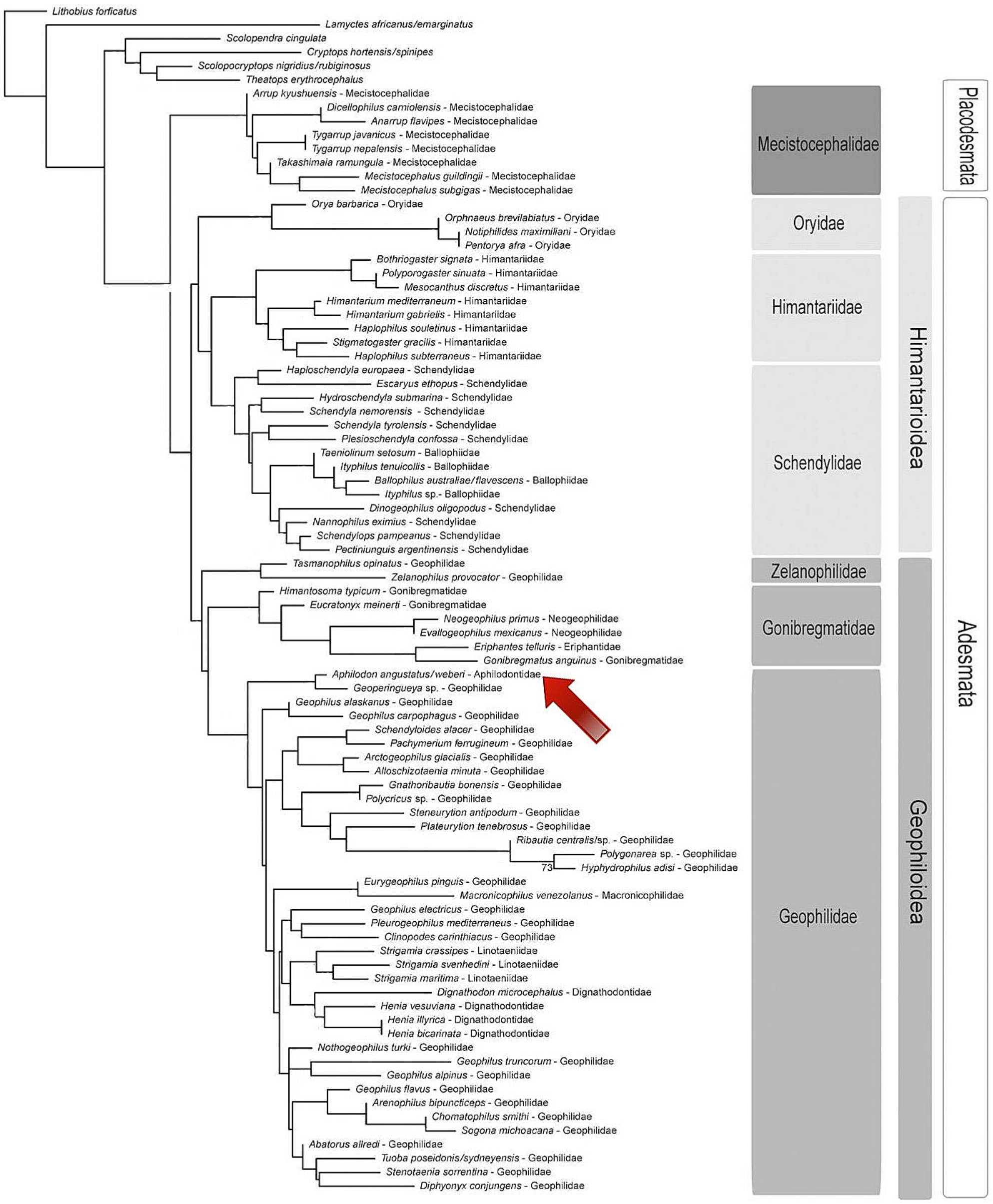

Figura 3. Hipótese filogenética de Geophilomorpha fornecida por Bonato *et al.* (2014) com base em dados morfológicos e moleculares. Parcialmente modificado pela inclusão da seta vermelha que identifica a posição de Aphilodontinae.

Em dissertação de mestrado, Calvanese (2017) abordou a taxonomia e relações filogenéticas das espécies de Aphilodontinae. Nesta pesquisa foram analisados o material tipo de *Aphilodon spegazzinii*, *A. angustatus*, *A. bidentatus*, *A. brevipes*, *A. caffrarius*, *A. longipes*, *A. macronyx*, *A. maritimus*, *A. pauciporus*, *A. porosus*, *A. transvaalicus*, *A. weberi, Mecophius neotropicus*, *Mecistauchenus micronyx* e *Philacroterium cribellatum*. Já os tipos de *A. modestus*, *A. intermedius*, *A. porosus* e *A. longipes* não foram localizados. Ao material consultado, foram comparadas sete potenciais espécies novas de Aphilodontinae recém-coletadas no sudeste do Brasil.

Todo o grupo foi submetido a análise filogenética baseada em morfologia, realizada com outros integrantes das outras cinco subfamílias de Geophilidae *sensu* Bonato *et al.* (2014) e dois integrantes de *Geoperingueyia*, incluindo o tipo *G. conjungens* Attems, 1928.

Como resultado, a análise recuperou a monofilia de Aphilodontinae, que foi dividida em dois principais clados, sendo um com todas as espécies sul-africanas e outro com todas as espécies Neotropicais. Calvanese (2017) advoga que *Aphilodon* pode ser utilizado para agrupar todas as espécies Neotropicais, incluindo espécies de *Mecophilus* e *Mecistauchenus*, enquanto *Philacroterium* pode suportar todas as espécies sul-africanas. Em adição, o autor argumentou que na subfamília pode ser anexado *Geoperingueyia*, que consiste em um gênero mais relacionado a Aphilodontinae que a outros integrantes de Geophilidae (Figura 4).

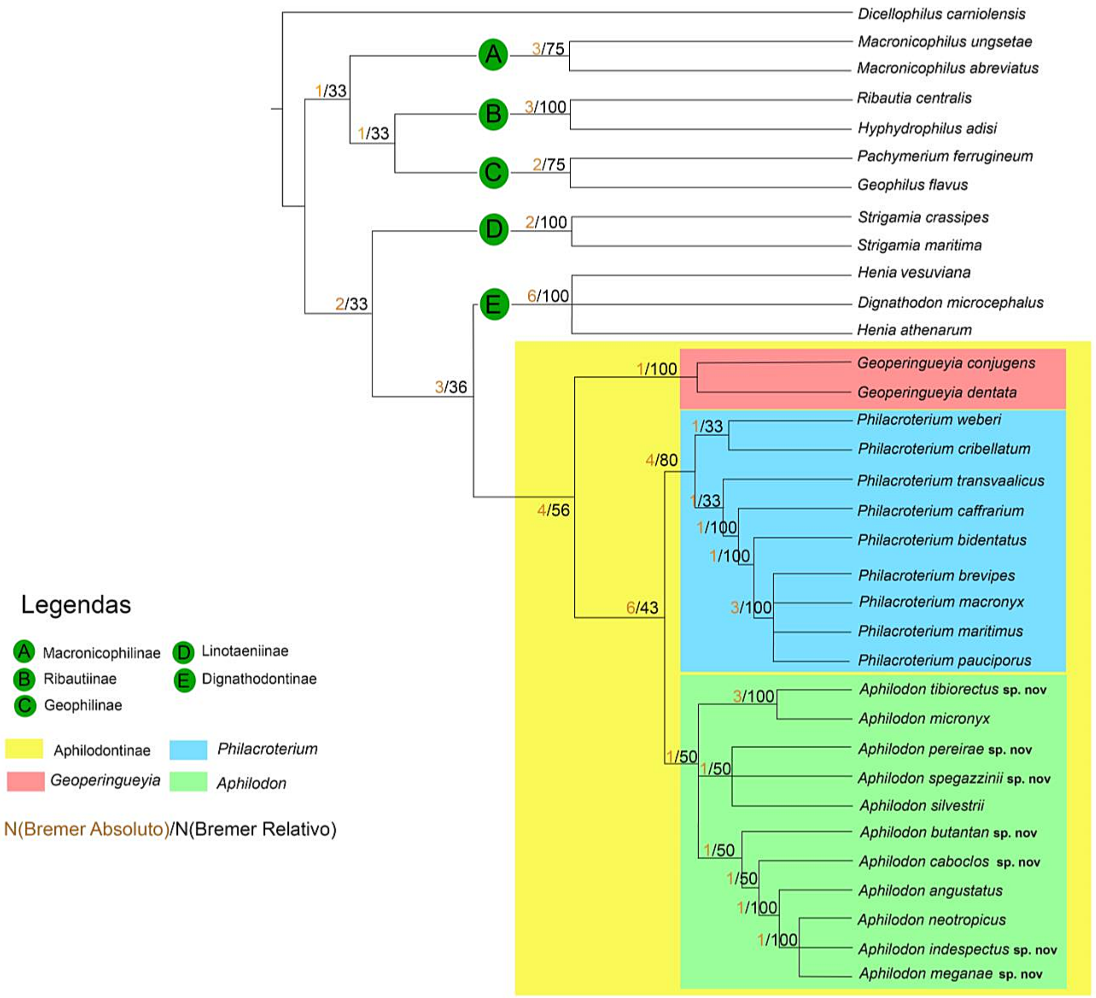

Figura 4. Hipótese filogenética de Aphilodontinae fornecida por Calvanese (2017) com base em dados morfológicos.

Após a conclusão do trabalho por Calvanese (2017) novas expedições de coleta foram conduzidas pelo Brasil, e um novo grupo de centípedes relacionado a Aphilodontinae passou a ser conhecido. Junto a este material foi identificada também uma nova espécie relacionada a *Mecophilus neotropicus*, que gerou desconfiança sobre a sinonímia de *Mecophilus* com *Aphilodon*, sugerida por interpretação da filogenia acima mencionada. Sobre estas amostras novas análises foram desenvolvidas, as quais se somaram as pesquisas de Calvanese (2017) e resultaram na monografia apresentada por Calvanese *et al.* (2019), relacionada a taxonomia das espécies Neotropicais de Aphilodontinae e que apresenta uma filogenia morfológica para a subfamília (Figura 5).

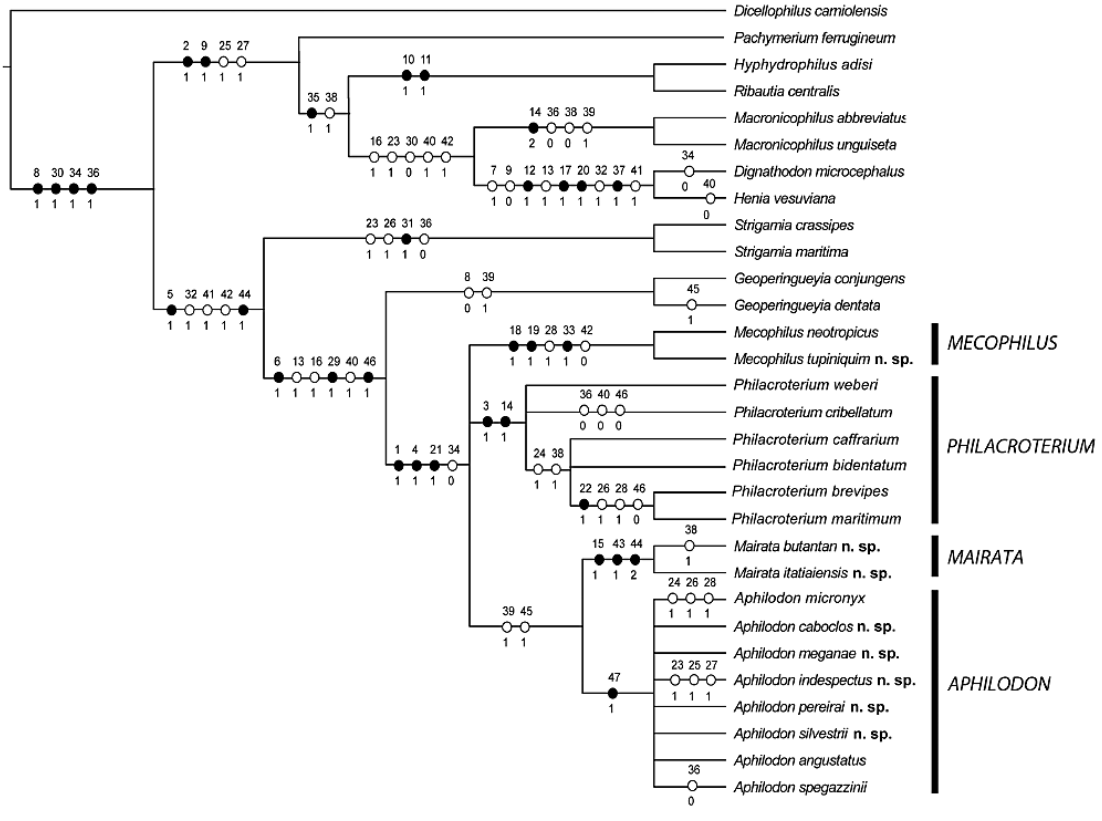

Figura 5. Hipótese filogenética de Aphilodontinae fornecida por Calvanese *et al.* (2019) com base em dados morfológicos. Círculos pretos indicam sinapomorfias não homoplásticas. Círculos brancos indicam sinapomorfias homoplásticas. O número de cada caractere é dado acima dos ramos e seu respectivo estado abaixo.

Neste trabalho os autores apresentaram redescrições para os tipos de *A. spegazzinii*, *A. angustatus* e *M. neotropicus*. *Mecistauchenus* passou a ser formalmente considerado sinônimo júnior de *Aphilodon*, e um neótipo foi designado para *A. micronyx*, sendo também registrado pela primeira vez machos para esta espécie. Um novo gênero Neotropical, *Mairata* Calvanese, Brescovit & Bonato, 2019 foi reportado. Em adição oito novos afilodontideos registrados para o sudeste do Brasil foram descritos, sendo duas espécies de *Mairata* (*M. butantan* Calvanese, Brescovit & Bonato, 2019 e *M. itatiaiensis* Calvanese, Brescovit & Bonato, 2019), cinco espécies de *Aphilodon* (*A. caboclus* Calvanese, Brescovit & Bonato, 2019, *A. indespectus* Calvanese, Brescovit & Bonato, 2019, *A. meganae* Calvanese, Brescovit & Bonato, 2019, *A. pereirai* Calvanese, Brescovit & Bonato, 2019 e *A. silvestrii* Calvanese, Brescovit & Bonato, 2019) e *Mecophilus tupiniquim* Calvanese, Brescovit & Bonato, 2019.

A análise filogenética apresentada por Calvanese *et al.* (2019) englobou boa parte das espécies Neotropicais conhecidas, seis espécies africanas até então alocadas em *Aphilodon* e *Philacroterium cribellatum*, espécie tipo de *Philacroterium*. Como grupo externo foram analisados representantes de *Geoperingueyia* e outras linhagens de Geophilidae. Como resultado, os autores recuperaram como monofélicos (*Geoperingueyia* + Aphilodontinae), Aphilodontinae, *Mecophilus* e *Mairata*. Como em Calvanese (2017), as espécies de *Aphilodon* foram recuperadas em dois principais clados, um com todas as espécies Neotropicais do gênero e outro englobando as espécies sul-africanas de *Aphilodon* e a espécie tipo de *Philacroterium*. Calvanese *et al.* (2019) então propuseram a transferência de todas as espécies sul-africanas de *Aphilodon* para *Philacroterium* e apresentaram Aphilodontinae com quatro gêneros, incluindo os Neotropicais *Aphilodon* com 10 espécies, *Mecophilus* com duas espécies e *Mairata* com duas espécies, mais o sul-africano *Philacroterium* com 11 espécies.

Embora a monografia apresentada por Calvanese *et al.* (2019) tenha representado um grande avanço para a sistemática de Aphilodontinae, algumas questões não puderam ser efetivamente respondidas baseadas apenas na morfologia do conjunto de espécies analisadas, dentre elas: 1) A relação entre *Geoperingueyia* e Aphilodontinae não foi totalmente elucidada porque oito das dez espécies conhecidas de *Geoperingueyia* não foram analisadas. 2) A relação evolutiva entre os gêneros de Aphilodontinae não foi resolvida, sendo recuperada uma politomia com (*Philacroterium* + *Mecophilus* + (*Mairata* + *Aphilodon*)). E mesmo a afinidade entre *Mairata* e *Aphilodon* foi estabelecida apenas por uma sinapomorfia homoplástica, e com baixo valor de suporte de ramo. 3) A história evolutiva das espécies de *Aphilodon* não foi bem resolvida, sendo a relação parentética do grupo recuperada como uma politomia. A este problema se acrescenta a falta de características morfológicas reconhecidas para definir este gênero, que foi garantido apenas por uma sinapomorfia.

2- Objetivos

A presente tese foi elaborada para apresentar um estudo mais refinado sobre a sistemática de Aphilodontinae, além de analisar os padrões de distribuição do grupo em paralelo com sua história evolutiva.

Sobre estas premissas, dois principais objetivos foram traçados: (1) apresentar uma hipótese filogenética para as espécies de Aphilodontinae utilizando caracteres moleculares e morfológicos, (2) realizar uma primeira avaliação sobre a biogeografia do grupo, baseada na estimativa de tempo de divergência entre espécies e análise de reconstrução de áreas ancestrais.

Como nós consideramos que algumas questões relacionadas à delimitação geográfica, morfologia e taxonomia de Aphilodontinae careciam ainda serem melhor elucidadas, quatro objetivos secundários foram adicionados a esta pesquisa: I) realizar expedições de coleta em diferentes biomas buscando por espécies novas, II) descrever novas espécies, III) avaliar a relação entre Aphilodontinae e *Geoperingueyia* e IV) avaliar novos caracteres morfológicos para diagnóstico e análise filogenética do grupo.

3- Organização da tese

Para facilitar o acesso aos dados, esta tese foi dividida em capítulos relacionados aos diferentes temas abordados. Com exceção do primeiro e último (relativos a Introdução e Considerações finais), cada capítulo traz um ou mais artigos que já foram ou serão encaminhados para revistas científicas. Abaixo segue uma breve descrição de cada tópico:

Capítulo 1. *Introdução geral.* Caracteriza os miriápodes e quilópodes e reporta a história de Geophilomorpha, Geophilidae e Aphilodontinae. Também apresenta os objetivos da tese e como está organizada.

Capítulo 2. *Novas espécies de Aphilodontinae.* Este capítulo é dividido em dois manuscritos, sendo que o primeiro traz a descrição de novas espécies de *Aphilodon* e o segundo traz a descrição de uma nova espécie de *Mecophilus*. Ambos os manuscritos foram produzidos para serem submetidos no periódico científico *Zootaxa*.

Manuscrito 1. "*Six new species of Aphilodon centipedes (Geophilidae: Aphilodontinae) from Brazil*". Neste trabalho seis novas espécies brasileiras de *Aphilodon* são descritas e ilustradas com base em machos e fêmeas, incluindo

Aphilodon foraminis **n. sp.**, *A. aiuruoca* **n. sp.** e *A. acutus* **n. sp.** registradas para o estado do Rio de Janeiro, e *A. rectitibia* **n. sp.** registrada para os estados de Minas Gerais, Rio de Janeiro e São Paulo. *Aphilodon cangaceiro* **n. sp.** é o primeiro registro de Aphilodontinae para o estado do Piauí e *Aphilodon bahianus* **n. sp.** é a primeira espécie de Aphilodontinae conhecida para o estado da Bahia.

Manuscrito 2. "*A new species of Mecophilus (Geophilidae: Aphilodontinae), with the first registration of the genus from the state of Rio de Janeiro, Brazil*". Apresenta uma espécie nova, *Mecophilus carioca* **n. sp.**, que é detalhadamente descrita com base em machos e fêmeas recentemente coletados no Parque Nacional de Itatiaia. Esta nova espécie representa o primeiro registro de *Mecophilus* para o estado do Rio de Janeiro, Brasil.

Capítulo 3. *Filogenia de Aphilodontinae.* Nesta seção é apresentado um manuscrito que traz uma hipótese filogenética para a subfamília, baseada em dados moleculares e morfológicos. Este trabalho foi produzido para ser submetido no periódico científico *Zoological Journal of the Linnean Society*.

Manuscrito 3. "*Combined molecular and morphological phylogeny of the Aphilodontinae centipedes (Geophilomorpha: Geophilidae)*". Neste trabalho são analisados 52 terminais, incluindo todas as espécies conhecidas de Aphilodontinae e *Geoperingueyia* (grupo irmão de Aphilodontinae). A matriz de dados morfológicos engloba 58 caracteres, dos quais onze caracteres foram pela primeira vez aplicados no grupo. Os dados moleculares analisados foram os marcadores mitocondriais COI e 16S rRNA e os nucleares 18S e 28S rRNAs.

Quatro análises filogenéticas foram conduzidas: máxima parcimônia cladística para caracteres morfológicos, máxima verossimilhança para partições moleculares analisadas separadamente, máxima verossimilhança para partições moleculares analisadas juntas e máxima verossimilhança para partições morfológicas e todas as partições moleculares analisadas juntas (evidência total).

Como resultado, de forma geral a monofilia de Aphilodontinae e também de seus quatro gêneros foi recorrentemente recuperada, sendo que os dados moleculares e a abordagem de evidência total demonstraram maior suporte de clados e foram mais hábeis em resolver algumas politomias entre gêneros e espécies. Os resultados apontam para a monofilia do clado (*Geoperingueyia* + Aphilodontinae), que dentro de

Geophilidae parece estar mais relacionado a Dignathodontinae. *Philacroterium* foi recuperado como mais basal dentro de Aphilodontinae, sendo grupo irmão de um clado com os gêneros Neotropicais. Neste clado, *Mecophilus* é grupo irmão de (*Aphilodon* + *Mairata*). Em adição, novas sinapomorfias morfológicas foram diagnosticadas para *Geoperingueyia* e *Aphilodon* e a sistemática de Aphilodontinae é discutida.

Capítulo 4. *Biogeografia de Aphilodontinae.* Este capítulo é composto por um manuscrito que aborda aspectos da biogeografia histórica de Aphilodontinae através de datação molecular e avaliação do efeito dos processos de vicariância e dispersão na diversificação do grupo. Este trabalho foi produzido para ser submetido no periódico científico *Journal of Biogeography*.

Manuscrito 4. "*Historical biogeography of the Aphilodontinae centipedes (Geophilomorpha, Geophilidae): Cretaceous West Gondwana vicariance followed by sympatric diversification shaped the current distribution of the genera*". Sob os preceitos da metodologia científica, avaliamos a hipótese de que o gênero sul-africano *Philacroterium* divergiu dos gêneros Neotropicais no Cretáceo, devido à separação da África continental e da América do Sul. Já o clado Neotropical se diversificou em uma mesma área biogeográfica, e expandiu seu território algumas vezes com casos de dispersão observados entre espécies de *Aphilodon* que apresentam ampla distribuição. Para testar esta hipótese, sequências moleculares relativas aos marcadores mitocondriais COI e 16S rRNA e nucleares 18S e 28S rRNAs foram usadas para estimar o tempo de divergência entre as espécies de Aphilodontinae em uma abordagem bayesiana. Já a reconstrução da distribuição em áreas ancestrais foi obtida por Statistical Dispersal Vicariance Analysis (S-DIVA), Dispersal Extinction Cladogenesis (DEC) e Statistical Dispersal Extinction Cladogenesis (S-DEC). As unidades de área utilizadas neste estudo foram baseadas em biorregiões propostas por Olson *et al.* (2001) e Morrone (2014).

Os resultados sugerem que Aphilodontinae apresentou uma distribuição ancestral na província do Atlântico (biorregião da América do Sul relativa ao bioma de Mata Atlântica) ou em um fragmento Gondwânico compreendendo parte da África continental e da América do Sul. No final do Cretáceo, por volta de 140 milhões de anos atrás, o gênero sul-africano *Philacroterium* separou-se dos outros Aphilodontinae por um evento vicariante inferido como a separação entre a África continental e a América do Sul. Os três gêneros Neotropicais *Aphilodon*, *Mecophilus* e *Mairata* se diversificaram na biorregião da província do Atlântico, e algumas espécies de *Aphilodon* alcançaram outras áreas, principalmente por dispersão.

Capítulo 5. *Considerações finais.* Apresenta as principais conclusões dos capítulos anteriores e traça um panorama para os principais desafios a serem enfrentados no campo da sistemática e biogeografia de Aphilodontinae.

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Capítulo 2. Novas espécies de Aphilodontinae

Six new species of *Aphilodon* centipedes (Geophilidae: Aphilodontinae) from Brazil

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Abstract

After recent sampling from Southeast and Northeast of Brazil, six new species of *Aphilodon* are described and illustrated based on males and females. From the Atlantic Forest biome, *Aphilodon foraminis* **n. sp.**, *A. aiuruoca* **n. sp.**, *A. acutus* **n. sp.** and *A. rectitibia* **n. sp.** were registered in the Parque Nacional de Itatiaia, state of Rio de Janeiro. *A. rectitibia* **n. sp.** was also reported from the municipalities of Eldorado and Salesópolis, state of São Paulo, and sampled from the Parque Nacional da Serra do Cipó, in the state of Minas Gerais. *Aphilodon cangaceiro* **n. sp.** is the first record of Aphilodontinae for the state of Piauí and was sampled in the Caatinga biome, from the Parque Nacional da Serra das Confusões. *Aphilodon bahianus* **n. sp.** is the first species of Aphilodontinae known from Bahia, being registered from the Atlantic Forest biome in the Parque Nacional do Descobrimento and Reserva Biológica de Una, and in the Caatinga biome from the Parque Municipal de Mucugê and Parque Nacional da Chapada da Diamantina. Additionally, some morphological characteristics of the known species of *Aphilodon* and its distribution are compared.

Key words: Geophilomorpha, biodiversity, Atlantic Forest, Caatinga, morphology, taxonomy.

Introduction

Aphilodontinae centipedes are characterized by the filiform body with less than 1 to 7 cm, forcipule composed by only three articles and sternites without ventral pores (Bonato *et al.* 2011). The taxonomy of the subfamily was recently reviewed by Calvanese *et al.* (2019), and four genera were considered, the South African *Philacroterium* Attems, 1926 and the Neotropical *Aphilodon* Silvestri, 1898, *Mecophilus* Silvestri, 1909 and *Mairata* Calvanese, Bonato & Brescovit, 2019.

Inside the Neotropics the most diverse is *Aphilodon*, with at the moment 10 species (Calvanese *et al.* 2019). Although this centipedes shown a high morphological variation, such as in the shape of forcipule, body size and number of leg-bearing segments (Attems 1929), Calvanese *et al.* (2019) clarified its diagnosis and identified one morphological novelty to delimit the genus, that is, the presence of a terminal spine in the ultimate legs. Currently, species of *Aphilodon* are known mainly from the Atlantic Forest biome in the Southeast of Brazil, but some species occur in the Atlantic Forest of Paraguay, Argentina Pampas, and Brazilian Cerrado (Silvestri 1909; Calvanese *et al.* 2019).

Here, six new species recently sampled of *Aphilodon* are described and illustrated based on both male and females. In addition, from these materials the knowledge about the occurrence of the genus is significantly expanded to the Northeast of Brazil, with first reports from the states of Bahia and Piauí and inside the Caatinga biome.

Material and Methods

All type-species were manually collected in an expedition recently carried out by the first author in 09/2017 and between 09/2018 and 01/2019 with license Sisbio number 60676–2. The sampled material was deposited in the Collection of Myriapodology of the Instituto Butantan, São Paulo (IBSP, curator: A.D. Brescovit). Also were evaluated some specimens deposited at the Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil (MZSP, curator R. Pinto da Rocha).

Measurements and morphological analysis were made on the cephalic plate, antennae, labrum, mandible, first and second maxillary, forcipular apparatus, leg-bearing segments of the trunk, ultimate leg-bearing segment, and post-pedal segmentation. Photographs were taken on a Leica DFC 500 digital camera coupled to a Leica MZ 16A stereomicroscope. Tiny structures or internal parts were analyzed using a

Leica DFC 500 digital camera coupled to a Leica DM4000B optical microscope. Assembling stacks of photos were made in a Leica Application Suite version 2.5.0. At least one specimen of each new species was examined by Scanning Electron Microscope (SEM), with a FEI Quanta 250 at the Laboratório de Biologia Celular of Instituto Butantan. The protocol for this procedure follows Calvanese *et al.* (2019).

The adult condition of the females was found by the full spermatheca and the gonopods fully developed. The adult condition of the males was found by the masses of spermatozoa and the gonopods fully developed.

Dissection procedures were based on Pereira (2000) and the terminology of morphological structures follows that of Bonato *et al.* (2010). All measurements are given in millimeters.

The abbreviations used in Table 1 and illustrations have its meaning displayed directly in the legends, and listed below:

AT—antenna
BT—basis of tarsungulum
CE—cephalic plate
CP—coxal pores
CX—coxosternite
CXS—coxosternite of the second maxillary
DE—denticle
DT—denticle of tarsungulum
DTI—denticle of tibia
FL—forcipule
FP—foraminal process
FPT—forcipular pretergite
MC—middle of coxosternite of the second maxillary
MS—metasternite
MT—metatergite
PS—presternite
PT—pretergite
TA—tarsungulum
TI—tibia
TR—transverse suture of cephalic plate

TS—terminal spine
TTF—trochanteroprefemur+femur
UMS—ultimate metasternite
UPS—ultimate presternite

Results

Geophilomorpha Pocock, 1895

Geophiloidea Leach, 1815

Geophilidae Leach, 1815

Aphilodontinae Silvestri, 1909 (1908)

Genus *Aphilodon* Silvestri, 1898

Type species. *Aphilodon spegazzinii* Silvestri, 1898, by monotypy.

Included species:
A. spegazzinii Silvestri, 1898
A. micronyx Brölemann, 1902
A. angustatus Silvestri, 1909
A. intermedius Silvestri, 1909
A. modestus Silvestri, 1909
A. caboclos Calvanese, Brescovit & Bonato, 2019
A. indespectus Calvanese, Brescovit & Bonato, 2019
A. meganae Calvanese, Brescovit & Bonato, 2019
A. pereirai Calvanese, Brescovit & Bonato, 2019
A. silvestrii Calvanese, Brescovit & Bonato, 2019
A. foraminis **n. sp.**
A. aiuruoca **n. sp.**
A. acutus **n. sp.**
A. rectitibia **n. sp.**

A. cangaceiro **n. sp.**
A. bahianus **n. sp.**

Diagnosis. Recently, one non homoplastic synapomorphy was reported to *Aphilodon* (Calvanese *et al.* 2019): in males and females, the ultimate legs with a terminal spine in the last article (tarsus 1) (Figs 3C, 9F, 11C), and this characteristic was here considered effective to differentiate species of *Aphilodon* from the other Aphilodontinae.

In addition, *Aphilodon* and the other Neotropical Aphilodontinae differs from the South African *Philacroterium* by have species with the lateral parts of labrum thin and membranous (in the *Philacroterium* species the pieces of labrum are only vestigial) and second maxillary telopodite with three articles (Figs 1A, 5A, 7A) (in the *Philacroterium* species the telopodite of second maxillary have only two articles). The Neotropical genera *Aphilodon* and *Mairata* differs from *Mecophilus* by having species with the length of the forcipular pretergite shorter than 1/8 of the cephalic plate length (Fig. 2B), and by the shape of the forcipular coxosternite and first sternite of leg-bearing segments, that are wider than long (Fig. 2C) (in *Mecophilus* these structures are elongated). *Aphilodon* differs from *Mairata* by having species with telopodite of second maxillary composed of three articles not reduced (Figs 1A, 5A, 7A) (in *Mairata* the article 3 is reduced), and ultimate legs only moderately thickened in males and slender in females (Fig. 2F–G) (in *Mairata* males have the ultimate legs remarkably thickened, and females have the tarsus 1 most wide in the distal part).

Morphological variation and occurrence. Some morphological characteristics of the species of *Aphilodon* and its geographical distribution are reported in Table 1 (data referring to the already described species were based on the genus review presented by Calvanese *et al.* 2019).

Aphilodon foraminis new species
(Figs 1–2)

Types. Holotype: f#, from Parque Nacional de Itatiaia, Itatiaia, Rio de Janeiro, Brazil, 03–17/12/2017, V. Calvanese & A. Silva col., deposited in IBSP 6442. Paratypes: 2f# (IBSP 6444) and 2m# (IBSP 6443), same data as holotype.

Other material examined. 12f# (IBSP 6445), 8m# (IBSP 6446), with the same data of types.

Etymology. The specific epithet means "hole" in Latin, which refers to the large foraminal process on the last article (tarsus 1) of the ultimate legs, characteristic of this species.

Diagnosis. *Aphilodon foraminis* **n. sp.** resemble *A. caboclos* and *A. rectitibia* **n. sp.** by the forcipular tarsungulum longer than the trochanteroprefemur+femur (Figs 1B−C, 2C), but differs from both species by having 39−43 leg-bearing segments (Fig. 2A) (both have > 49 leg-bearing segments), denticles of tarsungulum not apparent (Fig. 1B−C) (the denticle is apparent in *A. caboclos* and *A. rectitibia* **n. sp.**), tarsus 1 of the ultimate legs with a large terminal foraminal process (Fig. 1F) (this structure is small in the other species). Also, it differs from *A. rectitibia* **n. sp.** by having a second maxillary coxosternite not reduced in the middle part (Fig. 1A) (against a coxosternite reduced in the middle part of this species), and by the forcipule with the tibia not parallel to the base of tarsungulum (Fig. 1B−C) (in *A. rectitibia* **n. sp.** the tibia is straight, parallel to the base of the tarsungulum). From *A. caboclos* also differs by the cephalic plate wider than long and with a vestigial transverse suture present (Fig. 2B) (in *A. caboclos* the cephalic plate is longer than wider and without transverse suture).

Description of holotype. Female (IBSP 6442).

General aspect: body with 12 long and 0.5 maximum width (middle part of trunk). Color (preserved specimen in alcohol): head and forcipular segment ferrugineus, other leg-bearing segments and post-pedal segmentation are dark yellow (Fig. 2A−G).

Cephalic plate: wider than long, 0.43 long and 0.47 wide (length/width ratio 0.9), with a basal row of ca. 8 setae and a row of 8 setae in the middle (in the position corresponding to the transverse suture, which can be observed as vestigial). Other sparse setae also are present, mainly in the distal part. General morphology as in Fig. 2B.

Antennae: with 0.84 long (1.95 times as long as cephalic plate); articles II−XIII length/width ratio 0.5–0.7; last article length/width ratio 1.4, with 16 claviform specialized setae dorsal and 12 ventral. General morphology as in Fig. 2A−C.

Clypeus: 3+3 lateral setae; 2+2 post-antennal setae; 7+7 sub clypeal setae.

Mandible: pectinate lamellae with ca. 25 denticles.

First maxillary: left telopodite length 0.05 and width 0.03; distal article with 3 subapical sensilla; each medial projection of coxosternite with 2 apical and 2 subapical sensilla. General morphology as in Fig. 1A.

Second maxillary: 6 short setae distributed in a row along the posterior margin between the telopodites; coxosternite rectangular, with middle part not suppressed, maximum length/maximum width ratio 0.6; left telopodite length 0.08 (1.55 times as long as the telopodite of first maxillary) and maximum width (basal margin) 0.03; both telopodite with one apical and two subapical seta. General morphology as in Fig. 1A.

Forcipular segment: tergite sub rectangular, slightly wider at the base, length 0.11 (0.25 times as long as the cephalic plate) and width 0.46 (length/width ratio 0.25), with many sparse setae; coxosternite length 0.3 and width 0.48 (length/width ratio 0.6); telopodite length 0.32, not reaching the anterior margin of cephalic plate, with 2 denticles in the trochanteroprefemur+femur and 1 denticle in the tibia, all relatively large; denticle distal of trochanteroprefemur with 1 apical seta, denticle of femur bilobed, with 2 apical and one subapical setae, denticle of tibia bilobed and with 5 setae; tarsungulum without an apparent denticle and with 1.35 times the length of the trochanteroprefemur+femur. General morphology as in Figs 1B–C, 2B–C.

Legs: 41 pairs; first leg 0.75 times as long as the second leg, with procoxae extended to the midline of the body; leg 15 length 0.67. General morphology as in Fig. 2A−G.

Tergum from the first to penultimate leg-bearing segment: rectangular tergites, with two rows of ca. 10 setae and short sparse setae; tergite 15 length 0.21 and width 0.45 (length/width ratio 0.45); pretergite 15 with 0.3 times as long as the previous metatergite and with one row of 10 setae and short sparse setae. General morphology as in Fig. 2D.

Sternum from the first to penultimate leg-bearing segment: first sternite trapezoidal, other sternites subquadratic, with scattered setae; sternite 19 length 0.28 and width 0.31 (length/width ratio 0.9); presternite present throughout body and presternite 19 with 0.2 times as long as the previous metasternite. General morphology as in Fig. 2C, E.

Ultimate leg-bearing segment: metatergite wider than long, trapezoidal, length 0.16 and maximum width 0.19 (length/width ratio 0.85); sternite apparently only in the sides of the metasternite. General morphology as in Figs 1D, 2F

Ultimate legs: straight and slightly thickened compared to the previous ones, length 0.81 (1.4 as long as the penultimate leg); each coxopleuron with ca. 6 coxal pores opening sparsely; tarsus 1 approximately as long as the tibia. General morphology as in Figs 1D, 2F.

Postpedal segments: gonopods with 2+2 distal setae.

Sexual dimorphism. Males with ultimate legs a little thicker than in females (Figs 1E, 2G), and gonopod biarticulated, article 1 with 3–4 setae, article 2 with 4–5 scattered setae.

Variation. N= 25, including 15f# and 10m# (from a single locality): total body length: 11−15 mm. Pairs of legs: 39–43. Coxal pores: 5–8.

Natural history. The specimens were found in the high and low part of the Parque Nacional de Itatiaia, inside high forests or in forest refuges in open areas. The specimens were sampled mainly in ravines or near roots, buried about 5–10 cm.

Distribution. Brazil, state of Rio de Janeiro.

Aphilodon aiuruoca new species

(Figs 3–4)

Types. Holotype: f#, from Parque Nacional de Itatiaia, Itatiaia, Rio de Janeiro, Brazil, 03–17/12/2017, V. Calvanese & A. Silva col., deposited in IBSP 6447. Paratypes: 2f# (IBSP 6449) and 3m# (IBSP 6448), same data as holotype.

Other material examined. 2f# (IBSP 6450), with the same data of types.

Etymology. The specific epithet "aiuruoca" refers to the Aiuruoca river that springs in the Serra da Mantiqueira in the Parque Nacional de Itatiaia, the type locality. Aiuruoca, in Tupi language, means "house of parrots".

Diagnosis. *Aphilodon aiuruoca* **n. sp.** resembles *A. cangaceiro* **n. sp.** by the presence of the opening of the coxal pores only grouped to the lateral membrane of the metasternite (Figs 3B, 4G), but differs by having body length 7–10 mm (against 20–30 mm in *A. cangaceiro* **n. sp.**), 41–43 leg-bearing segments (Fig. 4A) (against 87–93), and metatergite, pretergite, metasternite and presternite with shape not elongated (Fig. 4D–E) (against elongated).

Description of holotype. Female (IBSP 6447).

General aspect: body with 8 long and 0.25 of maximum width (middle part of trunk). Color (preserved specimen in alcohol): head and forcipular segment pale yellow, other leg-bearing segments and post-pedal segmentation whitish (Fig. 4A–G).

Cephalic plate: longer than wide, 0.24 long and 0.2 wide (length/width ratio 1.2), with setae sparse all over its surface. General morphology as in Fig. 4B.

Antennae: 0.45 long (1.9 times as long as the cephalic plate); articles II−XIII length/width ratio 0.4–0.7; last article length/width ratio 2.5, with 12 claviform specialized setae dorsal and 8 ventral. General morphology as in Fig. 4A–B.

Clypeus: 3+3 lateral setae; 2+2 post-antennal setae; 4+4 sub clypeal setae.

Mandible: pectinate lamellae with ca. 10 denticles.

First maxillary: left telopodite length 0.034 and width 0.024; distal article with 2 subapical sensilla; each medial projection of coxosternite with 4 apical and 1 subapical sensilla.

Second maxillary: 8 little setae distributed in a row along the posterior margin between the telopodites; coxosternite sub rectangular, with middle part not suppressed, length/width ratio 0.6; left telopodite length 0.05 (1.5 times as long as the telopodite of first maxillary) and maximum width (basal margin) 0.02; both telopodite with one apical sensilla and two subapical setae.

Forcipular segment: tergite sub rectangular, slightly wider at the base, length 0.04 (0.16 times as long as the cephalic plate) and width 0.19 (length/width ratio 0.2), with a row of 10 setae; coxosternite length 0.18 and width 0.21 (length/width ratio 0.85); telopodite length 0.14, not reaching the anterior margin of the cephalic plate, with 2 denticles in the trochanteroprefemur+femur and 1 denticle in the tibia, all relatively large; denticle distal of trochanteroprefemur with 1 apical seta, denticle of femur bilobed, with 2 apical setae, denticle of tibia bilobed and with 4 setae; tarsungulum with a short denticle, and measuring 0.85 times as long as the trochanteroprefemur+femur. General morphology as in Figs 3A, 4B–C.

Legs: 43 pairs; first leg 0.8 times as long as the second leg, with procoxae extended to the midline of the body; leg 34 length 0.24. General morphology as in Figs 3A, 4A–G.

Tergum from the first to penultimate leg-bearing segment: rectangular tergites, with two rows of 6 setae; tergite 18 length 0.1 and width 0.24 (length/width ratio 0.4); pretergite 18 with 0.6 times as long as the previous metatergite and with one row of 6 setae. General morphology as in Fig. 4D.

Sternum from the first to penultimate leg-bearing segment: first sternite oval, other sternites elongated, with scattered setae; sternite 27 length 0.12 and width 0.11 (length/width ratio 1.1); presternite present throughout body and presternite 27 has a length 0.25 times as long as the previous metasternite. General morphology as in Fig. 4E.

Ultimate leg-bearing segment: metatergite wider than long, pentagonal, length 0.11 and maximum width 0.14 (length/width ratio 0.8); sternite apparently divided in the middle part. General morphology as in Figs 3B, 4G.

Ultimate legs: conical, tapering to the anterior part, length 0.56 (2.05 as long as penultimate leg); each coxopleuron with ca. 8 coxal pores opening close to the membrane between the coxopleuron and the metasternite; tarsus 1 measuring 2.1 times as long as tibia. General morphology as in Figs 3B, 4F–G.

Postpedal segments: gonopods with 3+3 distal setae (Figs 3B, 4G).

Sexual dimorphism. Male with ultimate legs a little thicker than in female, and gonopod biarticulated, article 1 with 3–6 setae, article 2 with 6–9 scattered setae.

Variation. N= 8, including 5f# and 3m# (from a single locality): total body length: 7−10 mm. Pairs of legs: 41–43. Coxal pores: 5–10.

Natural history. The specimens were found in the low part of the Parque Nacional de Itatiaia, inside high forests. The specimens were sampled near roots, in the most humid refuges of the environment, buried about 5–10 cm.

Distribution. Brazil, state of Rio de Janeiro.

Aphilodon acutus new species

(Figs 5–6)

Types. Holotype: f#, from Parque Nacional de Itatiaia, Itatiaia, Rio de Janeiro, Brazil, 03–17/12/2017, V. Calvanese & A. Silva col., deposited in IBSP 6451. Paratypes: 2f# (IBSP 6453) and 2m# (IBSP 6452), same data as holotype.

Other material examined. 4f# (IBSP 6454), with the same data of types.

Etymology. The specific epithet "acutus" means "sharp" in Latin, and refers to the large and sharpened denticle of tarsungulum, characteristic of this species.

Diagnosis. *Aphilodon acutus* **n. sp.** resembles *A. micronyx* and *A. rectitibia* **n. sp.** by having the coxosternite of the second maxillary distinctly shorter in the middle part (Fig. 5A). *Aphilodon acutus* **n. sp.** differs from the two species by having the cephalic plate longer than wide and without transverse suture apparent (Fig. 6B) (the two species have a cephalic plate wider than long and with an apparent transverse suture), and the denticle of tarsungulum is remarkably large (Figs 5B, 6C) (against a denticle relatively short in *A. micronyx* and *A. rectitibia* **n. sp.**).

Description of holotype. Female (IBSP 6451).

General aspect: body with 18 long and 0.4 maximum width (middle part of trunk). Color (preserved specimen in alcohol): head and forcipular segment ferrugineus, other leg-bearing segments and post-pedal segmentation pale yellow (Fig. 6A–G).

Cephalic plate: longer than wide, 0.37 long and 0.3 wide (length/width ratio 1.2), with setae mainly in three rows: anterior, medial and posterior. General morphology as in Fig. 6B.

Antennae: left antenna 0.93 long (2.5 times as long as the cephalic plate); articles II−XIII length/width ratio 0.6–1; last article length/width ratio 2, with 13 claviform specialized setae dorsal and 10 ventral. General morphology as in Fig. 6B–C.

Clypeus: 3+3 lateral setae; 2+2 post-antennal setae; 6+6 sub clypeal setae.

Mandible: pectinate lamellae with ca. 25 denticles.

First maxillary: left telopodite length 0.036 and width 0.033; distal article with 1 subapical sensilla; each medial projection of coxosternite with 3 subapical sensilla. General morphology as in Fig. 5A.

Second maxillary: 10 little setae distributed in a row along the posterior margin between the telopodites; coxosternite with suppressed middle part (middle 0.32 times as long as the lateral margins) and maximum length/maximum width ratio 0.7; left telopodite length 0.06 (1.6 times as long as the telopodite of first maxillary) and maximum width (basal margin) 0.024; right telopodite with one apical sensilla and two subapical setae and left with one apical and one subapical setae. General morphology as in Fig. 5A.

Forcipular segment: tergite sub trapezoidal, length 0.13 (0.35 times as long as the cephalic plate) and width 0.35 (length/width ratio 0.37), with 12 sparse setae; coxosternite length 0.28 and width 0.38 (length/width ratio 0.73); telopodite length 0.25, not reaching the anterior margin of the cephalic plate, with 2 denticles in the trochanteroprefemur+femur and 1 denticle in the tibia, all relatively large; denticle distal of trochanteroprefemur with 1 apical seta, denticle of femur bilobed, with 2 apical and 1 subapical setae, denticle of tibia bilobed and with 3 setae; tarsungulum with a large denticle, and 0.9 as long as the trochanteroprefemur+femur. General morphology as in Figs 5B, 6B–C.

Legs: 63 pairs; first leg 0.6 times as long as the second leg, with procoxae extended to the midline of the body; leg 50 length 0.5. General morphology as in Fig. 6A–G.

Tergum from the first to penultimate leg-bearing segment: rectangular tergites, with two rows of 6–7 setae; tergite 50 length 0.15 and width 0.4 (length/width ratio 0.37); pretergite 50 with length 0.4 times as long as the previous metatergite and one row of 6 setae. General morphology as in Fig. 6D.

Sternum from the first to penultimate leg-bearing segment: first sternite in a semi-circle shape, other sternites rectangular or sub quadratic, with scattered setae; sternite 50 length 0.18 and width 0.16 (length/width ratio 1.1); presternite tiny throughout body, not visible in some segments. General morphology as in Fig. 6C, E.

Ultimate leg-bearing segment: metatergite wider than long, trapezoidal, length 0.1 and maximum width 0.17 (length/width ratio 0.6); presternite not apparent. General morphology as in Figs 5C, 6G.

Ultimate legs: straight, length 0.9 (1.8 as long as penultimate leg); each coxopleuron with ca. 12 coxal pores, most part opening close to the membrane between the coxopleuron and the metasternite, but some pores are most scattered; tarsus 1 measuring 1.5 times as long as tibia. General morphology as in Figs 5C, 6G.

Postpedal segments: gonopods with 4+4 distal setae.

Sexual dimorphism. Males with ultimate legs a little thicker than in females (Fig. 6F), and gonopod biarticulated, article 1 with 2–3 setae, article 2 with 3–4 scattered setae.

Variation. N= 9, including 7f# and 2m# (from a single locality): total body length: 18−22 mm. Pairs of legs: 61–65. Coxal pores: 8–12.

Natural history. The specimens were found in the high and low part of the Parque Nacional de Itatiaia, in the humus or under logs or rocks, buried about 2–5 cm.

Distribution. Brazil, state of Rio de Janeiro.

Aphilodon rectitibia **new species**

(Figs 7–8)

Types. Holotype: f#, from Parque Nacional de Itatiaia, Rio de Janeiro, Brazil, 03–17/12/2017, V. Calvanese & A. Silva col., deposited in IBSP 6467. Paratypes: 2f# (IBSP 6468), same data as holotype and 1m# (IBSP 6469), Parque Nacional da Serra do Cipó, Santana do Riacho, Minas Gerais, Brazil, 01–11/09/2018, V. Calvanese & A. Silva col.

Other material examined. BRAZIL. *Rio de Janeiro*: Itatiaia, 14/11/1945, O. Schubart col., 1m#, 1f# (MZSP 3061). *São Paulo*: Eldorado (24º31'12"S, 48º06'29"W), 09/2015, 1f# (MZSP 3437); Salesópolis, 09−10/07/1992, 1m# (MZSP 0739); 09−10/07/1992, 1m#, 1f# (IBSP 4081); Estação Biológica de Boracéia (23°37'51"S, 45°52'11"W), 26/11/1968, Rabelo col., 1m# (MZSP 921).

Etymology. The epithet "rectitibia" is a reference to the peculiar shape of the forcipular tibia, the fusion of the Latin words tibia (structure) + rectus (morphology).

Diagnosis. *Aphilodon rectitibia* **n. sp.** resembles *A. micronyx* and *A. foraminis* **n. sp.** by having a cephalic plate wider than long and with an apparent transverse suture (Fig. 8B), but differs by having the forcipular tibia straighter, parallel to the basis of tarsungulum (Figs 7B–C, 8C). Also, differs from *A. micronyx* by the tarsungulum that is longer than the trochanteroprefemur+femur (Figs 7B–C, 8C) (in *A. micronyx* the trochanteroprefemur+femur is longer). From *A. foraminis* **n. sp.** also differs by having 51–55 leg-bearing segments (Fig. 8A) (against 39–43), coxosternite of the second maxillary distinctly shorter in the middle part (Fig. 7A) (against a coxosternite not reduced in the middle), 20–35 coxal pores (Figs 7D, 8G) (against 5–8), denticles of tarsungulum apparent (Fig. 7B–C) (against not apparent), and ultimate tarsus 1 with a terminal foraminis small (against a large foraminal process).

Description of holotype. Female (IBSP 6467).

General aspect: body with 27 long and 1 of maximum width (middle part of trunk). Color (preserved specimen in alcohol): head and forcipular segment ferrugineus, other leg-bearing segments and post-pedal segmentation dark yellow (Fig. 8A–G).

Cephalic plate: wider than long, 0.8 long and 0.9 wide (length/width ratio 0.9), with setae sparse. General morphology as in Fig. 8B.

Antennae: left antenna 2.2 long (2.75 times as long as cephalic plate); articles II−XIII length/width ratio 0.4–0.8; last article length/width ratio 2, with 30 claviform specialized setae dorsal and 22 ventral. General morphology as in Fig. 8B–C.

Clypeus: 4+4 lateral setae; 2+2 post-antennal setae; 10+11 sub clypeal setae.

Mandible: pectinate lamellae with ca. 35 denticles.

First maxillary: left telopodite length 0.06 and width 0.055; distal article with 2 subapical sensilla; each medial projection of coxosternite with 3 subapical sensilla. General morphology as in Fig. 7A.

Second maxillary: 16 short setae distributed in a row along the posterior margin between the telopodites; coxosternite rectangular, with middle part suppressed (ca. 0.4 times as long as the lateral margins), length/width ratio 0.54, left telopodite length 0.11 (1.8 times as long as the telopodite of the first maxillary) and maximum width (basal margin) 0.05; both telopodite with one sensilla apical and two subapical setae. General morphology as in Fig. 7A.

Forcipular segment: tergite trapezoidal, length 0.3 (0.35 times as long as the cephalic plate) and width 0.9 (length/width ratio 0.3), with short setae sparse; coxosternite length 0.67 and width 0.70 (length/width ratio 0.95); telopodite length 0.76, reaching the anterior margin of the cephalic plate, with 2 denticles in the trochanteroprefemur+femur and 1 denticle in the tibia, distal denticle of trochanteroprefemur reduced and denticles of femur and tibia moderately developed; distal denticle of trochanteroprefemur with 1 short apical seta, denticle of femur bilobed and with 2 apical and 2 subapical setae, denticle of tibia bilobed with 2 apical and 2 subapical setae; tarsungulum with a reduced denticle and 2.15 times as long as trochanteroprefemur+femur. General morphology as in Figs 7B–C, 8B–C.

Legs: 53 pairs; first leg 0.8 times as long as the second leg, with procoxae not extended to the midline of the body; leg 40 length 0.9. General morphology as in Fig. 8A–G.

Tergum from the first to penultimate leg-bearing segment: rectangular tergites, with sparse setae; tergite 23 length 0.35 and width 1 (length/width ratio 0.3); pretergite 23 with 0.3 times as long as the previous metatergite and with sparse setae. General morphology as in Fig. 8D.

Sternum from the first to penultimate leg-bearing segment: first sternite in a semi circle shape, other sternites rectangular or subquadratic with many scattered setae; sternite 23 length 0.33 and width 0.43 (length/width ratio 0.75); presternite present throughout the body, in segment 35 with 0.14 times as long as the previous metasternite and with 10 setae. General morphology as in Fig. 8E.

Ultimate leg-bearing segment: metatergite wider than long, trapezoidal, length 0.23 and maximum width 0.26 (length/width ratio 0.9); sternite apparent only in the sides of the metasternite. General morphology as in Fig. 8G.

Ultimate legs: straight, length 1.55 (1.6 as long as penultimate leg); each coxopleuron with ca. 30 coxal pores sparse; tarsus 1 measuring 1.75 times as long as tibia. General morphology as in Fig. 8F–G.

Postpedal segments: gonopods with 3+3 distal setae.

Sexual dimorphism. Male with ultimate legs thicker than in female (Fig. 7D), male gonopod biarticulate, article 1 with 8–10 setae, article 2 with 13–14 scattered setae.

Variation. N= 11, including 6f# and 5m# (from four localities): total body length: 20−50 mm. Pairs of legs: 51–55. Coxal pores: 20–35.

Natural history. The specimens were found in the high and low part of the Parque Nacional de Itatiaia and Parque Nacional da Serra do Cipó, inside the high forests or in forest refuges in open areas, and sampled in the humus or under logs or rocks, buried about 2–5 cm.

Distribution. Brazil, states of Minas Gerais, Rio de Janeiro and São Paulo.

Aphilodon cangaceiro new species

(Figs 9–10)

Types. Holotype: f#, from Parque Nacional da Serra das Confusões, Caracol, Piauí, Brazil, 03–17/01/2019, V. Calvanese & A. Silva col., deposited in IBSP 6455. Paratypes: 2f# (IBSP 6457) and 2m# (IBSP 6456), same data as holotype.

Other material examined. 3f# (IBSP 6459), 10m# (IBSP 6458), with the same data of types.

Etymology. The epithet is an adjective, means resident of cangaço, and is a reference to the locality of the type.

Diagnosis. *Aphilodon cangaceiro* **n. sp.** resembles *A. aiuruoca* **n. sp.** by having the opening of the coxal pores only grouped to the lateral membrane of the metasternite (Figs 9E, 10E), but differs by having body length between 20–30 mm (against 7–10 mm in *A. aiuruoca* **n. sp.**), number of leg-bearing segments between 87–93 (Fig. 10A) (against 41–43), and by the metatergite, pretergite, metasternite and presternite with shape elongated (Fig. 9B, D).

Description of holotype. Female (IBSP 6455).

General aspect: body with 24 long and 0.22 maximum width (middle part of trunk). Color (preserved specimen in alcohol): head and forcipular segment pale ferrugineus, other leg-bearing segments and post-pedal segmentation whitish (Fig. 10A−E).

Cephalic plate: longer than wide, 0.23 long and 0.17 wide (length/width ratio 1.35), with short setae sparse. General morphology as in Fig. 10B.

Antennae: left antenna 0.58 long (2.5 times as long as the cephalic plate); articles II−XIII length/width ratio 0.4–0.8; last article length/width ratio 2.4, with 8 claviform specialized setae dorsal and 6 ventral. General morphology as in Fig. 10B–C.

Clypeus: 3+3 lateral setae; 2+2 post-antennal setae; 5+4 sub clypeal setae.

Mandible: pectinate lamellae with ca. 15 denticles.

First maxillary: left telopodite length 0.028 and width 0.016; distal article with 2 subapical sensilla; each medial projection of coxosternite with one apical and 2 subapical sensilla.

Second maxillary: 9 little setae distributed in a row along the posterior margin between the telopodites; coxosternite rectangular, with middle part not suppressed, length/width ratio 0.6, left telopodite length 0.06 (2.1 times as long as the telopodite of first maxillary) and maximum width (basal margin) 0.027; both telopodite with one apical and two subapical setae.

Forcipular segment: tergite trapezoidal, length 0.075 (0.32 times as long as cephalic plate) and width 0.17 (length/width ratio 0.44), with 12 sparse setae; coxosternite length 0.21 and width 0.22 (length/width ratio 0.95); telopodite length 0.18, not reaching the anterior margin of the cephalic plate, with 2 denticles in the trochanteroprefemur+femur and 1 denticle in the tibia, all relatively large; denticle distal of trochanteroprefemur with 1 apical seta, denticle of femur bilobed with 1 apical and 2 subapical setae, denticle of tibia bilobed with 2 apical and 2 subapical setae; tarsungulum with a denticle and 0.86 times as long as the trochanteroprefemur+femur. General morphology as in Figs 9A, 10B–C.

Legs: 91 pairs; first leg 0.9 times as long as the second leg, with procoxae extended to the midline of the body; leg 17 length 0.25. General morphology as in Figs 9B–E, 10A–C.

Tergum from the first to penultimate leg-bearing segment: rectangular tergites or subquadratic, with two rows of 5–5 setae; tergite 17 length 0.13 and width 0.16 (length/width ratio 0.8); pretergite 17 with 0.74 times as long as the previous metatergite and with ca. 10 sparse setae. General morphology as in Fig. 9B.

Sternum from the first to penultimate leg-bearing segment: first sternite in a semi-circle shape relatively elongated, other sternites rectangular and elongated with scattered setae; sternite 17 length 0.18 and width 0.086 (length/width ratio 2.1);

presternite elongated throughout body, in segment 17 length 0.11 and width 0.19 (length/width ratio 0.55), 0.6 times as long as the previous metasternite and with two rows of ca. 5+5 setae. General morphology as in Fig. 9D.

Ultimate leg-bearing segment: metatergite wider than long, trapezoidal, length 0.06 and maximum width 0.13 (length/width ratio 0.4); presternite apparent and elongated, with 0.44 times as long as the previous metasternite. General morphology as in Fig. 9E.

Ultimate legs: straight, length 0.47 (2.2 as long as the penultimate leg); each coxopleuron with 4 coxal pores opening above to the metasternite; tarsus 1 measuring 1.6 times as long as tibia, and with a well developed terminal spine. General morphology as in Fig. 9E–F.

Postpedal segments: gonopods are very membranous, with 2+2 distal setae.

Sexual dimorphism. Male ultimate legs are thicker than in females (Fig. 10D–E), and gonopod biarticulated, article 1 with 2–4 setae, article 2 with 2–5 scattered setae.

Variation. N= 18, including 6f# and 12m# (from a single locality): total body length: 20−30 mm. Pairs of legs: 87–93. Coxal pores: 4–6.

Natural history. The specimens were found in the Parque Nacional da Serra das Confusões, in areas of Caatinga. All specimens were sampled in forest refuges that lie between large rocks, totally rolled up and forming a kind of ball drawn close to the roots.

Distribution. Brazil, state of Piauí.

Aphilodon bahianus **new species**

(Figs 11–12)

Types. Holotype: f#, from Parque Nacional do Descobrimento, Prado, Bahia, Brazil, 11–18/11/2018, V. Calvanese & A. Silva col., deposited in IBSP 6460. Paratypes: 2f# (IBSP 6462) and 2m# (IBSP 6461), same data as holotype.

Other material examined. BRAZIL. *Bahia*: Andaraí, Parque Nacional da Chapada da Diamantina, 29/11–05/12/2018, V. Calvanese & A. Silva col., 1m# (IBSP 6465); Mucugê, Parque Municipal de Mucugê, 29/11–05/12/2018, V. Calvanese & A. Silva col., 2m# (IBSP 6465); Prado, Parque Nacional do Descobrimento, 12/2018, V.

Calvanese & A. Silva col., 4f# (IBSP 6463); Una, Reserva Biológica de Una, 20–28/11/2018, V. Calvanese & A. Silva col., 5f#, 5m# (IBSP 6464).

Etymology. The epithet is an adjective and denotes the occurrence of the holotype, in the state of Bahia.

Diagnosis. *Aphilodon bahianus* **n. sp.** resembles *A. foraminis* **n. sp.** by having the denticle of tarsungulum not apparent (Figs 11A, 12C), but differs by having the cephalic plate longer than wide, and without apparent transverse suture (Fig. 12B), tarsungulum distinctly curved and shorter than the trochanteroprefemur+femur (Figs 11A, 12C), and ultimate tarsus 1 with a terminal foraminis small, and well developed terminal spinous (Fig. 11C).

Description of holotype. Female (IBSP 6460).

General aspect: body with 18 long and 0.34 maximum width (middle part of trunk). Color (preserved specimen in alcohol): head and forcipular segment ferrugineus, other leg-bearing segments and post-pedal segmentation yellowish (Fig. 12A–G).

Cephalic plate: longer than wide, 0.32 long and 0.29 wide (length/width ratio 1.1), with short setae sparse. General morphology as in Fig. 12B.

Antennae: left antenna 0.93 long (2.9 times as long as the cephalic plate); articles II−XIII length/width ratio 0.4–0.7; last article length/width ratio 1.9, with 6 claviform specialized setae dorsal and 4 ventral. General morphology as in Fig. 12B–C.

Clypeus: 2+2 lateral setae; 2+2 post-antennal setae; 6+5 sub clypeal setae.

Mandible: pectinate lamellae with ca. 20 denticles.

First maxillary: left telopodite length 0.029 and width 0.018; distal article with one subapical sensilla; each medial projection of coxosternite with one apical and 2 subapical sensilla.

Second maxillary: 12 short setae distributed in a row along the posterior margin between the telopodites; coxosternite rectangular, with middle part not suppressed (length/width ratio 0.55), left telopodite length 0.054 (1.8 times as long as the telopodite of first maxillary) and maximum width (basal margin) 0.023; both telopodite with one sensilla apical and two subapical setae.

Forcipular segment: tergite trapezoidal, length 0.09 (0.3 times as long as the cephalic plate) and width 0.29 (length/width ratio 0.3), with ca. 10 sparse setae; coxosternite length 0.30 and width 0.32 (length/width ratio 0.93); telopodite length 0.18, not reaching the anterior margin of the cephalic plate, with 2 denticles in the trochanteroprefemur+femur and 1 denticle in the tibia, all well developed; denticle distal of trochanteroprefemur with 1 apical seta, denticle of femur bilobed and with 1 apical and 3 subapical setae, denticle of tibia with 2 apical and 2 subapical setae; tarsungulum with a not defined denticle and 0.75 times as long as the trochanteroprefemur+femur. General morphology as in Figs 11A, 12B–C.

Legs: 65 pairs; first leg 0.95 times as long as the second leg, with procoxae extended to the midline of the body; leg 32 length 0.4. General morphology as in Fig. 12A–G.

Tergum from the first to penultimate leg-bearing segment: rectangular tergites, with two rows of 6–6 setae and short sparse setae; tergite 35 length 0.16 and width 0.33 (length/width ratio 0.5); pretergite 35 with 0.3 times as long as the previous metatergite and with a row of 6 sparse setae. General morphology as in Fig. 12D.

Sternum from the first to penultimate leg-bearing segment: first sternite in a semi circle shape, other sternites rectangular and elongated with scattered setae; sternite 35 length 0.2 and width 0.12 (length/width ratio 1.6); presternite present throughout body, in segment 35 with 0.15 times as long as the previous metasternite and with one row of 7 setae. General morphology as in Fig. 12C, E.

Ultimate leg-bearing segment: metatergite wider than long, trapezoidal, length 0.14 and maximum width 0.18 (length/width ratio 0.77); sternite apparent only in the sides of the metasternite. General morphology as in Fig. 12G.

Ultimate legs: straight, length 0.9, about 2 times as long as penultimate leg; each coxopleuron with ca. 7 coxal pores sparse; tarsus 1 measuring 1.7 times as long as tibia. General morphology as in Fig. 12G.

Postpedal segments: gonopods with 3+3 distal setae.

Sexual dimorphism. Ultimate legs are a little thicker in males than in females (Figs 11B, 12F). Male gonopod biarticulated, article 1 with 3–6 setae, article 2 with 2–4 scattered setae.

Variation. N= 22, including 12f# and 10m# (from four localities): total body length: 16−21 mm. Pairs of legs: 63–65. Coxal pores: 6–9.

Natural history. The specimens were found inside large forests or in forest refuges in open areas, near streams or in most humid refuges, buried about 5–10 cm or just below rotten logs.

Distribution. Brazil, state of Bahia.

Discussion

Because species of *Aphilodon* are tiny and difficult to access due to its underground habitat, often protected by the roots of trees or stones, most specimen sampling depends on manual and often time-consuming work. The many difficulties affected to establish the real diversity of the group in the analyzed areas, and the observed diversity were proportional to the sampling time spent. In this sense, the great diversity of species of *Aphilodon* observed in the Parque Nacional de Itatiaia in relation to the other areas here studied is related to the longer time of sampling spent in the area, 15 days, versus up to 7 days in the other locations of research. The lack of knowledge about the real diversity of Aphilodontinae can also be checked in Calvanese *et al.* (2019), where among the eight new species of the subfamily proposed by the authors, four are known only from São Roque, a small municipality in the state of São Paulo that has had its myriapods studied in the recent years (Calvanese *et al.* 2020).

The four new species sampled in the Parque Nacional de Itatiaia have large structural differences in the body shape and seem to transit through different microenvironments of the forest. Specimens of *Aphilodon aiuruoca* **n. sp.** has a less sclerotized body with a relatively shorter length, already representatives of *A. foraminis* **n. sp.** and *A. acutus* **n. sp.** despite having a relatively short body have a well sclerotized cuticle, which may indicate a greater ability to move around without drying out. *A. rectitibia* **n. sp.** has a relatively larger and sclerotized body being most frequently observed moving across the soil surface.

The first registration of *Aphilodon* from Bahia, Brazil, is not a surprise, since apparently this genus is abundant in the Atlantic Forest (Calvanese *et al.* 2019), a biome that extends in part of this state. However, besides the Atlantic Forest, *Aphilodon bahianus* **n. sp.** was also sampled from the Caatinga biome and transition areas, in four relatively distant municipalities, which may indicate a wide distribution for this species.

Aphilodon cangaceiro **n. sp.** is the first species of Aphilodontinae known to the state of Piauí, in the Caatinga biome. It is characterized by having some structures of the body elongated and an increase in the number of pairs of legs, which result in a longer body length. This kind of modification can be parallel verified also in other centipede endemic to the Caatinga, as reported by Calvanese *et al.* (*in. prep.*) to *Albissilvam* Calvanese, Brescovit & Bonato, the first Geophilidae known from this biome.

Finally, although the knowledge about the *Aphilodon* diversity has undergone a notorious upgrade with several new species described in recent years, the discovery of new species in a few visited areas makes it clear that many other Aphilodontinae are still awaiting registration at least from Brazil.

Acknowledgements

We express thanks for all the support provided by the Instituto de Biociências of Universidade de São Paulo (IB-USP) and Laboratório de Coleções Zoológicas (LCZ). We thank all the staff of the biological reserves visited, for the collaboration and support. Especially, thanks to the researcher Adele M.S.S. Silva for help in field collections. Thanks to Beatriz Mauricio for the help in SEM analysis. The anonymous reviewers we thank for the suggestions and corrections. This work was supported by CNPq grants 169641/2017–1 to VCC and CNPq granted PQ 303903/2019–8, to ADB.

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FIGURE CAPTIONS

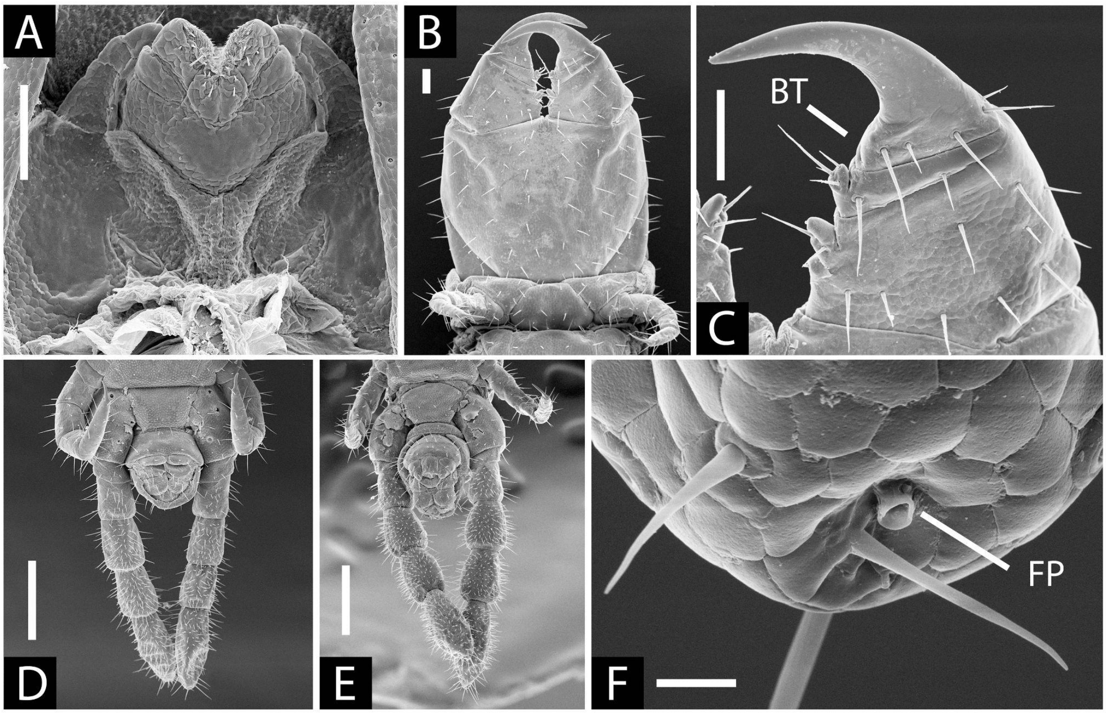

FIGURE 1. A−F, *Aphilodon foraminis* **n. sp.** Female (IBSP 6445): **A**, first and second maxillary, ventral; **B**, forcipular segment and first leg-bearing segment, ventral; **C**, left forcipule, ventral; **D**, terminal part of the body, ventral. Male (IBSP 6446): **E**, terminal part of body, ventral; **F**, terminal part of the left ultimate leg, ventral. **Abbreviations:** BT: basis of tarsungulum. FP: foraminal process. **Scale bars:** 0.05 (A−C), 0.2 (D−E), 0.005 (F) mm.

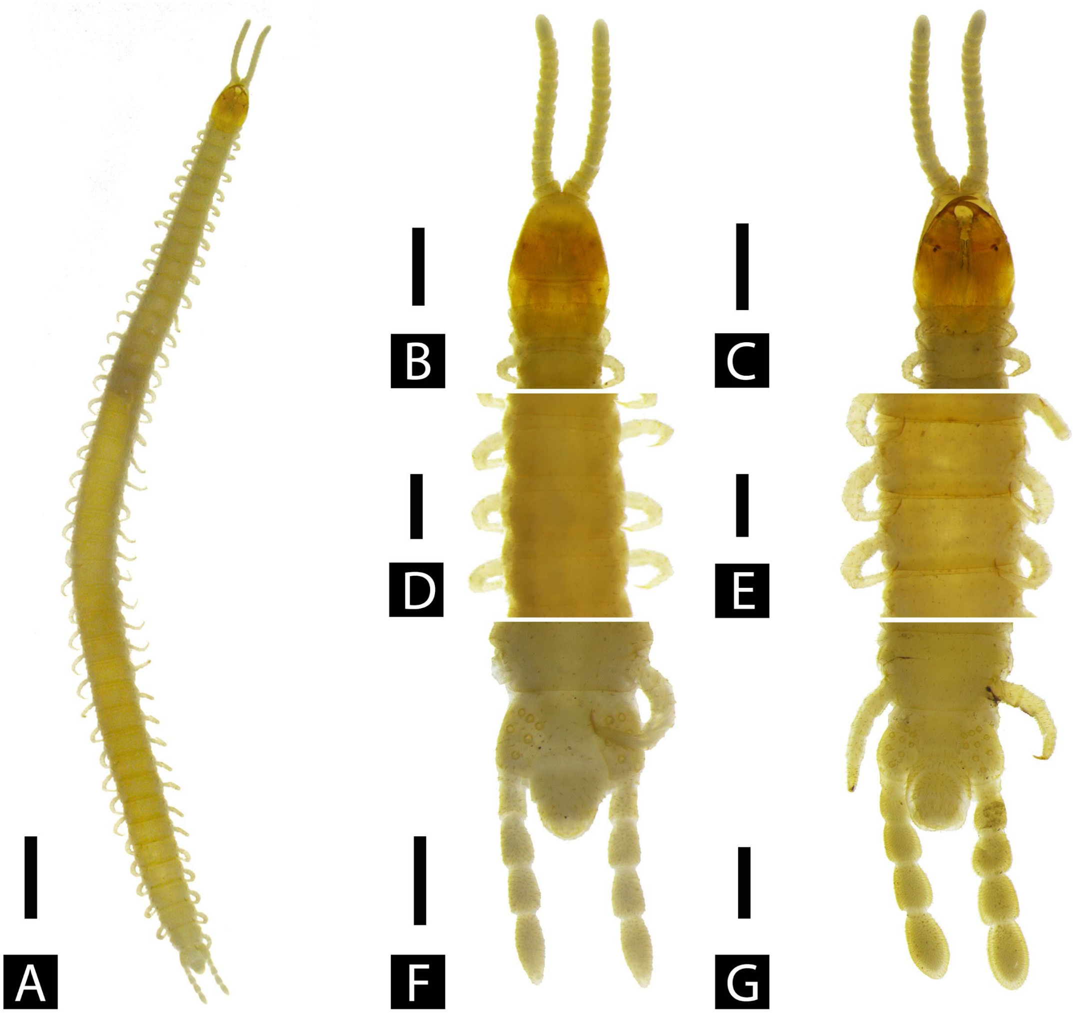

FIGURE 2. A−G, *Aphilodon foraminis* **n. sp.** Holotype female (IBSP 6442): **A**, habitus, ventral; **B**, anterior part of body, dorsal; **C**, same, ventral; **D**, leg-bearing segment 15−16, dorsal; **E**, same, ventral; **F**, terminal part of body, ventral. Paratype male (IBSP 6443): **G**, terminal part of body, ventral. **Scale bars:** 1 (A), 0.5 (B−C), 0.25 (D−G) mm.

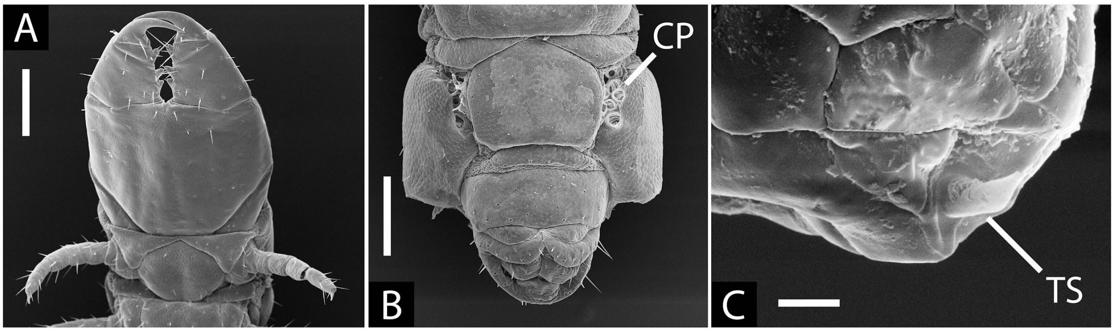

FIGURE 3. A−C, *Aphilodon aiuruoca* **n. sp.** Female (IBSP 6450): **A**, forcipular segment and first leg-bearing segment, ventral; **B**, terminal part of body, except ultimate telopodites, ventral; **C**, terminal part of the left ultimate leg, ventral. **Abbreviations**: CP: coxal pores. TS: terminal spine. **Scale bars:** 0.1 (A−B), 0.002 (C) mm.

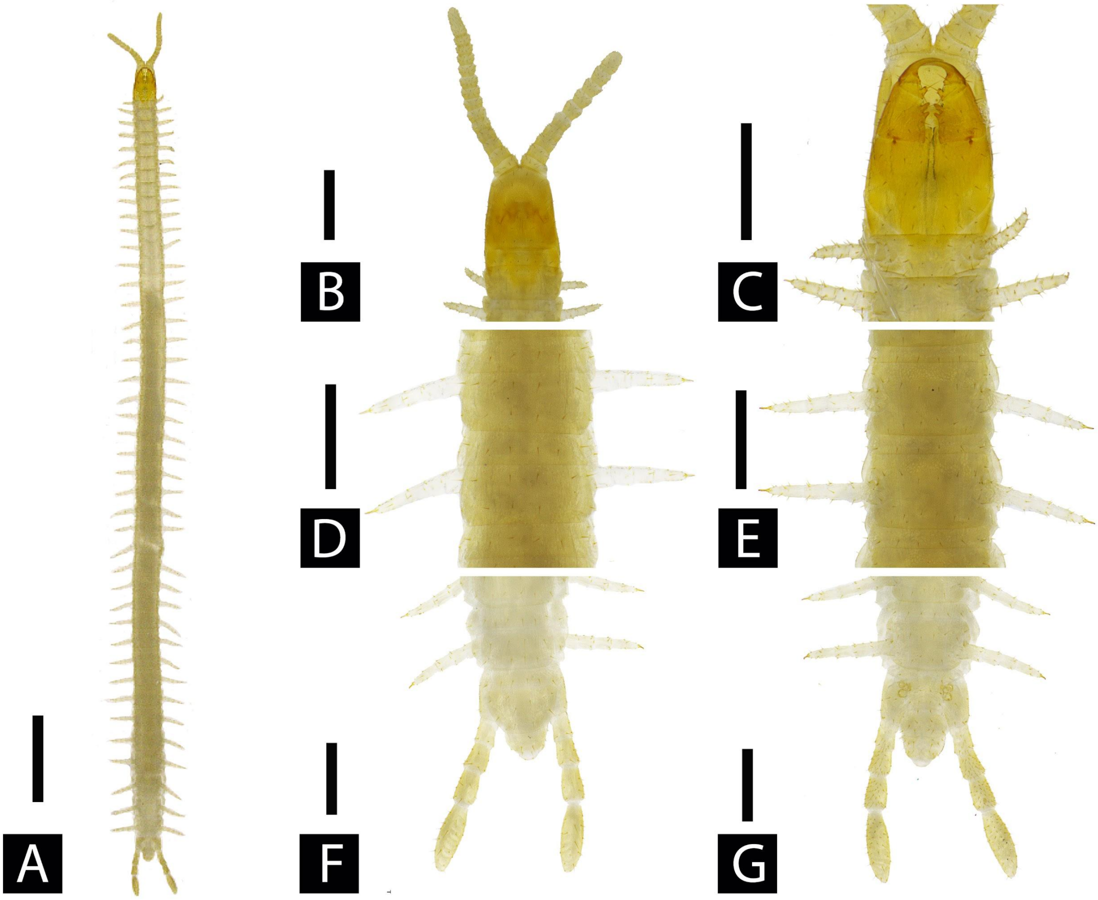

FIGURE 4. A−G, *Aphilodon aiuruoca* **n. sp.** Holotype female (IBSP 6447): **A**, habitus, ventral; **B**, anterior part of body, dorsal; **C**, same, ventral; **D**, leg-bearing segment 15−16, dorsal; **E**, same, ventral; **F**, terminal part of body, dorsal; **G**, same, ventral. **Scale bars:** 1 (A), 0.2 (B−G) mm.

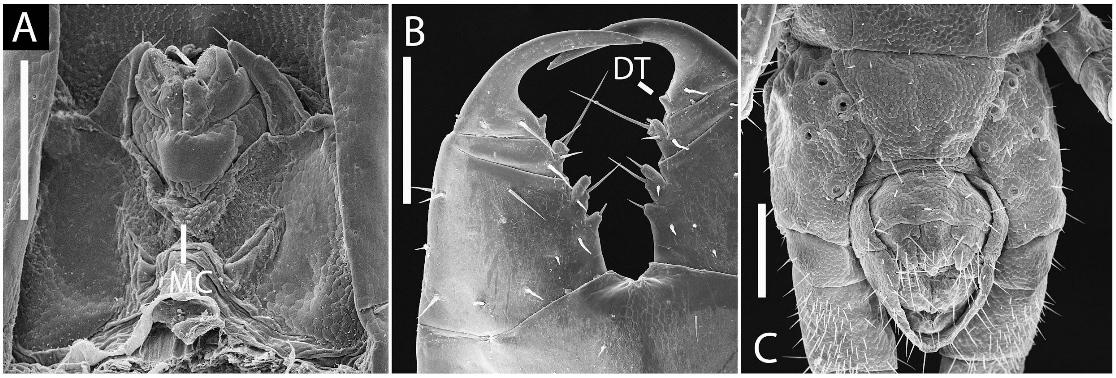

FIGURE 5. A−C, *Aphilodon acutus* **n. sp.** Paratype male (IBSP 6452): **A**, first and second maxillary, ventral; **B**, forcipule, ventral; **C**, terminal part of body, except ultimate telopodites, ventral. **Abbreviation:** DT: denticle of tarsungulum. MC: middle of coxosternite of the second maxillary. **Scale bars:** 0.1 (A−C) mm.

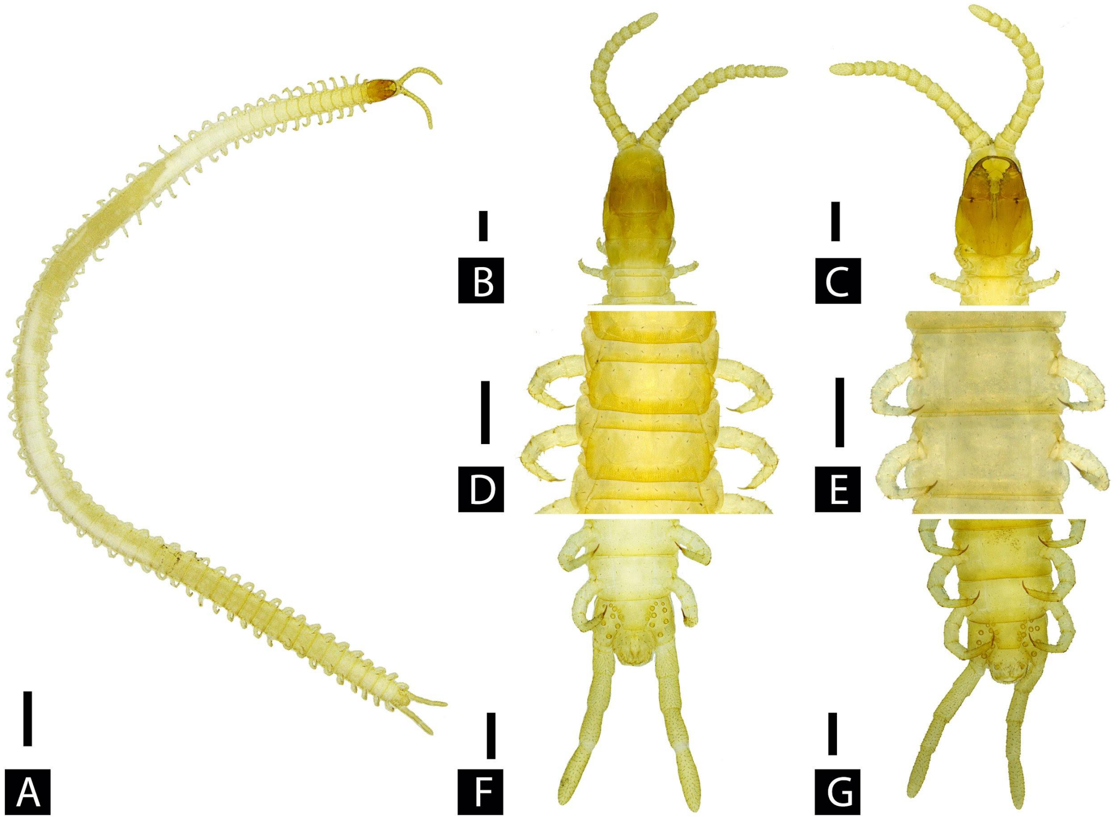

FIGURE 6. A−G, *Aphilodon acutus* **n. sp.** Paratype male (IBSP 6452): **A**, habitus, ventral; **B**, anterior part of body, dorsal; **C**, same, ventral; **D**, leg-bearing segment 15−16, dorsal; **E**, same, ventral; **F**, terminal part of body, ventral. *Aphilodon acutus* **n. sp.** Holotype female (IBSP 6451): **G**, terminal part of body, ventral. **Scale bars:** 1 (A), 0.2 (B−G) mm.

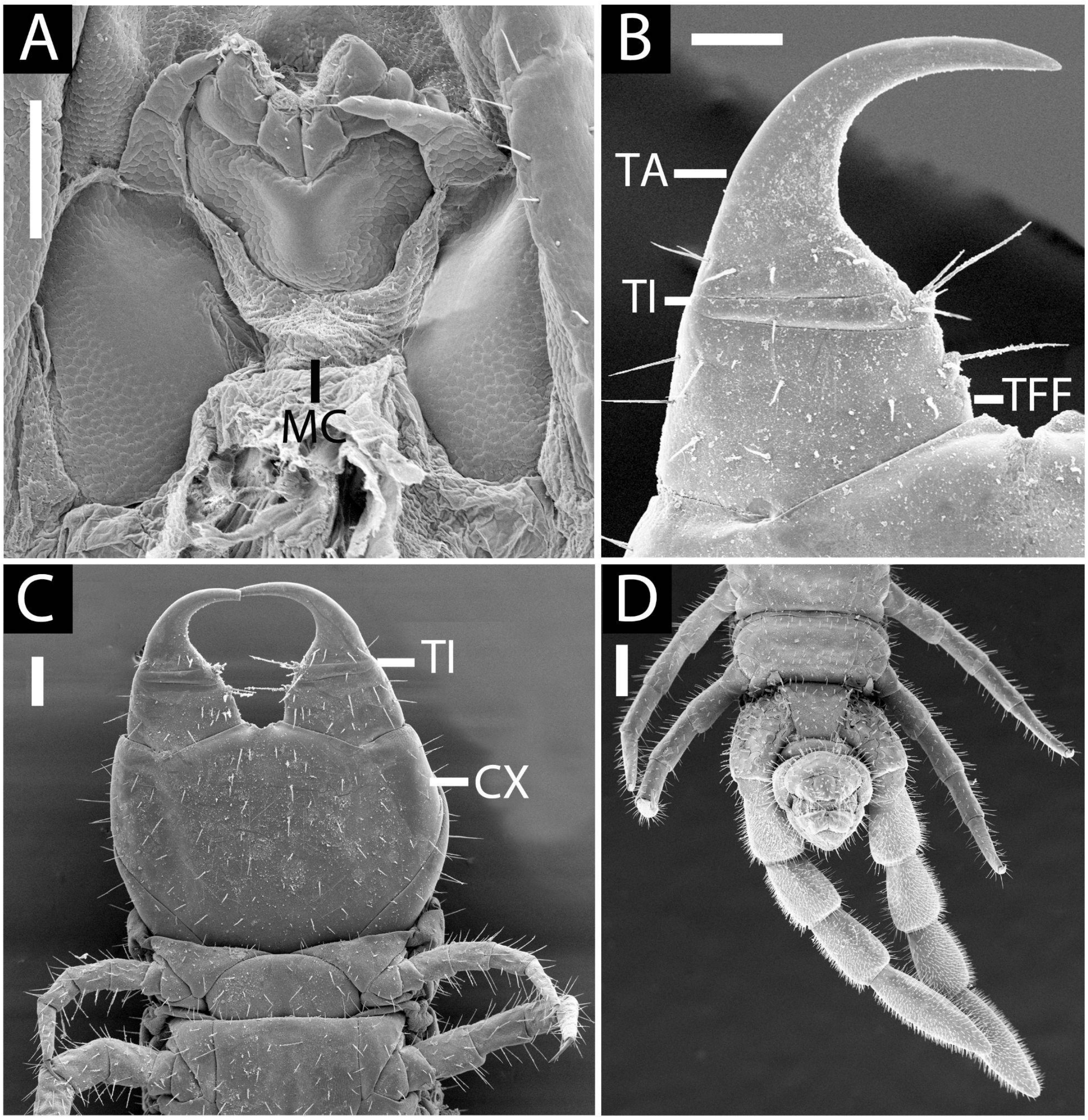

FIGURE 7. A−D, *Aphilodon rectitibia* **n. sp.** Female (IBSP 4081): **A**, first and second maxillary, ventral; **B**, right forcipular telopodite, ventral. Paratype male (IBSP 6469): **C**, forcipular segment and first leg-bearing segment, ventral; **D**, terminal part of body, ventral. **Abbreviations**: CX: coxosternite. MC: middle of coxosternite of the second maxillary. TA: tarsungulum. TFF: trochanteroprefemur+femur. TI: tibia. **Scale bars:** 0.1 (A−D) mm.

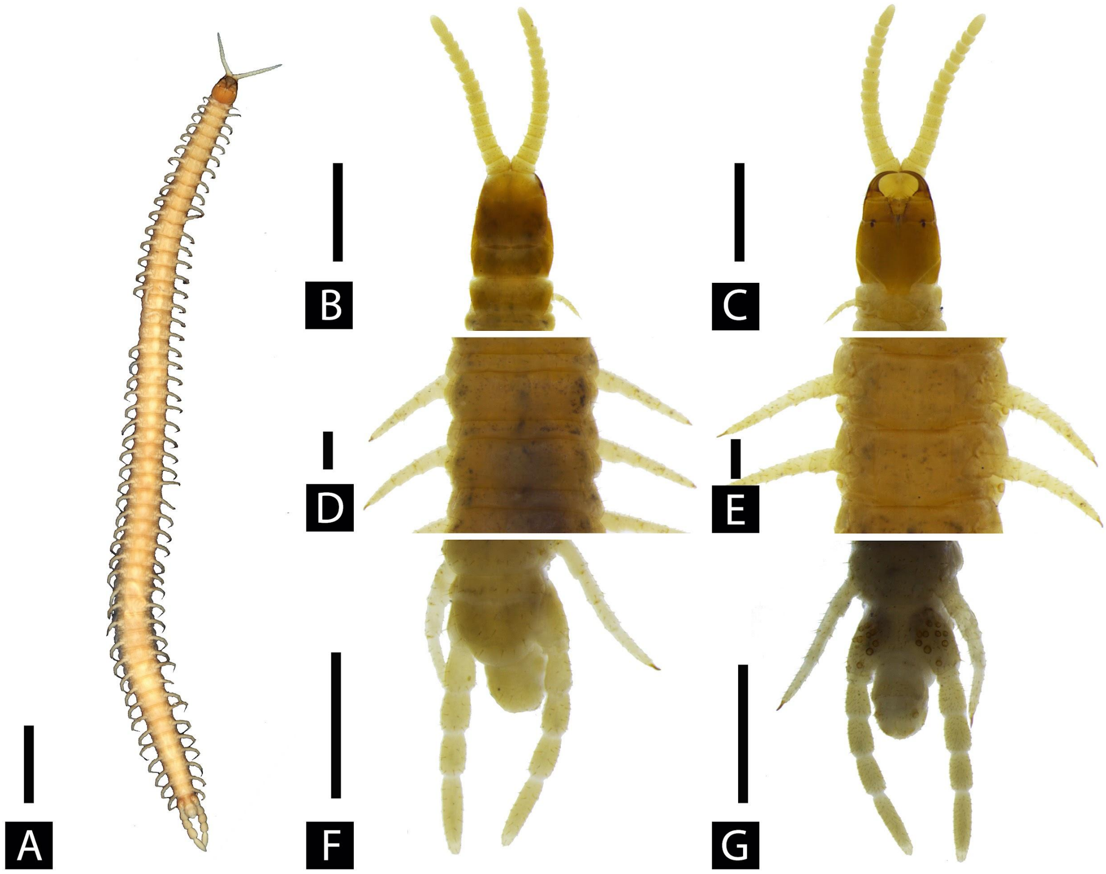

FIGURE 8. A–G, *Aphilodon rectitibia* **n. sp.** Male (MZSP 3061): **A**, habitus, ventral. Holotype female (IBSP 6467): **B**, anterior part of body, dorsal; **C**, same, ventral; **D**, leg-bearing segment 15–16, dorsal; **E**, same, ventral; **F**, terminal part of body, dorsal; **G**, same, ventral. **Scale bars:** 2 (A), 1 (B–C, F–G), 0.2 (D–E) mm.

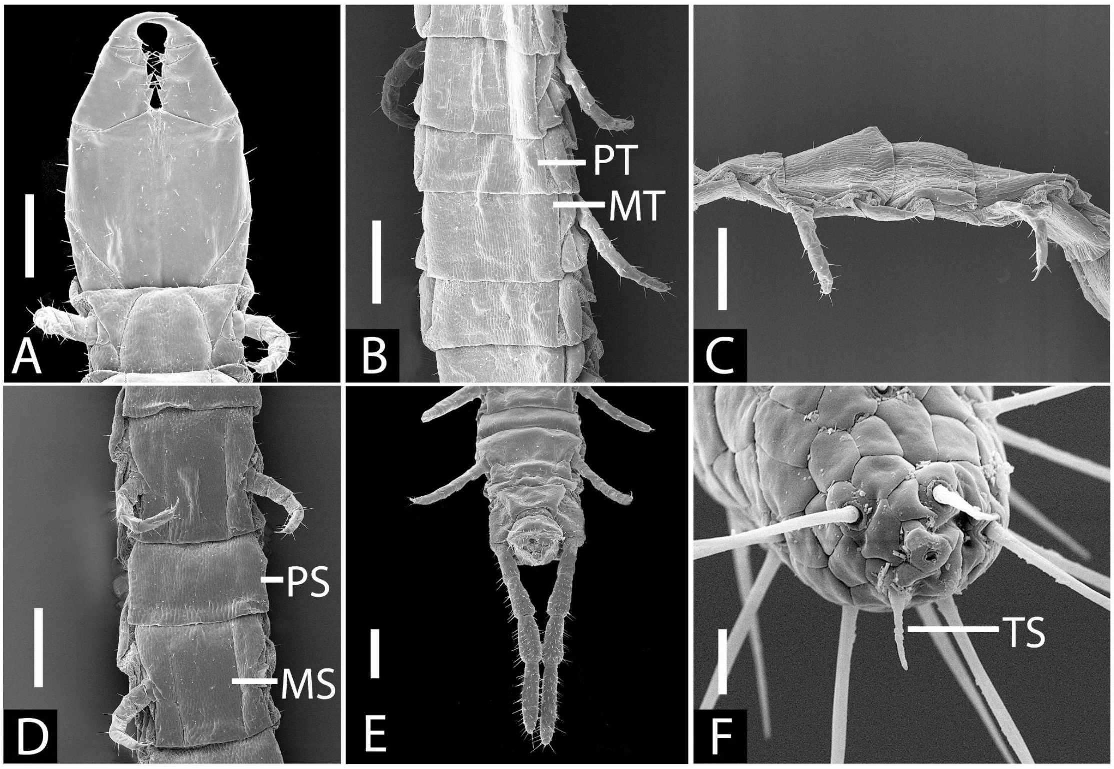

FIGURE 9. A–F, *Aphilodon cangaceiro* **n. sp.** Female (IBSP 6459): **A**, anterior part of body, ventral; **B**, leg-bearing segment 11–12, dorsal; **C**, leg-bearing segment 33–34, lateral; **D**, leg-bearing segment 44–45, ventral; **E**, terminal part of body, ventral; **F**, terminal part of the left ultimate leg, ventral. **Abbreviations**: MS: metasternite. MT: metatergite. PS: presternite. PT: pretergite. TS: terminal spine. **Scale bars:** 0.1 (A–E), 0.005 (F) mm.

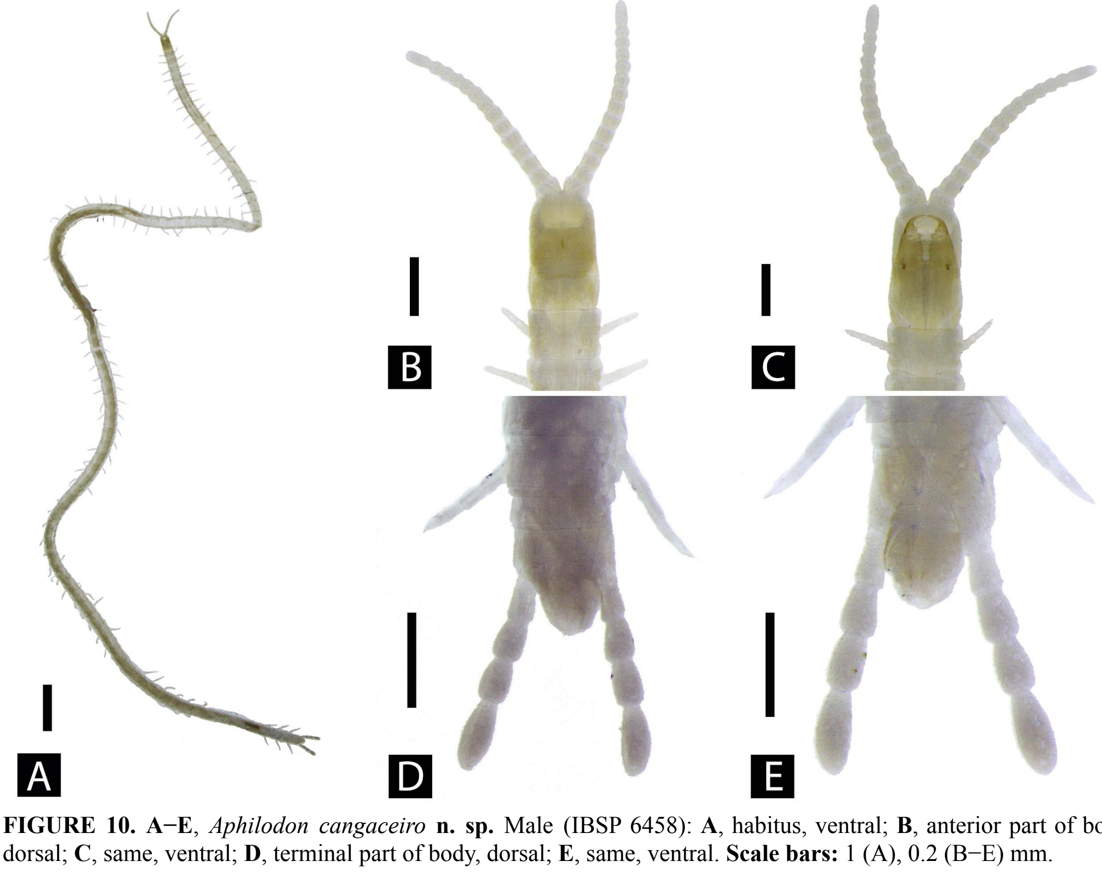

FIGURE 10. A–E, *Aphilodon cangaceiro* **n. sp.** Male (IBSP 6458): **A**, habitus, ventral; **B**, anterior part of body, dorsal; **C**, same, ventral; **D**, terminal part of body, dorsal; **E**, same, ventral. **Scale bars:** 1 (A), 0.2 (B–E) mm.

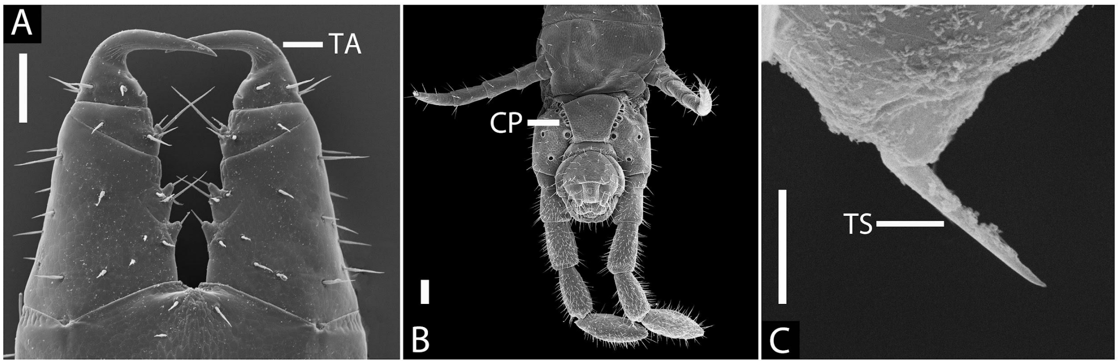

FIGURE 11. A−C, *Aphilodon bahianus* **n. sp.** Paratype male (IBSP 6461): **A**, forcipule, ventral; **B**, terminal part of body, ventral; **C**, terminal part of the left ultimate leg, lateral. **Abbreviations**: CP: coxal pores. TA: tarsungulum. TS: terminal spine. **Scale bars:** 0.05 (A−B), 0.005 (C) mm .

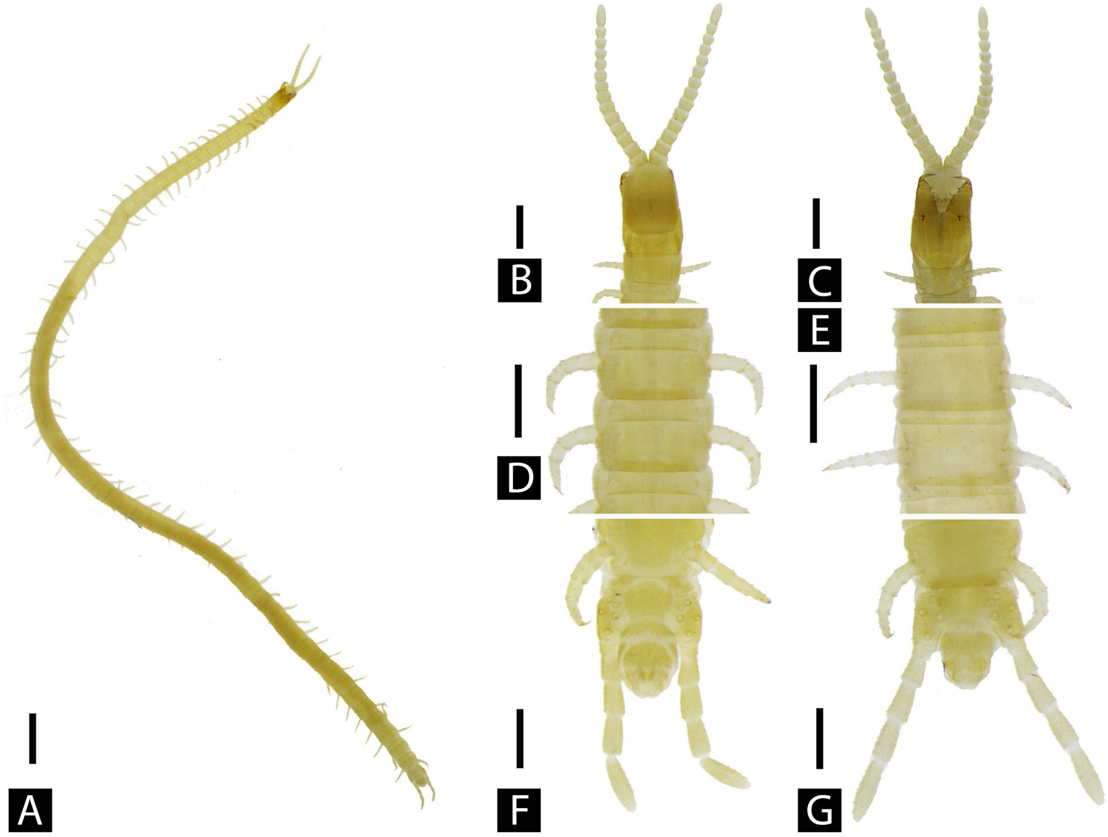

FIGURE 12. A−G, *Aphilodon bahianus* **n. sp.** Paratype male (IBSP 6461): **A**, habitus, ventral; **B**, anterior part of body, dorsal; **C**, same, ventral; **D**, leg-bearing segment 15−16, dorsal; **E**, same, ventral; **F**, terminal part of body, ventral. *Aphilodon bahianus* **n. sp.** Holotype female (IBSP 6460): **G**, terminal part of body, ventral. **Scale bars:** 1 (A), 0.2 (B−G) mm.

Table 1. Some characteristics of the known species of *Aphilodon* and its occurrence. Abbreviations: AT: antenna, CE: cephalic plate, CXS: coxosternite of the second maxillary, DE: denticle, DT: denticle of tarsungulum, DTI: denticle of tibia, FL: forcipule, FPT: forcipular pretergite, TA: tarsungulum, TTF: trochanteroprefemur+femur, TR: transverse suture of cephalic plate, UMS: ultimate metasternite, UPS: ultimate presternite.

Species	**Body length (mm)**	**Pair of legs**	**CE length /width**	**TR**	**AT /CE length**	**FPT exposed**	**CXS shorter in middle**	**DE of FL**	**TA /TTF length**	**DT**	**UPS exposed**	**UMS length /width**	**Coxal pores**	**Occurrence**
Aphilodon spegazzinii Silvestri, 1898	20–45	61−65	ca. 1.1	no	3−4	yes	no	all 3 large	< 1	small	yes	ca. 1.4	17−31 sparse	Argentina: Buenos Aires
Aphilodon micronyx Brölemann, 1902	30–70	57–61	0.8–0.9	yes	2−3	yes	yes	all 3 small	< 1	small	yes	ca. 0.9	27−34 sparse	Brazil: Minas Gerais, Paraná, Rio de Janeiro, Santa Catarina, São Paulo
Aphilodon angustatus Silvestri, 1909	10–20	51–59	1.1–1.2	no	ca. 2	yes	no	all 3 large	< 1	small	yes	ca. 0.7	6−8 sparse	Argentina: Misiones Brazil: Mato Grosso, Paraná, São Paulo Paraguay: Paraguari
Aphilodon intermedius Silvestri, 1909	20	57	ca. 1.2	no	ca. 2	yes	no	all 3 large	< 1	small	yes	ca. 0.3	ca. 20 sparse	Argentina: Misiones
Aphilodon modestus Silvestri, 1909	9	45	ca. 1.1	no	ca. 2	yes	no	two large, DTI small	< 1	small	yes	ca. 0.3	ca. 5 sparse	Paraguay: Tacurú Pucú
Aphilodon caboclos Calvanese, Brescovit & Bonato, 2019	28–40	51–53	ca. 1.2	no	2−3	yes	no	all 3 large	> 1	small	yes	ca. 0.4	7–11 sparse	Brazil: São Paulo
Aphilodon indespectus Calvanese, Brescovit & Bonato, 2019	10–20	39–41	ca. 1.1	no	ca. 2	yes	no	all 3 small	< 1	small	yes	ca. 0.5	10−15 sparse	Brazil: São Paulo
Aphilodon meganae Calvanese, Brescovit & Bonato, 2019	7–8	37–39	ca. 1.2	no	ca. 2	no	no	all 3 large	< 1	small	yes	ca. 0.7	7−10 sparse	Brazil: São Paulo
Aphilodon pereirai Calvanese, Brescovit & Bonato, 2019	20–70	73–77	1.3–1.4	no	2−2.7	yes	no	all 3 large	< 1	small	no	ca. 0.7	22−44 sparse	Brazil: São Paulo
Aphilodon silvestrii Calvanese, Brescovit & Bonato, 2019	20–60	69–71	ca. 1.3	no	1.3−2	yes	no	all 3 large	< 1	small	yes	ca. 0.8	23−35 sparse	Brazil: São Paulo
Aphilodon foraminis **n. sp.**	10–15	39–43	ca. 0.9	yes	ca. 2	yes	no	all 3 large	>1	absent	yes	ca. 0.8	5–8. sparse	Brazil: Rio de Janeiro
Aphilodon aiuruoca **n. sp.**	7–10	41–43	ca. 1.2	no	ca. 2	yes	no	all 3 large	< 1	small	yes	ca. 0.8	5−10 close to metasternite	Brazil: Rio de Janeiro
Aphilodon acutus **n. sp.**	18–22	61–65	ca. 1.2	no	ca. 2.5	yes	yes	all 3 large	< 1	large	no	ca. 0.6	8−12 sparse	Brazil: Rio de Janeiro
Aphilodon rectitibia **n. sp.**	20–50	51–55	ca. 0.9	yes	ca. 2.7	yes	yes	all 3 small	> 1	small	yes	ca. 0.8	ca. 30 sparse	Brazil: Rio de Janeiro, Minas Gerais, São Paulo
Aphilodon cangaceiro **n. sp.**	20–30	87–93	ca. 1.3	no	ca. 2.5	yes	no	all 3 large	< 1	small	yes	ca. 0.4	4−6 close to metasternite	Brazil: Piauí
Aphilodon bahianus **n. sp.**	15–20	63–65	ca. 1.1	no	ca. 2.9	yes	no	all 3 large	< 1	absent	yes	ca. 0.7	6−9 sparse	Brazil: Bahia

A new species of *Mecophilus* (Geophilidae: Aphilodontinae), with the first registration of the genus from the state of Rio de Janeiro, Brazil

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Abstract

Mecophilus carioca **n. sp.** is described based on males and females recently sampled in the Parque Nacional de Itatiaia, and is the first record of the genus from the state of Rio de Janeiro, Brazil. The specimens were analyzed in both light microscopy and scanning electron microscopy, and photos and schematic illustrations are provided. In order to differentiate the new species, we checked the types of other species of *Mecophilus* and compared the main variation observed within the genus. Moreover, the known distribution of the evaluated species is presented in a map.

Key words: Atlantic Forest, biodiversity, Geophilomorpha, morphology, taxonomy.

Introduction

Mecophilus Silvestri, 1909 is a poorly known centipede genus endemic to the Brazilian Atlantic Forest biome (Silvestri 1909; Calvanese *et al.* 2019). This remarkable group calls attention by presenting the members of Aphilodontinae with the lowest number of leg-bearing segments, known as 35 to males and females, and shorter body length, between 7–8 mm (Silvestri 1909; Calvanese *et al.* 2019). On the other hand, the main morphological novelties retrieved to the group are related with the elongation of structures in the forcipular segment or along the trunk (Bonato *et al.* 2011; Calvanese *et al.* 2019).

At the moment two species were recorded to *Mecophilus*, the type species *Mecophilus neotropicus* Silvestri, 1909, that is based on one male from Foz do Iguaçu, state of Paraná, Brazil and *Mecophilus tupiniquim* Calvanese, Brescovit & Bonato, 2019, recently described from São Roque, state of São Paulo, Brazil, based on males and females.

Here we present a third species for the genus, *Mecophilus carioca* **n. sp.**, described and illustrated based on male and female specimens. The new species is the first report of *Mecophilus* from the state of Rio de Janeiro, Brazil.

Material and Methods

The specimens of the new species were manually sampled from the Parque Nacional de Itatiaia, municipality of Itatiaia, Rio de Janeiro (license Sisbio number 60676–2). The material was deposited in the Collection of Myriapodology of the Instituto Butantan, São Paulo (IBSP, curator: A.D. Brescovit). Also, for comparison we analyze representatives of the other two known species of *Mecophilus* deposited in the IBSP and in Museo Civico di Storia Naturale Giacomo Doria, Genova, Italy (MSNG, curator: R. Poggi).

The morphological analysis and measurements were made on the cephalic plate, antennae, labrum, mandible, first and second maxillary, forcipular apparatus, leg-bearing segments of the trunk, ultimate leg-bearing segment and post-pedal segmentation. Male and female specimens of the new species were photographed on a Leica DFC 500 digital camera coupled to a Leica MZ 16A stereomicroscope or using a Leica DFC 500 digital camera coupled to a Leica DM4000B optical microscope. Assembling stacks of photos were made in a Leica Application Suite version 2.5.0. Also, one male and one female (IBSP 6473) were examined by Scanning Electron Microscope (SEM), with a FEI Quanta 250 at the Laboratório de Biologia Celular of Instituto Butantan based on the protocol provided by Calvanese *et al.* (2019). Schematic drawings were made with an Intuos Pro digital table on photographs and posteriorly edited in Inkscape 0.92.

Adult females were characterized by the full spermatheca, and the adult condition of the males by the masses of spermatozoa and the gonopods fully developed.

Dissection procedures were based on Pereira (2000) and the terminology of morphological structures follows that of Bonato *et al.* (2010). All presented measurements are given in millimeters, and refer to the maximum externally visible length of the structures.

A map with the known distribution of the *Mecophilus* species was made using the software Quantum Geographic Information System, QGIS 3.20 software (QGIS 2021), from disponible data of sampling localities of specimens.

The abbreviations used in Table 1 and illustrations have its meaning displayed directly in the legends, and listed below:

AAS—anterior accessory spine
AP—anal pore
CA—calyx of venom gland
AT—antenna
CP—cephalic plate
CS—claviform setae
CXS—second maxillary coxosternite
DF—denticle of femur
DT—denticle of tarsungulum
DTF—denticle of trochanteroprefemur
FCX—forcipular coxosternite
FMT—telopodite of first maxillary
FP—foraminal process
FPT—forcipular pretergite
FS—sternite of first leg-bearing segment
FTE—forcipular tergite
GE—glandular epithelium
GF—female gonopod
MAL—left mandible
MP—membranous part
PAS—posterior accessory spine
PL—penultimate leg
QP—most sclerotized part
SA—setae type “A”
SB—setae type “B”
SC—scutellum

SE—setae
SMT—telopodite of second maxillary
SP—stigmatopleurite
S1—sclerite “1”
S2—sclerite “2”
S3—sclerite “3”
S4—sclerite “4”
TA—tarsungulum
TTF—trochanteroprefemur+femur
UL—ultimate legs

Results

Geophilomorpha Pocock, 1895

Geophiloidea Leach, 1815

Geophilidae Leach, 1815

Aphilodontinae Silvestri, 1909 (1908)

Genus *Mecophilus* Silvestri, 1909

Type species. *Mecophilus neotropicus* Silvestri, 1909, by monotypy.

Included species:
M. neotropicus Silvestri, 1909
M. tupiniquim Calvanese, Brescovit & Bonato, 2019
M. carioca **n. sp.**

Diagnosis. The genus *Mecophilus* was recently retrieved through phylogenetic analysis (Calvanese *et al.* 2019), and three morphological novelties unique to the group were reported. Based on this analysis and through new measurements carried out on the type material of *Mecophilus* and on the specimens of the new species, we present the following diagnosis to the genus: forcipular pretergite elongate, with at least 1/5 the length of the cephalic plate (Figs 1B, 4A) (against a structure shorter than 1/8 the length of the cephalic plate for other Aphilodontinae), forcipular coxosternite longer than wide (Figs 1C, 4B) (against a coxosternite wider than long for other Aphilodontinae), and sternite of first leg-bearing segment longer than wide (Figs 1C, 4B) (against a wider than long first sternite for other Aphilodontinae).

Morphological variation and occurrence. Some morphological characteristics of the three species of *Mecophilus* and its known geographical distribution are reported in Table 1.

***Mecophilus carioca* new species**
(Figs 1–6)

Type specimens. Holotype: f#, from Parque Nacional de Itatiaia, Itatiaia, Rio de Janeiro, Brazil, 12/2017, V. Calvanese & A. Silva col., deposited in IBSP 6470. Paratypes: 1f# (IBSP 6471) and 1m# (IBSP 6472), same data as holotype.

Other material analyzed. 1f# 2m# (IBSP 6473; 1m# 1f# used for SEM), with the same data of types.

Etymology. The epithet is an adjective, and is a tribute to the capital of the state of Rio de Janeiro, the wonderful city. “Carioca” comes from two Tupi words: kara'iwa (white man) and oka (house), which together mean “house of the white man”. The local indigenous people used the name after the foundation of Rio de Janeiro to refer to the inhabitants of the city.

Diagnosis. *Mecophilus carioca* **n. sp.** resemble *M. tupiniquim* by the position of the forcipule denticle of tarsungulum, close to the basal margin of the structure (Fig. 4C) (in *M. neotropicus* the denticle is positioned in the middle of the article) and cephalic plate with shape sub rectangular (Fig. 1B) (in *M. neotropicus* the shape of the cephalic plate is trapezoidal). *Mecophilus carioca* **n. sp.** differs from *M. tupiniquim* by the smaller length of the tarsungulum denticle (Fig. 4C) (the denticle is large in *M. tupiniquim*), distal denticle of trochanteroprefemur and denticle of femur are positioned not close to each other (Figs 1C, 4B) (in *M. tupiniquim* the distal denticles of the trochanteroprefemur and the denticle of femur are close together), and the ultimate legs in females without terminal claw (Figs 1A, F, 6A–B) (in *M. tupiniquim* females have a well developed terminal claw).

Description of holotype. Female (IBSP 6470).

General aspect: body with 6.6 long and 0.26 maximum width (middle part of trunk). Color (preserved specimen in alcohol): head and forcipular segment ferrugineus, other leg-bearing segments and post-pedal segmentation whitish (Fig. 1A−G).

Cephalic plate: longer than wide, with 0.3 long and 0.2 wide (length/width ratio 1.5), with setae sparse. General morphology as in Fig. 1B.

Antennae: left antennae 0.52 long (ca. 1.75 times as long as the cephalic plate); articles II−XIII length/width ratio 0.3–0.95; last article length/width ratio 1.9, with 6 claviform specialized setae dorsal and 4 ventral. Morphology and chaetotaxy as in Figs 1A–C, 2A–C.

Clypeus: lateral setae not observed; 1+1 post-antennal setae; 3+3 sub clypeal setae, morphology as in Fig. 2D.

Mandible: pectinate lamellae with ca. 20 elongated denticles, morphology as in Fig. 3B.

First maxillary: left telopodite length 0.016 and width 0.018; distal article with 2 apical and 1 subapical sensille; each medial projection of coxosternite with 3 subapical sensille. Morphology as in Fig. 3A–B.

Second maxillary: coxosternite rectangular, concave in the middle of posterior margin (ca. 0.3 times as long as the lateral margins), length/width ratio 0.6, left telopodite length 0.018 (1.1 times as long as the telopodite of first maxillary) and maximum width (basal margin) 0.01; both telopodite with 2 little lateral sensille in the article 1, and 1 short apical and 2 most basal setae in the article 3. Morphology as in Fig. 3A–D.

Forcipular segment: tergite trapezoidal, length 0.14 (0.45 times as long as the cephalic plate) and width 0.23 (length/width ratio 0.6), with one row of 6 setae and short sparse setae; pretergite length 0.066 (0.22 times as long as the cephalic plate) and width 0.10 (length/width ratio 0.66); coxosternite length 0.3 and width 0.24 (length/width ratio 1.25); telopodite length 0.25, reaching but not surpassing the anterior margin of the cephalic plate, with 2 well developed denticles in the trochanteroprefemur+femur and 1 reduced denticle in the tibia; denticle of trochanteroprefemur with 1 short apical setae, denticle of femur bilobed and with 2 subapical and one apical setae, denticle of tibia with 1 apical and 2 subapical setae; tarsungulum with a reduced denticle and 0.6 times as long as trochanteroprefemur+femur. Right calyx inside the leg-bearing segment 9, left calyx inside the leg-bearing segment 10. Morphology as in Figs 1C, 4A–D.

Legs: 35 pairs; first leg 0.9 times as long as the second leg, with procoxae not extended to the middle of the body; leg 15 length 0.26. Morphology as in Figs 1A–G, 5A–B, D–E.

Tergum from the first to penultimate leg-bearing segment: rectangular tergites, with two rows of 8 setae and short sparse setae; tergite 15 length 0.095 and width 0.23 (length/width ratio 0.4); pretergite 15 with 0.4 times as long as the previous metatergite and with one row of 8 setae. Morphology as in Figs 1D, 5A.

Sternum from the first to penultimate leg-bearing segment: first sternite rectangular (length/width ratio 1.2), other sternites rectangular, with a few large setae scattered; sternite 16 length 0.15 and width 0.12 (length/width ratio 1.25); presternite exposed throughout body, in segment 16 with 0.12 times as long as the previous metasternite and with one row of 8 setae. Morphology as in Figs 1E, 5C.

Ultimate leg-bearing segment: metatergite wider than long, in a pentagonal shape, length 0.078 and maximum width 0.113 (length/width ratio 0.7); sternite apparent and slightly suppressed in the middle part. Morphology as in Figs 1F, 6A.

Ultimate legs: straight, length 0.8 (with 2.8 as long as the penultimate leg); each coxopleuron with 4–5 large coxal pores, opening most close to the metasternite; tarsus 1 measuring 0.9 times as long as tibia. Morphology as in Figs 1F, 6A.

Postpedal segments: gonopods membranous and without setae (Fig. 6D).

Sexual dimorphism. Males with ultimate legs just a little more thickened (Fig. 1G), with six articles (without tarsus 2, Fig. 6C) and with ca. 2.3 as long as the penultimate leg. Post pedal segmentation with greater amount of setae. Gonopod biarticulated, article 1 with 5–6 setae, article 2 with 5–7 scattered setae. General morphology of the terminal part of the body as in Fig. 1G.

Variation. N= 6, including 3f# and 3m# (from a single locality): total body length: 6.5−7 mm. Leg-bearing segments: 33–35 (only one male with 33, other with 35). Coxal pores in each coxopleuron: 4–6.

Natural history. The specimens were manually collected in the Atlantic Forest biome, at the soil of the forest (Fig. 7B–E), normally housed between the roots of trees, buried about 4−10 cm, accessed for sampling mainly in ravines or recent landslides.

Mecophilus carioca **n. sp.** is known only from the Parque Nacional de Itatiaia, where the specimens were sampled in the lowest part of the park.

Distribution. Brazil, state of Rio de Janeiro (Fig. 7A).

Discussion

Most diverse among centipedes, the order Geophilomorpha too have the most variable body shapes, both in the number of body segments, which varies between 27 to 191, as in the total length that varies in adults between 5 mm to more than 200 mm (Bonato & Minelli 2008). Species of *Mecophilus* are among the smallest geophilomorph centipedes, with a body length of less than 1 cm in adults, and *M. neotropicus* and *M. tupiniquim* have specimens with 35 leg-bearing segments. Here, we present the species of Aphilodontinae with the smallest known number of leg-bearing segments, and in *M. carioca* **n. sp.** the number varies between 33–35. In other genera of Aphilodontinae, appear the existence of a considerable amplitude in the body length, just in *Aphilodon meganae* Calvanese, Brescovit & Bonato, 2019, the body have less than 41 leg-bearing segments and length up to 1 cm in adults (Calvanese *et al.* 2019).

The forcipular segment, that has been very informative to delimit *Mecophilus* by presenting several autapomorphic components (Calvanese *et al.* 2019) is also useful in the identification of species. This part of the body is very peculiar in *M. neotropicus*, that has a proportionally more elongated forcipular tergite, pretergite and coxosternite than in other species of *Mecophilus* (see Table 1). Also, the three known species can be quickly differentiated by the position of the denticle of tarsungulum (in the middle of article in *M. neotropicus* and basal in *M. tupiniquim* and *M. carioca* **n. sp.**), and its expression (conspicuous in *M. neotropicus* and *M. tupiniquim* and reduced in *M. carioca* **n. sp.**).

Unlike other Neotropical Aphilodontinae (including species of *Aphilodon* Silvestri, 1898 and *Mairata* Calvanese, Brescovit & Bonato, 2019) that male and female have ultimate legs with six articles (tarsus 2 not present) and without terminal claw, at least in *Mecophilus tupiniquim* and *M. carioca* **n. sp.**, male ultimate legs have six articles and female ultimate legs have seven articles. Despite the absence of a terminal claw in males of *Mecophilus*, the presence of a terminal claw in females of *M. tupiniquim* constitutes an important difference from *M. carioca* **n. sp.**, in which females have no claws. Also, through scanning electron microscopy analysis, we checked the terminal part of the last article of the ultimate pair of legs of males of *Mecophilus* *tupiniquim* and males and females of *M. carioca* **n. sp.** to check for any evidence of the pretarsus (such as in *Aphilodon* where the structure is modified in a small terminal spine, see Calvanese *et al* 2019: 22, fig. 13A), but nothing was found (Fig. 6A–C).

Acknowledgements

We express thanks for all the support provided by the Instituto de Biociências of Universidade de São Paulo (IB-USP) and Laboratório de Coleções Zoológicas (LCZ). We thank all the staff of the Parque Nacional de Itatiaia for the collaboration and support. Especially thanks to Adele M.M.S. Silva for help in field collections. Thanks to Beatriz Mauricio for the help in the SEM analysis. The anonymous reviewers we thank for the suggestions and corrections. This work was supported by CNPq grants 169641/2017–1 to VCC and CNPq granted PQ 303903/2019–8, to ADB.

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FIGURE CAPTIONS

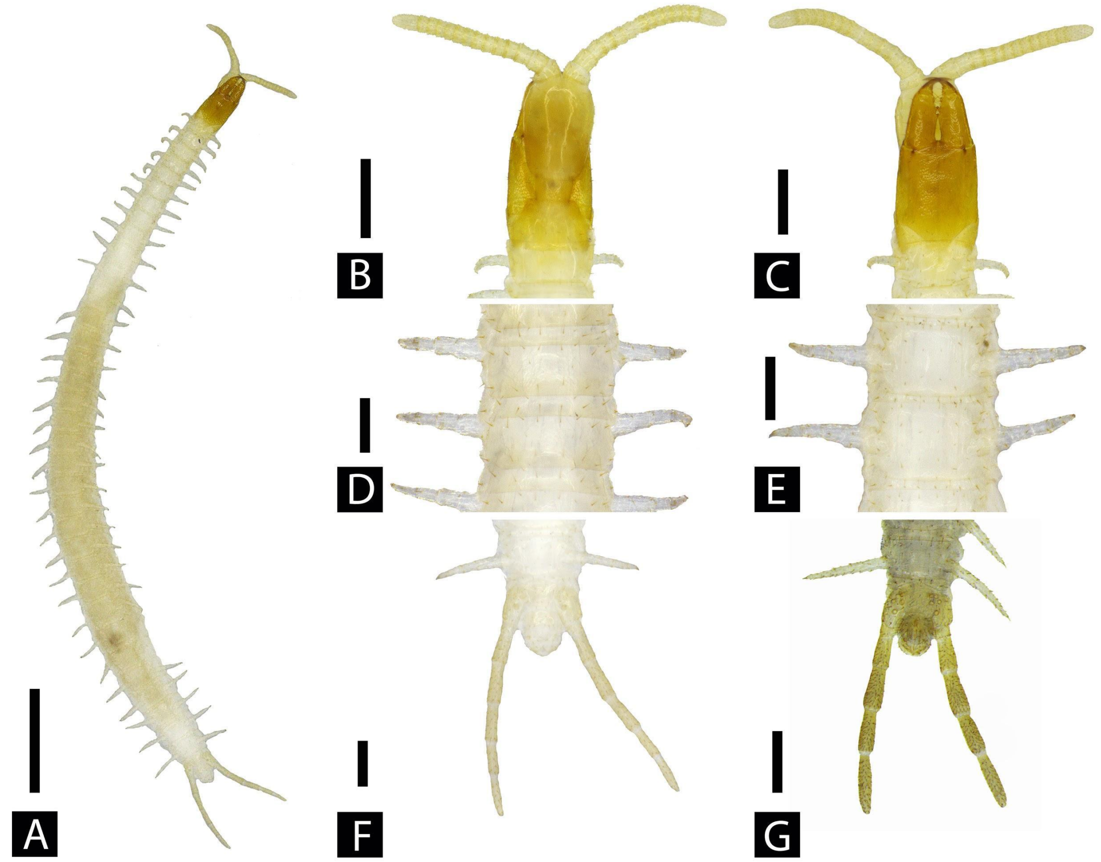

FIGURE 1. A−G, *Mecophilus carioca* **n. sp.** Holotype female (IBSP 6470): **A**, habitus, ventral; **B**, anterior part of body, dorsal; **C**, same, ventral; **D**, leg-bearing segment 15−16, dorsal; **E**, same, ventral; **F**, terminal part of body, ventral. Paratype male (IBSP 6472): **G**, terminal part of body, ventral. **Scale bars:** 1 (A), 0.2 (B−C, F−G), 0.1 (D−E) mm.

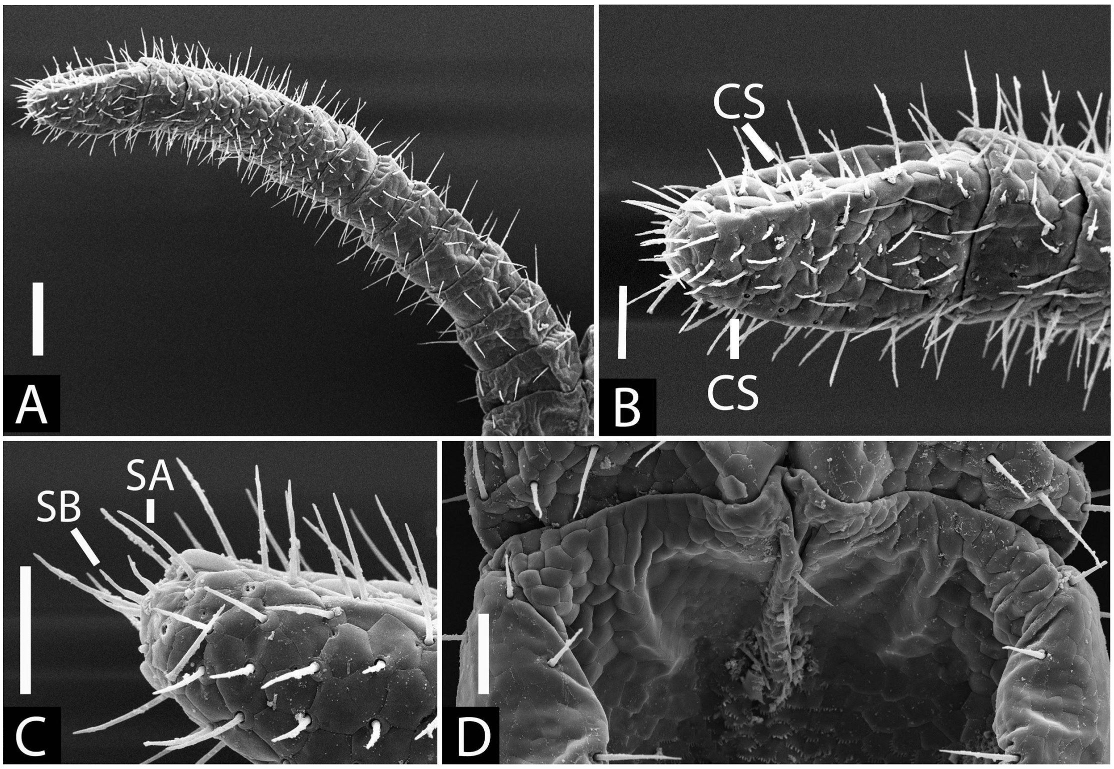

FIGURE 2. A−D, *Mecophilus carioca* **n. sp.** Female (IBSP 6473): **A**, right antenna, ventral; **B**, terminal part of the right antenna, ventral; **C**, terminal part of the last article of left antenna, ventral; **D**, clypeus, ventral. **Abbreviations:** CS: claviform setae. SA: setae type "A". SB: setae type "B". **Scale bars:** 0.05 (A), 0.02 (B−D) mm.

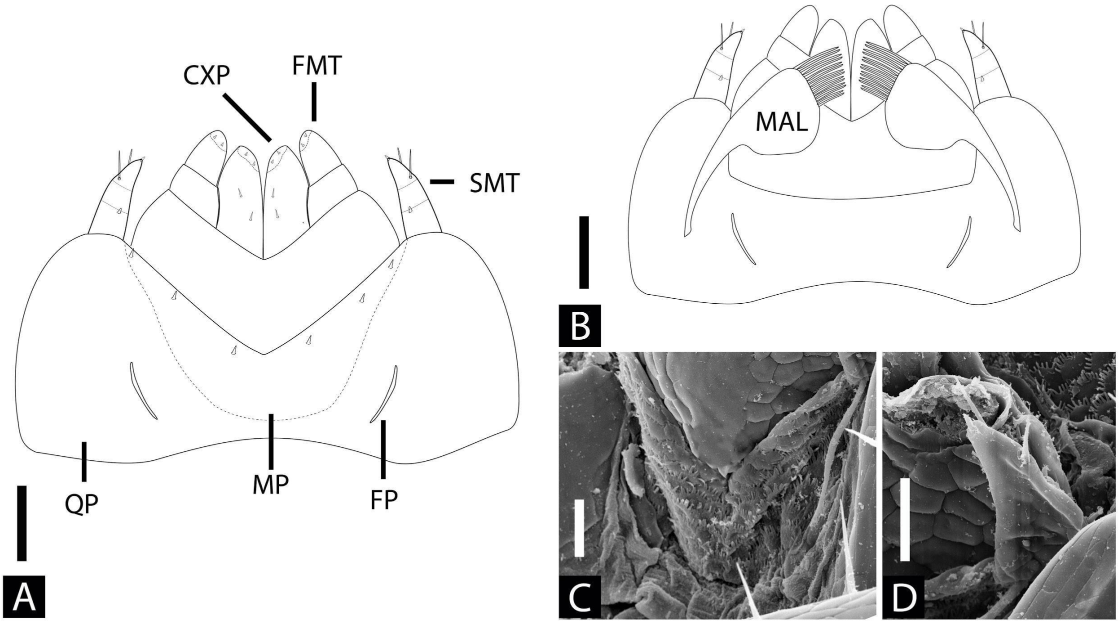

FIGURE 3. A−D, *Mecophilus carioca* **n. sp.** Female (IBSP 6473): **A**, first and second maxillary, ventral; **B**, maxillary complex and mandibles, dorsal; **C**, middle of the second maxillary coxosternite, ventral; **D**, left telopodite of second maxillary, ventral. **Abbreviations:** CXP: medial projections of coxosternite. FMT: telopodite of first maxillary. FP: foraminal process. MAL: left mandible. MP: membranous part. QP: most sclerotized part. SMT: telopodite of second maxillary. **Scale bars:** 0.02 (A−B), 0.01 (C−D) mm.

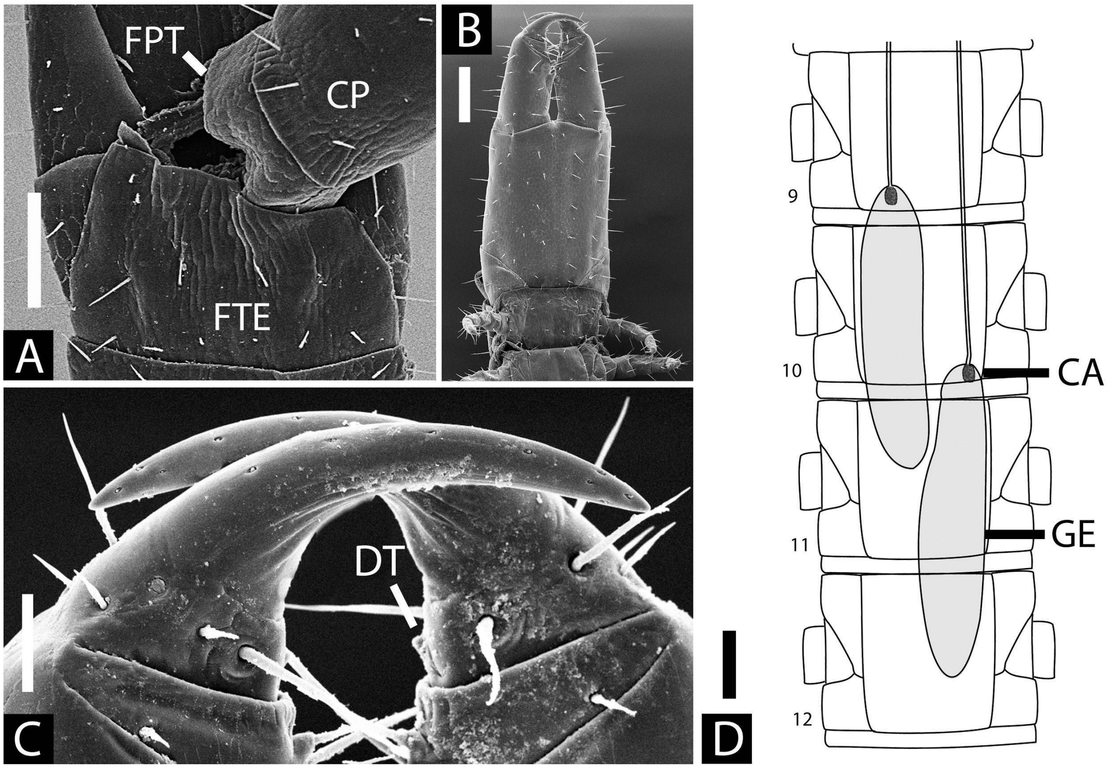

FIGURE 4. A–D, *Mecophilus carioca* **n. sp.** Female (IBSP 6473): **A**, forcipular tergite and pretergite, dorsal; **B**, forcipular segment and first pair of legs, ventral; **C**, tarsungulum, ventral; **D**, leg-bearing segments 9–12 and venom glands, ventral. **Abbreviations:** CA: calyx of venom gland. CP: cephalic plate. DT: denticle of tarsungulum. GE: glandular epithelium. FPT: forcipular pretergite. FTE: forcipular tergite. **Scale bars:** 0.05 (A), 0.1 (B, D), 0.02 (C) mm.

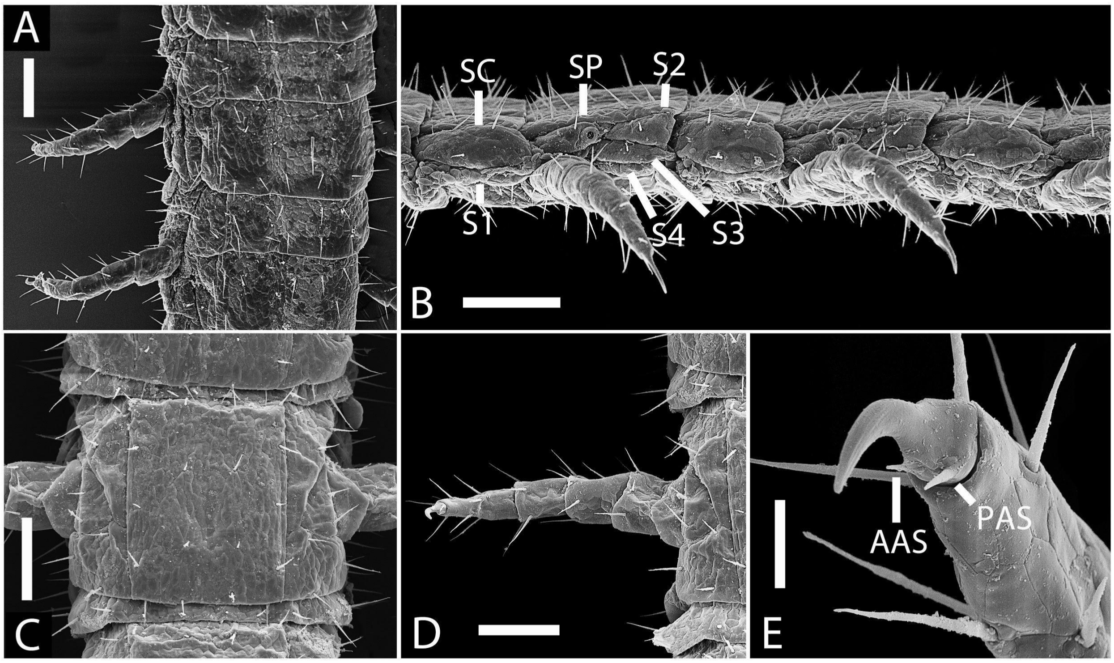

FIGURE 5. A–E, *Mecophilus carioca* **n. sp.** Female (IBSP 6473): **A**, leg-bearing segment 13–14, dorsal; **B**, leg-bearing segment 22–23, lateral; **C**, leg-bearing segment 8, ventral; **D**, right leg 18, ventral; **E**, apex of the left leg 25, latero ventral. **Abbreviations:** AAS: anterior accessory spine. PAS: posterior accessory spine. SC: scutellum. SP: stigmatopleurite. S1: sclerite "1". S2: sclerite "2". S3: sclerite "3". S4: sclerite "4". **Scale bars:** 0.05 (A–D), 0.01 (E) mm.

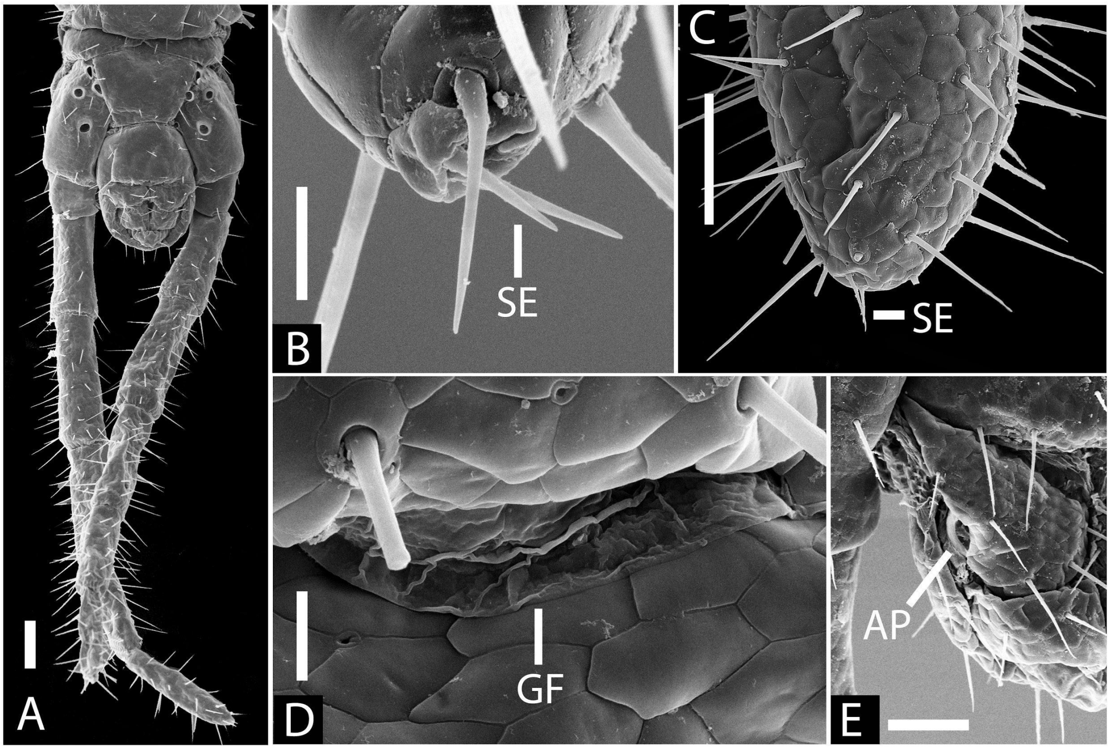

FIGURE 6. A−E, *Mecophilus carioca* **n. sp.** Female (IBSP 6473): **A**, terminal part of body, ventral; **B**, terminal part of the left ultimate leg, ventral; **D**, right gonopod, ventral; **E**, part of the post pedal segmentation, ventral. Male (IBSP 6473): **C**, terminal part of the left ultimate leg, ventral. **Abbreviations:** AP: anal pore. GF: female gonopod. SE: setae. **Scale bars:** 0.05 (A), 0.005 (B, D), 0.02 (C, E) mm.

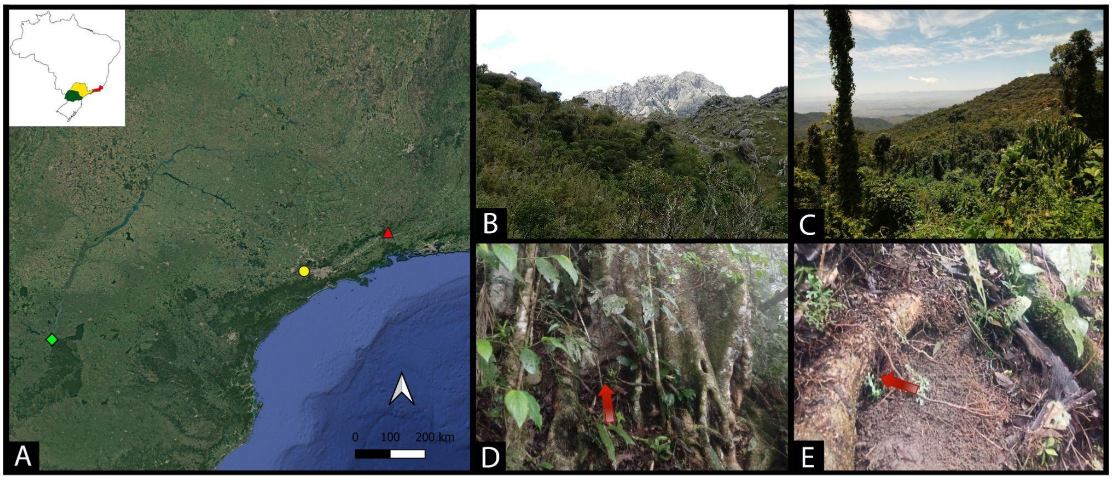

FIGURE 7. A−E, known occurrence of species of *Mecophilus* and habitat of *M. carioca* **n. sp.** in the Parque Nacional de Itatiaia: **A**, map with the known distribution of species. At the top on the map of Brazil, green indicates the state of Paraná, in yellow the state of São Paulo and in red the state of Rio de Janeiro. Below, green diamond indicates the known occurrence of *M. neotropicus* Silvestri, 1909, yellow circle indicates the occurrence of *M. tupiniquim* Calvanese, Brescovit & Bonato, 2019 and red triangle indicates the occurrence of *M. carioca* **n. sp.**; **B** and **C**, general aspect of the Parque Nacional de Itatiaia; **D** and **E**, local sampling of *M. carioca* **n. sp.**, red arrows indicate the sampling point.

Table 1. Some characteristics of the species of *Mecophilus* and its occurrence. Abbreviations: AT: antenna, CP: cephalic plate, CXS: second maxillary coxosternite, DF: denticle of femur, DT: denticle of tarsungulum, DTF: denticle of trochanteroprefemur, FCX: forcipular coxosternite, FPT: forcipular pretergite, FS: sternite of first leg-bearing segment, FTE: forcipular tergite, OC: occurrence, PL: penultimate leg, TA: tarsungulum, TTF: trochanteroprefemur+femur, UMS: ultimate metasternite, UL: ultimate legs.

Species	**Recorded specimens /depositary**	**Body length (mm)**	**Pair of legs**	**CP length /width**	**CP shape**	**AT /CP length**	**FPT /CP length**	**FPT length /width**	**FTE /CP length**	**FCX length /width**	**DTF and DF spaced**	**DT length**	**DT position**	**FS length /width**	**UL /PL length**	**UL terminal claw**	**Coxal pores**	**OC**
Mecophilus neotropicus Silvestri, 1909	1m# (MCSG)	8	35	ca. 1.4	trapezoid	1.7	0.55	ca. 1	0.83	1.4	yes	large	middle	1.5	male: ca. 1.7 female: ?	male: no female: ?	5+5	Brazil: Paraná, Foz do Iguaçu
Mecophilus tupiniquim Calvanese, Brescovit & Bonato, 2019	1m#, 4f# (IBSP)	7–7.5	35	ca. 1.5	rectangular	ca. 2.4	ca. 0.25	ca. 0.6	ca. 0.35	ca. 1.2	no	large	basal	ca. 1.2	male: ca. 1.9 female: 1.8−2	male: no female: yes	6−7 + 6−7	Brazil: São Paulo, São Roque
Mecophilus carioca **n. sp.**	3m#, 3f# (IBSP)	6.5–7	33–35	ca. 1.5	rectangular	ca. 1.75	ca. 0.25	ca. 0.7	ca. 0.45	ca. 1.2	yes	small	basal	ca. 1.2	male: ca. 2.3 female: ca. 2.8	male: no female: no	4−6 + 4−6	Brazil: Rio de Janeiro, Itatiaia

Capítulo 3. Filogenia de Aphilodontinae

Combined molecular and morphological phylogeny of the Aphilodontinae centipedes (Geophilomorpha: Geophilidae)

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Abstract

Currently represented by 32 species and four genera, Aphilodontinae centipedes are known from South Africa and part of South America. In this contribution for the first time is presented a phylogeny of the subfamily based on molecular and morphological data. Morphological matrix is composed of 52 terminals, including all known species of Aphilodontinae and *Geoperingueyia* (sister group of Aphilodontinae) and 58 characters, of which 11 characters were at first time used for the group. Molecular data aimed at the mitochondrial 16S rRNA and cytochrome c oxidase subunit I (COI) and nuclear 28S and 18S rRNAs, and all known genera of Aphilodontinae were represented. Four phylogenetic analyses were conducted: maximum cladistic parsimony to morphological characters, maximum likelihood to molecular partitions analyzed separately, maximum likelihood to molecular partitions analyzed together and maximum likelihood of morphological and molecular datasets analyzed together (total evidence). Most analysis recovered the monophyly of Aphilodontinae and corroborates with its four genera, but molecular analysis and total evidence approach were better able to resolve some polytomies between the genera and species. The results point to the monophyly of a clade with *Geoperingueyia* and Aphilodontinae, which within Geophilidae seems to be most related to Dignathodontinae. *Philacroterium* was recovered as the most basal in

Aphilodontinae, as a sister group of a clade with the Neotropical genera. In this arrangement *Aphilodon* and *Mairata* are most nested, and sister to *Mecophilus*. Additionally, new morphological synapomorphies were diagnosed to *Geoperingueyia*, Aphilodontinae and *Aphilodon*, and the systematics of the subfamily is discussed.

Key words: Chilopoda-evolution-systematic-total evidence.

Introduction

The subfamily Aphilodontinae represents a group of centipedes associated with terrestrial environments highly cryptics, such as between plant roots, rock grooves or fossorial cavities (Silvestri, 1909; Pereira *et al.*, 2007). Because they are little observed in the field, aphilodontines were little accessed in the past literature, however current researches reported an assiduous occurrence of the group for a large part of South America and South Africa (Calvanese *et al.*, 2019; Calvanese & Brescovit, 2021a,b). When adults, these centipedes have body length less than 1 to 7 cm and between 33−93 leg-bearing segments (Calvanese & Brescovit, 2021a,b), and are diagnosed by some peculiar characteristics in the buccal appendages, as the vestigial pieces of the labrum and short telopodite in the second maxillary. However, the classical feature used in the group diagnosis occurs in the poison inoculating apparatus, where the forcipular trochanteroprefemur and forcipular femur are fused, resulting in a forcipule with only three articles (Silvestri, 1909a,b; Attems, 1929).

Since the proposition as family Aphilodontidae by Silvestri (1909a), the taxa has been considered valid (Attems, 1929, 1947; Verhoeff, 1937, 1938; Lawrence, 1955, 1963; Pereira *et al.*, 2007; Bonato *et al.*, 2011), and recently it was accessed as Aphilodontinae, a subfamily of Geophilidae (Calvanese *et al.*, 2019). Currently, four genera composes the group (Calvanese *et al.*, 2019), which is represented in South Africa by *Philacroterium* Attems, 1926 and in South America by *Aphilodon* Silvestri, 1898, *Mecophilus* Silvestri, 1909 and *Mairata* Calvanese, Brescovit & Bonato, 2019. The South African species were mainly worked in the middle of the 20th century, when many works addressed the diversity of Geophilomorpha in this country (as Attems, 1926, 1928, 1929; Verhoeff, 1937, 1938; Lawrence, 1955, 1963), and at the moment *Philacroterium* counts 11 species.

Although the first species of Aphilodontinae have been described from Argentina and Brazil (Silvestri, 1898, 1909a,b; Brölemann, 1902), only six Neotropical species were known until the group review provided by Calvanese *et al.* (2019), and recent descriptions of new species (Calvanese & Brescovit, 2021a,b). Currently, the Neotropical genera have known occurrences partially sympatric, and *Aphilodon* embraces 16 species, *Mecophilus* three species and *Mairata* two species.

If the knowledge about Aphilodontinae morphology has advanced in the recent past, the molecular evolution inside the group has been poorly accessed, and so far only one species, *Philacroterium weberi* (Silvestri, 1909), has had sequences evaluated in phylogenies (see Edgecombe *et al.*, 1999; Murienne *et al.*, 2010; Bonato *et al.*, 2014).

In this contribution, we present a phylogenetic study to Aphilodontinae based on morphological and molecular data, analyzed together under maximum likelihood and embracing all the 32 species known to the subfamily. The morphological dataset has as basis the matrix provided by Calvanese *et al.* (2019), with addition of 22 terminals, and 11 characters not yet applied to the subfamily. The molecular dataset comprises sequences of 28S, 18S, 16S rRNAs and cytochrome c oxidase subunit I (COI), and have representatives of the four genera of Aphilodontinae. In order to better understand the behavior of each data source and its phylogenetic implications, the morphological dataset and the molecular partitions were also evaluated separately. In this process, maximum cladistic parsimony was used to search only in the morphological matrix, and the molecular partitions were analyzed by maximum likelihood together and separately.

Based on the results, we present a most complete phylogeny to Aphilodontinae and discuss the contribution of the morphology and molecules to this hypothesis. New morphological synapomorphies were defined at different hierarchical taxonomic levels related to Aphilodontinae, and some new insights on the general morphology are provided. In addition, the affinity of *Geoperingueyia* Attes, 1926 with Aphilodontinae, and its positions inside Geophilidae are also accessed and discussed.

Material and Methods

Morphological sampling

This research was based on analysis of Geophilomorpha specimens from eight zoological collections (curators are in parentheses): IBSP, Instituto Butantan, São Paulo, Brazil (A.D. Brescovit); INPA, Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (M.L. de Oliveira); MNRJ, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (A.B. Kury); MSNG, Museo di Storia Naturale Giacomo Doria, Genova, Italy (R. Poggi); NHMW, Museum of Natural History of Vienna, Austria (N. Akkari); PD, Minelli-Bonato collection, Dipartimento di Biologia, Università di Padova, Italy (L. Bonato); SAM, South African Museum, South Africa (S.V. Noort); KZN, KwaZulu-Natal Museum, Pietermaritzburg, South Africa (D. Herbert).

Specimens from 29 of the 32 species of Aphilodontinae were analyzed, of which 14 belong to *Aphilodon*, three to *Mecophilus*, two to *Mairata* and 10 to *Philacroterium*. From *Geoperingueyia*, the sister group of Aphilodontinae (Bonato *et al.*, 2014; Calvanese *et al.*, 2019), specimens of four species were evaluated. Other six species of *Geoperingueyia*, one of *Philacroterium* and two of *Aphilodon* have not had biological material consulted because of the inaccessibility of types or other related specimens, and its morphologies were verified from previous literature, especially the original descriptions. The cast of analyzed specimens also have representatives of other subfamilies of Geophilidae (*sensu* Bonato *et al.*, 2014), and includes: one species of *Dignathodon* Meinert, 1870, one of *Henia* C.L. Koch, 1847, two of *Strigamia* Gray, 1843, one of *Hyphydrophilus* Pereira, Minelli & Barbieri, 1994, one of *Ribautia* Brölemann, 1909, one of *Pachymerium* C.L. Koch, 1847 and two species of *Macronicophilus* Silvestri, 1909. *Dicellophilus carniolensis* (C.L. Koch, 1847), a representative of the basal Mecistocephalidae was used as root (details about species used in the analysis are presented in Appendix 1).

Specimens from 43 species had their general morphology analyzed in a Leica DFC 500 digital camera coupled to a Leica MZ 16A stereomicroscope and using a Leica DFC 500 digital camera coupled to a Leica DM4000B optical microscope (see Appendix 1). Measurements and morphological analysis were made on the cephalic plate, antennae, labrum, mandible, first and second maxillary, forcipular apparatus, leg-bearing segments of the trunk, ultimate leg-bearing segment, and post-pedal segmentation. This procedure took into account dissection procedures proposed by Pereira (2000) and were conducted at the Laboratório de Coleções Zoológicas of the Instituto Butantan.

Specimens of 32 species were also analyzed by Scanning Electron Microscope (SEM) (see Appendix 1), with a FEI Quanta 250 at the Laboratório de Biologia Celular of Instituto Butantan, following the protocol specified by Calvanese *et al.* (2019).

Molecular sampling

The mitochondrial cytochrome c oxidase subunit I (COI) and 16S rRNA, and the nuclear 28S and 18S rRNA were targeted to assess the evolution of Aphilodontinae. In this procedure, new sequences obtained from 17 species of Aphilodontinae were integrated with DNA sequences from one species of Aphilodontinae and from other six species of Geophilomorpha available in the GeneBank (details in Appendix 1).

The new sequences were obtained from freshly collected 100% ethanol-preserved specimens, previously identified by morphology by one of us (V.C.C.). For DNA extraction, priority was given to parts with abundant muscle tissue, such as the legs, however very small specimens had the whole body used. The procedure of DNA extraction was based on Fetzner (1999), following the steps: 1) the tissues preserved in 100% alcohol were dried, cut into small pieces and placed in a 1.8 ml tube, then were added 300 µl of lysis solution (10 mM Tris base, 100 mM EDTA, 2% SDS pH 8) and 5 µl of proteinase K (20 mg/l). The samples remained overnight at 55 °C. 2) The material was cooled at ambient temperature for about 1h, then 300 µl of ammonium acetate was added and the tube was vortexed, next the solution was incubated in a -20 °C freezer for thirty minutes. Subsequently the samples were centrifuged for 10 minutes at 13,000 rpm at 4 °C and then the supernatant was transferred to new tubes of 1.5 ml. Next, 600 µl of absolute isopropanol was added to the new tubes, and each sample was manually inverted to mix the solution, then the material was cooled for one hour in a -20 °C freezer. 3) The samples were centrifuged for 10 minutes at 13,000 rpm at 4 °C and the resulting supernatant was discarded. Next, 600 µl of 70% ethanol was added to each tube and the material was again centrifuged. The ethanol was discarded, and the samples were dried in a vacuum pump for 30 minutes. Finally, the DNA was resuspended in 50 µl TE (10 mM Tris base, 0.1 mM EDTA pH 8) and stored in a refrigerator (2 to 8 °C).

The fragments of the genes of interest were amplified with oligonucleotide primers whose sequences are shown in Table 1. In this procedure, for a final volume of 20 µl of PCR reaction, were added 1 µl of purified DNA, 10.45 µl of milli-Q water, 4 µl of buffer (Tris-HCI 200 mM, KCL 500 mM, pH 8,4), 0.5 µl magnesium chloride (25 mM), 2 µl de dNTP (2 mM), 1 µl of light chain primer, 1 µl of heavy chain primer and 0.05 µl of DNA taq Polymerase. The amplification of the fragments of interest was carried out in a thermocycler (Techne 300, Analytical) respecting the following temperature regime: for the mitochondrial COI and 16S the program consisted of an initial denaturation step (3 min at 98 °C) followed by 35 cycles including denaturation at 98 °C for 10 s, annealing at 45 °C for 30 s, and extension at 72 °C for 20 s, with a final extension step at 72 °C for 3 min. For the nuclear 18S and 28S the program consisted of an initial denaturation step (3 min at 98 °C) followed by 35 cycles including denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension step at 72 °C for 3 min. PCR products were verified by 1% agarose gel electrophoresis and purified with the Agencourt AMPure XP (Beckman Coulter, Inc.) following the manufacturer's protocol.

Table 1. List of primer sequences, authors and final length of the obtained alignments.

Target sequence	Primer name	Authors	Sequence of the primer	Alignment obtained
COI (mitochondrial)	LCO1490	Folmer *et al.* (1994)	5′-GGTCAACAAATCATAAAGATATTGG-3′	609 pb
	HCOoutout	Prendini *et al.* (2005)	5′-GTAAATATATGRTGDGCTC-3′	
16S rRNA (mitochondrial)	16S ptFN	Pinto da Rocha *et al.* (2014)	5′-GACTGTGCAAAGGTAGCATAATC-3′	409 pb
	16S BR	Palumbi (1996)	5′-CCGGTCTGAACTCAGATCACGT-3′	
18S rRNA (nuclear)	18Sc	Apakupakul *et al.* (1999)	5′-CGGTAATTCCAGCTCCAATAG-3′	1,517 pb
	Worm B	Littlewood & Olson (2001)	5′-CTTGTTACGACTTTTACTTCC-3′	
28S rRNA (nuclear)	28s D1AF	Arango & Wheeler (2007)	5′-CCCSCGTAAYTTAGGCATAT-3′	1,747 pb
	28SD3AP	Reyda & Olson (2003)	5′-CAAGTACCGTGAGGGAAAGTTG-3′	
	28SD4BR	Edgecombe & Giribet (2006)	5′-CCTTGGTCCGTGTTTCAAGAC-3′	
	28S BR	De Ley *et al.* (1999)	5′-TCGGAAGGAACCAGCTACTA-3′	

The sequencing reactions were performed from the purified DNA in a tube contained 5 µl of Milli Q water, 1.5 µl of DNA PCR product, 2 µl of buffer (200 mM TrisHCP pH9.0 with 5 mM MgCl2), 0.5 µl of the primer and 1 µl ABI BigDye Terminator v.3.0 (Applied Biosystems). The procedures for reactions involved an initial denaturation step for 3 min at 95 °C, and 25 cycles (95 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min). Final products are then cycle-sequenced in forward and reverse directions in an ABI 3100 Genetic Analyzer at the Centro de Sequenciamento do Departamento de Química, Instituto de Química of the Universidade de São Paulo.

The quality of each chromatogram and the assemblage of contiguous sequences were assessed using the package Consed/Phred Phrap (Ewing & Green, 1998; Gordon *et al.*, 1998). To check for possible contamination, BLAST searches (Altschul *et al.*, 1997) were implemented via the NCBI website (http://www.ncbi.nlm.nih.gov). All new sequences obtained in this research were deposited in GenBank (numbers of access are presented in Appendix 1).

Phylogenetic analysis

The phylogenetic relationships of Aphilodontinae were evaluated on four data sets: 1) only morphological data, analyzed by Maximum Cladistic Parsimony (MP); 2) each molecular partition was analyzed separately by Maximum Likelihood (ML); 3) the four molecular partitions analyzed together by ML; 4) total evidence of morphological and molecular data analyzed together by ML.

For each topology the support value of the clades was obtained on the metrics of bootstrap (BS) (Felsenstein, 1985). As in Bonato *et al.* (2014) for convenience, the clades with bootstrap < 50% are referred as poorly supported, between 50–85% as moderately supported, and > 85% as strongly supported. The analysis of results and discussions are made on the strict consensus topology of morphological characters from MP, with character optimization in ACCTRAN (accelerated transformation) algorithm. Also, based on ML analysis of the concatenated molecular data and total evidence analysis. However, the topologies obtained for each genetic marker can be accessed with the values of support on Appendix 4.

Morphological data. The morphological characters evaluated here were obtained in the literature (53 characters) or first proposed (5 ch.). The unpublished ones were built following the logic proposed by Sereno (2007). All dataset is presented in Appendix 2, embracing 58 characters related to the cephalic plate (1 ch.), clypeus (2 ch.), labrum (6 ch.), first maxillary (3 ch.), second maxillary (9 ch.), maxillary complex (1 ch.), forcipular apparatus (19 ch.), trunk (5 ch.), and ultimate legs (12 ch.). The matrix of morphological data can be accessed in Appendix 3.

T.N.T. 1.5 (Goloboff & Catalano, 2016) was used for heuristic searches for the most parsimonious trees. Equal weighing (EW) was applied with “new technologies”, with 10,000 random addition sequences (RAS) followed by rounds of tree bisection and reconnection (TBR), holding 100 trees per replication (commands: mult=tbr, repl 10000 hold 100). In all runs, the minimum length for collapsing was equal to 0. To evaluate the robustness of the dataset, 1000 replicates of bootstrap were applied to the strict consensus topology.

Molecular data. Sequences were aligned using Mafft (Katoh *et al.*, 2009) with 1000 cycles of iterative refinement incorporating local pairwise alignment information with the L-INS-i algorithm. A gap-opening penalty and a gap-extension penalty were used with default values (1.53 and 0.123, respectively). Editions of sequences were realized in the software BioEdit 7.0 (Hall, 2004), where the final alignments of each partition were edited so that all sequences were approximately the same length by removing the longest ends.

Substitution models were specified independently for each of the gene partitions using jModelTest version 2.1.7 (Darriba *et al.*, 2012) under the Akaike information criterion (AIC) (Posada & Buckley, 2004). However, the application of the models in the analysis depended on the availability found in the software used for phylogenetic searches, and in the absence of the specific selected model, more similar models were adopted based on the scheme proposed by Posada & Crandall (1998).

The best topology of each partition was estimated by ML using RAxML 2.0 beta (Edler *et al.*, 2019) with a GTR + G model (Yang, 1993). All partitions were also analyzed together, being concatenated in RAxML 2.0 beta and analyzed by maximum likelihood with a GTR + G model applied to each partition. 1000 replicates of bootstrap were applied to each topology.

Combined analysis. Morphological and molecular datasets were analyzed in combination, applying a GTR + G model to each molecular partition and a MK model (Lewis, 2011) to the morphological data. To evaluate the robustness of the dataset, 1000 replicates of bootstrap were applied to the topology obtained.

Results

The search for the most parsimonious phylogeny on only the morphological characters resulted in 66 topologies with 107 steps (consistency index= 0.57, retention index= 0.89). The strict consensus topology (Fig. 1) has 109 steps (consistency index= 0.56, retention index= 0.89), and counts with 47% of the clades with a BS < 50%, 19% with a BS between 50−85% and 33% of clades with a BS > 85%. All 58 morphological characters employed were considered phylogenetic informative.

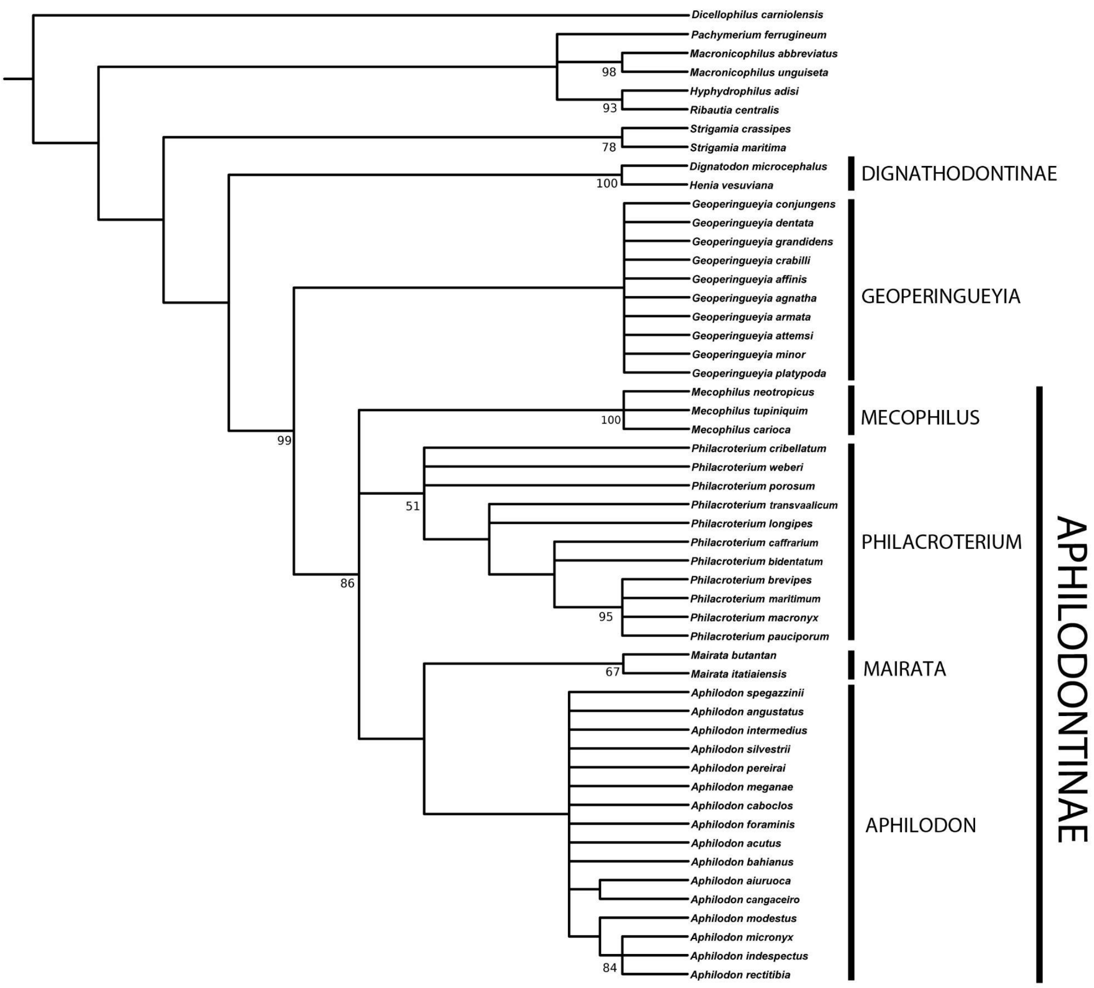

FIGURE 1. Topology of the strict consensus under equal weights from 66 most parsimonious trees obtained by MP analysis of the morphological dataset. Bootstrap values greater than or equal to 50% are given close to the nodes. Classification of Aphilodontinae and most related taxa are displayed.

When molecular data were analyzed separately, COI topology has 31.5% of clades with BS < 50%, 47.4% with BS between 50−85% and 21% has a BS > 85%. 16S topology has 23.5% of clades with BS < 50%, 35.3% with BS between 50−85% and 41.1% has a BS > 85%. 18S topology has 20% of clades with BS < 50%, 40% with BS between 50−85% and 40% has a BS > 85%. 28S topology has 0% of clades with BS < 50%, 60% with BS between 50−85% and 40% has a BS > 85%. Topologies obtained for each of the four genetic fragments analyzed are presented in Appendix 4.

The topology from the four molecular partitions analyzed together (Fig. 2) presented 19% of clades with BS < 50%, 52.4% with BS between 50−85% and 28.6% with a BS > 85%.

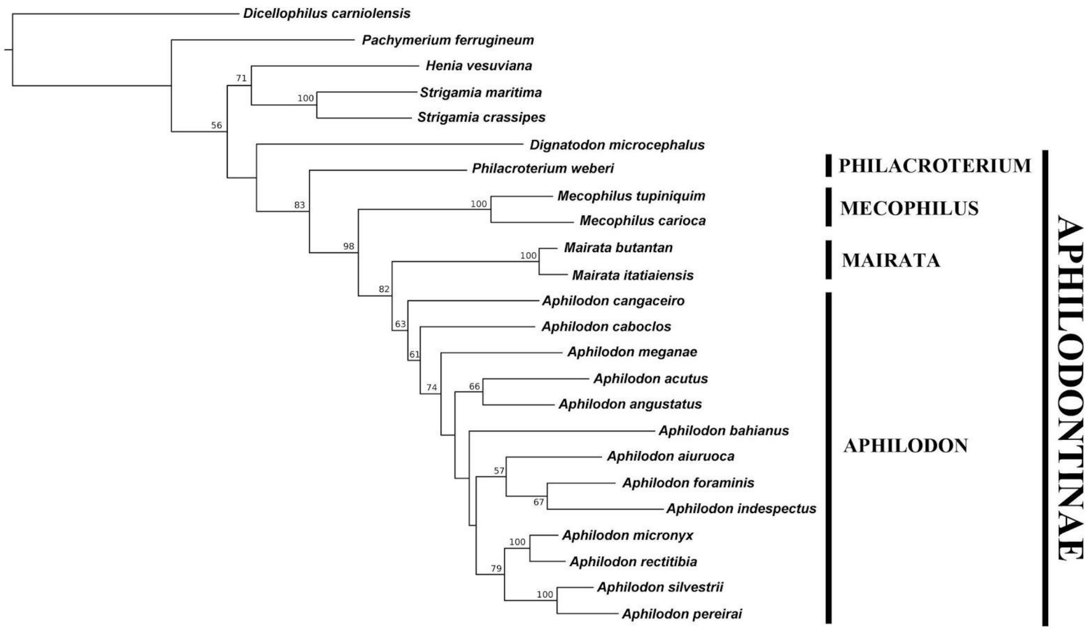

FIGURE 2. Topology from ML analysis of the all four genes concatenated. LnL = -36017.832169. Bootstrap values greater than or equal to 50% are given close to the nodes. Classification of Aphilodontinae and most related taxa are displayed.

When morphological and molecular data were analyzed together in a combined analysis, a greater number of clades were resolved in the topology (Fig. 3), and 30.4% of clades has BS < 50%, 36.1% has a BS between 50−85% and 33.5% a BS > 85%. The BS ≥ 50% are reported to the clades in the strict consensus topology of morphological characters (Fig. 1), to the topology of all four molecular partitions analyzed together (Fig. 2) and to the total evidence topology (Fig. 3).

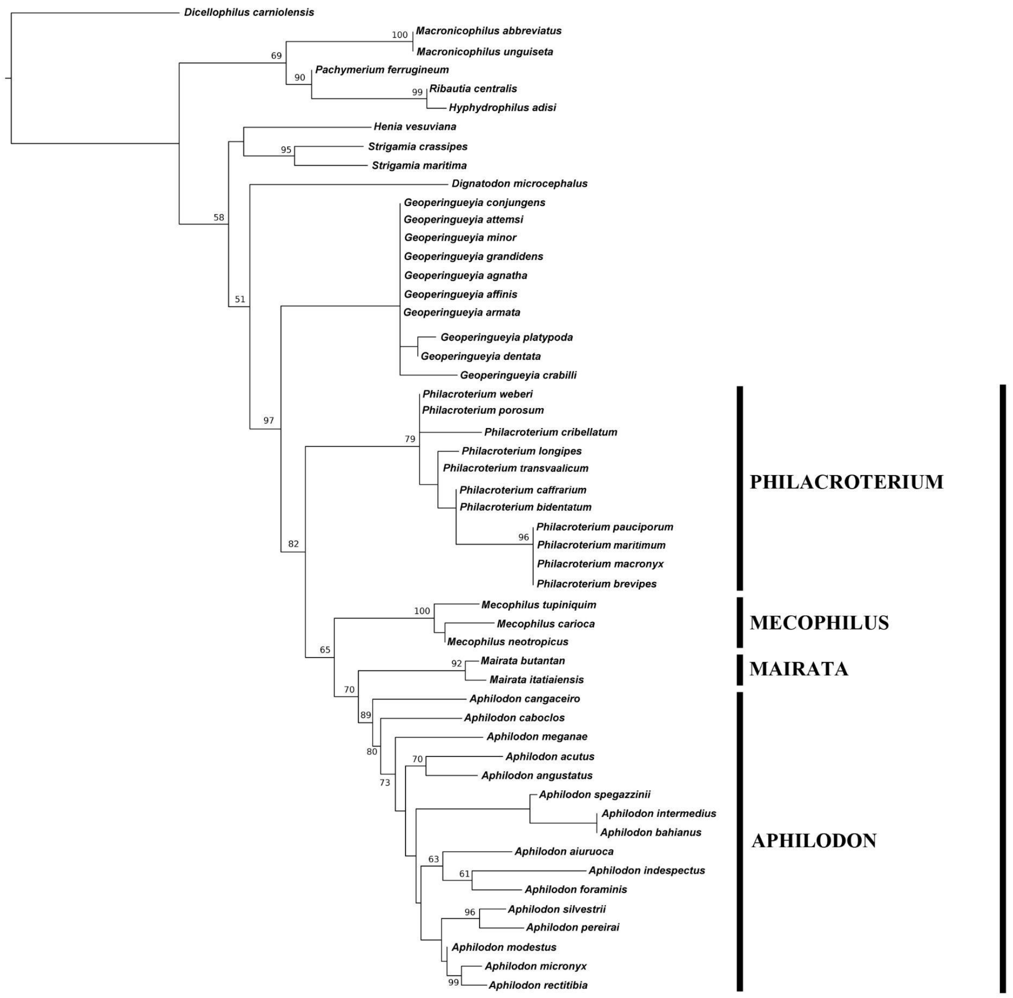

FIGURE 3. Topology from ML analysis of total evidence dataset. LnL = -36566.979847. Bootstrap values greater than or equal to 50% are given close to the nodes. Classification of Aphilodontinae and most related taxa are displayed.

The results indicate that inside Geophilidae, a monophyletic clade with Aphilodontinae and *Geoperingueyia* are most related to Dignathodontinae, but the relationship of these groups has not been fully clarified. MP of morphological characters recovers this clade poorly supported (BS= 30%), but with three non homoplastic synapomorphies related to the reduction of the length of the second maxillary telopodite (ch. 16, Fig. 4A), the position of the calyx of venom gland along the body (ch. 41, Figs 4C−D) and the pleurites of leg-bearing segments most elongated (ch. 44, Fig. 4B). However, ML analysis of the concatenated molecular dataset recovers *Dignathodon microcephalus* (Lucas, 1846) most nested to Aphilodontinae (BS= 40%), and *Henia vesuviana* (Newport, 1844) most related to species of Linotaeniinae (BS= 71%) (*Geoperingueyia* was not evaluated in this dataset). In the total evidence analysis this clade is maintained with the inclusion of *Geoperingueyia* together with

Aphilodontinae, and its relations with *Dignathodon microcephalus* is retrieved with BS= 51%.

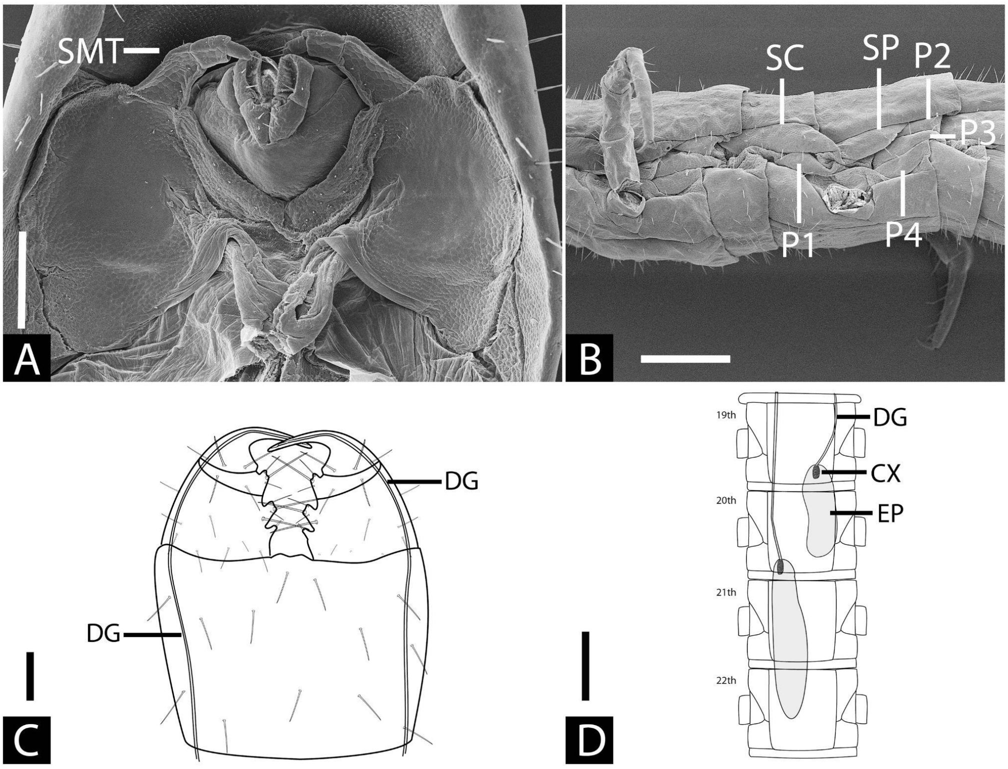

FIGURE 4. A−D, synapomorphic characters recovered to the clade (Dignathodontiane + (*Geoperingueyia* + Aphilodontinae)) on MP morphological analysis. *Aphilodon micronyx* Brölemann, 1902 (IBSP): **A**, second maxillary, ventral, ch. 16 state 1 (telopodite reduced in length). *Aphilodon pereirai* Calvanese, Brescovit & Bonato, 2019 (IBSP): **B**, leg-bearing segments 18 and 19, lateral, ch. 44 state 1 (elongated pleurites); **C**, forcipular segment, elongated ducts of the venom gland, ventral; **D**, leg-bearing segments 19−22, ventral, ch. 41 state 1 (presence of the calyx of venom gland along the body). **Abbreviations**: CX: calyx of the venom gland. DG: duct of the venom gland. EP: glandular epithelium. P1: pleurite "1". P2: pleurite "2". P3: pleurite "3". P4: pleurite "4". SC: scutellum. SMT: second maxillary telopodite. SP: stigmathopleutite. **Scale bars:** 0.1 (A, C), 0.5 (B, D) mm.

MP of morphological characters recovers together Aphilodontinae and *Geoperingueyia* in a clade supported by seven non homoplastic synapomorphies, including the reduction in sclerotization of the lateral and central parts of the labrum (chs. 5 and 8, both ambiguous, Figs 5A−B), lateral parts of the labrum without projections (ch. 6, Fig. 5B), middle of the labrum without projections (ch. 9, Figs 5A−B), forcipule with apical and subapical setae in the distal denticle of throchanteroprefemur and denticles of femur and tibia (ch. 37, Figs 5E−G), forcipule not markedly conic (ch. 40, Fig. 5F) and absence of a terminal claw in the ultimate legs of males (ch. 57). The molecular relationship of the group was not accessed due to non-obtention of *Geoperingueyia* sequences, however the clade was maintained in the analysis under total evidence with BS= 97%. In the MP of morphological characters and in the total evidence analysis, species of *Geoperingueyia* were grouped in a low supported clade (BS < 50%). The first analysis recovers to the genus a single non homoplastic synapomorphy related to the morphology of the foraminal process in the coxosternite of the second maxillary, that is transversely elongated (ch. 17, Fig. 5D).

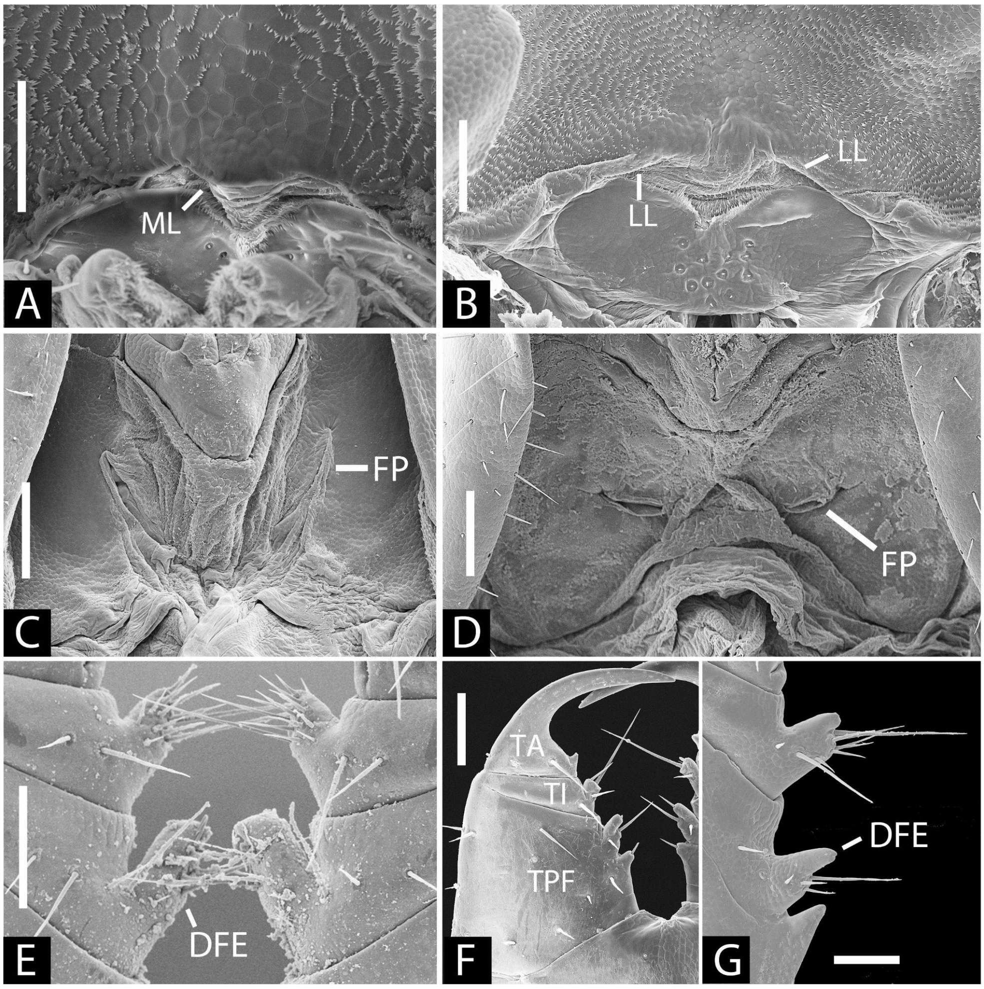

FIGURE 5. A−G, morphology of *Geoperingueyia* and Aphilodontinae, and some features recovered as synapomorphies on MP morphological analysis. *Aphilodon pereirai* Calvanese, Brescovit & Bonato, 2019 (IBSP): **A**, middle of labrum, ventral, ch. 9 state 1 (absence of projections in the middle part); **C**, coxosternite of second maxillary, ventral, ch. 17 state 0 (foraminal process not transversely elongated). *Aphilodon micronyx* Brölemann, 1902 (IBSP): **B**, labrum, ventral, ch. 6 state 1 (absence of projections in the lateral parts). *Geoperingueyia dentata* Verhoeff, 1938 (MNRJ): **D**, coxosternite of second maxillary, ch. 17 state 1 (foraminal process transversely elongated); **E**, forcipular denticles of femur and tibia, ventral, ch. 37 state 1 (presence of setae in the forcipular denticles). *Aphilodon acutus* Calvanese & Brescovit, 2021 (IBSP): **F**, right forcipule, ventral, ch. 27 state 1 (forcipular trochanteroprefemur and femur not articulated). *Maiata itatiaiensis* Calvanese, Bonato & Brescovit, 2019 (IBSP): **G**, forcipular distal denticle of trochanteroprefemur, denticle of femur and denticle of tibia, ventral, ch. 36 state 1 (forcipular denticle of femur with a bilobed shape). **Abbreviations**: DFE: denticle of forcipular femur. FP: foraminal process. LL: lateral part of labrum. ML: middle of labrum. TA: tarsungulum. TI: tibia. TPF: trochanteroprefemur+femur. **Scale bars:** 0.05 (A, E, G), 0.1 (B−D, F) mm.

Aphilodontinae was recovered as a natural group and with a BS > 80% in morphological and molecular analysis and with the two datasets combined. In addition, the genera *Aphilodon*, *Mecophilus*, *Philacroterium* and *Mairata* were considered monophyletic both in the MP of morphological character as in the ML analysis of the concatenated molecular dataset, being maintained in the total evidence analysis. Under MP analysis, Aphilodontinae have six non homoplastic synapomorphies, including a marked descletotization of the lateral and central parts of the labrum (chs. 5 and 8, both ambiguous, Figs 5A−B), the presence of sub-clypeal setae (ch. 2), middle part of labrum rudimentary and not clearly separated from the clypeus (ch. 7, Figs 5A−B), fusion of the trochanteroprefemur and femur in the forcipule (ch. 27, Figs 5F−G) and also in forcipule, the denticle of femur bilobed (ch. 36, Figs 5F−G). The subfamily has one homoplastic synapomorphy, the absence of pores in the sternite (ch. 45).

MP of morphological data recovered a polytomy with *Philacroterium*, *Mecophilus*, and the most nested *Aphilodon* and *Mairata* (BS < 50%). ML analysis of the concatenated molecular dataset and total evidence analysis indicate *Philacroterium* as a sister group of other aphilodontines (BS= 83% and BS= 82%, respectively). *Mecophilus* as sister to *Aphilodon* and *Mairata* was recovered in ML analysis of the concatenated molecular data with a BS= 98% and in total evidence with BS= 65%. A clade with *Aphilodon* and *Mairata* was best supported when molecular data were implemented, with BS= 82% in the ML analysis of the concatenated molecular dataset and BS= 70% in the total evidence analysis.

The analysis from MP recovers to the species of the South Africa *Philacroterium* two non homoplastic synapomorphies: lateral pieces of the labrum not distinctly from the clypeus (ch. 4, Fig. 6A) and telopodite of the second maxillary with only two articles (ch. 18, Figs 6B−E). This analysis indicated *Philacroterium cribellatum* Attems, 1928 (type species of the genus), *Philacroterium porosum* (Verhoeff, 1937) and *Philacroterium weberi* (Silvestri, 1909) in a basal polytomy with one other clade. This clade, have species with the pores grouped to the metasternite in the ultimate legs (ch. 49, homoplastic), and embraces *Philacroterium transvaalicum* (Lawrence, 1963) and *P. longipes* (Lawrence, 1955) sister to *P. caffrarium* (Verhoeff, 1937), *P. bidentatum* (Lawrence, 1955) and a monophyletic clade with *P. brevipes* (Verhoeff, 1938), *P. macronyx* (Lawrence, 1955), *P. maritimum* (Lawrence, 1963) and *P. pauciporum* (Lawrence, 1963), that is supported by the reduction of the distal denticle of trochanteroprefemur (ch. 31, homoplastic). A clade with *P. brevipes*, *P. macronyx*, *P. maritimum* and *P. pauciporum* was well supported with one non homoplastic synapomorphy related to the fusion of the femur and tibia articles in the forcipule, and other three homoplastic synapomorphies, including the reduction of the denticles of femur and tibia (ch. 33 and 35) and the presence of a terminal claw in the ultimate legs of males (ch. 57). The relationship between species of *Philacroterium* was not accessed by molecular data due to the inaccessibility of the material, and the only species of the genus analyzed, *P. weberi*, was recovered as a sister lineage of the other Neotropical genera of the subfamily. However, the same interspecific relationship obtained on morphological data to *Philacroterium* was maintained in the total evidence analysis.

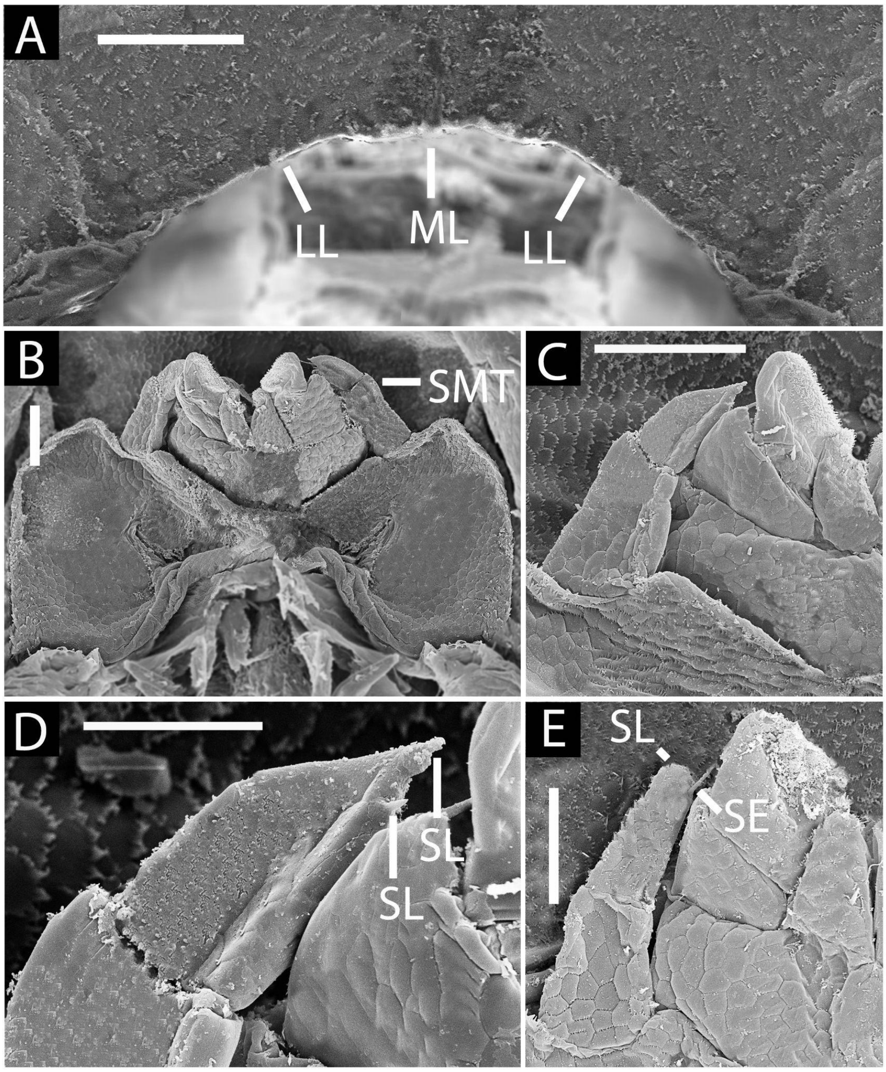

FIGURE 6. A−E, morphology of *Philacroterium* and some features recovered as synapomorphic on MP morphological analysis. *Philacroterium maritimum* (Lawrence, 1963) (KZN): **A**, labrum, ventral, ch. 4 state 1 (absence of a clear separation between the clypeus and the lateral parts of the labrum); **E**, telopodite of the second maxillary, ventral, ch. 18, state 1 (only two articles in the telopodite). *Philacroterium weberi* (Silvestri, 1909) (KZN): **B**, maxillary complex, ventral; **C**, right telopodite of second maxillary, ch. 18, state 1 (two articles in the telopodite); **D**, apical article of the second maxillary telopodite, ventral. **Abbreviations**: LL: lateral part of labrum. ML: middle of labrum. SE: seta. SMT: second maxillary telopodite. SL: sensilla. **Scale bars:** 0.05 (A−C, E), 0.01 (D) mm.

Mecophilus BS was equal to 100% in all analyses. MP of morphological characters recovered to the genus three non homoplastic synapomorphies, including the elongation of the forcipular pretergite (ch. 23, Fig. 7A), elongation of the forcipular coxosternite (ch. 24, Fig. 7D) and elongation of the first sternite (ch. 43, Fig. 7E). Other five homoplastic synapomorphies were diagnosed, including elongated cephalic plate (ch. 1, Fig. 7A), elongated throchanteroprefemur in the forcipule (ch. 26, Fig. 7D), denticle of forcipular tibia reduced (ch. 35) and ultimate legs in females with tarsus 2 not markedly shorter than the tarsus 1 (ch. 53, Fig. 7F). MP of the morphological characters recovered a polytomy between the three species of the genus, but in the ML analysis of total evidence *Mecophilus tupiniquim* Calvanese, Brescovit & Bonato, 2019 is most basal to *M. neotropicus* Silvestri, 1909 and *M. carioca* Calvanese & Brescovit, 2021.

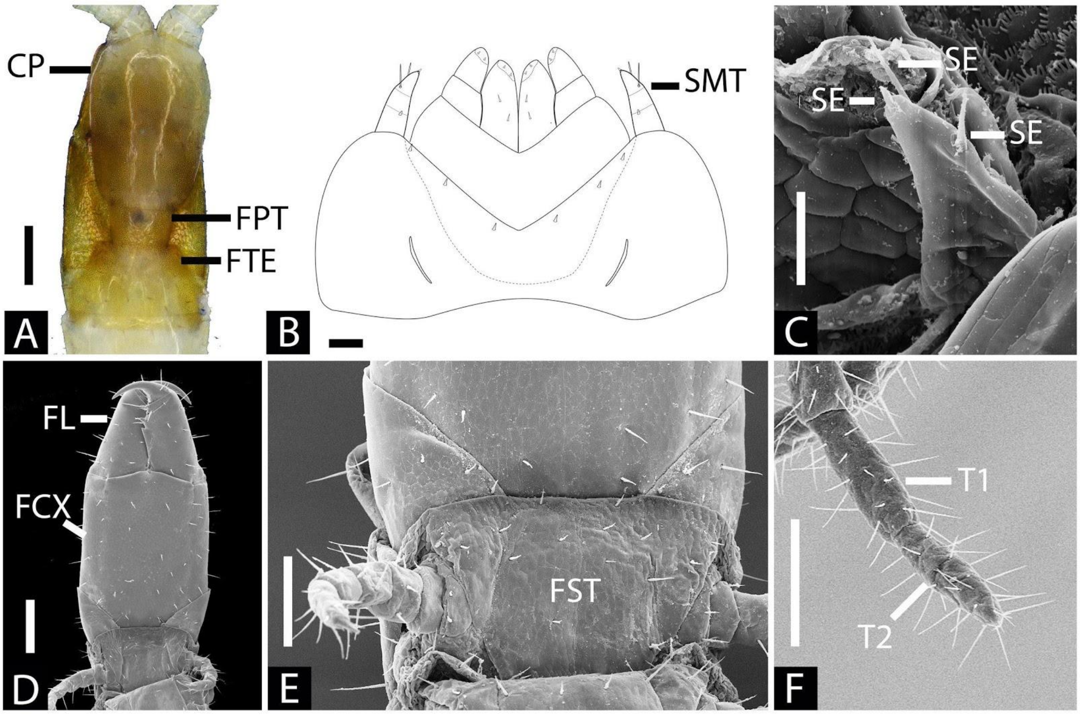

FIGURE 7. A−F, morphology of *Mecophilus* and some features recovered as synapomorphies on MP morphological analysis. *Mecophilus carioca* Calvanese & Brescovit, 2021 (IBSP): **A**, cephalic plate and forcipular segment, dorsal, ch. 1 state 1 (cephalic plate elongated), ch. 23 state 1 (forcipular pretergite elongated); **B**, maxillary complex, ventral; **C**, left telopodite of the second maxillary, ventral; **D**, forcipular segment, ventral, ch. 24 state 1 (forcipular coxosternite elongated); **E**, first leg-bearing segment, ventral, ch. 43 state 1 (first sternite elongated); **F**, terminal part of the right ultimate leg (female), latero-ventral, ch. 53 state 0 (ultimate legs in females with tarsus 2 not markedly shorter than the tarsus 1). **Abbreviations**: CP: cephalic plate. FCX: forcipular coxosternite. FL: forcipule. FST: first sternite. FPT: forcipular pretergite. FTE: forcipular tergite. SE: setae. SMT: second maxillary telopodite. T1: ultimate leg tarsus 1. T2: ultimate leg tarsus 2. **Scale bars:** 0.1 (A, D, F), 0.01 (B−C), 0.05 (E) mm.

Aphilodon was best supported in ML analysis of the concatenated molecular data, with a BS= 63% and in total evidence with BS= 89%. Morphological dataset analyzed separately recovered the group as monophyletic, but the BS was < 50%. MP of morphological characters recovers this genus with two non homoplastic synapomorphies, the peculiar arrangement between the setae in the telopodite of second maxillary (ch. 22, Figs 8A−C) and the presence of a terminal spine in the ultimate legs (ch. 58, Fig. 8D). In the MP analysis, ten species of the genus were recovered in a not resolved polytomy together with two clades: *Aphilodon aiuruoca* Calvanese & Brescovit, 2021 and *A. cangaceiro* Calvanese & Brescovit, 2021, that shares coxal pores opening close to the metasternite in the ultimate legs (ch. 49, homoplastic) and *A. modestus* Silvestri, 1909 sister to *A. micronyx* Brölemann, 1902, *A. indespectus* Calvanese, Brescovit & Bonato, 2019 and *A. rectitibia* Calvanese & Brescovit, 2021, that have the forcipular denticle of tibia reduced (ch. 35, homoplastic). *A. micronyx*, *A. indespectus* and *A. rectitibia* share reductions also in the distal denticle of trochanteroprefemur and denticle of femur (ch. 31 and 33, homoplastics).

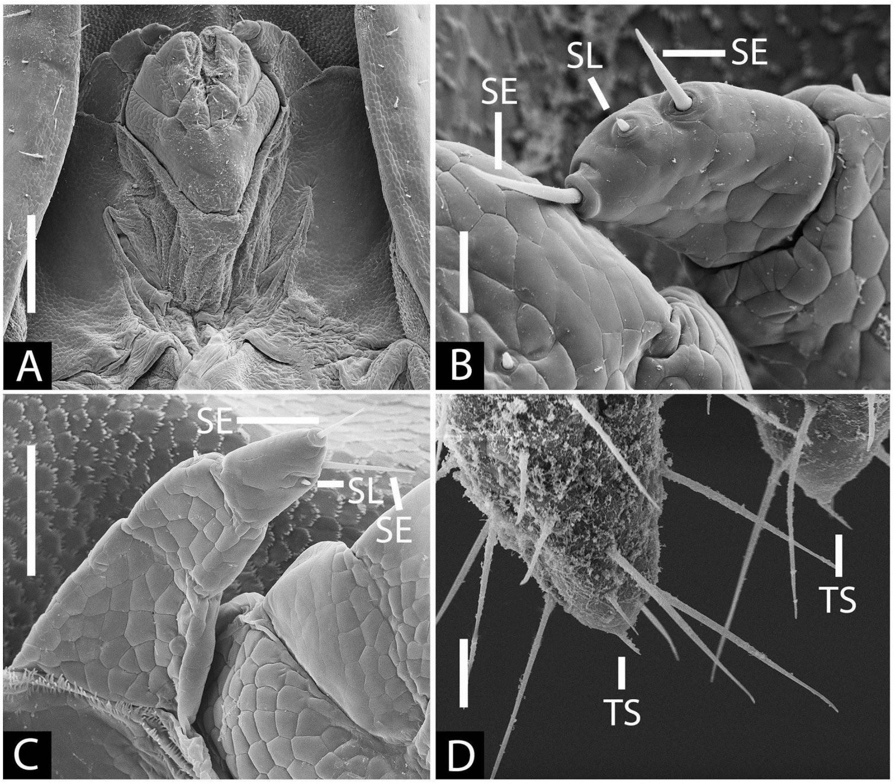

FIGURE 8. A−D, morphology of *Aphilodon* and features recovered as synapomorphies on MP morphological analysis. *Aphilodon pereirai* Calvanese, Brescovit & Bonato, 2019 (IBSP): **A**, maxillary complex, ventral; **B**, article 3 of the left second maxillary telopodite, ventral, ch. 22 state 1 (apex of the article 3 with two setae and one sensilla); **C**, right telopodite of second maxillary, ventral. *Aphilodon bahianus* Calvanese & Brescovit, 2021 (IBSP): **D**, apex of the ultimate legs, ventral, ch. 58 state 1 (presence of a terminal spine). **Abbreviations**: SE: seta. SL: sensilla. TS: terminal spine. **Scale bars:** 0.1 (A), 0.01 (B, D), 0.05 (C) mm.

Inside *Aphilodon*, ML analysis of the concatenated molecular dataset and total evidence analysis recovers *A. cangaceiro* followed by *A. caboclos* Calvanese, Brescovit & Bonato, 2019 and *A. meganae* Calvanese, Brescovit & Bonato, 2019 as most basal.

These datasets retrieves two large clades within the genus: *A. angustatus* Silvestri, 1909 and *A. acutus* Calvanese & Brescovit, 2021 together with moderate support (in both dataset BS between 50–85%), and with low support (BS < 50%) *A. bahianus* Calvanese & Brescovit, 2021 most basal to two other clada: (1) *A. aiuruoca* sister to the most nested *A. foraminis* and *A. indespectus*, and (2) *A. micronyx* and *A. rectitibia* sister to *A. silvestrii* Calvanese, Brescovit & Bonato, 2019 and *A. pereirai* Calvanese, Brescovit & Bonato, 2019. In the analysis of total evidence, also with a low support (BS < 50%) were grouped together with *A. bahianus*, *A. spegazzinii* and *A. intermedius*. The other clade was constant in both analyzes, and in ML analysis of the concatenated molecular dataset *A. aiuruoca* sister to *A. foraminis* and *A. indespectus* presented a BS= 57% and in the total evidence analysis a BS= 63%. On the other side, a clade with *A. micronyx* and *A. rectitibia* sister to *A. silvestrii* and *A. pereirai* have on ML analysis of the concatenated molecular dataset a BS= 79%. In the total evidence analysis *A. modestus* becomes part of this group, which has a BS < 50%. Both groupments, *A. micronyx* and *A. rectitibia* as *A. silvestrii* and *A. pereirai* have a BS > 85% in ML analysis of the concatenated molecular dataset and in the total evidence analysis.

The two known species of *Mairata* share a monophyletic clade moderately supported in the MP analysis of morphological characters (BS= 67%), but well supported in the molecular and combined datasets, with a BS > 85% to both. Three synapomorphies were recovered in the MP analysis, including an article 3 of the telopodite of second maxillary shorter and narrow (ch. 19, Figs 9A−C), female ultimate legs conical and with the distal part largest (ch. 54, Fig. 9E) and ultimate legs in males with tarsus 1 in a globose shape (ch. 55, Figs 9D−E).

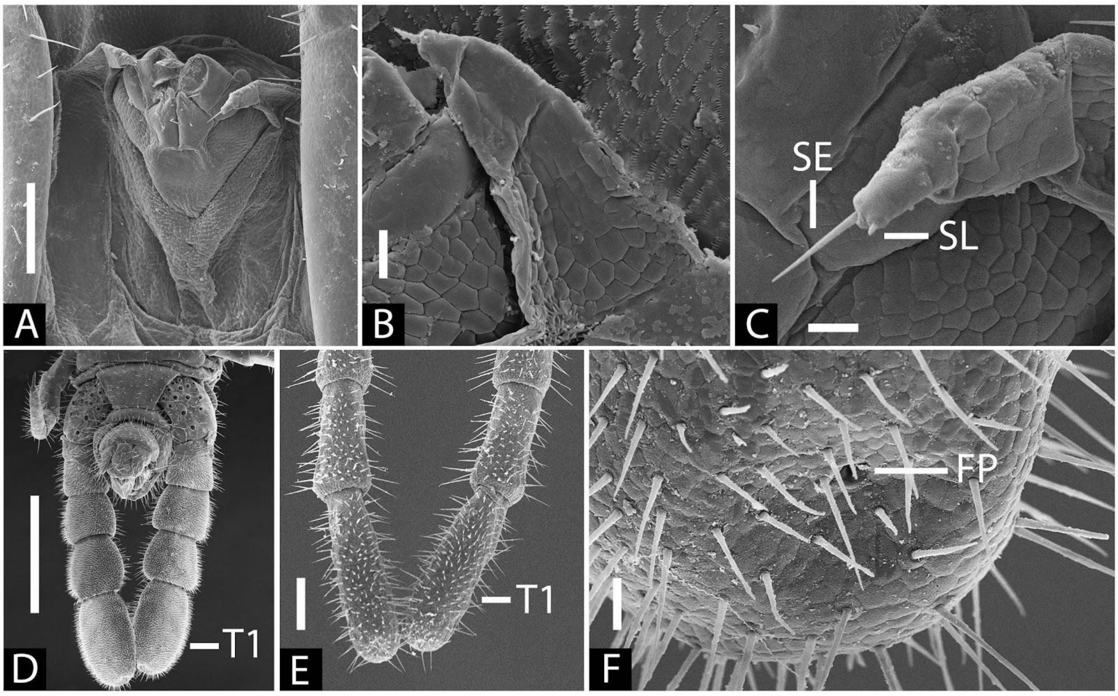

FIGURE 9. A−F, morphology of *Mairata* and some features recovered as synapomorphies on MP morphological analysis. *Mairata itatiaiensis* Calvanese, Brescovit & Bonato, 2019 (IBSP): **A**, maxillary complex, ventral; **C**, apex of the second maxillary telopodite, latero-ventral, ch. 19 state 1 (telopodite article 3 shortened); **D**, posterior part of body, ventral, ch. 55 state 2 (male with tarsus 1 thickened, in globose shape). *M. itatiaiensis* Calvanese, Brescovit & Bonato, 2019 (IBSP): **B**, left telopodite of second maxillary, ventral; **E**, terminal part of the ultimate legs, latero-ventral, ch. 54 state 1 (female with tarsus 1 distinctly widening at the distal tip); **F**, distal part of the tarsus 1 in the ultimate leg, latero-ventral. **Abbreviations**: FP: foraminal process. SE: setae. SL: sensilla. T1: tarsus 1. **Scale bars:** 0.1 (A, E), 0.01 (B−C, F), 0.5 (D) mm.

Discussion

Dataset

As the final results of an extensive effort made in the recent years on the study of the morphology of Aphilodontinae in the Butantan Institute, Brazil (see Calvanese *et al.*, 2019; Calvanese & Brescovit, 2021a,b), we present a phylogenetic matrix of morphological data that encompasses all species of Aphilodontinae and its sister group *Geoperingueyia*. Based on these data, it was possible to establish the position of Aphilodontinae within Geophilidae, and to delimit the subfamily and its four genera through non-homoplastic morphological synapomorphies.

In relation to the molecules, the knowledge about the group has advanced considerably, and within Aphilodontinae all genera were represented in the combined molecular matrix presented here. However, although *Aphilodon*, *Mecophilus* and *Mairata* count with a representativeness > 66% in this matrix, *Philacroterium* was represented only by one species. Also, to *Geoperingueyia* no genetic material was evaluated in this research. These issues are related to the scarcity of sequences available to the South African Geophilomorpha in data banks such as the GenBank, and that could not be sampled to genetic material access in this study.

New insights on the morphology of Aphilodontinae

Aphilodontinae centipedes are impressive for the peculiar morphology of their structures linked to food. However, analyzing the evolutionary history of the group, we can observe that these changes emerged gradually in the lineage of Dignathodontinae, *Geoperingueyia* and Aphilodontinae. Thus, on the basis of this clade, changes appeared in the telopodite of the second maxillary, which became smaller and lost its terminal claw, and in the forcipule, where the venom gland was allocated to the trunk segments. Furthermore, in *Geoperingueyia* and Aphilodontinae there are progressive reductions in the labrum, which are extreme in Aphilodontinae, where the lateral and central pieces seem to have lost their functions and are only vestigial. In contrast, forcipular denticles are well developed in most of *Geoperingueyia* and Aphilodontinae, with a particular arrangement of sensory setae. Also, in the species of Aphilodontinae there is the fusion of one or two articles in the forcipule. These modifications in the structures with primary functions related to feeding may indicate a change in eating habits not only to aphilodontines, but for a very ancient clade which also includes Dignathodontinae and *Geoperingueyia*.

Among the different body parts used in the systematics of Aphilodontinae, the second maxillary has been highly informative mainly at the genus level (Silvestri, 1909b; Attems, 1929; Calvanese *et al.*, 2019). In fact, members of the four genera can be separated only from characteristics in the telopodite of the second maxillary, including the number of articles (two in *Philacroterium* versus three in the other genera), morphology (species of *Mairata* have the third article of the telopodite reduced in length and width) and chaetotaxy. *Philacroterium* species usually have one or two small apical sensilla, and a largest subapical seta in the telopodite (Figs 6C−E). This arrangement is very similar to that observed in *Mairata*, where a small apical sensilla and a larger subapical seta are observed (Figs 9A−C). In *Mecophilus* the telopodites are very membranous and with three setae in the third article, one short apical and two large at the basis of the article (Figs 7B−C). Already species of *Aphilodon* have two large setae with a short sensilla between, in the apical or subapical region of the telopodite (Figs 8A−C). However, although informative, the morphology and chaetotaxy of the second maxillary telopodite sometimes is different between the pair of telopodites in the same individual. We presume that the observed variation can be related to injuries suffered, once in species of Aphilodontinae, the telopodite of the second maxillary consists of a not sclerotized and apparently fragile structure involved in a laborious task, such as handling food.

The denticles of forcipule may vary in expression between different species of Aphilodontinae, but Calvanese *et al.* (2019) report the total absence of denticles only to *A. indespectus*. However, from a re-analysis of the type material, we conclude that although the denticles of the forcipula are quite small, they can be recognized from SEM in specimens of *A. indespectus*.

Over time, different authors have added to the diagnosis of Aphilodontinae the characteristic absence of ventral pores (Silvestri, 1909; Attems, 1929; Bonato *et al.*, 2011), which can be found in other Geophilidae, including *Geoperingueyia* (Pereira, 1981). However, Verhoeff (1937) described *P. porosum* with ventral pores. This species does not have a review reported in the literature, and when we analyzed the type material (deposited in the KZN), no ventral pore was observed. Despite the analysis of the material, no specimen was dissected, clarified or analyzed in SEM, and due to the poor quality of conservation of the specimens it is difficult to specify if any segment has in fact no ventral pore. Therefore, future sampling and analysis of new representatives of the species from the type locality would be ideal to clarify this issue.

In the ancestral geophilids, males and females probably had the ultimate pair of legs with seven articles and terminal claws, and in different times and at different lineages, these structures were reduced. In all analyzed specimens that do not have a terminal claw, and even in species of *Aphilodon* that have a terminal spine in the tarsus 1, an orifice was observed in the apex of the ultimate leg (Fig. 9F), which probably is the point of insertion of muscular fibers.

Systematic of Aphilodontinae

Aphilodontinae was here supported as a monophyletic group both in the molecular and morphological data. If the morphological characters were effective to delimiting Aphilodontinae and its four genera (Calvanese *et al.*, 2019), the same was not true for resolving the relationship between the genera or among the species of the most diverse *Aphilodon*. However, the inclusion of genetic material in the phylogenetic analysis made possible access to this relation, and the evolutionary relationship between the genera, and between species of *Aphilodon* were better explained.

As in other works (Bonato *et al.*, 2014; *Calvanese et al.*, 2019) the affinity between Aphilodontinae and *Geoperingueyia* was confirmed. However, we consider precipitate the transfer of *Geoperingueyia* to Aphilodontinae mainly due to the lack of review of a large number of genera of Geophilidae, which may come to indicate a new relationship of affinity of this genus within the family.

The close relationship of Aphilodontinae species with other geophilids has been discussed since the proposition of the taxon as a family by Silvestri (1909), with the transfer of *Aphilodon*, previously considered by the same author as a Geophilidae component (Silvestri, 1898). Many authors recognized the proximity of members of Aphilodontinae to other Geophilidae, and along the time Aphilodontinae was classified inside to this family (Attems, 1929; Verhoeff, 1937, 1938; Lawrence, 1955, 1963). Despite this, in the recent past, many authors presented the group as a family, separated from Geophilidae (Edgecombe & Giribet, 2007; Pereira *et al.*, 2007; Bonato *et al.*, 2011). Bonato *et al.*, (2014) clarifies this issue based on phylogeny and retrivies Aphilodontinae in the same clade with other Geophilidae. In this work, from phylogenetic analysis on morphological data, Aphilodontinae and Dignathodontinae are close lineages inside Geophilidae. However, in the same work, the relationship between the two subfamilies was not recovered with molecular data or from total evidence. In our analysis, the close relationship between Aphilodontinae and Dignathodontinae inside Geophilidae was recovered from both morphology and molecular data, and three morphological synapomorphies were diagnosed for a clade with Dignathodontinae, Aphilodontinae and *Geoperingueyia*.

Conclusion

In this contribution we were able to advance on four main fronts related to the systematics of Aphilodontinae: (1) the first approach to the molecular evolution of the subfamily was assessed on representatives of its four genera. The first molecular sequences from the Neotropical species of Aphilodontinae are presented. (2) Based on molecular data, the evolutionary relationship between the genera of Aphilodontinae became better understood, as well as the relationships between the species of *Aphilodon*, the most diverse genus of the subfamily. (3) The knowledge about the morphology of Aphilodontinae and its evolution can be improved through the analysis of all species of the group. New morphological synapomorphies were recognized for Aphilodontinae and *Aphilodon*. (4) The relationship of Aphilodontinae and

Geoperingueyia can be assessed by comparison of all species of the two groups under phylogenetic analysis. For the first time a morphological synapomorphy was attributed to *Geoperingueyia*, sister group of Aphilodontinae.

Acknowledgements

We express thanks for all the support provided by the Instituto de Biociências of Universidade de São Paulo (IB-USP) and Laboratório de Coleções Zoológicas (LCZ) of Instituto Butantan. Thanks to Manuel Antunes and Beatriz Vieira, staff of the Laboratório de Evolução Molecular (LEM), for all the support in the process of obtaining molecular sequences. Beatriz Mauricio (Laboratório de Biologia Celular, Instituto Butantan, São Paulo) for helping with SEMs. The anonymous reviewers we thank for the suggestions and corrections. This work was supported by CNPq grants 169641/2017−1 to VCC and CNPq granted PQ 303903/2019−8, to ADB.

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Supplementary material

Appendix 1. Analyzed material.

Appendix 2. Morphological characters used in the phylogenetic analysis.

Appendix 3. Matrix of morphological dataset.

Appendix 4. Individual topologies of each genetic marker obtained from molecular phylogenetic analysis.

Appendix 1. Analyzed material.

Table 1: terminals and data source used in the phylogenetic analysis.

Taxa	**ID number (morphological analysis)**	**Optical microscope / stereomicroscope**	**SEM**	**DNA sequence**	**COI**	**16S**	**18S**	**28S**
Aphilodontinae								
***Aphilodon spegazzinii* Silvestri, 1898**	f#, m# (MSNG Vase type XXVI)	+	+	-	-	-	-	-
***Aphilodon micronyx* Brölemann, 1902**	f#, m# (IBSP 6175)	+	+	Original	+	+	+	+
***Aphilodon angustatus* Silvestri, 1909**	f#, m# (IBSP 4065)	+	+	Original	+	+	+	+
***Aphilodon intermedius* Silvestri, 1909**	-	Published	-	-	-	-	-	-
***Aphilodon modestus* Silvestri, 1909**	-	Published	-	-	-	-	-	-
***Aphilodon caboclos* Calvanese, Brescovit & Bonato, 2019**	f#, m# (IBSP 4073)	+	+	Original	+	+	+	-
***Aphilodon indespectus* Calvanese, Brescovit & Bonato, 2019**	f#, m# (IBSP 3656)	+	+	Original	+	+	-	-
***Aphilodon meganae* Calvanese, Brescovit & Bonato, 2019**	f#, m# (IBSP 4070)	+	+	Original	+	+	+	+
***Aphilodon pereirai* Calvanese, Brescovit & Bonato, 2019**	f#, m# (IBSP 3784)	+	+	Original	+	+	+	+
***Aphilodon silvestrii* Calvanese, Brescovit & Bonato, 2019**	f# (IBSP 4080) m# (IBSP 3066)	+	+	Original	+	+	+	+
***Aphilodon foraminis* Calvanese & Brescovit, 2021**	f# (IBSP 6444) m# (IBSP 6443)	+	+	Original	+	+	+	+

Table 1: continuation.

Taxa	**ID number (morphological analysis)**	**Optical microscope / stereomicroscope**	**SEM**	**DNA sequence**	**COI**	**16S**	**18S**	**28S**
Aphilodontinae								
Aphilodon aiuruoca **Calvanese & Brescovit, 2021**	f# (IBSP 6449) m# (IBSP 6448)	+	+	Original	+	+	+	+
Aphilodon acutus **Calvanese & Brescovit, 2021**	f# (IBSP 6453) m# (IBSP 6452)	+	+	Original	+	+	+	+
Aphilodon cangaceiro **Calvanese & Brescovit, 2021**	f# (IBSP 6457) m# (IBSP 6456)	+	+	Original	+	-	+	-
Aphilodon bahianus **Calvanese & Brescovit, 2021**	f# (IBSP 6462) m# (IBSP 6461)	+	+	Original	+	+	+	+
Aphilodon rectitibia **Calvanese & Brescovit, 2021**	f# (IBSP 6468) m# (IBSP 6469)	+	+	Original	+	+	+	-
Mairata butantan **Calvanese, Brescovit & Bonato, 2019**	f#, m# (IBSP 3663)	+	+	Original	+	+	+	+
Mairata itatiaiensis **Calvanese, Brescovit & Bonato, 2019**	f#, m# (IBSP 6178)	+	+	Original	+	+	+	+
Mecophilus neotropicus **Silvestri, 1909**	m# (MSNG Vase type XXVI)	+	-	-	-	-	-	-
Mecophilus tupiniquim **Calvanese, Brescovit & Bonato, 2019**	f# (IBSP 6172) m# (IBSP 6173)	+	+	Original	+	+	+	+
Mecophilus carioca **Calvanese & Brescovit, 2021**	f#, m# (IBSP 6473)	+	+	Original	+	+	+	+

Table 1: continuation.

Taxa	**ID number (morphological analysis)**	**Optical microscope / stereomicroscope**	**SEM**	**DNA sequence**	**COI**	**16S**	**18S**	**28S**
Aphilodontinae								
***Philacroterium cribellatum* Attems, 1928**	m# (NHMW 7044) f# (SAM 7522)	+	-	-	-	-	-	-
***Philacroterium weberi* (Silvestri, 1909)**	m#, f# (KZN 8142)	+	+	Published	-	-	AF173264.1	HM453302.1
***Philacroterium caffrarium* (Verhoeff, 1937)**	m#, f# (KZN 6779)	+	-	-	-	-	-	-
***Philacroterium brevipes* (Verhoeff, 1938)**	m#, f# (KZN 1022)	+	+	-	-	-	-	-
***Philacroterium bidentatum* (Lawrence, 1955)**	f# (KZN 5078)	+	-	-	-	-	-	-
***Philacroterium longipes* (Lawrence, 1955)**	-	Published	-	-	-	-	-	-
***Philacroterium maritimum* (Lawrence, 1963)**	f# (KZN 6822)	+	+	-	-	-	-	-
***Philacroterium porosum* (Verhoeff, 1937)**	f# (SAM 8355)	+	-	-	-	-	-	-
***Philacroterium macronyx* (Lawrence, 1955)**	f# (KZN 404)	+	+	-	-	-	-	-
***Philacroterium pauciporum* (Lawrence, 1963)**	f# (KZN 7847)	+	-	-	-	-	-	-
***Philacroterium transvaalicum* (Lawrence, 1963)**	m#, f# (KZN 6703)	+	-	-	-	-	-	-
“Geophilidae *lato sensu*”								
***Geoperingueyia affinis* Verhoeff, 1938**	-	Published	-	-	-	-	-	-
***Geoperingueyia agnatha* Verhoeff, 1940**	-	Published	-	-	-	-	-	-

Table 1: continuation.

Taxa	ID number (morphological analysis)	Optical microscope / stereomicroscope	SEM	DNA sequence	COI	16S	18S	28S
“Geophilidae *lato sensu*”								
***Geoperingueyia armata* Verhoeff, 1938**	-	Published	-	-	-	-	-	-
***Geoperingueyia affinis* Verhoeff, 1938**	-	Published	-	-	-	-	-	-
***Geoperingueyia agnatha* Verhoeff, 1940**	-	Published	-	-	-	-	-	-
***Geoperingueyia attemsi* Lawrence, 1955**	-	Published	-	-	-	-	-	-
***Geoperingueyia conjungens* Attems, 1928**	f# (SAM 0820) m# (SAM 3387)	+	-	-	-	-	-	-
***Geoperingueyia crabilli* Pereira, 1981**	f#, m# (PD G 2897)	+	-	-	-	-	-	-
***Geoperingueyia dentata* Verhoeff, 1938**	m#, f# (MNRJ 15443)	+	+	-	-	-	-	-
***Geoperingueyia grandidens* Lawrence, 1963**	m# (SAM 8906)	+	-	-	-	-	-	-
***Geoperingueyia minor* Verhoeff, 1940**	-	Published	-	-	-	-	-	-
***Geoperingueyia platypoda* Lawrence, 1963**	-	Published	-	-	-	-	-	-
Dignathodontinae								
***Dignatodon microcephalus* (Lucas, 1846)**	f#, m# (PD G 5001)	+	+	Published	KF569289.1	KF569221.1	KF569245.1	-
***Henia vesuviana* (Newport, 1844)**	f# (PD G 1525) m# (PD G 5258)	+	+	Published	AY288754.1	KF569223.1	AF173255.1	-

Table 1: continuation.

Taxa	**ID number (morphological analysis)**	**Optical microscope / stereomicroscope**	**SEM**	**DNA sequence**	**COI**	**16S**	**18S**	**28S**
Linotaeniinae								
Strigamia crassipes **(C.L Koch, 1835)**	f#, m# (PD G 2892)	+	+	Published	JN306680.1	LT702892.1	LT702905.1	KF569282.1
Strigamia maritima **(Leach, 1817)**	f#, m# (PD G 1207)	+	+	Published	AY288753.1	AY288733.1	AF173265.1	HM453303.1
Ribautiinae								
Hyphydrophilus adisi **Pereira, Minelli & Barbieri, 1994**	f#, m# (PD G 435)	+	+	-	-	-	-	-
Ribautia centralis **Silvestri, 1907**	f#, m# (INPA 058)	+	+	-	-	-	-	-
Macronicophilinae								
Macronicophilus abbreviatus **Pereira, Foddai & Minelli, 2000**	1m# (INPA 048) 1f# (IBSP 6188)	+	-	-	-	-	-	-
Macronicophilus unguiseta **Pereira, Foddai & Minelli, 2000**	f#, m# (INPA 057)	+	-	-	-	-	-	-
Geophilinae								
Pachymerium ferrugineum **(C.L. Koch, 1835)**	f#, m# (PD G 1045)	+	+	Published	MG319175.1	AF370863.1	AY288702.1	HM453301.1
Root taxon / Mecistocephalidae								
Dicellophilus carniolensis **(C.L. Koch, 1847)**	f# (PD G 5441) m#,(PD G 5363)	+	+	Published	KF569305.1	HM453225.1	HM453237.1	HM453285.1

Appendix 2. Morphological characters used in the phylogenetic analysis.

Below is presented a list with the 58 morphological characters used in our phylogenetic analysis on Maximum Cladistic Parsimony and Maximum Likelihood searches on total evidence. Bibliographic references are given for characters already employed in previous phylogenetic analysis. Characters that have been modified from the literature are identified with one asteristic, and unpublished characters are identified by two asterisks. Associated parameters and comments are based on the strict consensus topology obtained in the MP analysis, with character optimization performed on the ACCTRAN (accelerated transformation) algorithm. Values of length (L), consistency index (CI) and retention index (RI) are indicated.

1*- Cephalic plate: length / width (Bonato *et al.*, 2014, ch. 4, modified): (0) < 1.3, (1) ≥ 1.4. L= 3, CI= 0.33, RI= 0.66. The intervals to the discretization of the character states have been amplified from Bonato *et al.* (2014).

Cephalic plate is elongated in *Dicellophilus carniolensis* (root), *Pachymerium ferrugineum*, *Hyphydrophilus adisi* and *Ribautia centralis*. The analyzed species of Macronicophilinae, Linotaeniinae, Dignathodontinae and *Geoperingueyia* have the cephalic plate shorter. In Aphilodontinae the most part too have a shorter cephalic plate, with exception of *Mecophilus* species, that have a most elongated structure.

2- Clypeus: medial part: “sub-clypeal” setae (Calvanese *et al.*, 2019, ch. 1): (0) absent, (1) present. L= 1, CI= 1, RI= 1.

The presence of the sub-clypeal setae is a non-homoplastic synapomorphy of Aphilodontinae.

3- Clypeus: lateral setae (Calvanese *et al.*, 2019, ch. 2): (0) present, (1) absent. L= 1, CI= 1, RI= 1.

The loss of the lateral setae of the clypeus is a non-homoplastic synapomorphy that groups *Pachymerium ferrugineum*, *Hyphydrophilus adisi*, *Ribautia centralis* and species of Macronicophilinae.

4*- Labrum: lateral pieces: separation from clypeus (Calvanese *et al.*, 2019, ch. 3, modified): (0) distinct at SEM or microscopy with transmitted light, (1) indistinct at

SEM or microscopy with transmitted light. L= 1, CI= 1, RI= 1. The delimitation of structures have been modified from Calvanese *et al.* (2019).

The separation of the lateral pieces from the clypeus is not observed only in the species of *Philacroterium*.

5*- Labrum: lateral pieces (Calvanese *et al.*, 2019, ch. 4, modified): (0) distinctly sclerotized, like the clypeus, (1) distinctly sclerotized but less than the clypeus, (2) only membranous. L= 2, CI= 1, RI= 1. The delimitation of structures was modified from Calvanese *et al.* (2019).

In the most part of Geophilomorpha the lateral pieces are as sclerotized as the clypeus, in *Geoperingueyia* the lateral pieces are a little less sclerotized than the clypeus and in Aphilodontinae, when apparent, the lateral pieces are only membranous.

6*- Labrum: lateral pieces: projections (Bonato *et al.*, 2014, ch. 37, modified): (0) present, (1) absent. L= 1, CI= 1, RI= 1. The characterizations of structures have been modified from Bonato *et al.* (2014).

The loss of projections in the lateral pieces of the labrum is a synapomorphy of a clade with *Geoperingueyia* and Aphilodontinae.

7- Labrum: mid-part: separation from clypeus (Calvanese *et al.*, 2019, ch. 6): (0) distinct at microscopy with transmitted light, (1) indistinct at microscopy with transmitted light. L= 1, CI= 1, RI= 1.

The separation of the mid-part of the labrum from the clypeus is clearly observed in most Geophilomorpha, but is not observed only in the members of Aphilodontinae.

8- Labrum: mid-part:** (0) distinctly sclerotized, like the clypeus, (1) distinctly sclerotized but less than the clypeus, (2) only membranous. L= 2, CI= 1, RI= 1.

Usually the mid part of the labrum is well sclerotized, like the clypeus. In species of *Geoperingueyia* the mid part is less sclerotized, but only in Aphilodontinae this region is membranous.

9- Labrum: mid-part:** (0) with projections, (1) totally smooth. L= 1, CI= 1, RI= 1.

The loss of projections in the external part of the mid-part of the labrum is a synapomorphy of a clade with *Geoperingueyia* and Aphilodontinae.

10- First maxillary: width of coxal projection / width of telopodite (Bonato *et al.*, 2014, ch. 71): (0) < 1, (1) > 1.1. L= 2, CI= 0.5, RI= 0.5.

Coxal projections well developed, wider than telopodites, are characteristic of species of Dignathodontinae.

11- First maxillary: scute filaments (Bonato *et al.*, 2014, ch. 77): (0) absent, (1) present. L= 2, CI= 0.5, RI= 0.75.

The presence of scute filaments was not observed only in *Dicellophilus carniolensis* (root) and species of *Geoperingueyia*.

12- First maxillary: lappets on coxosternite and / or telopodites (Edgecombe *et al.*, 1999, ch. 27): (0) absent, (1) present. L= 1, CI= 1, RI= 1.

The presence of lappets on coxosternite was observed only in *Pachymerium ferrugineum*, *Hyphydrophilus adisi*, *Ribautia centralis* and species of Macronicophilinae.

13- Second maxillary: coxosternite: hyaline, weakly sclerotized isthmus (Bonato *et al.*, 2014, ch. 80): (0) absent, (1) present. L= 1, CI= 1, RI= 1.

The coxosternite mid-part as a most hyaline and weakly sclerotized isthmus was considered a non-homoplastic synapomorphy of Ribautiinae clade, including *Hyphydrophilus adisi* and *Ribautia centralis.*

14- Second maxillary: coxosternite: statumen (Bonato *et al.*, 2014, ch. 87): (0) absent, (1) present. L= 1, CI= 1, RI= 1.

The presence of a statumen was verified only in species of Ribautiinae, including *Hyphydrophilus adisi* and *Ribautia centralis.*

15- Second maxillary: coxosternite: anterior margin medially overlapping the coxosternite of the first maxillary (Bonato *et al.*, 2014, ch. 81): (0) present; (1) absent. L= 1, CI= 1, RI= 1.

The condition of the coxosternite anterior margin medially overlapping the coxosternite of the first maxillary was considered a non-homoplastic synapomorphy of species of Dignathodontinae.

16- Maxillary complex: second maxillary telopodite length / coxosternite width (Bonato *et al.*, 2014, ch. 89): (0) > 0.3; (1) < 0.3. L= 1, CI= 1, RI= 1.

Usually in Geophilomorpha, the telopodite of the second maxillary is well developed, being considered proportionally smaller in relation to the length of the coxosternite only in species of Dignathodontinae, *Geoperingueyia* and Aphilodontinae.

17- Second maxillary: metameric pores:** transversely elongated: (0) absent, (1) present. L= 1, CI= 1, RI= 1.

Usually in Geophilomorpha, the metameric pore present in the coxosternite is longitudinally elongated. Only species of *Geoperingueyia* have a transversely elongated structure.

18- Second maxillary: telopodite: number of articles (Calvanese *et al.*, 2019, ch. 14): (0) 3, (1) 2, (2) 4. L= 2, CI= 1, RI= 1.

Most Geophilomorpha have the telopodite of the second maxillary with 3 articles. An exception are species of *Philacroterium* that have only two articles in the structure, a condition that was recovered as a non-homoplastic synapomorphy of the group. On the other hand, only species of *Macronicophilus* have four articles in the telopodite.

19- Second maxillary: telopodite: width of article 3 / width of article 2 (Calvanese *et al.*, 2019, ch. 15): (0) > 0.7, (1) < 0.4. L= 1, CI= 1, RI= 1.

Only species of *Mairata* have the article 3 of the second maxillary telopodite remarkably reduced in relation to the article 2.

20- Second maxillary: pretarsus: claw (Calvanese *et al.*, 2019, ch. 16): (0) present, (1) absent. L= 2, CI= 0.5, RI= 0.75.

Usually in Geophilomorpha, the telopodite of the second maxillary has a terminal claw. Exceptions are species of Macronicophilinae, Dignathodontinae, *Geoperingueyia* and Aphilodontinae where the terminal claw is not observed.

21- Second maxillary: pretarsus: stout tubercle with 1−2 short spines (Foddai & Minelli, 2000, ch. 28): (0) absent, (1) present. L= 1, CI= 1, RI= 1.

Only species of Dignathodontinae have a stout tubercle with 1−2 short spines in the apex of the second maxillary telopodite.

22- Second maxillary: telopodite article 3 chaetotaxy: two apical setae with one sensilla between:** (0) absent, (1) present. L= 1, CI= 1, RI= 1.

Species of *Aphilodon* have a peculiar chaetotaxy in the article 3 of the second maxillary telopodite, where two setae and one sensilla more or less apical can be observed.

23- Forcipular pretergite: length / width (Calvanese *et al.*, 2019, ch. 18): (0) < 0.2, (1) > 0.5. L= 1, CI= 1, RI= 1.

A most elongated pretergite was considered a non-homoplastic synapomorphy of *Mecophilus*.

24- Forcipular coxosternite: length / width (Calvanese *et al.*, 2019, ch. 19): (0) < 0.9, (1) > 1.1. L= 1, CI= 1, RI= 1.

A most elongated coxosternite was considered a non-homoplastic synapomorphy of *Mecophilus*.

25- Forcipular coxosternite: chitin-lines reaching or almost reaching the condyles (Bonato *et al.*, 2014, ch. 121): (0) absent, (1) present. L= 1, CI= 1, RI= 1.

Chitin-lines reaching or almost reaching the condyles was considered a non-homoplastic synapomorphy of Dignathodontinae.

26- Forcipular trochanteroprefemur (Bonato *et al.*, 2014, ch. 126): length / width: (0) > 1.3; (1) < 1.3. L= 3, CI= 0.33, RI= 0.66.

A most elongated forcipular trochanteroprefemur was observed only in *Pachymerium ferrugineum*, *Hyphydrophilus adisi*, *Ribautia centralis* and species of *Mecophilus*.

27- Forcipule: functional articulation between trochanteroprefemur and femur (Calvanese *et al.*, 2019, ch. 21): (0) present, (1) absent. L= 1, CI= 1, RI= 1.

Usually in the forcipule, centipedes have a functional articulation between the trochanteroprefemur and femur. In all species of Aphilodontinae this functional articulation is not observed, being this condition considered as a non-homoplastic synapomorphy of the group.

28- Forcipule: functional articulation between femur and tibia (Calvanese *et al.*, 2019, ch. 21): (0) present, (1) absent. L= 1, CI= 1, RI= 1.

Only *Philacroterium brevipes*, *P. macronyx*, *P. maritimum* and *P. pauciporum* don't have a functional articulation between the forcipular femur and tibia.

29- Forcipule: trochanteroprefemur: basal denticle (Bonato *et al.*, 2014, ch. 128): (0) absent, (1) present. L= 2, CI= 0.5, RI= 0.5.

The basal denticle of trochanteroprefemur was observed only in *Dicellophilus carniolensis* (root), *Hyphydrophilus adisi* and *Ribautia centralis.*

30- Forcipule: trochanteroprefemur: distal denticle (Bonato *et al.*, 2014, ch. 129, modified; Calvanese *et al.*, 2019, ch. 23): (0) present, (1) absent. L= 3, CI= 0.33, RI= 0.60.

The distal denticle of trochanteroprefemur was not observed only in the species of Macronicophilinae, Linotaeniinae and Dignathodontinae.

31- Forcipule: trochanteroprefemur: distal denticle: length / width (Calvanese *et al.*, 2019, ch. 24): (0) > 0.6, (1) < 0.4. L= 3, CI= 0.33, RI= 0.77.

When present, the distal denticle of trochanteroprefemur is usually conspicuous, being reduced in parallel in one species of *Geoperingueyia* and some species of *Philacroterium* and *Aphilodon*.

32- Forcipule: femur: denticle (Calvanese *et al.*, 2019, ch. 25): (0) present, (1) absent. L= 2, CI= 0.5, RI= 0.83.

The denticle of femur was not observed only in *Pachymerium ferrugineum, Hyphydrophilus adisi*, *Ribautia centralis,* species of Macronicophilinae, and species of Dignathodontinae.

33- Forcipule: femur: denticle: length / width (Calvanese *et al.*, 2019, ch. 26): (0) > 0.6, (1) < 0.4. L= 3, CI= 0.33, RI= 0.75.

When present, the denticle of femur is usually conspicuous, being reduced in parallel in species of Linotaeniinae and some species of *Philacroterium* and *Aphilodon*.

34- Forcipule: tibia: denticle (Calvanese *et al.*, 2019, ch. 27): (0) present, (1) absent. L= 2, CI= 0.50, RI= 0.83.

The denticle of tibia was not observed only in *Pachymerium ferrugineum, Hyphydrophilus adisi*, *Ribautia centralis,* species of Macronicophilinae, and species of Dignathodontinae.

35- Forcipule: tibia: denticle: length / width (Calvanese *et al.*, 2019, ch. 28): (0) > 0.6, (1) < 0.4. L= 3, CI= 0.33, RI= 0.8.

When present, the denticle of tibia is usually conspicuous, being reduced in parallel in species of *Mecophilus* and in some species of *Philacroterium* and *Aphilodon*.

36- Forcipule: femur: denticle: bilobed:** (0) no, (1) yes. L= 1, CI= 100, RI= 100.

Only species of Aphilodontinae have a bilobed denticle of femur, being this characteristic considered a non-homoplastic synapomorphy of the subfamily.

37- Forcipule: denticles on femur and tibia: sub-apical setae (Calvanese *et al.*, 2019, ch. 29): (0) absent, (1) present. L= 1, CI= 1, RI= 1.

Only species of *Geoperingueyia* and Aphilodontinae have subapical setae in the denticles of forcipular femur and tibia, being this characteristic considered a non-homoplastic synapomorphy of this group.

38- Forcipule: tarsungulum: basal projection (Calvanese *et al.*, 2019, ch. 30): (0) absent, (1) present. L= 3, CI= 0.33, RI= 0.5.

Only *Dicellophilus carniolensis* (root), species of Macronicophilinae and species of Dignathodontinae don't have the basal projection of tarsungulum.

39- Forcipule: tarsungulum: large basal denticle (Bonato *et al.*, 2014, ch. 131): (0) absent, (1) present. L= 1, CI= 1, RI= 1.

Only species of Linotaeniinae have a distinctly large basal denticle of tarsungulum.

40- Forcipule: tarsungulum / trochanteroprefemur width (Bonato *et al.*, 2014, ch. 130): (0) > 0.5; (1) < 0.5. L= 1, CI= 1, RI= 1.

Only in species of *Geoperingueyia* and Aphilodontinae the tarsungulum is not markedly narrower in the basis than the trochanteropraefemur, being this characteristic considered a non-homoplastic synapomorphy of this group.

41- Forcipule: calix of gland: position (Bonato *et al.*, 2014, ch. 125): (0) inside of the forcipular segment, (1) along the body. L= 1, CI= 1, RI= 1.

Usually centipedes present the calix of the venom gland inside the forcipular apparatus. The presence of the calix of venom gland along the body was recovered as a non-homoplastic synapomorphy of a clade with Dignathodontinae, *Geoperingueyia* and Aphilodontinae.

42- First leg-bearing segment: procoxae (Calvanese *et al.*, 2019, ch. 32): (0) not separating sternite and forcipular coxosternite, (1) separating sternite and forcipular coxosternite. L= 2, CI= 0.5, RI= 0.75.

The first pair of legs with more developed procoxae, that extended to the middle part of the body partially separating the sternum from the forcipular coxosternite, was recovered as a non-homoplastic synapomorphy of a clade with Linotaeniinae, Dignathodontinae, Aphilodontinae and *Geoperingueyia*.

43- First leg-bearing segment: sternite: length / width (Calvanese *et al.*, 2019, ch. 33): (0) ≤ 0.7, (1) ≥ 1.1. L= 1, CI= 1, RI= 1.

Only species of *Mecophilus* have a most elongated sternite in the first leg-bearing segment.

44- Leg-bearing segments: distinctly elongated anterior and posterior pleurites in a segment (Bonato *et al.*, 2014, ch. 141): (0) no; (1) yes. L= 1, CI= 1, RI= 1.

The condition of the anterior and posterior pleurites distinctly elongated was recovered as a non-homoplastic synapomorphy of a clade with Dignathodontinae, Aphilodontinae and *Geoperingueyia*.

45- Leg-bearing segments: ventral pore-fields (Edgecombe *et al.*, 1999, ch. 61): (0) absent, (1) present, at least on some segments. L= 2, CI= 0.5, RI= 0.94.

The absence of ventral pore-fields was observed only in *Dicellophilus carniolensis* (root) and in species of Aphilodontinae.

46- Leg-bearing segments: metasternites: sub-central pore-fields extending also to sub-marginal areas (Bonato *et al.*, 2014, ch. 162): (0) present, (1) absent. L= 3, CI= 0.33, RI= 0.5.

When present, the pores of metasternite are extended also to sub-marginal areas only in *Pachymerium ferrugineum*, species of *Strigamia* and species of *Geoperingueyia.*

47- Ultimate leg-bearing segment: metasternite: length / width (Bonato *et al.*, 2014, ch. 169): (0) ≥ 1, (1) ≤ 0.8. L= 6, CI= 0.16, RI= 0.28.

The proportion of metasternite was very variable, and although most species of Aphilodontinae have a wider than long structure, other morphology were observed in parallel within *Philacroterium* and *Aphilodon*.

48- Ultimate leg-bearing segment: coxopleuron: coxal organs opening in ventral pouches (Edgecombe *et al.*, 1999, ch. 110): (0) absent (1) present. L= 1, CI= 1, RI= 1.

Only in species of Dignathodontinae the coxal organs open in ventral pouches.

49- Ultimate leg-bearing segment: coxopleuron: coxal pores: distribution (Calvanese *et al.*, 2019, ch. 38): (0) sparse, (1) grouped together close to the metasternite. L= 6, CI= 0.16, RI= 0.66.

Usually in Geophilidae, coxal organs are sparse on the coxopleuron surface. However, the aspect grouped next to the lateral margin of the metasternite was observed in parallel in several groups, including *Hyphydrophilus adisi* and *Ribautia centralis,* species of Dignathodontinae, one species of *Geoperingueyia*, one species of *Mairata*, some species of *Philacroterium* and some species of *Aphilodon*.

50- Ultimate leg-bearing segment: leg: tarsal articles in females (Calvanese *et al.*, 2019, ch. 39): (0) 2, (1) 1. L= 3, CI= 0.33, RI= 0.88.

Usually, in Geophilomorpha females have ultimate legs with seven articles (tarsus 2 present). The structure with only six articles by the loss of the tarsus 2 was recovered in parallel as homoplastic synapomorphy of Macronicophilinae, *Geoperingueyia* and between *Aphilodon* and *Mairata*.

51- Ultimate leg-bearing segment: leg: tarsal articles in males (Calvanese *et al.*, 2019, ch. 40): (0) 2, (1) 1. L= 4, CI= 0.25, RI= 0.57.

Usually, Geophilomorpha males have ultimate legs with seven articles. A transition to a structure with only six articles, by the loss of tarsus 2, was recovered in parallel as homoplastic synapomorphy of species of Macronicophilinae, and between *Geoperingueyia* and Aphilodontinae, with one reversion inside *Philacroterium*.

52- Ultimate leg-bearing segment: leg: width of ultimate leg / width of penultimate leg in males (Calvanese *et al.*, 2019, ch. 41): (0) about 1, (1) distinctly > 1. L= 1, CI= 1, RI= 1.

The males ultimate legs, proportionally wider than the predecessor legs, was recovered as a non-homoplasy synapomorphy of a clade with Linotaeniinae, Dignathodontinae, Aphilodontinae and *Geoperingueyia*.

53*- Ultimate leg-bearing segment: leg: length of tarsus 2 / length of tarsus 1 in females (Calvanese *et al.*, 2019, ch. 42, modified): (0) > 0.8 (1) < 0.6. L= 2, CI= 0.5, RI= 0.8. The delimitation of structures was modified from Calvanese *et al.* (2019).

When present, a shorter tarsus 2 can be observed in species of Linotaeniinae, Dignathodontinae and *Philacroterium*.

54- Ultimate leg-bearing segment: leg: shape of single tarsal article in females (Calvanese *et al.*, 2019, ch. 43): (0) narrowing uniformly at both ends, (1) distinctly widening at the distal tip. L= 1, CI= 1, RI= 1.

Only in *Mairata* females have the single tarsal article distinctly widening at the distal tip, being this characteristic considered a non-homoplastic synapomorphy of this genus.

55- Ultimate leg-bearing segment: leg: shape of the single tarsal article in males (Calvanese *et al.*, 2019, ch. 44): (0) slender, (1) thickened, in elliptical shape, (2) thickened, in globose shape. L= 2, CI= 1, RI= 1.

Between the Geophilidae that have males with a single tarsal article, Macronicophilinae species have a slender structure. Most *Geoperingueyia* and Aphilodontinae have a thickened, elliptical tarsus 1. Only in *Mairata* males have the single tarsal article most thickened, in a globose shape, being this characteristic considered as non-homoplastic synapomorphy of this genus.

56- Ultimate leg-bearing segment: leg: claw in females (Calvanese *et al.*, 2019, ch. 45): (0) present, (1) absent. L= 5, CI= 0.2, RI= 0.8.

Usually, in Geophilomorpha females have ultimate legs with terminal claws. The absence of the structure was observed in some species of *Geoperingueyia*, one species of *Mecophilus*, one species of *Philacroterium* and all species of *Mairata* and *Aphilodon*.

57- Ultimate leg-bearing segment: leg: claw in males (Calvanese *et al.*, 2019, ch. 46): (0) present, (1) absent. L= 4, CI= 0.25, RI= 0.76.

Usually in Geophilomorpha males have ultimate legs with well developed terminal claws. The absence of the structure was considered a non-homoplastic synapomorphy of *Geoperingueyia* and Aphilodontinae, with some posterior reversions observed in *Philacroterium.*

58- Ultimate leg-bearing segment: leg: terminal spine (Calvanese *et al.*, 2019, ch. 47): (0) absent, (1) present. L= 1, CI= 1, RI= 1.

Only species of *Aphilodon* have a terminal spine in the ultimate legs of both males and females, being this characteristic considered a non-homoplastic synapomorphy of this genus.

Appendix 3. Matrix of morphological dataset.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58
Dicellophilus carniolensis **(C.L. Koch, 1847)**	1	?	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	-	0	0	0	0	0	0	0	-	-	0	0	0
Aphilodon spegazzinii **Silvestri, 1898**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	0	0	0	1	1	1	-	0	1	1	1	1
Aphilodon micronyx **Brölemann, 1902**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	1	0	1	0	1	1	1	1	0	1	1	1	0	1	0	-	1	0	0	1	1	1	-	0	1	1	1	1
Aphilodon angustatus **Silvestri, 1909**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	0	1	1	1	-	0	1	1	1	1
Aphilodon intermedius **Silvestri, 1909**	0	1	0	0	2	1	1	2	1	0	?	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	0	?	1	1	-	?	1	?	1	?
Aphilodon modestus **Silvestri, 1909**	0	1	0	0	2	1	1	2	1	0	?	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	1	1	1	1	0	1	1	1	0	1	0	-	1	0	0	1	1	1	-	0	1	1	1	?
Aphilodon silvestrii **Calvanese, Brescovit & Bonato, 2019**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	0	1	1	1	-	0	1	1	1	1
Aphilodon pereirai **Calvanese, Brescovit & Bonato, 2019**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	0	1	1	1	-	0	1	1	1	1
Aphilodon indespectus **Calvanese, Brescovit & Bonato, 2019**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	1	0	1	0	1	1	1	1	0	1	1	1	0	1	0	-	1	0	0	1	1	1	-	0	1	1	1	1
Aphilodon meganae **Calvanese, Brescovit & Bonato, 2019**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	0	1	1	1	-	0	1	1	1	1
Aphilodon caboclos **Calvanese, Brescovit & Bonato, 2019**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	0	1	1	1	-	0	1	1	1	1
Aphilodon foraminis **Calvanese & Brescovit, 2021**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	0	1	1	1	-	0	1	1	1	1
Aphilodon acutus **Calvanese & Brescovit, 2021**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	0	1	1	1	-	0	1	1	1	1
Aphilodon aiuruoca **Calvanese & Brescovit, 2021**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	1	1	1	1	-	0	1	1	1	1
Aphilodon cangaceiro **Calvanese & Brescovit, 2021**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	1	1	1	1	-	0	1	1	1	1
Aphilodon bahianus **Calvanese & Brescovit, 2021**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	0	1	1	1	-	0	1	1	1	1
Aphilodon rectitibia **Calvanese & Brescovit, 2021**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	1	0	1	0	1	1	1	1	0	1	1	1	0	1	0	-	1	0	0	1	1	1	-	0	1	1	1	1
Mairata butantan **Calvanese, Brescovit & Bonato, 2019**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	1	1	1	1	-	1	2	1	1	0
Mairata itatiaiensis **Calvanese, Brescovit & Bonato, 2019**	0	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	0	1	1	1	-	1	2	1	1	0
Mecophilus neotropicus **Silvestri, 1909**	1	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	1	1	1	0	1	1	1	1	1	0	-	1	0	0	?	1	1	?	?	1	?	1	0
Mecophilus tupiniquim **Calvanese, Brescovit & Bonato, 2019**	1	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	1	1	1	0	1	1	1	1	1	0	-	1	0	0	0	1	1	0	-	1	0	1	0
Mecophilus carioca **Calvanese & Brescovit, 2021**	1	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	1	1	1	0	1	1	1	1	1	0	-	1	0	0	0	1	1	0	-	1	1	1	0
Philacroterium cribellatum **Attems, 1928**	0	1	0	1	-	1	1	2	1	0	1	0	0	0	0	1	0	1	-	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	0	0	0	0	0	1	1	-	-	0	0	0
Philacroterium weberi **(Silvestri, 1909)**	0	1	0	1	-	1	1	2	1	0	1	0	0	0	0	1	0	1	-	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	0	0	1	1	1	-	1	0	1	0
Philacroterium caffrarium **(Verhoeff, 1937)**	0	1	0	1	-	1	1	2	1	0	1	0	0	0	0	1	0	1	-	1	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	1	0	1	1	1	-	1	0	1	0
Philacroterium brevipes **(Verhoeff, 1938)**	0	1	0	1	-	1	1	2	1	0	1	0	0	0	0	1	0	1	-	1	0	0	0	0	0	1	1	1	0	0	1	0	1	0	1	1	1	1	0	1	1	1	0	1	0	-	1	0	1	0	1	1	1	-	1	0	0	0

Matrix of morphological dataset: continuation.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58
***Philacroterium bidentatum* (Lawrence, 1955)**	0	1	0	1	-	1	1	2	1	0	1	0	0	0	0	1	0	1	-	1	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	1	0	?	?	1	-	?	0	?	0
***Philacroterium maritimum* (Lawrence, 1963)**	0	1	0	1	-	1	1	2	1	0	1	0	0	0	0	1	0	1	-	1	0	0	0	0	0	1	1	1	0	0	1	0	1	0	1	1	1	1	0	1	1	1	0	1	0	-	1	0	1	0	?	?	1	-	?	0	?	0
***Philacroterium porosum* (Verhoeff, 1937)**	0	1	0	1	-	1	1	2	1	0	1	0	0	0	0	1	0	1	-	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	?	-	1	0	0	?	?	1	?	?	?	0	?	0
***Philacroterium macronyx* (Lawrence, 1955)**	0	1	0	1	-	1	1	2	1	0	1	0	0	0	0	1	0	1	-	1	0	0	0	0	0	1	1	1	0	0	1	0	1	0	1	1	1	1	0	1	1	1	0	1	0	-	1	0	1	?	1	1	?	?	1	?	0	0
***Philacroterium pauciporum* (Lawrence, 1963)**	0	1	0	1	-	1	1	2	1	0	1	0	0	0	0	1	0	1	-	1	0	0	0	0	0	1	1	1	0	0	1	0	1	0	1	1	1	1	0	1	1	1	0	1	0	-	1	0	1	?	?	1	?	?	?	?	?	0
***Philacroterium transvaalicum* (Lawrence, 1963)**	0	1	0	1	-	1	1	2	1	0	1	0	0	0	0	1	0	1	-	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	1	0	1	1	1	-	1	0	1	0
***Philacroterium longipes* (Lawrence, 1955)**	0	1	0	1	-	1	1	?	1	0	?	0	0	0	0	1	0	1	-	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	-	1	0	1	0	?	?	1	-	?	1	?	0
***Dignatodon microcephalus* (Lucas, 1846)**	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	1	1	0	0	0	1	1	0	0	0	1	-	1	-	1	-	0	0	0	-	0	1	1	0	1	1	-	1	1	1	0	1	1	1	0	-	0	0	0
***Geoperingueyia conjungens* Attems, 1928**	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1	1	0	1	0	0	1	1	1	-	0	1	0	1	0
***Geoperingueyia dentata* Verhoeff, 1938**	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1	1	0	1	0	0	1	1	1	-	0	1	1	1	0
***Geoperingueyia grandidens* Lawrence, 1963**	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1	1	0	1	0	0	?	1	1	?	?	1	?	1	0
***Geoperingueyia crabilli* Pereira, 1981**	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	?	0	1	1	1	0	1	1	0	1	0	1	1	1	1	-	0	1	0	0	0
***Geoperingueyia affinis* Verhoeff, 1938**	0	0	0	0	1	1	0	1	1	0	?	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1	1	0	?	0	0	?	1	1	?	?	1	?	1	0
***Geoperingueyia agnatha* Verhoeff, 1940**	0	0	0	0	1	1	0	1	1	0	?	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1	1	0	1	0	0	?	1	1	?	?	1	?	1	0
***Geoperingueyia armata* Verhoeff, 1938**	0	0	0	0	1	1	0	1	1	0	?	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1	1	0	?	0	0	?	?	?	?	?	?	?	?	0
***Geoperingueyia attemsi* Lawrence, 1955**	0	0	0	0	1	1	0	1	1	0	?	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1	1	0	1	0	0	1	?	?	-	0	?	0	1	0
***Geoperingueyia minor* Verhoeff, 1940**	0	0	0	0	1	1	0	1	1	0	?	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1	1	0	1	0	0	?	1	1	-	?	1	?	1	0
***Geoperingueyia platypoda* Lawrence, 1963**	0	0	0	0	1	1	0	1	1	0	?	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1	1	0	0	0	0	1	1	1	-	0	1	1	1	0
***Henia vesuviana* (Newport, 1844)**	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	1	1	0	0	0	1	1	0	0	0	1	-	1	-	1	-	0	0	0	-	0	1	1	0	1	1	1	1	1	1	0	0	1	1	-	-	0	0	0
***Hyphydrophilus adisi* Pereira, Minelli & Barbieri 1994**	1	0	1	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	-	1	-	0	0	1	0	0	0	1	0	0	1	1	1	0	1	0	0	0	0	-	-	0	0	0
***Macronicophilus abbreviatus* Pereira, Foddai & Minelli, 2000**	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	0	1	0	0	0	0	0	1	0	0	0	1	-	1	-	1	-	0	0	0	-	0	0	0	0	0	1	1	0	0	0	1	1	0	-	0	0	0	0	0
***Macronicophilus unguiseta* Pereira, Foddai & Minelli, 2000**	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	0	1	0	0	0	0	0	1	0	0	0	1	-	1	-	1	-	0	0	0	-	0	0	0	0	0	1	1	0	0	0	1	1	0	-	0	0	0	0	0
***Pachymerium ferrugineum* (C.L. Koch, 1835)**	1	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-	1	-	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	-	-	0	0	0
***Ribautia centralis* Silvestri, 1907**	1	0	1	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	-	1	-	0	0	1	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	-	-	0	0	0
***Strigamia crassipes* (C.L. Koch, 1835)**	0	0	0	0	?	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	-	0	1	0	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0	0	0	1	1	-	-	0	0	0
***Strigamia maritima* (Leach, 1817)**	0	0	0	0	?	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	-	0	1	0	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0	0	0	1	1	-	-	0	0	0

Appendix 4. Individual topologies of each genetic marker obtained from molecular phylogenetic analysis.

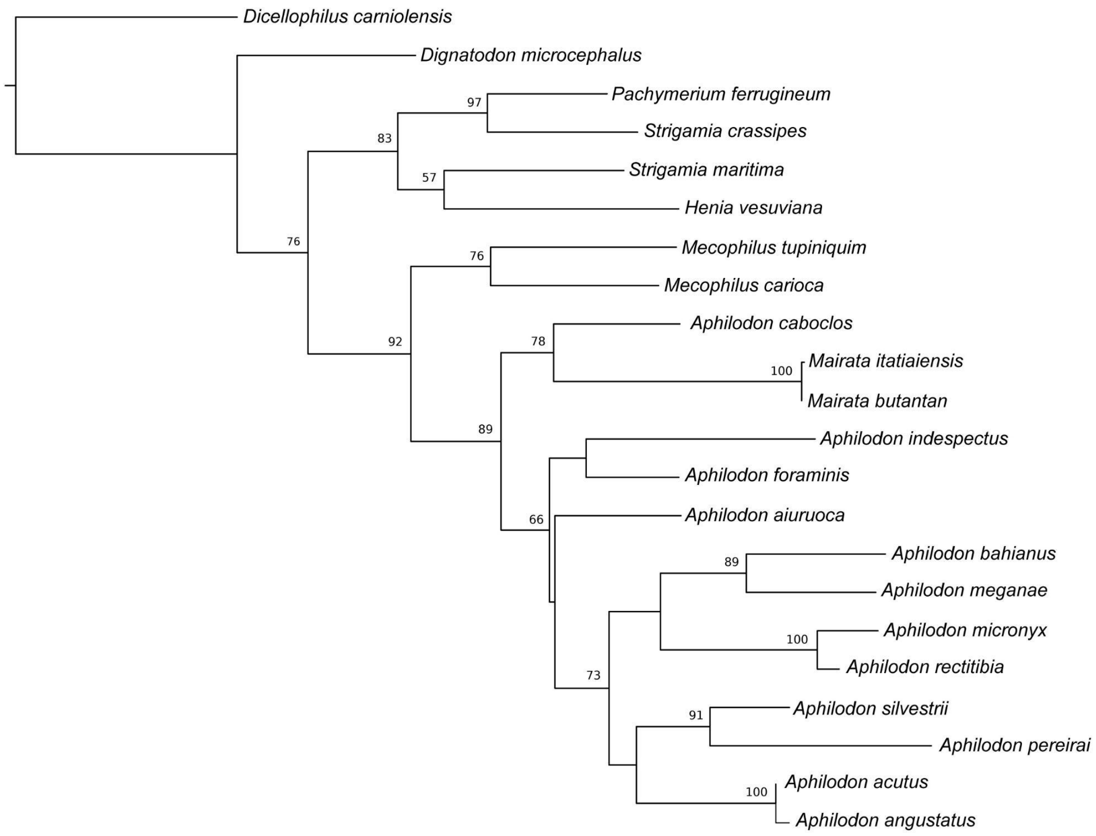

FIGURE 1. Topology from ML analysis of the mitochondrial 16S. LnL= -4629.6088. Bootstrap values greater than or equal to 50% are given close to the nodes.

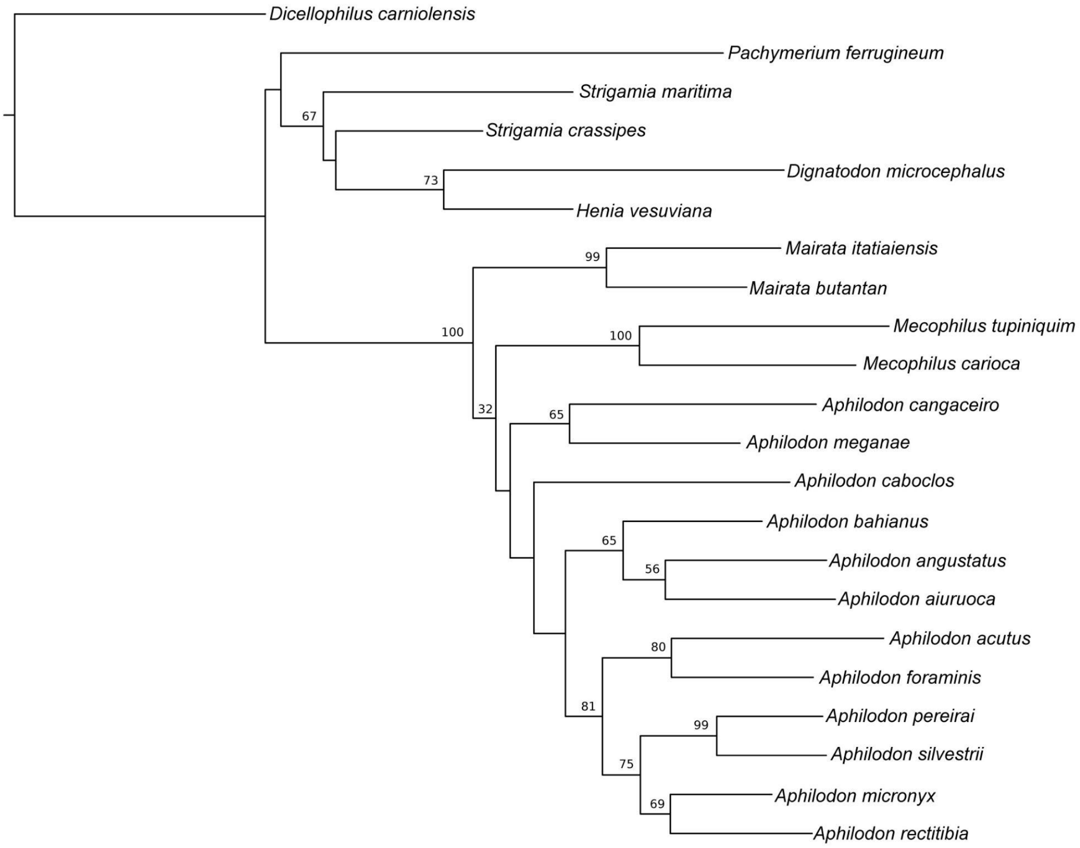

FIGURE 2. Topology from ML analysis of the mitochondrial cytochrome c oxidase subunit I (COI). LnL= -6510.5010. Bootstrap values greater than or equal to 50% are given close to the nodes.

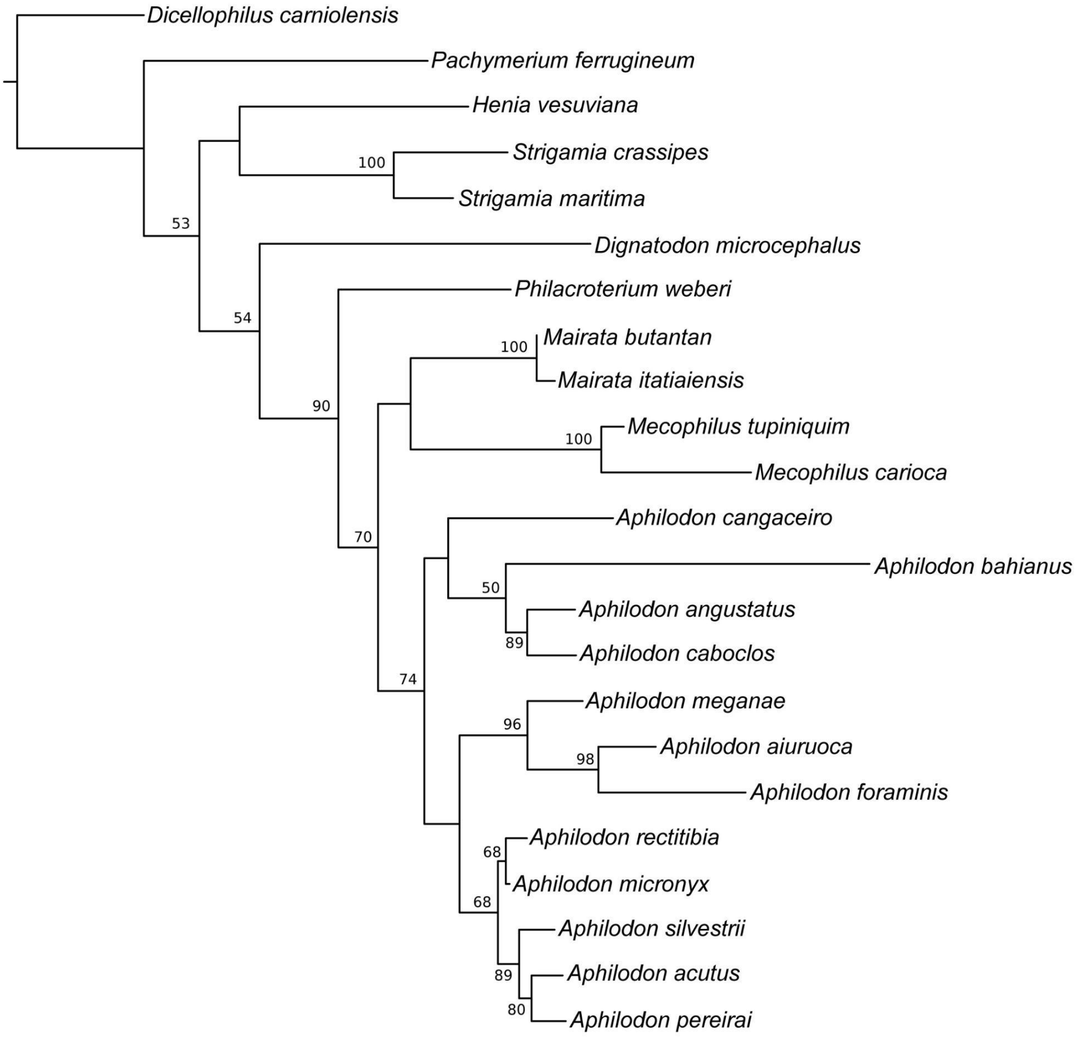

FIGURE 3. Topology from ML analysis of the nuclear 18S. LnL= -11851.7324. Bootstrap values greater than or equal to 50% are given close to the nodes.

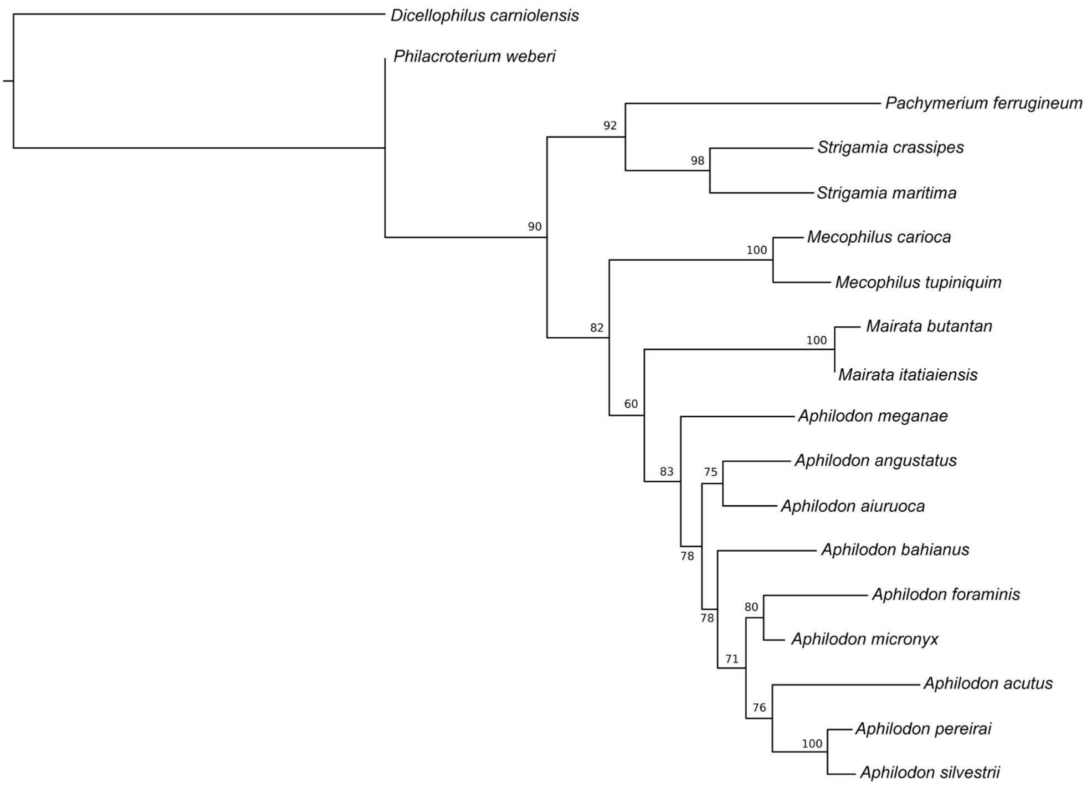

FIGURE 4. Topology from ML analysis of the nuclear 28S. LnL= -12010.9426. Bootstrap values greater than or equal to 50% are given close to the nodes.

Capítulo 4. Biogeografia de Aphilodontinae

Historical biogeography of the Aphilodontinae centipedes (Geophilomorpha, Geophilidae): Cretaceous West Gondwana vicariance followed by sympatric diversification shaped the current distribution of the genera

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Abstract

Aim. We tested the hypothesis that ancient vicariance in the Cretaceous followed by sympatric diversification shaped the current distribution of the genera of Aphilodontinae centipedes.

Location. South America, South Africa.

Methods. Molecular sequences of the mitochondrial cytochrome oxidase I (COI) and 16S rRNA, and nuclear 18S and 28S rRNAs were used to estimate the divergence times of the species of Aphilodontinae in a Bayesian framework, with an uncorrelated log-normal relaxed clock and two points of calibrations. Ancestral areas were reconstructed with Statistical Dispersal Vicariance Analysis (S-DIVA), Dispersal Extinction Cladogenesis (DEC) and Statistical Dispersal Extinction Cladogenesis (S-DEC).

Results. The results suggest that Aphilodontinae separated from Dignathodontinae and Linotaeniinae in the Mesozoic around 170 million years ago (Ma), probably due to a vicariant event, inferred as the separation between Laurasia and Gondwana. Most likely, Aphilodontinae had an ancestral range in the Atlantic province, a bioregion of South America, or in a Gondwanan fragment comprising continental Africa and South America. In late Cretaceus, around 140 Ma, the South African genus *Philacroterium* split apart from the other Aphilodontinae by a vicariant event inferred as the separation between the continental Africa and South America. The three known Neotropical genera, *Aphilodon*, *Mecophilus* and *Mairata* diversified in the Atlantic province bioregion, and some species of *Aphilodon* reached other areas mainly through dispersion.

Main conclusions. Our results support the hypothesis that West Gondwana vicariance in Cretaceus separated the South African from the South American Aphilodontinae, and the Neotropical genera diversified first in the same bioregion, having rarely incorporated other regions in their distribution. This pattern of distribution corroborates what is expected from the group, first because members of Aphilodontinae have low dispersion capacity and are strongly associated with the microenvironment where they live. Second, the body plan between the Neotropical Aphilodontinae is quite different, which allows the exploration of different niches in the same general environment.

Key words: ancestral area reconstruction, evolution, molecular dating.

Introduction

The advent of most accessible techniques to date divergences between lineages coupled with an improvement of knowledge about the history of geological areas has led to a recent increase in the number of works that combine molecular dating with the reconstruction of ancestral areas for testing biogeographic hypotheses (Riddle et al., 2008; Kodandaramaiah, 2010). Despite criticism related to the accuracy achieved, divergence times analysis can be used to compare cladogenesis events with the timing of the rising of geographical barriers, being an important instrument for discerning the impact of historic events in the current distribution of a taxon (Anderson et al., 2009). Currently, reconstruction of distribution over ancestral areas can be done based on event-based methods, that attempt to identify historical events as vicariance, dispersal, extinction and within-area speciation, taking into account the individual history of a clade (Crisci, 2001; Posadas et al., 2006). The temporal demarcation on a clade embodies in the evolutionary question a widespread constant that can serve as a parameter for comparison with hypotheses of reconstruction of ancestral areas. Thus, when based on dated phylogenies, both the reconstruction of ancestral areas and the inference of vicariance or dispersions are better evaluated, since they can also be compared with other widely known scientific data, such as geologic or historical events.

Centipedes are an old lineage of arthropods which emerged from a myriapod common ancestor in the middle of Paleozoic, about 440 million years ago (Murienne et al., 2010). After emergence, the group experienced a relatively rapid diversification, where its six main lineages, currently recognized as orders, diverged in a period less than 50 million years (Murienne et al., 2010; Fernández, 2014). Currently, five orders still have representatives alive and more than 3,000 species of the group are spread worldwide, with exceptions only in the poles, some oceanic islands and deeper deserts (Bonato et al., 2011).

Nowadays, the most diverse order, Geophilomorpha, has a world-wide distribution and its ancestral distribution in the Pangea supercontinent makes the group interesting for studies involving historical biogeography, since expressive events may have shaped its evolution. However, until now only a few works investigated the biogeography of the group, among them Bonato et al. (2017) estimated the time of divergence between species of *Strigamia* Gray, 1843, a genus of Geophilidae with Holarctic occurrence. Biogeography of the Gondwanic Geophilomorpha is still poorly known, and in fact, the taxonomy and occurrence of most groups presents numerous gaps related to the lack of sampling of different regions or even the lack of revision of the material already described over vast areas. In this scenario, Aphilodontinae, a subfamily of Geophilidae, stands out for having its species recently reviewed (Calvanese et al., 2019), and the phylogenetic relationships ancored on molecular and morphological evidence (Calvanese & Brescovit, 2021c), which opens the way for the study of deeper relationships, such as a biogeography assessment.

Currently Aphilodontinae have four genera, of which *Philacroterium* Attems, 1926 counts 11 species spread over South Africa (Calvanese et al., 2019). Inside the South America, 16 species are known to *Aphilodon* Silvestri, 1898, *Mecophilus* Silvestri, 1909 counts 3 species, and *Mairata* Calvanese, Brescovit & Bonato, 2019 have 2 species (Calvanese & Brescovit, 2021c). The phylogeny based on total evidence proposed by Calvanese & Brescovit (2021c) resolves the Neotropical genera in a monophyletic clade, being *Aphilodon* and *Mairata* closely related. Figure 1 shows some characteristics of Aphilodontinae, including general morphology and diagnosed morphological synapomorphies for the main groups.

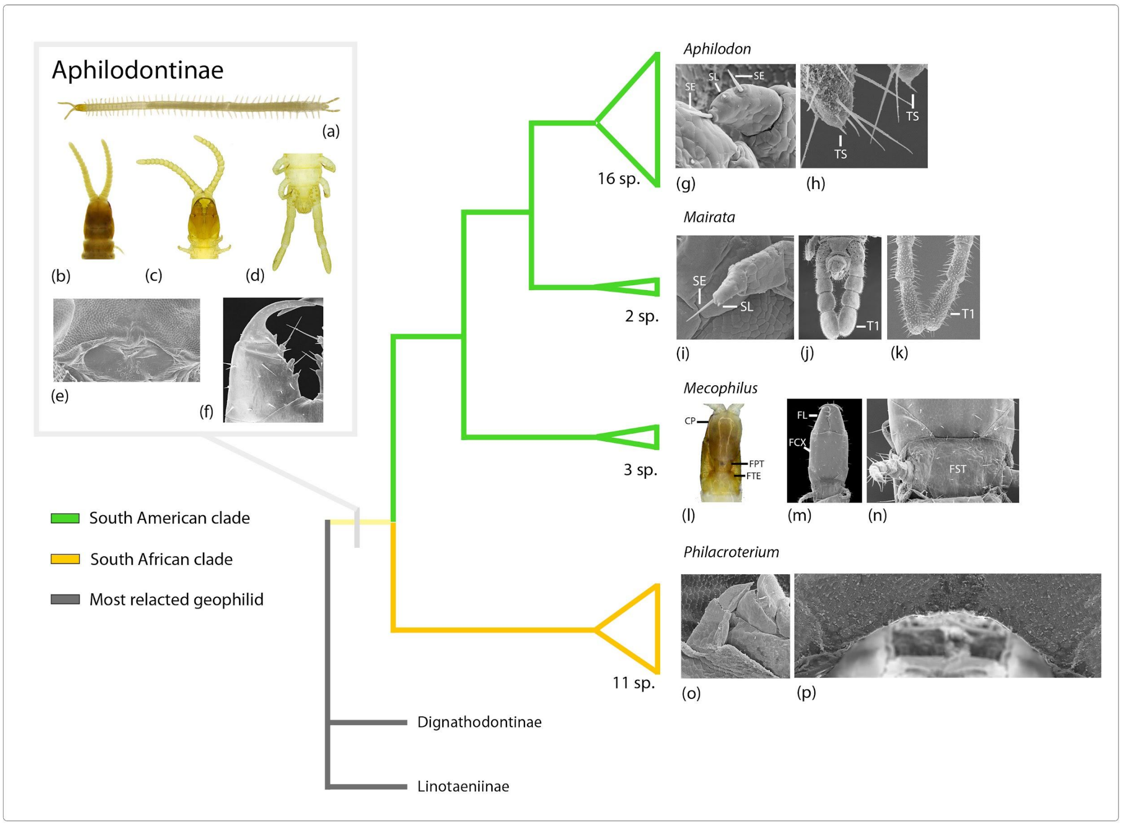

FIGURE 1. Relationships of Aphilodontinae based on phylogenetic analysis of molecular and morphological characters, from Calvanese & Brescovit (2021c). The number of species and distribution data were obtained from the same work. **Morphological aspects of Aphilodontinae:** (a) habitus, ventral; (b) anterior part of body, dorsal; (c) same, ventral; (d) terminal part of body, ventral; (e) middle and lateral parts of labrum reduced (synapomorphy of Aphilodontinae according Calvanese et al., 2019), ventral; (f) forcipule with only three articles (synapomorphy of Aphilodontinae according to Calvanese et al., 2019), ventral. **Morphological synapomorphies of *Aphilodon* (according to Calvanese & Brescovit, 2021c):** (g) second maxillary telopodite and associated chaetotaxy, ventral. SE: setae, SL: sensillum; (h) terminal spine present in the tarsus of ultimate legs, ventral. TS: terminal spine. **Morphological synapomorphies of *Mairata* (according to Calvanese & Brescovit, 2021c):** (i) second maxillary telopodite with article 3 reduced, ventral. SE: setae, SL: sensillum; (j)−(k): ultimate legs tarsus 1 of males and females (respectively) enlarged, ventral. T1: tarsus 1. **Morphological synapomorphies of *Mecophilus* (according to Calvanese & Brescovit, 2021c):** (l) elongated forcipular pretergite, ventral. CP: cephalic plate, FTE: forcipular tergite, FPT: forcipular pretergite; (m) elongated forcipular coxosternite, ventral. FL: forcipule, FCX: forcipular coxosternite; (n) elongated first sternite of the body, ventral. FST: first sternite. **Morphological synapomorphies of *Philacroterium* (according to Calvanese & Brescovit, 2021c):** (o) second maxillary telopodite with only two articles, ventral; (p) labrum with lateral pieces only vestigial, ventral. Figures (a)−(e) were obtained in Calvanese & Brescovit (2021a), figures (f)−(p) were obtained in Calvanese & Brescovit (2021c).

Inside the Neotropics, *Mecophilus* and *Mairata* are endemic to the Brazilian Atlantic Forest biome (Calvanese et al., 2019). *Aphilodon* has 14 species in this biome, of which *Aphilodon angustatus* Silvestri, 1909 also occurs in the Brazilian Amazonia, Cerrado and Pantanal and *A. bahianus* Calvanese & Brescovit, 2021 also occurs in the Caatinga Brazilian biome. Just *A. spegazzinii* Silvestri, 1898 is restricted to the Argentina Pampas, and *A. cangaceiro* Calvanese & Brescovit, 2021 is restricted to the Caatinga (Calvanese & Brescovit, 2021a).

As the most part of Geophilomorpha, specimens of Aphilodontinae have a low capacity of dispersion, especially due to the small size of the body which can vary between 0.6–7 cm in adults, and its association with specific microhabitats in the first layers of the soil. Given these characteristics and its current distribution, we hypothesize that the South African *Philacroterium* diverged from the Neotropical genera in the Cretaceous, due to the separation of the continental Africa and South America. Already the Neotropical clade has diversified in the same biogeographic area (Atlantic forest biome), and expanded its territory a few times with cases of dispersion observed between species of *Aphilodon* that have wide-distribution.

To test this hypothesis we performed the divergence time estimation for species of Aphilodontinae, reconstructed their ancestral range and evaluated the contribution of vicariance and dispersal to the diversification of Aphilodontinae. Finally, we confront the results with historical events and propose a biogeographic hypothesis for the subfamily.

Material and Methods

Datasets

Sequences related to the mitochondrial cytochrome oxidase I (COI) and 16S rRNA, and the nuclears 18S and 28S rRNAs were objectives. All disponible sequences of Aphilodontinae, mostly produced by Calvanese & Brescovit (2021c), were analyzed, including 13 species of *Aphilodon*, two of *Mecophilus*, one of *Philacroterium* and two species of *Mairata*. For out-groups were used one species of Dignathodontinae (*Dignathodon microcephalus* Lucas, 1846) and one species of Linotaeniinae (*Strigamia crassipes* C.L. Koch, 1835), which are most related to Aphilodontinae inside Geophilidae (Calvanese et al., 2019; Calvanese & Brescovit, 2021c). To this set, a species of Gonibregmatidae, also of Geophilomorpha and a species of *Cormocephalus* Newport, 1844, of Scolopendromorpha, were implemented for nodal calibration in the divergence times analysis. A full list of the samples used in this research and respective Genbank accession numbers are summarized in Table 1, in the Supplementary information.

Divergence Times

The alignments of sequences corresponding to each genetic marker were performed in CLUSTALW at default parameters with MEGA 6 (Tamura et al., 2013). All partitions analyzed had a GTR+I+Γ substitution model selected in JMODELTEST v.2.1.7 (Darriba et al., 2012). The molecular clock hypothesis was tested in MEGA 6 (Tamura et al., 2013) and because all partitions were rejected (P-value < 0.01) we adopted an uncorrelated lognormal relaxed clock model (Drummond et al., 2006).

The estimates of dating divergence were performed under Bayesian inference in BEAST v.2 (Bouckaert et al., 2014), with all four partitions analyzed unlinked in relation to the site and clock models. Constranges were set based on the best supported phylogenetic relationships of the major groups of Aphilodontinae obtained in the phylogenetic analysis under molecular and morphological characters, previously provided by Calvanese & Brescovit (2021c). Thus, here were considered as monophyletic the following clusters: Aphilodontinae, (*Mecophilus* + (*Aphilodon* + *Mairata*)), *Aphilodon*, *Mecophilus* and *Mairata*.

A Yule tree prior was used to model branching times. To sample the topologies, a Monte Carlo Markov chain of 50,000,000 generations was implemented with a sampling frequency every 10,000 generations. After assessing EES convergence with TRACER 1.6 (Rambaut et al., 2016), 10% of the trees were discarded as burn-in, and the remaining set of trees was merged in a consensus tree in TreeAnnotator v.1.4.3 (BEAST package). The .XML file is available under request.

Nodal calibration

The dataset was calibrated with two points, the most basal refers to the minimum age of divergence between Scolopendromorpha and Geophilomorpha according to the reported age of the fossil *Mazoscolopendra* Mundel, 1979 (for more informations see Mundel, 1979), dating 306 Ma, and was modelled with a normal distribution and an average of 30 Ma. The second point of calibration was based on a previous estimation proposed by Fernández et al. (2014) and set the split of Himantarioidea and Geophiloidea to about 170 Ma, this prior was modelled with a normal distribution and an average of 25 Ma.

Biogeographical analysis

Based on the known distribution range of each species (Table 1, in Supplementary material), terminals were associated with biogeographic regions available (more details in Figs 1–4, in the Supplementary material). Neotropical occurrences were scored in relation to the regionalization scheme proposed by Morrone et al. (2014), using the shapefile provided by Loewenberg-Neto (2014), including: Atlantic province (A), Araucaria Forest province (B), Paraná Forest province (C), Chacoan province (D), Cerrado province (E), Rondônia province (F), Xingu-Tapajós province (G) and the

Caatinga province (H). The South African areas were based on the ecoregions defined by WWF (Olson et al., 2001), including the Lowland Fynbos (I), Montane Fynbos biome (J), Coast forest Mosaic (K). The Holarctic distribution of Linotaeniinae and Dignathodontinae was associated with only one unit (L).

To access the biogeographical history of Aphilodontinae, we utilized a combination of three analytical approaches, all implemented in the software RASP 3.02 (Yu et al., 2015): (1) Statistical Dispersal Vicariance Analysis (S-DIVA) (Yu et al., 2010) with the maximum number of ancestral areas restricted to two for each node, and 50,000 post burn-in trees from BEAST analysis; (2) Dispersal Extinction Cladogenesis (DEC) (Ree et al., 2005; Ree & Smith, 2008) with the maximum number of ancestral areas restricted to two for each node, and the consensus tree from BEAST analysis; (3) Statistical Dispersal Extinction Cladogenesis (S-DEC) (Yu et al., 2015) with the maximum number of ancestral areas restricted to two for each node, and 50,000 post burn-in trees from BEAST analysis.

Results

Divergence time

The chronogram with the divergence time of species of Aphilodontinae is presented in Fig. 2. This topology places the origin of the stem group of Aphilodontinae at a mean age of 172.86 Ma (95% highest posterior density interval, HPD, 135.9–210 Ma). The estimated divergence time between *Philacroterium* and the most nested *Mecophilus*, *Mairata* and *Aphilodon* was 140.1 Ma (95% HPD, 105.7–173.1 Ma). The divergence of *Mecophilus* from *Mairata* and *Aphilodon* was estimated to be 128 Ma (95% HPD, 96.8–159.4 Ma). *Mairata* and *Aphilodon* divergence was estimated to 115.2 Ma (95% HPD, 86.4–144.7 Ma).

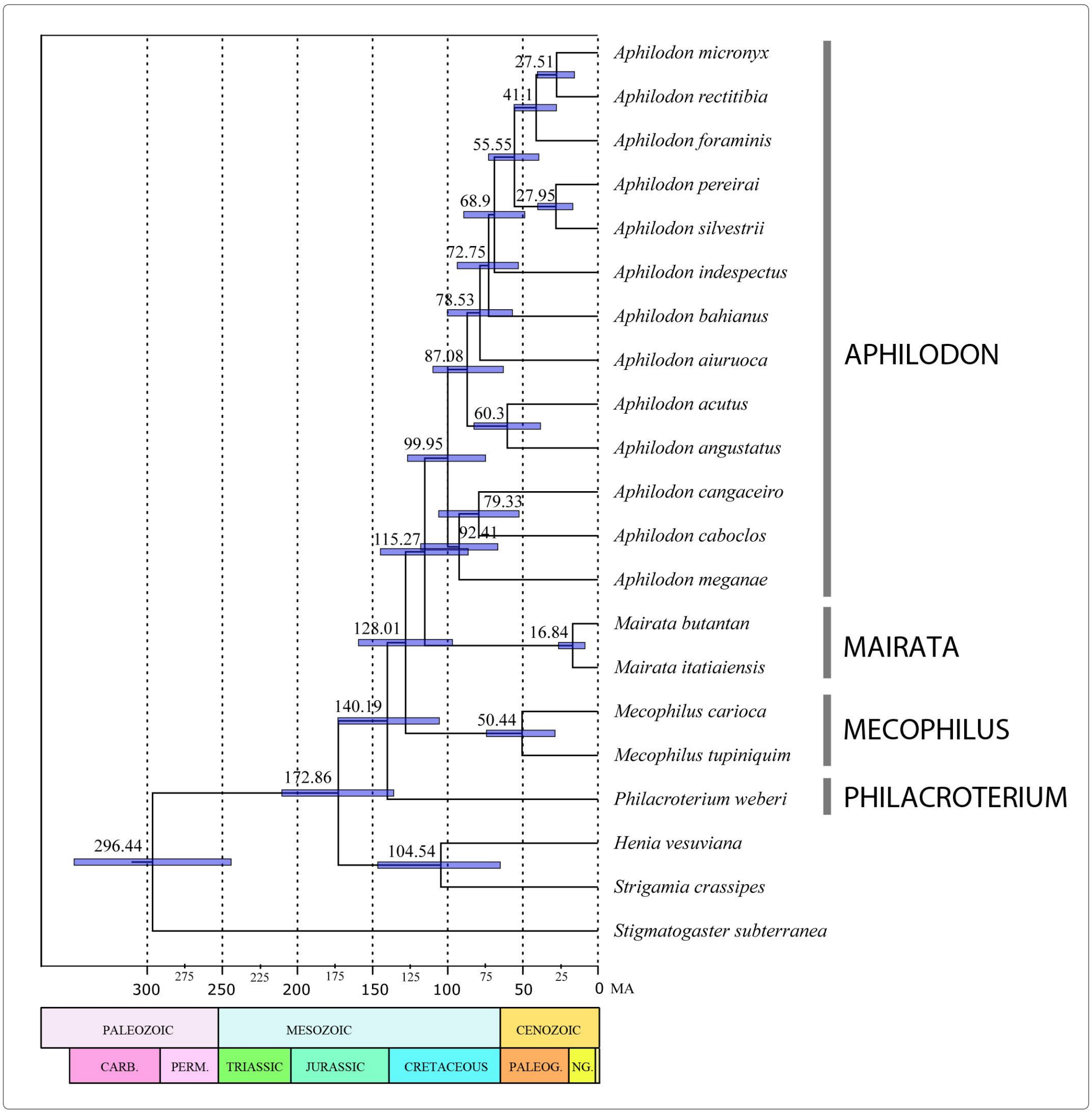

FIGURE 2. Chronogram with the divergence times of the subfamily Aphilodontinae, derived from the BEAST analysis. Median ages estimated are shown associated to each node, and the 95% credibility intervals are represented as horizontal blue bars.

Ancestral area reconstruction

The results obtained in the analysis of S-DIVA, DEC and S-DEC are shown in Figure 3. S-DIVA recovered with the same marginal probability four possible ancestral ranges for Dignathodontinae + Linotaeniinae + Aphilodontinae: Atlantic province + Holarctic region, Lowland fynbos + Holarctic region, Montane fynbos + Holarctic region and Coast forest mosaic + Holarctic region. A retraction range to the Holarctic region of Linotaeniinae + Dignatodontinae and a dispersion of Aphilodontinae to the Atlantic Forest province + Montane fynbos biome followed by a vicariant event divided the groups.

With the same marginal probability three possible ancestral ranges for the Aphilodontinae were inferred: Atlantic province + Montane fynbos, Atlantic province + Lowland fynbos, Atlantic province + Coast forest mosaic. The stem lineage of *Philacroterium weberi* (Silventri, 1909) dispersed to two other areas and occupied its current distribution in three South African biomes, and the Neotropical Aphilodontinae stem lineage settled in the Atlantic province. One vicariance event was associated with the division of these two clades.

Mecophilus, *Mairata* and *Aphilodon* diversified in the same area, the Atlantic province, being the former two genera endemic to this region. In the most part, the species of *Aphilodon* remained in the Atlantic province, however, one dispersion of the ancestor of *A. cangaceiro* and *Aphilodon caboclos* Calvanese, Brescovit & Bonato, 2019 to the Atlantic province + Caatinga province followed by a vicariance event that caused the disruption between these two areas and species was inferred. *Aphilodon angustatus* Silvestri, 1909 is largely the most widespread species of *Aphilodon*, and six different process of dispersion from the Atlantic province were inferred, to the Araucaria Forest province, Paraná Forest province, Chacoan province, Cerrado province, Rondônia province and Xingu-Tapajós province. Other expansions of territory by dispersion were diagnosed from the Atlantic province: *A. bahianus*, to the Caatinga province, *Aphilodon rectitibia* Calvanese & Brescovit, 2021 to the Araucaria Forest and *Aphilodon micronyx* Brölemann, 1902 to the Araucaria Forest province and Paraná Forest province.

DEC and S-DEC analysis were congruent with each other and showed only three most large differences in relation to the results obtained in S-DIVA: (1) DEC and S-DEC inferred the distribution of the ancestor of Aphilodontinae + Dignathodontinae + Linotaeniinae in an area corresponding to the Atlantic province + Holarctic region. A vicariant event was inferred to have separated Aphilodontinae from Dignathodontinae + Linotaeniinae. (2) The ancestral area inferred to the Aphilodontinae crown group was the Atlantic province. The *Philacroterium weberi* ancestor dispersed to the Montane fynbos, Lowland fynbos and Coast Forest mosaic, and a vicariant event separated this lineage from another group that remained in the Atlantic province. (3) The *A. cangaceiro* ancestor dispersed to the Atlantic province + Caatinga, and then this species was isolated from the Atlantic province in the Caatinga province by vicariance.

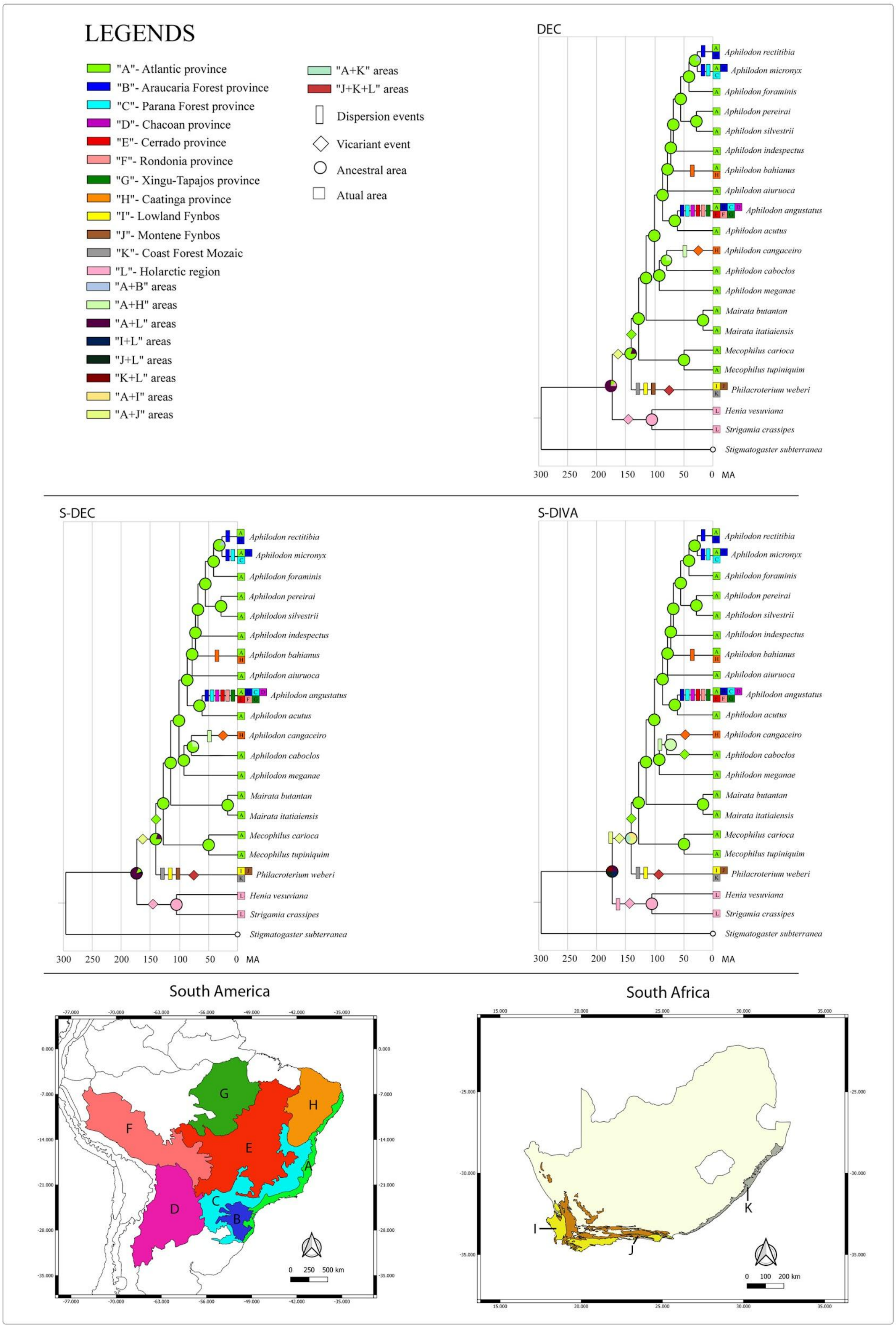

FIGURE 3. Reconstruction of historical biogeography for Aphilodontinae using Statistical Dispersal Vicariance Analysis (S-DIVA), Dispersal Extinction Cladogenesis (DEC) and Statistical Dispersal Extinction Cladogenesis (S-DEC). Further details are provided in the figure.

Discussion

Historical biogeography of Aphilodontinae

Most probably, Aphilodontinae ancestors have separated from the sister lineages Dignathodontinae and Linothaeniinae as a result of a vicariance event. The divergence of the gondwanic Aphilodontinae from the mostly Holarctic Linotaeniinae and Dignathodontinae, estimated in the Mesozoic, corroborated the timing of separation of Gondwana and Laurasia (Blakey, 2008).

The original area of Aphilodontinae was some point of West Gondwana in an area that currently corresponds to the Atlantic province and easterm coast of the African continent. The movement of the tectonic plates that led to the separation of the South American continent from mainland Africa in Cretaceous (Blakey, 2008) was inferred as the possible event responsible for the separation of two ancestral lineages, the South African and Neotropical Aphilodontinae (*sensu* Calvanese & Brescovit, 2021c).

Currently, the genus *Philacroterium* is distributed throughout all coasts of South Africa, and only *Philacroterium transvaalicum* (Lawrence, 1963) and *Philacroterium caffrarium* (Verhoeff, 1937) occur further inland. Seven of eleven species are known from only one biome. *Philacroterium weberi* is the most widespread species with records in three different biomes, however a better understanding of the biogeographical history inside *Philacroterium* cannot be addressed in depth in this work due to the inaccessibility of genetic material for a greater number of South African species of Aphilodontinae.

The diversification of the three Neotropical genera was recovered as sympatric inside the Atlantic province, occurring between 130–115 Ma. Although the three Neotropical genera share the same biogeographic province, in general they present a very different body plan, both in relation to the size of the body as the morphology of the oral appendages used for feeding. Thus, we can hypothesize that each lineage specialized in a specific microenvironment, without necessarily overlapping niches.

Although the distribution of *Mecophilus* and *Mairata* presented here overlaps almost completely in the Atlantic province, a single record of *Mecophilus neotropicus* (not incorporated in the analysis due to unavailability of genetic material) placed this genus also in Paraná Forest Province. It is also important to consider the high degree of ignorance regarding the true number of species of these genera, which results from several factors such as diminished body size, cryptic habitat and minimal mobility in the environment. Thus, specific sampling for Aphilodontinae specimens have been carried out only in recent years (Calvanese et al., 2019; Calvanese et al., 2020), and of the three known species of *Mecophilus*, two were based on specimens recent collected, as for *Mairata*, of which the two known species have been collected and described in recent years (Calvanese et al., 2019; Calvanese & Brescovit, 2021a,b). The bias regarding the group's current distribution is intensified by the fact that the recently collected specimens from both genera were sampled in the same expeditions. That is, it shares the same known distribution because only in these locations we implemented specific methodologies for sampling.

The most widespread *Aphilodon* presents greater diversity in the Atlantic province, an area recovered as primary for this genus. The first range expansion may have come with the dispersion of the ancestor of *A. cangaceiro* to an area that we currently identify as Atlantic province + Caatinga province, being later isolated in the Caatinga province by an event of vicariance. The other twelve species of *Aphilodon* analyzed occur in the Atlantic province, and among them, only four expanded their distribution to other biogeographic regions. *A. micronyx* and *A. rectitibia*, considered sister lineages, stand out in Aphilodontinae due to the relatively large body length, which is also better chitinized and can enable greater dispersion capacity. Our biogeographic analysis points to an independent dispersion of these species for Araucaria Forest province and *A. micronyx* also for Paraná Forest province. *A. bahianus*, registered further north in the Atlantic province than other representatives of *Aphilodon*, expanded its distribution to Caatinga province by dispersion, occupying an area further south of this province, in a region adjacent to the Atlantic province.

The most widespread species of *Aphilodon* is *A. angustatus* and the three models of biogeographic analysis used here recover the Atlantic province as the ancestral area of origin for this species. Apparently, this species expanded its territory from successive dispersions and currently also occurs in the Araucaria Forest province, Paraná Forest province, Chacoan province, Cerrado province, Rondônia province and Xingu-Tapajós province.

However, *A. angustatus* specimens have a short and unquitinized body, with a dispersion capacity considered low. Despite being among the first known species of the genus, and by far the most revised, its morphological delimitation is fragile. Different from the most part of species of Aphilodontinae, which can be recognized by unique morphological traits, *A. angustatus* is diagnosed by a unique combination of traits (Silvestri, 1909; Pereira, et al., 2007; Calvanese et al., 2019). This can generate doubts about the plesiomorphic or synapomorphic conditions of the morphological characteristics that historically define *A. angustatus*. This can directly interfere with the analysis presented here, since most of the occurrence records obtained were based on the currently available morphological diagnosis. Thus, the possibility of *A. angustatus* comprising several cryptic lineages becomes a challenge that can be better solved with a more in-depth study of the real diversity and distribution of this species using phylogeographic tools.

Finally, although we consider that the proposed hypothesis regarding the diversification of Aphilodontinae genera can be tested, much remains to be done in the field of biogeography for this group. The real dimension of the diversity of the subfamily must be better evaluated from specific sampling in a larger territory, in order to quantify both the real number of species and the degree of endemicity of the species. At the moment only a small part of the tip of the iceberg can be assessed, from this still unknown group of centipedes.

Acknowledgements

We would like to thank for all the support provided by the Instituto de Biociências da Universidade de São Paulo (IB-USP) and Laboratório de Coleções Zoológicas (LCZ). Special thanks to Silvio S. Nihei, André Luiz G. Carvalho and Fabio Laurindo da Silva for the great teachings in the area of biogeography. This work was supported by CNPq grants 169641/2017–1 to VCC and PQ 303903/2019–8 to ADB.

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Supplementary information 1. Species and vouchers analyzed.

Supplementary Information 2. Current distribution of the evaluated species of Aphilodontinae plotted on the bioregions.

Supplementary information 1. Species and vouchers analyzed.

Table 1: terminals used in the analysis, occurrence data and genetic material analyzed.

Taxa	**Occurrence data**	**COI**	**16S**	**18S**	**28S**
Aphilodontinae					
***Aphilodon micronyx* Brölemann, 1902**	BRAZIL. Minas Gerais: Caratinga, RPPN Feliciano Abdalla, Reserva Muriqui (19°43’38”S, 41°49’17”W), 15−18/11/2007, C. Sampaio col., 1♀ (MNRJ 30013). Paraná: Rio Negro: S/N. Rio de Janeiro: Itatiaia, 15−18/11/2007, C. Sampaio col., 1♀ (MNRJ 30013); Parque Nacional de Itatiaia (22°22′31″S, 44°39′44″W), 03−17/12/2017, V. C. Calvanese & A. Silva col., 1♀ (IBSP 6174) [neotype], 2♂ and 2♀ (IBSP 6175), 5♀ (IBSP 6176), 1♀ (IBSP 6184), 1♀ (IBSP 6185), 1♀ (IBSP 6186), 1♀ (IBSP 6187). Santa Catarina: S;/N. São Paulo: Serra Negra, Alto da Serra (22°36’44”S, 46°42’02”W), 09⁄1927, R. Spitz col., 1♂ (MZUSP 551); Monte Alegre do Sul (22°40’57”S, 46°40’53”W), 21⁄01⁄1947, 1♂ (MZUSP 3124).	+	+	+	+
***Aphilodon angustatus* Silvestri, 1909**	ARGENTINA. Misiones: Corrientes, San Pedro, 1909, F. Silvestri col., 1♀, 1♂ [syntypes] (MCSG). BRAZIL. Mato Grosso: Poconé, Pirizal (16°16’S, 56°22’W), 29/02/2004, J.L. Silva col., 1♀ (PD 981). Minas Gerais: Serra da Canastra (IBSP). Pará: Curionópolis, 2014, BioEspeleo col. (IBSP). Paraná: Foz do Iguaçu (IBSP); Sengés (IBSP). São Paulo: Bananal (22°41’01” S, 44°19’24”W), 2010, J.M. Pereira col., 3♀, 1♂ (IBSP 3655); Caucaia do Alto, Reserva Morro Alto (23°41’S, 47°1’W), 07/2016, V.C. Calvanese col., 2♀, 1♂ (IBSP 4065); Iporanga, Parque Estadual do Alto do Ribeira (PETAR) (24°35’08”S, 48°35’35”W), 2010, J.M. Pereira col., 3♀, 2♂ (IBSP 3657); Paraná, Sengés (24°06’46”S, 49°27’49” W), 5♀ (IBSP 878). PARAGUAY: Paraguarì.	+	+	+	+
***Aphilodon caboclos* Calvanese, Brescovit & Bonato, 2019**	BRAZIL: São Paulo: Apiaí, Parque Estadual Turístico do Alto Ribeira (PETAR), Núcleo Caboclos (24°26’57”S, 48°35’10”W), 07/2016, V. Calvanese & A. Silva col., 1♀ [holotype] (IBSP 4071), 1♂ [paratype] (IBSP 4072), 1♀, 1♂ (IBSP 4073).	+	+	+	-
***Aphilodon indespectus* Calvanese, Brescovit & Bonato, 2019**	BRAZIL. São Paulo: Bananal (22°41’01”S, 44°19’24”W), 2010, J. M. Pereira col., 3♀, 3♂ (IBSP 3654); São Roque, Parque Ecológico da Mata da Câmara (23°31’41”S, 47°6’51”W), 06/2014, V. Calvanese col., 1♀ [holotype] (IBSP 3653), 3♀ and 4♂ [paratypes] (IBSP 3653).	-	+	-	-
***Aphilodon meganae* Calvanese, Brescovit & Bonato, 2019**	BRAZIL: São Paulo: Iporanga, Reserva Betary (24°35’14”S, 48°35’41”W), 07/2016, V. Calvanese & A. Silva col., 1♀ [holotype] (IBSP 4068), 4♀ and 2♂ [paratypes] (IBSP 4070); São Roque, Parque Ecológico da Mata da Câmara (23°31’41”S, 47°6’51”W), 12/04/2015, V. Calvanese col., 1♂ (IBSP 4082).	+	+	+	+

Table 1. Continuation.

Taxa	Occurrence data	COI	16S	18S	28S
Aphilodontinae					
***Aphilodon pereirai* Calvanese, Brescovit & Bonato, 2019**	BRAZIL. Minas Gerais: Monte Verde, 09/2015, V. Calvanese & A. Silva col., 1♀ [holotype] (IBSP 4067), 2♀ and 2♂, [paratypes] (IBSP 4078), 13♀ and 5♂ (IBSP 3784).	+	+	+	+
***Aphilodon silvestrii* Calvanese, Brescovit & Bonato, 2019**	BRAZIL. São Paulo: São Roque, Parque Ecológico da Mata da Câmara (23°31'41"S, 47°6'51"W), 06/2014, V. Calvanese col., 1♀ [holotype] (IBSP 3065), 3♂ [paratypes] (IBSP 3066); 07/2015, V. Calvanese col., 7♀ [paratypes] (IBSP 4080).	+	+	+	+
***Aphilodon foraminis* Calvanese, Bonato & Brescovit, 2021**	BRAZIL. Rio de Janeiro: Itatiaia, Parque Nacional de Itatiaia, 03-17/12/2017, V. Calvanese & A. Silva col., 1♀ [holotype] (IBSP 6442), 2♀ [paratypes] (IBSP 6444) and 2♂ [paratypes] (IBSP 6443), 12♀ (IBSP 6445) 8♂, (IBSP 6446).	+	+	+	+
***Aphilodon aiuruoca* Calvanese, Bonato & Brescovit, 2021**	BRAZIL. Rio de Janeiro: Itatiaia, Parque Nacional de Itatiaia, 03-17/12/2017, V. Calvanese & A. Silva col., 1♀ [holotype] (IBSP 6447), 2♀ [paratypes] (IBSP 6449) and 3♂ [paratypes] (IBSP 6448), 2♀ (IBSP 6450).	+	+	+	+
***Aphilodon acutus* Calvanese, Bonato & Brescovit, 2021**	BRAZIL. Rio de Janeiro: Itatiaia, Parque Nacional de Itatiaia, 03-17/12/2017, V. Calvanese & A. Silva col., 1♀ [holotype] (IBSP 6451), 2♀ [paratypes] (IBSP 6453) and 2♂ [paratypes] (IBSP 6452), 4♀ (IBSP 6454).	+	+	+	+
***Aphilodon cangaceiro* Calvanese, Bonato & Brescovit, 2021**	BRAZIL. Piaui: Caracol, Parque Nacional da Serra das Confusões, 03-17/01/2019, V. Calvanese & A. Silva col., 1♀ [holotype] (IBSP 6455), 2♀ [paratypes] (IBSP 6457) and 2♂ [paratypes] (IBSP 6456), 4♀ (IBSP 6454), 3♀ (IBSP 6459) 10♂, (IBSP 6458).	+	-	+	-
***Aphilodon bahianus* Calvanese, Bonato & Brescovit, 2021**	BRAZIL. Bahia: Andaraí, Parque Nacional da Chapada da Diamantina, 29/11-05/12/2018, V. Calvanese & Adele Silva col., 1 ♂ (IBSP 6465); Mucugê, Parque Municipal de Mucugê, 29/11-05/12/2018, V. Calvanese & Adele Silva col., 2♂ (IBSP 6465); Prado, Parque Nacional do Descobrimento, 12/2018, V. Calvanese & Adele Silva col., 1♀ [holotype] (IBSP 6460), 2♀ [paratypes] (IBSP 6462) and 2♂ [paratypes] (IBSP 6461), 4♀ (IBSP 6463); Una, Reserva Biológica de Una, 20-28/11/2018, V. Calvanese & Adele Silva col., 5♀, 5♂ (IBSP 6464).	+	+	+	+

Table 1. Continuation.

Taxa	**Occurrence data**	**COI**	**16S**	**18S**	**28S**
Aphilodontinae					
***Aphilodon rectitibia* Calvanese, Bonato & Brescovit, 2021**	BRAZIL. Minas Gerais: Santana do Riacho, Parque Nacional da Serra do Cipó, 01-11/09/2018, V. Calvanese & A. Silva col., Rio de Janeiro: Itatiaia, 14/11/1945, 1♂ [paratype] (IBSP 6469) O. Schubart col., 1♂ and 1♀ (MZSP 3061). Parque Nacional de Itatiaia, Rio de Janeiro, Brazil, 03-17/12/2017, V. Calvanese & A. Silva col., 1♀ [holotype] (IBSP 6467), 2♀ [paratypes] (IBSP 6468). São Paulo: Eldorado (24º31'12"S, 48º06'29"W), 09/2015, 1♀ (MZSP 3437); Salesópolis, 09−10/07/1992, 1♂ (MZSP 0739); Salesópolis, 09−10/07/1992, 1♂ and 1♀ (IBSP 4081); Salesópolis, Estação Biológica de Boracéia (23° 37' 51"S 45° 52' 11"W), 1♂ 26/11/1968, Rabelo col. (MZSP 921).	+	+	+	-
***Mairata butantan* Calvanese, Brescovit & Bonato, 2019**	BRAZIL: São Paulo: Cotia, Caucaia do Alto, Reserva Morro Alto (23°41’11”S, 47°1’20”W), 12/06/2016, V. Calvanese col., 2♂ (IBSP 3662); São Roque, Parque Ecológico da Mata da Câmara (23°31’41”S, 47°6’51”W), 06/2014, V. Calvanese & A. Silva col., 1♀ [holotype] (IBSP 3664), 2♀ and 2♂ [paratypes] (IBSP 3663); 12/04/2015, V. Calvanese col., 3♀ and 1♂ (IBSP 4079).	+	+	+	+
***Mairata itatiaiensis* Calvanese, Brescovit & Bonato, 2019**	BRAZIL: Rio de Janeiro: Itatiaia, Parque Nacional de Itatiaia (22°22′31″S, 44°39′44″W), 4−17/12/2017, V. Calvanese & A. Silva col., 1♂ (IBSP 6179), 1♀ (IBSP 6180), 1♂ (IBSP 6181), 2♀ (IBSP 6182) 1♀ (IBSP 6183); 16/12/2017, V. Calvanese & A. Silva col., 1 ♀ [holotype] (IBSP 6177), 2♀ and 2♂ [paratypes] (IBSP 6178).	+	+	+	+
***Mecophilus tupiniquim* Calvanese, Brescovit & Bonato, 2019**	BRAZIL: São Paulo: São Roque, Morro do Cruzeiro (23°32’18”S, 47°8’43”W), 15/11/2017, V. Calvanese col., 1♀ [holotype] (IBSP 6172), 4♂ [paratypes] (IBSP 6173).	+	+	+	+
***Mecophilus carioca* Calvanese, Bonato & Brescovit, 2021**	BRAZIL: Rio de Janeiro: Itatiaia, Parque Nacional de Itatiaia (22°22′31″S, 44°39′44″W), 12/2017, V. Calvanese & A. Silva col., 1♂ (IBSP 6179), 1♀, 2♂ (IBSP 6473), 1 ♀ [holotype] (IBSP 6470), 1♀ [paratype] (IBSP 6471),1♂ [paratype] (IBSP 6472).	+	+	+	+
***Philacroterium weberi* (Silvestri, 1909)**	SOUTH AFRICA. Western Cape: Cape Town, 1906, 1♂ (MNSG “Vase Type XI”) [one of syntypes]; 1956, 1♀ (NHMW 7045); Knysna, Storms River Mouth, 01⁄1961, 3♀ and 2♂ (KZN 8142). Eastern Cape: Port St. Johns, Umngazi Mouth, 1♀ (KZN 6238).	-	-	AF173264.1	HM453302.1

Table 1. Continuation.

Taxa	**Occurrence data**	**COI**	**16S**	**18S**	**28S**
Dignathodontinae					
***Henia vesuviana* (Newport, 1844)**	ITALY: Piemonte: 1♀ and 1♂ (PD G 1525 and PD G 5258).	AY288754.1	KF569223.1	AF173255.1	-
Linotaeniinae					
***Strigamia crassipes* (Koch, 1835)**	ITALY: Vittorio Veneto, 1♀ and 1♂ (PD G 2892).	JN306680.1	LT702892.1	LT702905.1	KF569282.1
Táxon used for nodal calibration					
Geophilomorpha / Himantariidae					
***Stigmatogaster subterranea* (Shaw, 1789)**	-	KF569302.1	KF569237.1	KF569260.1	KF569281.1
Scolopendromorpha / Scolopendridae					
***Cormocephalus hartmeyeri* (Kraepelin, 1908)**	-	KF676531.1	KF676491.1	KF676445.1	AY210812.1

Supplementary information 2. Current distribution of the evaluated species of Aphilodontinae plotted on the bioregions. *Colour of the biogeographical areas*: Atlantic province: light green. Araucaria Forest province: blue. Paraná Forest province: light blue. Chacoan province: purple. Cerrado province: red. Rondônia province: salmon. Xingu-Tapajós province: dark green. Caatinga province: orange. Lowland Fynbos: yellow. Montane Fynbos: brown. Coast forest Mosaic: grey. The Neotropical regionalization was based on the bioregions proposed by Morrone et al. (2014). The South African areas were based on the ecoregions adopted by WWF (Olson et al., 2001).

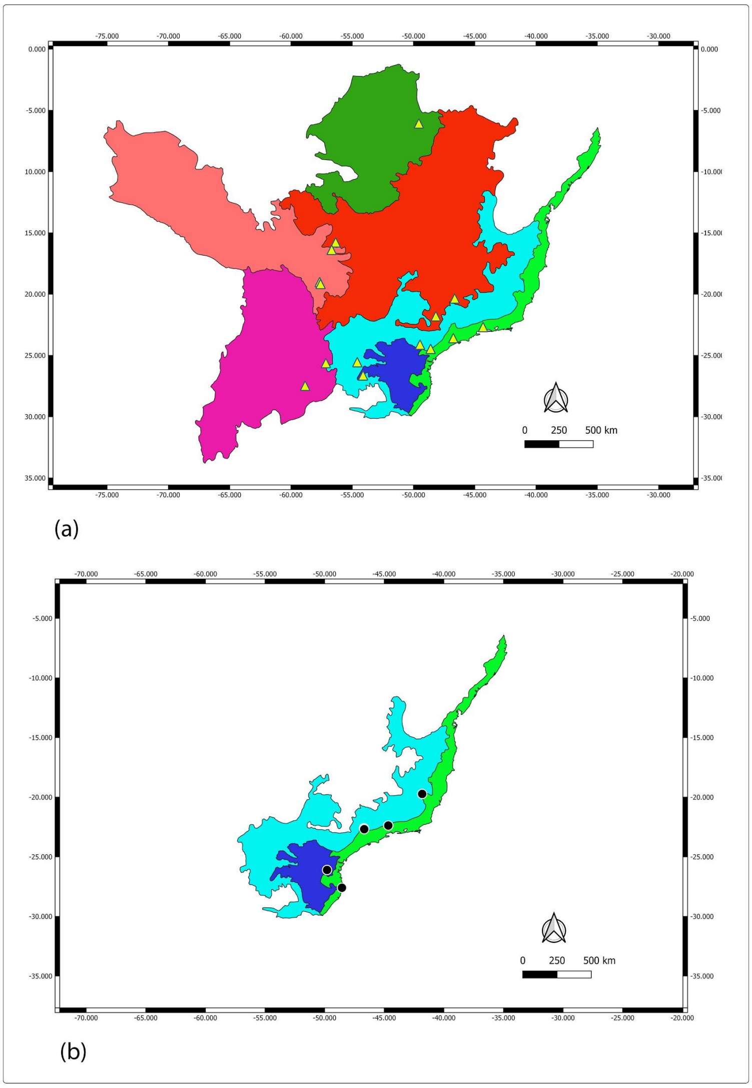

FIGURE 1. (a) distribution of *Aphilodon angustatus* Silvestri 1909 with the occurrence records indicated by yellow triangles; (b) distribution of *Aphilodon micronyx* Brölemann, 1902 with the occurrence records indicated by black dots.

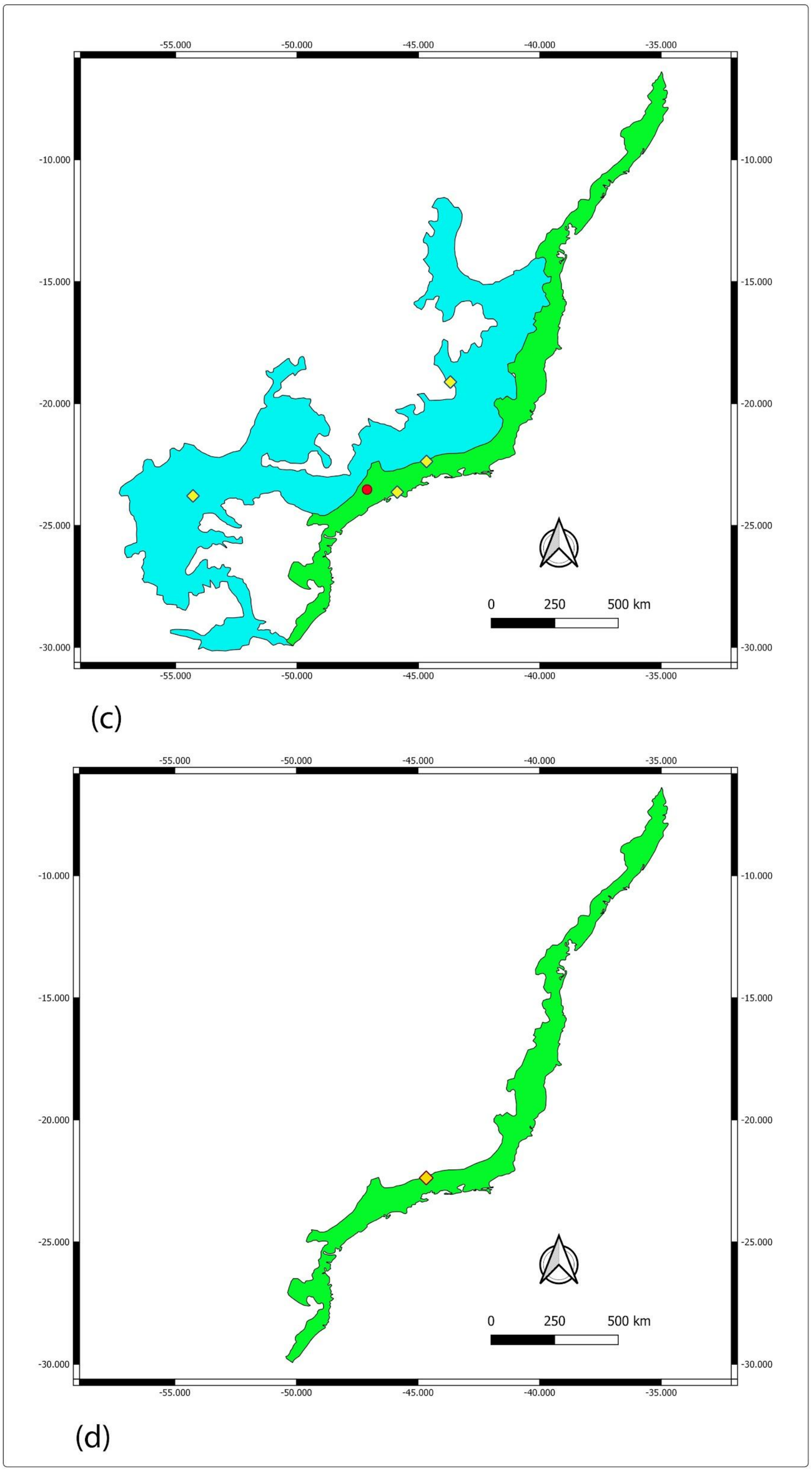

FIGURE 2. (c) distribution of *Aphilodon rectitibia* Calvanese, Brescovit & Bonato, 2019, with the occurrence records indicated by yellow losang and *Aphilodon silvestrii* Calvanese, Brescovit & Bonato, 2019 with the occurrence records indicated by a red circle; (d) distribution of *Aphilodon acutos* Calvanese & Brescovit, 2021, *A. aiuruoca* Calvanese & Brescovit, 2021 and *A. foraminis* Calvanese & Brescovit, 2021, known only to the same localization, indicated by a yellow losang.

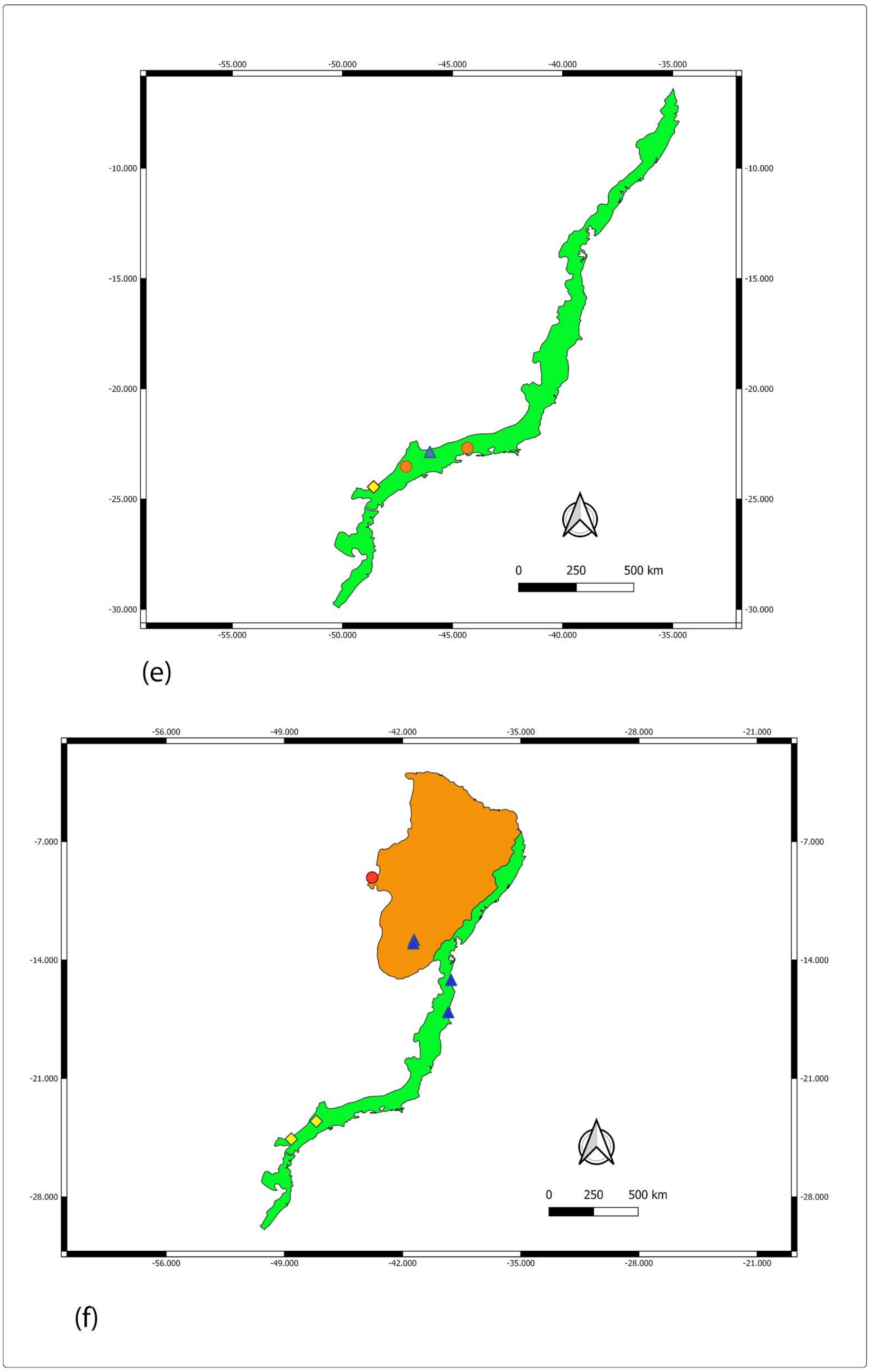

FIGURE 3. (e) distribution of *Aphilodon indespectus* Calvanese, Brescovit & Bonato, 2019, with the occurrence records indicated by a red circle, *Aphilodon caboclos* Calvanese, Brescovit & Bonato, 2019 with the occurrence records indicated by a yellow losang and *A. pereirai* Calvanese, Brescovit & Bonato, 2019 with the occurrence records indicated by a blue triangle; (f) distribution of *Aphilodon meganae* Calvanese, Brescovit & Bonato, 2019 with the occurrence records indicated by a yellow losang, *A. bahianus* Calvanese & Brescovit, 2021 with the occurrence records indicated by a blue triangle and *A. cangaceiro* Calvanese & Brescovit, 2021 with the occurrence records indicated by a red circle.

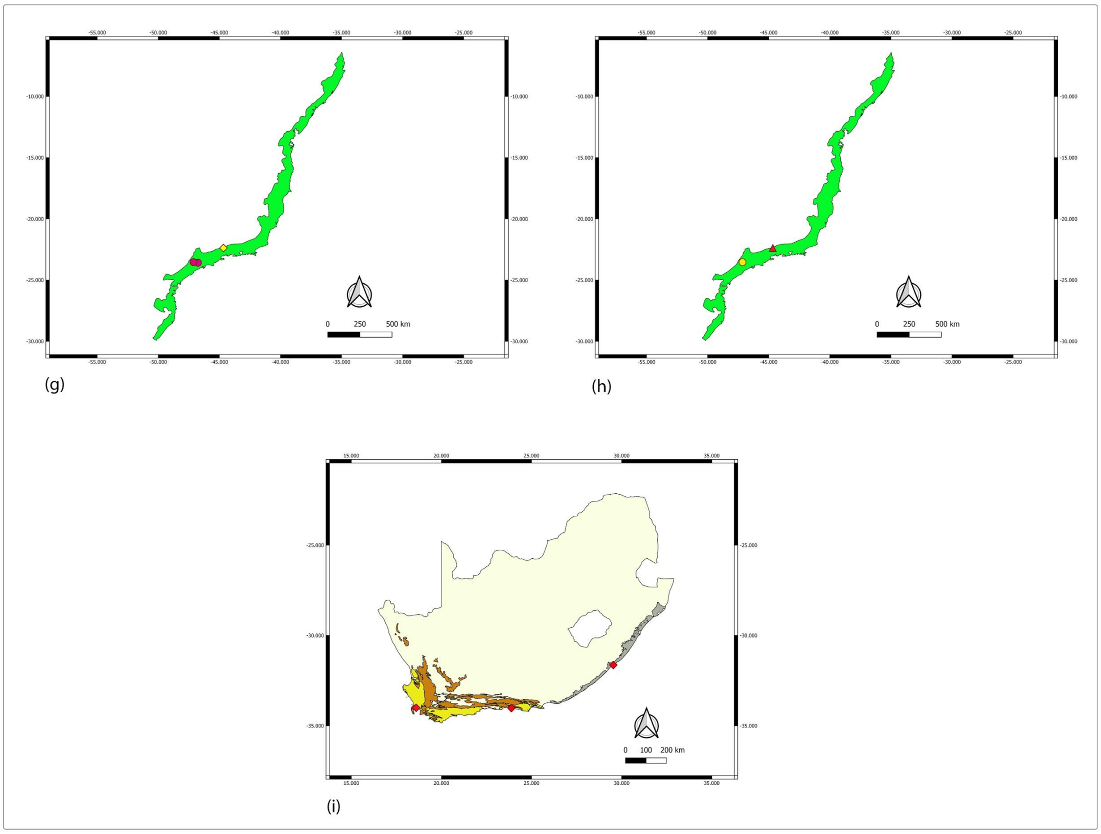

FIGURE 4. (g) distribution of *Mairata butantan* Calvanese, Brescovit & Bonato, 2019 with the occurrence records indicated by a purple circle and *Mairata itatiaiensis* Calvanese, Brescovit & Bonato, 2019, with the occurrence records indicated by a yellow losang; (h) distribution of *Mecophilus carioca* Calvanese & Brescovit, 2021 with the occurrence records indicated by a purple triangle and *Mecophilus tupiniquim* Calvanese, Brescovit & Bonato, 2019, with the occurrence records indicated by a yellow circle; (i) distribution of *Philacroterium weberi* (Silvestri, 1909) with the occurrence records indicated by a red circle.

CAPÍTULO 5. Considerações finais

I- ***Principais conclusões***

Em análise filogenética, a monofilia de Aphilodontinae e de seus quatro gêneros, incluindo *Aphilodon* Silvestri, 1898, *Mecophilus* Silvestri, 1909, *Philacroterium* Attems, 1926 e *Mairata* Calvanese, Bonato & Brescovit, 2019, foram corroboradas tanto sobre dados morfológicos como sobre dados moleculares, contudo, em análise de evidência total os agrupamentos apresentaram maior resolução.

Os dados moleculares foram mais informativos para estabelecer as relações entre os gêneros de Aphilodontinae e entre espécies de *Aphilodon*, recuperados em politomias na análise de dados morfológicos isolados. Contudo, dados morfológicos foram obtidos para uma quantidade maior de terminais e também foram informativos para delimitar os principais grupos.

Aphilodontinae: quatro sinapomorfias morfológicas não homoplásticas e não ambíguas foram diagnosticadas, incluindo a presença de cerdas subclipeares; parte média do labrum rudimentar e não claramente separada do clípeo; na forcípula, fusão do trochanteroprefemur e fêmur; e também na forcípula, dentículo do fêmur bilobado.

Aphilodon: duas sinapomorfias morfológicas não homoplásticas e não ambíguas foram diagnosticadas, incluindo a peculiar quetotaxia do terceiro artículo do telopodito da segunda maxila; e presença de um espinho terminal no último par de pernas.

Mecophilus: três sinapomorfias morfológicas não homoplásticas e não ambíguas foram diagnosticadas, incluindo um pretergito forcipular alongado; coxosternito forcipular alongado; e esternito do primeiro segmento portador de pernas alongado.

Philacroterium: duas sinapomorfias morfológicas não homoplásticas e não ambíguas foram diagnosticadas, incluindo as peças laterais do labrum não distintas do clípeo; e telopodito da segunda maxila com apenas dois artículos.

Mairata: três sinapomorfias morfológicas não homoplásticas e não ambíguas foram diagnosticadas, incluindo o terceiro artículo do telopodito da segunda maxila com formato curto e estreito; tarso 1 do último par de pernas com formato globoso em machos; e tarso 1 do último par de pernas mais largo em parte posterior em fêmeas.

Dentre as subfamílias de Geophilidae, Aphilodontinae foi considerada mais relacionada a Dignathodontinae e Linotaeniinae. Contudo, constitui uma linhagem irmã de *Geoperingueyia* Attems, 1926, gênero atualmente alocado apenas em Geophilidae, com quem compartilha três sinapomorfias morfológicas não homoplásticas e não ambíguas.

A inclusão de *Geoperingueyia* em Aphilodontinae foi por hora refutada, pois embora constituam linhagens irmãs, se distinguem em aspectos morfológicos considerados chave para delimitação de Aphilodontinae, como na forcípula, a fusão do trochanteroprefemur e fêmur. Em adição, a baixa representatividade de gêneros de Geophilidae revisados e testados em filogenias torna difícil estabelecer com segurança o posicionamento de *Geoperingueyia* dentro da família. Contudo, o gênero *Geoperingueyia* foi considerado monofilético baseado em uma sinapomorfia morfológica não homoplástica e não ambígua: processo foraminal do coxosternito da segunda maxila transversalmente alongado.

O conhecimento sobre a distribuição de Aphilodontinae no Brasil foi ampliado para os estados da Bahia, Pará e Piauí, e atualmente a subfamília é registrada em todas as regiões deste país. Pela primeira vez, Aphilodontinae foi reportada para Caatinga e Amazônia, sendo agora conhecida em todos os biomas brasileiros.

A análise de biogeografia apresentada para o grupo corroborou com a hipótese de que a dissolução da Gondwana, no Cretáceo, separou os Aphilodontinae entre sul-africanos e sul-americanos. Posteriormente, o grupo Neotropical diversificou em uma mesma biorregião, raramente incorporando outras regiões em sua distribuição. Esse padrão de ocorrência é compatível com o que se espera do grupo, já que os Aphilodontinae têm baixa capacidade de dispersão e estão fortemente associados ao microambiente em que vivem. Em adição, existe uma ampla variação no plano corporal entre os membros da subfamília, o que permite a exploração de diferentes nichos no mesmo ambiente geral.

O nosso conjunto de dados indica que em Geophilidae, um ancestral dos atuais Aphilodontinae divergiu de um ancestral de Dignathodontinae a aproximadamente 170 milhões de anos, em decorrência da fragmentação da Pangea. A cerca de 140 milhões de anos atrás, com a dissolução da Gondwana e consequente distanciamento dos continentes da África e América do Sul, Aphilodontinae foi dividida em dois clados. A linhagem africana se restringe a África do Sul e constitui um único gênero, *Philacroterium*, que atualmente conta com 11 espécies. A divisão dos três gêneros sul-americanos ocorreu entre 115–130 milhões de anos, de forma simpátrica em uma biorregião correspondente ao que conhecemos como bioma de Mata Atlântica. Atualmente são conhecidas 16 espécies de *Aphilodon*, três de *Mecophilus* e duas de *Mairata*. Os dois últimos gêneros permaneceram em sua biorregião original, contudo, através de dispersão algumas espécies de *Aphilodon* alcançaram outras áreas, e atualmente representantes deste gênero podem ser encontrados em um amplo território do Brasil, parte da Argentina e parte do Paraguai.

II- ***Sobre a sistemática e biogeografia de Aphilodontinae***

Historicamente a sistemática de Aphilodontinae vêm sendo baseada em critérios morfológicos para sua elaboração (Silvestri, 1909; Attems, 1929; Calvanese *et al.*, 2019). Neste trabalho, avançamos consideravelmente sobre o conhecimento relacionado a variação morfológica que ocorre no grupo, e apresentamos uma matriz filogenética que abrange todas as espécies conhecidas de Aphilodontinae e de seu grupo irmão *Geoperingueyia*. Este conjunto de dados permite estabelecer a posição de Aphilodontinae dentro de Geophilidae, e delimitar a subfamília e seus quatro gêneros por meio de sinapomorfias morfológicas não homoplásticas e não ambíguas.

Em relação às moléculas, nós apresentamos as primeiras sequências de DNA para os Aphilodontinae Neotropicais. Agora, todos os gêneros conhecidos da subfamília dispõe de material sequenciado, incluindo mais de 66% das espécies de *Aphilodon*, de *Mecophilus* e de *Mairata*. Por outro lado, *Philacroterium* foi representado em nossa matriz molecular por apenas uma de suas 11 espécies, e para *Geoperingueyia* nenhum material genético foi avaliado. Essas questões estão relacionadas à escassez de sequências disponíveis para os geofilomorfos sul-africanos em bancos de dados como o GenBank, e que cujas espécies não puderam ser acessadas em campo em nosso estudo.

Neste sentido, o maior desafio considerado para um futuro avanço do conhecimento sobre a diversidade e distribuição de Aphilodontinae ainda é a falta de amostragem. Esta questão se torna mais instigante a partir dos resultados de Calvanese *et al.* (2020) e manuscritos 1 e 2 aqui apresentados, que reportam diversas espécies em áreas onde se mantém um esforço intenso para coleta.

Futuras questões podem ainda ser endereçadas a espécies de Aphilodontinae que apresentam ampla distribuição (como *Aphilodon angustatus* Silvestri, 1909), que contrasta com um padrão de alta endemicidade reportado para a maioria das espécies do grupo (Calvanese *et al.*, 2019). Neste sentido, a existência de espécies crípticas (que não podem ser diferenciadas por morfologia) pode ser levada em conta, de modo que estudos filogeográficos específicos possam ser úteis.

Por fim, com um progressivo avanço sobre a sistemática e distribuição dos Aphilodontinae, outras hipóteses relacionadas a biogeografia do grupo podem ser no futuro testadas. Como exemplo, se destacam questões sobre a diversificação das espécies sul-africanas de *Philacroterium* e relação entre Aphilodontinae e *Geoperingueyia*, menos acessados em nosso trabalho.

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Abstract

Systematics and biogeography of Aphilodontinae Silvestri, 1909, with the first molecular approach to the subfamily (Geophilomorpha: Geophilidae)

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Advisor: Antonio Domingos Brescovit

Abstract of the PhD Thesis submitted to the Graduate Program of Zoology of the Institute of Biosciences of University of São Paulo, USP, as part of the requisites for the completion of a PhD degree in Biological Sciences.

Aphilodontines are centipedes characterized by the filiform body with less than 1 to 7 cm and between 33–93 leg-bearing segments, forcipule composed by only three articles and sternites without ventral pores. With four genera, the subfamily is known in South Africa by *Philacroterium* Attems, 1926 and in South America by *Aphilodon* Silvestri, 1898, *Mecophilus* Silvestri, 1909 and *Mairata* Calvanese, Bonato & Brescovit, 2019. This work presents new species and deals with the phylogenetic relationships and biogeography of Aphilodontinae, based mainly on morphological and molecular data.

Six new species of *Aphilodon* recently sampled in Brazil are presented. From the Atlantic Forest biome, *Aphilodon foraminis* **n. sp.**, *A. aiuruoca* **n. sp.**, *A. acutus* **n. sp.** and *A. rectitibia* **n. sp.** were registered in the state of Rio de Janeiro. *A. rectitibia* **n. sp.** was also reported from the states of São Paulo and Minas Gerais. *Aphilodon cangaceiro* **n. sp.** is the first record of Aphilodontinae for the state of Piauí and was sampled in the Caatinga biome. *Aphilodon bahianus* **n. sp.** is the first Aphilodontinae from Bahia, being registered from the Atlantic Forest and Caatinga biomes. Also, *Mecophilus carioca* **n. sp.**, is described based on material recently sampled from the Atlantic Forest, and is the first record of the genus from the state of Rio de Janeiro.

At the first time a phylogeny based on morphological and molecular data is presented to the subfamily. Morphological dataset is composed of 52 terminals, including all known species of Aphilodontinae and *Geoperingueyia* (sister group of Aphilodontinae) and 58 characters. Molecular data aimed at the mitochondrial COI and 16S rRNA and the nuclears 18S and 28S rRNAs, and all genera of Aphilodontinae were represented. Phylogenetic analysis were performed using four approaches: maximum parsimony cladistic to the morphological characters, maximum likelihood to the molecular partitions analyzed separately, maximum likelihood to the molecular partitions analyzed together, and maximum likelihood of morphological characters and all molecular partitions analyzed together (total evidence). Different analysis recovered the monophyly of Aphilodontinae and its four genera, however, molecular data and the total evidence approach were better able to resolve some polytomies between the genera and species. Furthermore, new morphological synapomorphies were diagnosed to Aphilodontinae, *Aphilodon* and *Geoperingueyia*, and some aspects about the evolution of the subfamily were discussed.

A biogeographical analysis based on molecular dating and ancestral area reconstruction is presented. In this research, sequences of the mitochondrial COI and 16S, and nuclears 18S and 28S were used to estimate the divergence times of the species of Aphilodontinae in a Bayesian framework, with an uncorrelated log-normal relaxed clock and two points of calibrations. Ancestral areas were reconstructed in a maximum likelihood approach in a Statistical Dispersal Vicariance Analysis (S-DIVA), Statistical Dispersal Extinction Cladogenesis (S-DEC) and Dispersal Extinction Cladogenesis (DEC). The results indicate that the subfamily originated approximately 170 million years ago, when the clade diverged from the other Geophilidae by the fragmentation of Pangea. About 140 million years ago *Philacroterium* diverged from other Aphilodontinae due to the distancing from the continents of Africa and South America. The Neotropical *Mecophilus*, *Aphilodon* and *Mairata* diversified into the same biogeographical area, and only *Aphilodon* expanded its territory by the dispersion of some species.

Key words: Arthropoda, biodiversity, Chilopoda, evolution, molecular dating, new species, phylogenetic analysis, taxonomy.

Resumo

Sistemática e biogeografia de Aphilodontinae Silvestri, 1909, com a primeira abordagem molecular para a subfamília (Geophilomorpha: Geophilidae)

Doutorando: Victor de Carvalho Calvanese

Orientador: Antonio Domingos Brescovit

Este resumo submetido ao Programa de Pós-Graduação em Zoologia do Instituto de Biociências da Universidade de São Paulo, USP, é parte dos requisitos necessários para a conclusão do grau de Doutor em Ciências Biológicas.

As centopéias de Aphilodontinae são caracterizadas pelo corpo filiforme com menos de 1 a 7 cm de comprimento e entre 33–93 segmentos portadores de pernas, forcípula composta por apenas três artículos e esternitos sem poros ventrais. Com quatro gêneros, a subfamília é conhecida para África do Sul por *Philacroterium* Attems, 1926 e América do Sul por *Aphilodon* Silvestri, 1898, *Mecophilus* Silvestri, 1909 e *Mairata* Calvanese, Bonato & Brescovit, 2019. O presente trabalho apresenta novas espécies e trata das relações filogenéticas e biogeografia de Aphilodontinae, com base principalmente em dados morfológicos e moleculares.

Seis novas espécies de *Aphilodon* recentemente amostradas no Brasil são apresentadas. No bioma de Mata Atlântica, *Aphilodon foraminis* **n. sp.**, *A. aiuruoca* **n. sp.**, *A. acutus* **n. sp.** e *A. rectitibia* **n. sp.** foram registradas no estado do Rio de Janeiro. *A. rectitibia* **n. sp.** também foi relatada para os estados de São Paulo e Minas Gerais. *Aphilodon cangaceiro* **n. sp.** é o primeiro registro de Aphilodontinae para o estado do Piauí e foi amostrado no bioma de Caatinga. *Aphilodon bahianus* **n. sp.** é o primeiro Aphilodontinae conhecido da Bahia, com registros em Mata Atlântica e Caatinga. Além disso, *Mecophilus carioca* **n. sp.** é descrito com base em material recentemente coletado em Mata Atlântica, sendo o primeiro registro do gênero no estado do Rio de Janeiro.

Pela primeira vez uma filogenia baseada em dados morfológicos e moleculares é apresentada para Aphilodontinae. A matriz morfológica é composta por 52 terminais, incluindo todas as espécies conhecidas de Aphilodontinae e *Geoperingueyia* (grupo irmão de Aphilodontinae) e 58 caracteres. Análises moleculares foram direcionadas aos marcadores mitocondriais COI e 16S rRNA e aos nucleares 18S e 28S rRNAs, e todos os gêneros de Aphilodontinae foram representados. Quatro análises filogenéticas foram conduzidas: máxima parcimônia para caracteres morfológicos, máxima verossimilhança para partições moleculares analisadas separadamente, máxima verossimilhança para partições moleculares analisadas em conjunto e máxima verossimilhança para caracteres morfológicos e todas as partições moleculares analisadas juntas (evidência total). Diferentes análises recuperaram a monofilia de Aphilodontinae e também de seus quatro gêneros, contudo, dados moleculares e a abordagem de evidência total resolveram melhor a relação entre gêneros e espécies. Além disso, novas sinapomorfias morfológicas foram diagnosticadas para Aphilodontinae, *Aphilodon* e *Geoperingueyia*, e alguns aspectos relacionados à evolução da subfamília são discutidos.

Uma análise de biogeografia baseada em datação molecular e reconstrução de áreas ancestrais é apresentada. Nesta pesquisa, sequências dos marcadores COI, 16S, 18S e 28S foram utilizadas para estimar o tempo de divergência entre as espécies de Aphilodontinae por Inferência Bayesiana, com um relógio log-normal relaxado não correlacionado e dois pontos de calibração. As áreas ancestrais foram reconstruídas em uma abordagem de máxima verossimilhança sobre os modelos de Statistical Dispersal Vicariance Analysis (S-DIVA), Statistical Dispersal Extinction Cladogenesis (S-DEC) e Dispersal Extinction Cladogenesis (DEC). Os resultados indicam que a subfamília divergiu de outros Geophilidae há aproximadamente 170 milhões de anos, pela fragmentação da Pangea. Há cerca de 140 milhões de anos, *Philacroterium* divergiu de outros Aphilodontinae pelo distanciamento da África e América do Sul. *Mecophilus, Aphilodon* e *Mairata* diversificaram em uma mesma biorregião, e apenas *Aphilodon* expandiu seu território pela dispersão de algumas de suas espécies.

Palavras-chave: Arthropoda, biodiversidade, Chilopoda, datação molecular, evolução, filogenética, novas espécies, taxonomia.