UNIVERSIDADE DE SÃO PAULO
FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DE RIBEIRÃO PRETO
PROGRAMA DE PÓS-GRADUAÇÃO EM ENTOMOLOGIA

Perlidae from Brasil: reducing Linnean e Wallacean shorfalls

Perlidae do Brasil: reduzindo os déficits Linneano e Wallaceano

Lucas Henrique de Almeida

Tese apresentada à Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo, como parte das exigências para obtenção do título de Doutor em Ciências, obtido no Programa de Pós-Graduação em Entomologia

Ribeirão Preto - SP

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Orientador: Prof. Dr. Pitágoras da Conceição Bispo

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NOTA

Essa tese é parte dos requisitos para obtenção do título de Doutor pelo Programa de Pós-graduação em Entomologia da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo. Assim sendo, não é considerada uma publicação científica que segue os requisitos do Código Internacional de Nomenclatura Zoológica (ICZN). Portanto, os nomes das espécies novas propostos aqui não são válidos.

DISCLAIMER

This thesis is part of the requirements for obtention of the Ph.D degree in the Graduate Program in Entomology of the Faculty of Philosophy, Sciences, and Languages of Ribeirão Preto, University of São Paulo. Therefore, this contribution is not considered a scientific publication that follows the requirements of the International Commission of Zoological Nomenclature (ICZN). Therefore, the names of the new species proposed here are not valid.

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Abstract

The order Plecoptera is composed of aquatic insects that inhabit mainly low and medium-order lotic environments, which are also important indicators of environmental quality. Among the plecopterans, the Perlidae family has the largest number of known species (about 30%) and is also the most diverse in the Neotropical Region and in Brazil. In Brazil, the family is represented by the genera *Anacroneuria*, *Enderleina*, *Kempnyia*, and *Macrogynoplax*. Despite recent advances in the study of Brazilian perlids, shortfalls in knowledge about diversity remain present. In this study, we studied the Brazilian fauna of Perlidae (Plecoptera) in order to reduce biodiversity shortfalls, especially the Linnean (lack of knowledge about existing species) and Wallacean shortfalls (lack of knowledge about the geographic distribution of species). Here, we describe six new species of Perlidae: *Anacroneuria duarte* sp. nov., *A. sallesi* sp. nov., *Enderleina castro* sp. nov., *Kempnyia guarani* sp. nov., *K. tupiniquim* sp. nov., and *K. zwicki* sp. nov. We proposed the synonymy of *Kempnyia sordida* with *K. obtusa*, we recorded for the first time *Anacroneuria pakitza* and *Macrogynoplax yupanqui* in Brazil, we performed several new records in different states, and we count several new occurrence points for dozens of species. In addition, we organized all the existing taxonomic knowledge about Perlidae from Brazil, generating a list with information on described semaphoronts and scientific articles that cited the 152 known species. Through the compiled occurrence data, we were able to discuss the sampling effort carried out from a political and phytogeographic perspective, highlighting the need to continue to expand the sampled locations. We also discussed the difficulties faced by the taxonomy of Perlidae from Brazil, highlighting the presence of problematic species, the difficult species delimitation, impediments in the association of nymphs, and the few phylogenetic, biogeographic, and ecological studies. We conclude by presenting the perspectives of future studies on Brazilian perlids.

Keywords: Stonefly, biodiversity, shortfalls, taxonomy

Resumo

A ordem Plecoptera é composta por insetos aquáticos habitantes de ambientes lóticos de baixas e médias ordens, sendo também importantes indicadores de qualidade ambiental. Dentre os plecópteros, a família Perlidae possui o maior número de espécies conhecidas (cerca de 30%), sendo também a mais diversa da Região Neotropical e do Brasil. No Brasil, a família é representada pelos gêneros *Anacroneuria*, *Enderleina*, *Kempnyia* e *Macrogynoplax*. Apesar dos recentes avanços no estudo dos perlídeos brasileiros, os déficits no conhecimento sobre a diversidade permanecem bastante presentes. Neste estudo, nós estudamos a fauna brasileira de Perlidae (Plecoptera) com o objetivo de reduzir os déficits da biodiversidade, especialmente o déficit Linneano (limitação do conhecimento sobre as espécies existentes) e o déficit Wallaceano (limitação do conhecimento sobre a distribuição geográfica das espécies). Aqui, nós descrevemos seis novas espécies de Perlidae: *Anacroneuria duarte* nov. sp., *A. sallesi* nov. sp., *Enderleina castro* nov. sp., *Kempnyia guarani* nov. sp., *K. tupiniquim* nov. sp. e *K. zwicki* nov. sp. Propomos a sinonímia de *Kempnyia sórdida* com *K. obtusa*, registramos a primeira ocorrência de *Anacroneuria pakitza* e *Macrogynoplax yupanqui* para o Brasil, obtivemos diversos novos registros para diferentes estados e contabilizamos inúmeros novos pontos de ocorrência para dezenas de espécies. Além disso, organizamos todo o conhecimento taxonômico existente sobre Perlidae do Brasil, gerando uma lista com informações sobre semaforontes descritos e artigos científicos que citam as 152 espécies conhecidas. Através dos dados de ocorrência compilados, foi possível discutir o esforço amostral realizado, considerando uma perspectiva política e fitogeográfica, evidenciando a necessidade de continuar a expandir os locais amostrados. Discutimos também sobre as dificuldades existentes na taxonomia de Perlidae do Brasil, evidenciando a presença de espécies problemáticas, a difícil delimitação de espécies, impedimentos na associação de ninfas, e os poucos estudos filogenéticos, biogeográficos e ecológicos. Finalizamos apresentando as perspectivas dos próximos estudos sobre os perlídeos brasileiros.

Palavras-chave: Plecoptera, biodiversidade, déficits, taxonomia

SUMÁRIO

Introdução Geral

Plecoptera Burmeister, 1839

Os insetos aquáticos da ordem Plecoptera são notórios habitantes de ambientes lóticos de baixas e médias ordens (até 4ª ordem, *sensu* Strahler, 1957) com águas limpas, rápidas, frias e bem oxigenadas. Em geral, os imaturos desta ordem são aquáticos e os adultos terrestres alados. Em função desta característica, as espécies da ordem sofrem os efeitos negativos das atividades antrópicas, tanto sobre os ambientes aquáticos (redução da qualidade da água e da integridade física do ambiente lótico), quanto sobre os ambientes terrestres (degradação das margens e da bacia, remoção da vegetação, erosão, etc). Assim, os plecópteros, juntamente com Ephemeroptera e Trichoptera, são importantes indicadores do grau de conservação dos ecossistemas lóticos e da bacia hidrográfica (Hellawell, 1978; 1986; Armitage *et al*., 1983). Sendo, portanto, de grande relevância como indicadores da qualidade ambiental.

Nos rios e riachos, as ninfas são comumente encontradas no fundo de pedra, troncos de árvores, musgo e folhiço (Froehlich & Oliveira, 1997), em regiões de corredeira ou remanso. Quando jovens, as ninfas alimentam-se de material particulado fino e, conforme o crescimento, passam a ser predadoras, raspadoras ou fragmentadoras (Stark *et al*., 2009). As trocas gasosas são realizadas através de brânquias presentes no tórax e/ou no final do abdômen ou através do tegumento. As brânquias filamentosas são também importantes como carater taxonômico, variando entre famílias e gêneros (Fig 1) (Shepard & Stewart, 1983; Stewart & Stark, 2002). Movimentos ondulatórios laterais do corpo são responsáveis pela natação das ninfas, sendo possível graças a presença de forte musculatura oblíqua entre os segmentos do corpo (Zwick, 1980; 2000). Devido as diferentes condições ambientais, o tempo de desenvolvimento das ninfas

pode variar de espécie para espécie, durando de três meses até anos (Romero, 2001) e de 12 a 36 instares (Pennak, 1978).

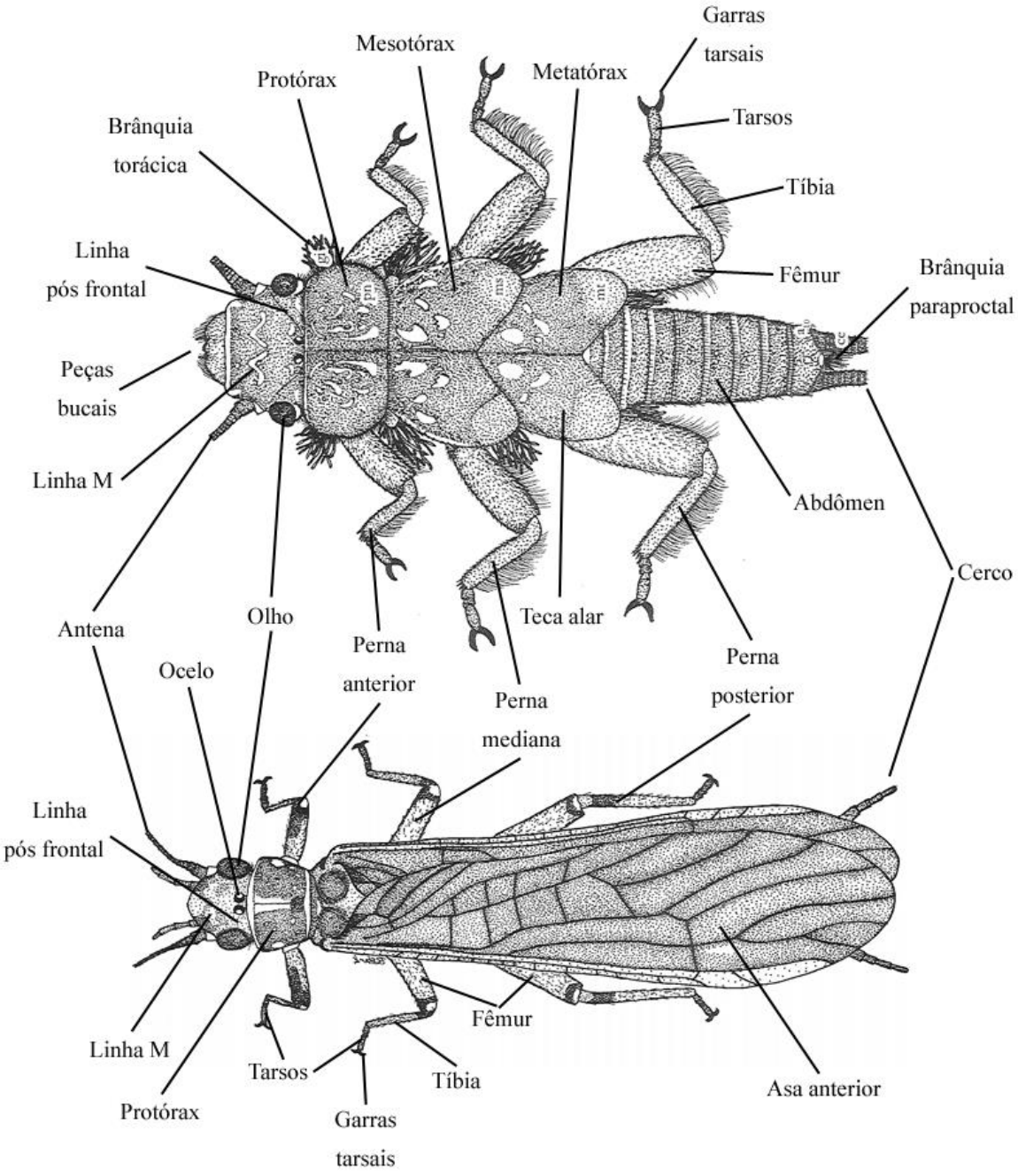

Figura 1. Morfologia da ninfa e adulto de Plecoptera, modificado de Stark *et al*. (2009).

Os adultos, por sua vez, vivem normalmente de uma a quatro semanas e são comumente encontrados nas proximidades dos rios e riachos, uma vez que são voadores fracos e acabam percorrendo pequenas distâncias. Durante a fase adulta, alguns plecópteros não se alimentam, geralmente apenas bebem água, estes possuem as peças bucais atrofiadas, como por exemplo, os integrantes da família Perlidae (Hynes, 1976). Apesar disso, alguns plecópteros apresentam hábitos alimentares variados quando adultos, podendo consumir algas, líquens, frutos, botões foliares e pólen (Hynes, 1976; Sato & Kato, 2017).

Os plecópteros são insetos hemimetábolos, com corpo geralmente achatado dorso-ventralmente, dois cercos no final do abdômen, asas posteriores que se dobram em forma de leque quando em repouso sobre o corpo e tamanho variando de 4 até 60 mm (Zwick, 1980; Stewart & Harper, 1996; Lecci & Froehlich, 2006; Froehlich, 2012). A cabeça é prognata e ampla articulação com o pronoto, possui dois olhos compostos bem desenvolvidos, dois ou três ocelos, antenas filiformes, multiarticulada e longas (Stark *et al*., 2009). As pernas apresentam tarsos tri-segmentados (Stark *et al.*, 2009). Quanto à coloração, variam entre marrom escuro, cinza e amarelo, e em algumas espécies, cores vibrantes como o verde, vermelho ou roxo podem ser observadas, como por exemplo, em integrantes da família Chloroperlidae e Eustheniidae (Olifiers, 2005). Onze segmentos formam o abdômen, dos quais, dez são distintos e visíveis. O 11º segmento apresenta um par de cercos que podem ser reduzidos para um ou dois segmentos em algumas espécies, porém, geralmente são longos e multissegmentados, além disso, apresenta também um par de paraproctos e as vezes um epiprocto (Hynes, 1976; Stewart & Harper, 1996; Froehlich, 2012).

Os adultos apresentam dois pares de asas articuladas do tipo membranosas que se dobram sobre o abdômen em repouso. As asas caracterizam-se por possuírem *arculus*, e a veia

RP e M fundidas na base (Béthoux, 2005). A asa anterior é relativamente estreita e longa, diferente da asa posterior, que é um pouco mais curta e larga devido a presença de um lobo anal bem desenvolvido. A asa posterior dobra-se em forma de leque quando em repouso (Pennak, 1978), fato que deu origem ao nome da ordem, *pleco* = entrelaçar, dobrar; *pteron* = asa (Froehlich, 2012). Adicionalmente, algumas espécies possuem asas reduzidas (Illies, 1963) ou ausentes (McLellan, 2001).

A placa subgenital tem origem no oitavo esternito nas fêmeas e no nono nos machos. Em alguns grupos, os machos apresentam uma placa subgenital com uma estrutura chamada martelo, que geralmente é cônico e esclerosado e apresenta papel importante para o acasalamento. O martelo é responsável, em algumas espécies de Arctoperlários, por dar início a troca de sinal sonoro quando batido ou atritado contra o substrato (comportamento que leva o nome de *"drumming"*), objetivando o encontro de uma parceira para o acasalamento. A frequência emitida pela batida ou atrito feito pelo macho é específica, sendo identificada e respondida pelas fêmeas virgens da espécie, resultando no encontro seguido de cópula (Stewart, 1997).

As fêmeas geralmente não possuem estrutura adaptada para deposição, porém, nos grupos em que ele é presente, é formado por um prolongamento dos esternos oito e nove, ou apenas do oito (Zwick, 1980). A colocação dos ovos, na maioria das espécies, ocorre durante o voo, iniciando-se pelo acúmulo de ovos na porção final do abdómen, dando origem a uma massa ou aglomerado de ovos. Posteriormente, quando chega a hora da postura dos ovos, a fêmea mergulha o abdômen na água e a massa de ovos é liberada, afundando e prendendo-se em algum substrato (Hynes, 1976; Pennak, 1978; Zwick, 2000).

Histórico taxonômico e classificação de Plecoptera

Em seu trabalho *Systema Naturae*, Linnaeus (1758) reuniu os plecópteros na ordem Neuroptera juntamente aos atuais representantes das ordens Ephemeroptera, Megaloptera, Neuroptera, Odonata e Trichoptera. Posteriormente, Geoffroy (1762) agrupou as espécies atualmente conhecidas como plecópteros em um novo gênero chamado *Perla* (Zwick, 1980). No século seguinte, Burmeister (1839) criou a ordem Plecoptera, separando-as das demais levando em conta as asas posteriores levemente mais curtas que as anteriores e a presença de um lobo anal desenvolvido na asa posterior que se dobra. Pela semelhança das asas anteriores, Burmeister também relacionou a ordem Plecoptera com Orthoptera e posteriormente com Megaloptera e Trichoptera (Zwick, 2009).

Nos dias de hoje, a posição de Plecoptera na filogenia de Hexapoda é bastante discutida, com hipóteses sugerindo que a ordem possa ser grupo irmão de todos os Neoptera ou de todos os Polyneoptera, enquanto outras sugerem parentesco próximo com Embioptera ou Phasmatodea (Zwick, 2009). Após a consolidação de Polyneoptera como um grupo monofilético no trabalho de Misof *et al*. (2014), trabalhos utilizando marcadores moleculares e filogenômica surgiram tentando discutir o posicionamento e o grau de parentesco interno das ordens. Alguns destes trabalhos sugerem que Plecoptera esteja posicionado entre os primeiros eventos de cladogênese de Polyneoptera, com Zoraptera + Dermaptera como grupo-irmão de Plecoptera + Demais Polyneopeta (Misof *et al*., 2014), como grupo-irmão de Dermaptera e estes como grupo-irmão de todos os outros Polyneoptera (Song *et al*., 2016), ou ainda como grupo irmão de todos os outros Polyneoptera (Ding *et al*., 2019). Dessa forma, o grau de parentesco e a posição de Plecoptera entre os Polyneoptera deverá ser discutida e esclarecida nos próximos anos.

Já os grupos dentro de Plecoptera começaram a ser continuamente discutidos a partir do início do século XX. Enderlein (1909) e Klapálek (1909) dividiram a ordem em duas subordens a partir de características do aparelho bucal. Klapálek criou Setipalpia ou Subpalpia, que reunia os grupos com palpo maxilar subcilíndrico e Filipalpia com palpos filiformes. Ao passo que Enderlein, diferente de Klapálek, sugeriu a divisão da ordem com base na presença e ausência de mandíbulas nos adultos, nomeando as subordens de Systellognatha e Holognatha (Zwick, 2000). Anteriormente, Burmeister (1839) já havia dividido a ordem em dois grupos utilizando os mesmos caracteres que Klapálek e Enderlein, configurando uma sinonímia entre os sistemas de classificação.

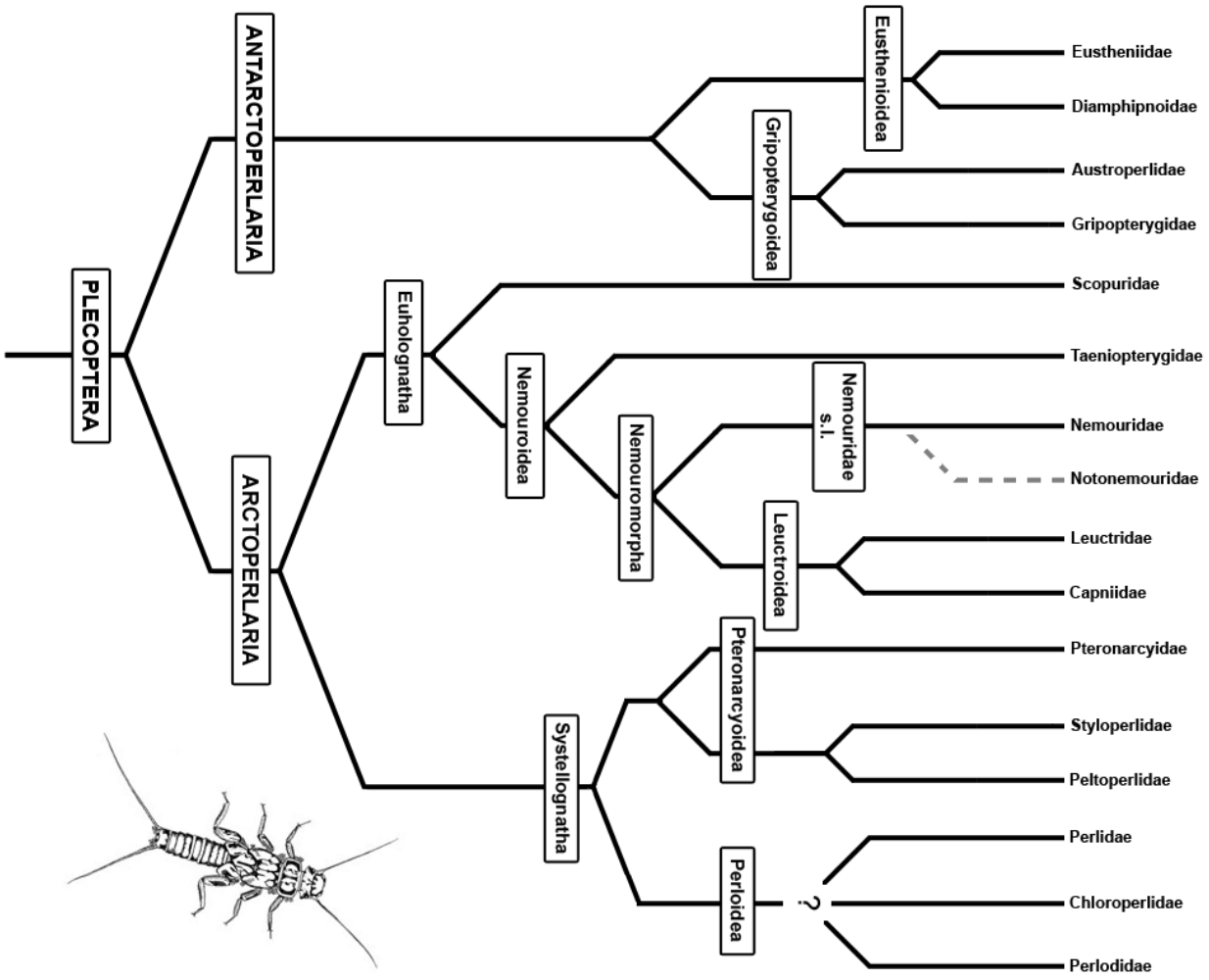

Figura 2. Filogenia das famílias de Plecoptera, modificado de Zwick (2000).

Anos após Klapálek e Enderlein, Frison (1935) notou inconsistência na classificação, pois nem todos os plecópteros com palpos longos possuíam também mandíbulas reduzidas na fase adulta. Então, Illies (1965), a partir da divisão de Filipalpia, criou Archiperlaria, uma terceira subordem com plecópteros de grande porte, coloração brilhante, além de outros caracteres. Segundo Zwick (1974), apenas a subordem Setipalpia possuiria caracteres derivados, como alongamento dos tarsos e mandíbulas reduzidas, ao passo que Filipalpia e Archiperlaria estariam unidas pela ausência de determinados caracteres.

Zwick (2000) apresentou uma proposta de classificação morfológica que ainda hoje é utilizada como base de comparação e discussão. Nesta classificação, Zwick dividiu os plecópteros em duas subordens, Arctoperlaria de origem na Laurásia e Actarctoperlaria de origem gondwânica austral (Fig 2). Recentemente, McCulloch *et al*. (2016) utilizaram marcadores moleculares e corroboraram a divisão em duas subordens propostas por Zwick, com idade de divergência estimada em 121 milhões de anos atrás na divisão da Pangeia em Gondwâna e Laurásia no período Jurássico. Letsch *et al*. (2021), por outro lado, utilizando dados moleculares mais robustos, encontraram outra explicação para a divisão entre Arctoperlaria e Antarctoperlaria, tendo ela ocorrido ao norte da Pangeia. Na proposta de Letsch *et al*. (2021), Arctoperlaria e Antarctoperlaria teriam respectivamente por volta de 255 e 184 milhões de anos de idade, divergindo muito antes do proposto por McCulloch *et al*. (2016). Arctoperlaria é dividida em duas infraordens, cada uma com seis famílias, sendo Euholognatha representada por Capniidae, Leuctridae, Nemouridae, Notonemouridae, Scopuridae e Taeniopterygidae; e Systellognatha por Chloroperlidae, Kathroperlidae Perlidae, Perlodidae, Peltoperlidae, Pteronarcyidae e Styloperlidae (Zwick, 2000; South *et al*., 2021). O nome Arctoperlaria se refere ao fato da maioria de seus representantes serem encontrados na Região Holártica ou Hemisfério Norte.

Apesar disso, duas famílias possuem ocorrência no Hemisfério Sul, sendo elas Notonemouridae e Perlidae (Pessacq *et al*., 2019). O nome Antarctoperlaria, por sua vez, remete a distribuição circum-artártica apresentada por seus representantes, estes são encontrados em todo o Hemisfério Sul, com exceção da África. Antarctoperlaria é dividida em duas superfamílias, Eusthenioidea com duas famílias (Diamphipnoidae e Eustheniidae) e Gripopterygoidea, também com duas famílias (Austroperlidae e Gripopterygidae) (Zwick, 2000).

Após o trabalho de McCulloch *et al*. (2016), outros trabalhos abordaram, através de informações moleculares, as relações filogenéticas dentro da ordem Plecoptera (Chen *et al*. 2018; Ding *et al*., 2019; Cao *et al*., 2019; South *et al*., 2021; Letsch *et al*., 2021). McCulloch *et al*. (2016) e Ding *et al*. (2019) utilizaram grande parte dos grupos de Plecoptera, recuperando as duas superfamílias de Antarctoperlaria (Eusthenioidea e Gripopterygoidea) e ambas as infraordens de Arctoperlaria (Euholognatha e Systellognatha). Porém, entre os arctoperlários, nem todas as superfamílias propostas por Zwick (2000) foram recuperadas, apenas Nemouroidea (Euholognatha) e Perloidea + Pteronarcyoidea (Systellognatha). Apesar disso, Chen *et al*. (2018) realizando abordagem focada em Systellognatha, não conseguiram recuperar Pteronarcyoidea como monofilético, indicando que trabalhos são necessários e poderão esclarecer o parentesco dentro de Systellognatha nos próximos anos (Fig 3). South *et al*. (2021) e Letsch *et al*. (2021), já incluindo a décima sétima família de Plecoptera, recuperaram Perloidea como monofilético, encontrando também a mesma relação de parentesco entre as quatro famílias que compõem o grupo (Fig 3).

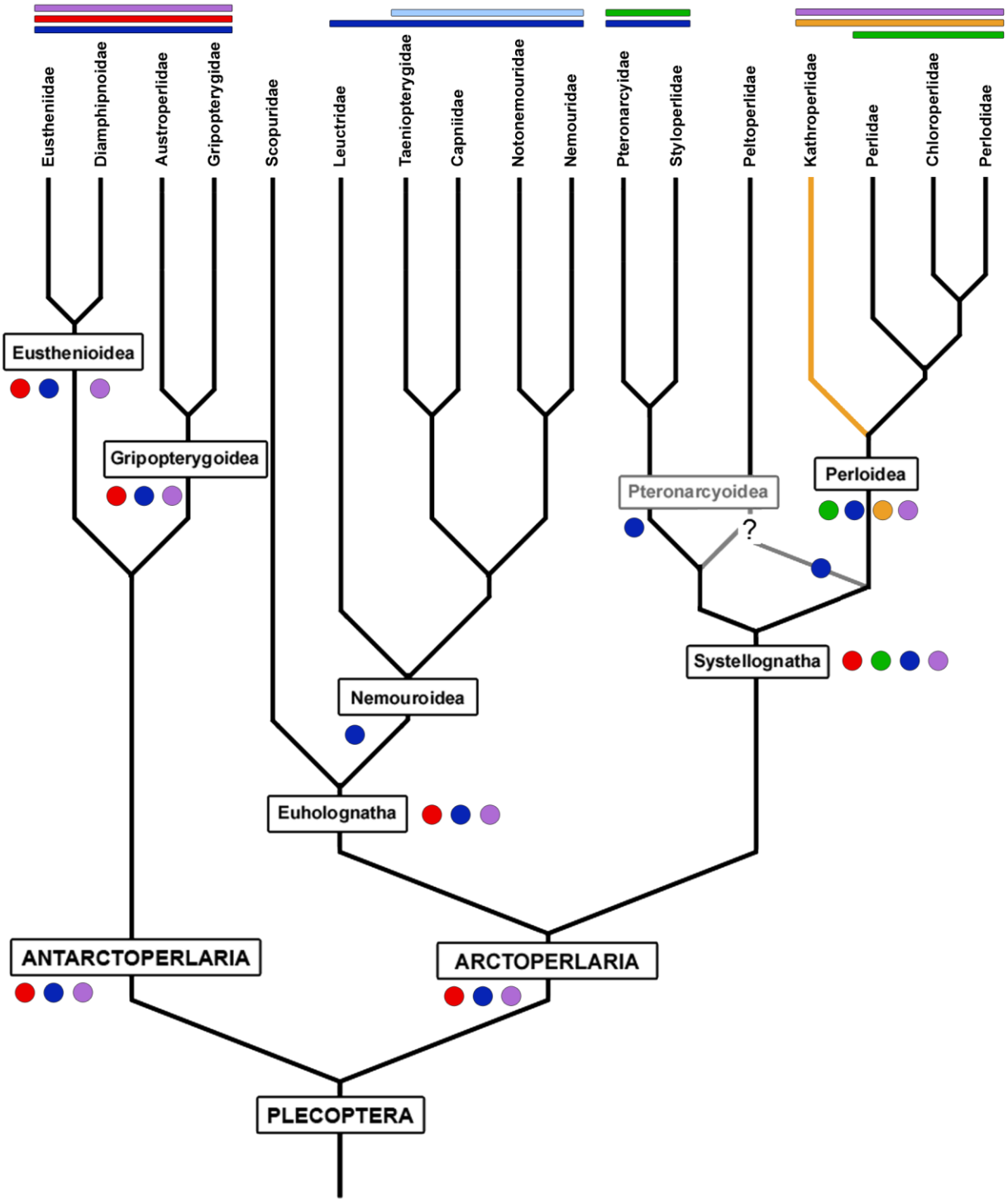

Figura 3. Relacionamentos e grupos de Plecoptera mais vezes suportados por preposições filogenéticas moleculares tendo como base de comparação a hipótese morfológica de Zwick (2000), com informações de McCulloch *et al*. (2016) em vermelho, Chen *et al*. (2018) em verde, Ding *et al*. (2019) em azul escuro, Cao *et al*. (2019) em azul claro, South *et al*. (2021)

em amarelo e Letsch *et al*. (2021) em roxo. Círculos e barras indicam grupos sustentados e relacionamentos resgatados nas hipóteses filogenéticas.

Diversidade de Plecoptera

A ordem Plecoptera possui mais de 3700 espécies descritas, distribuídas em 17 famílias e habita todas as regiões do mundo, com exceção da Antártica (DeWalt & Ower, 2019; South *et al*., 2021). Os registros fósseis mais antigos datam do período Carbonífero Superior, cerca de 300 milhões de anos atrás (Béthoux *et al*., 2012). Como dito anteriormente, a maior diversidade de plecópteros encontra-se em riachos de águas limpas, rápidas e frias, no entanto, é possível encontra-los também em riachos preservados de águas mais quentes (DeWalt & Ower, 2019).

Tabela 1. Total de espécies descritas e taxa de descrição no período de 1980 até 2018. Dados retirados de DeWalt & Ower (2019).

Região	Espécies descritas (Total)	Espécies descritas (1980 – 2018)	Taxa de descrição anual de espécies
Global	3718	1657	43.7
Asia-Temperada	1179	854	22.5
Américas Central e do Sul	528	343	9.0
Asia-Tropical	498	278	7.3
América do Norte	765	250	6.6
Australasia	307	173	4.6
Europa	489	119	3.1
África	80	17	0.4

A região do mundo com o maior número de espécies descritas é a Ásia Temperada (Tabela 1), na qual se inclui a China. Nos últimos anos, pesquisadores chineses publicaram muito mais em revistas científicas de língua inglesa que anteriormente, além disso, o número de

trabalhos publicados aumentou drasticamente, refletindo também na maior taxa de descrição anual de espécies (DeWalt & Ower, 2019). A segunda maior taxa de descrição de espécies pertence as regiões das Américas Central e do Sul, com nove espécies novas descritas por ano e mais de 500 no total. Provavelmente o valor total de espécies da região deva estar subestimando, uma vez que muitas áreas ainda não foram amostradas e até mesmo áreas consideradas bem conhecidas apresentam resultados relevantes a cada novo estudo (Almeida & Bispo, 2020). O mesmo acontece na África, apesar do clima desfavorável em grande parte do continente (Fochetti & Tierno de Figueroa, 2008).

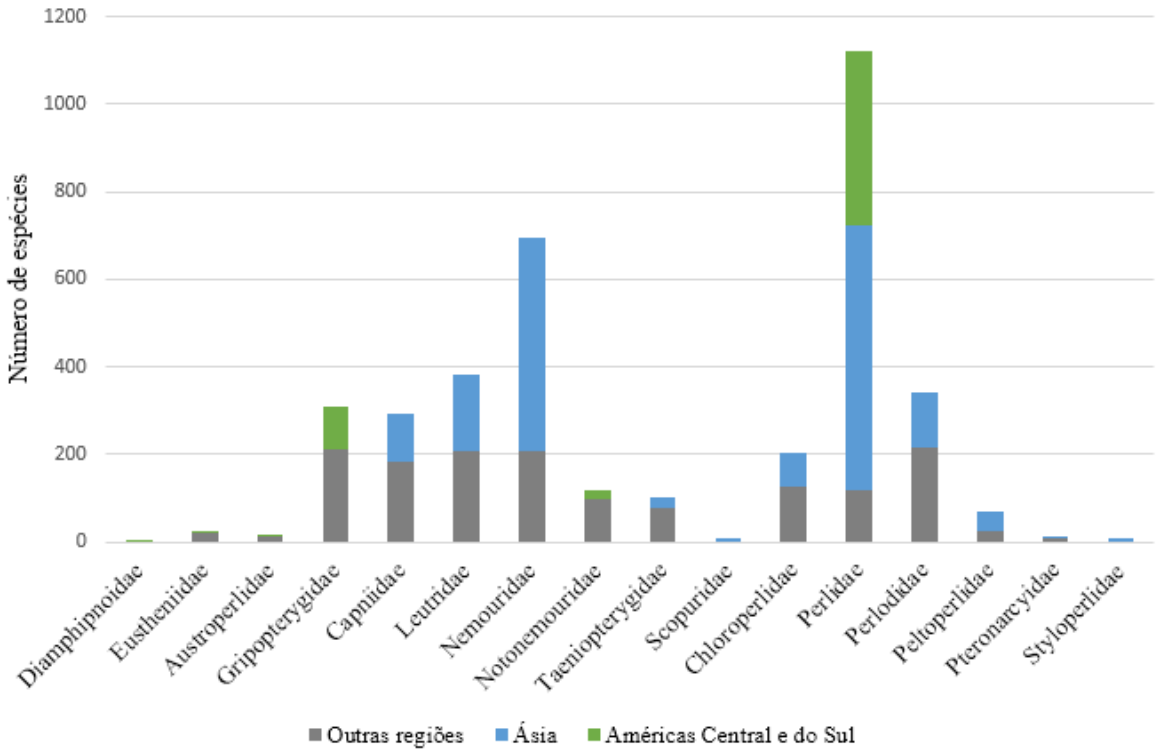

Figura 4. Riqueza de espécies de Plecoptera com ênfase na Ásia e nas Américas Central e do Sul. Adaptado de DeWalt & Ower (2019).

Globalmente, a taxa de descrição de novas espécies de Plecoptera é elevada e não se encontra em declínio. Por exemplo, a Ásia somada as Américas Central e do Sul serão

responsáveis, segundo estimativas, por aproximadamente 810 das 1.400 novas espécies que se espera descrever no mundo até 2050 (DeWalt & Ower, 2019). Estas predições podem ser explicadas pela presença das famílias mais diversas de arctoperlários na Ásia (Perlidae, Nemouridae, Leuctridae, Perlodidae e Capniidae) e pela presença de Perlidae nas Américas Central e do Sul, além de Gripopterigydae, a família mais diversa de Antarctoperlaria, na América do Sul. Mesmo pertencendo a Arctoperlaria, Perlidae, a família mais diversa da ordem, é representada sozinha por cerca de 400 espécies nas Américas Central e do Sul, somando aproximadamente o triplo da diversidade de Gripopterigydae (Fig 4) (Fochetti & Tierno de Figueroa, 2008; DeWalt & Ower, 2019).

Apesar do potencial de descobertas de novas espécies demonstrado pelas Américas Central e do Sul, a região com o segundo maior número de espécies descritas de Plecoptera é a América do Norte. Tradicionalmente, a América do Norte foi uma das regiões mais estudadas, juntamente a Europa, e provavelmente apresentarão poucas novas espécies nos próximos anos (DeWalt & Ower, 2019). Por serem bem estudadas, estas regiões serviram como base para se avaliar a vulnerabilidade das espécies existentes, e segundo Sánchez-Baoya & Wyckhuysmeet (2019), 35% das espécies de Plecoptera destas regiões estão em declínio, 29% estão ameaçadas e 19% estão sendo extintas local ou regionalmente. Segundo os autores, estas taxas podem ser maiores para grupos mais sensíveis. Assim, as perspectivas de futuro da taxonomia de Plecoptera mesclam-se entre muitas novas espécies a seres descobertas e descritas, e a constante ameaça de alterações climáticas e ambientais resultante de fontes naturais ou antrópicas.

Contexto brasileiro

No Brasil, a ordem Plecoptera totaliza aproximadamente 200 espécies descritas (Pessacq *et al*., 2019), alocadas em duas famílias, Gripopterygidae e Perlidae (Pessacq *et al*., 2019). Cada uma delas possui quatro gêneros, sendo Gripopterygidae representada por *Gripopteryx* Pictet, 1841, *Paragripopteryx* Enderlein 1909, *Tupiperla* Froehlich, 1969 e *Guaranyperla* Froehlich, 2001, e Perlidae por *Anacroneuria* Klapálek, 1909, *Enderleina* Jewett, 1960, *Kempnyia* Klapálek, 1914 e *Macrogynoplax* Enderlein, 1909. Atualmente, existem pelo menos nove espécies novas sendo descritas para a família Gripopterygidae (*Gripopteryx, Paragripopteryx* e *Tupiperla*) e outras 15 para a família Perlidae (*Anacroneuria* e *Kempnyia*).

Após os trabalhos de Jewett (1959, 1960), Illies (1963, 1966), Froehlich (1969, 1984a, 1988, 1990, 1994, 1998) e Zwick (1972, 1973), o conhecimento sobre os plecópteros no Brasil, melhorou consideravelmente. Por outro lado, assim como para outras ordens de insetos aquáticos, algumas regiões do Brasil apresentam escassez de informações taxonômicas sobre a ordem Plecoptera. No Norte do Brasil, o conhecimento vem melhorando principalmente devido aos trabalhos de Ribeiro-Ferreira (1996), Froehlich (2002, 2003), Ribeiro-Ferreira & Froehlich (1999, 2001) e Ribeiro & Rafael (2005, 2007), Ribeiro & Gorayeb (2015, 2016), Hamada *et al*. (2016), Hamada & Silva (2019), Rippel *et al*. (2019a, 2019b) e Menezes *et al*. (2020). O Nordeste brasileiro apresenta trabalhos antigos (Navás, 1926; Klapálek, 1922) e novos estudos têm sido restritos, principalmente, ao Estado da Bahia (Lecci & Froehlich, 2011; Righi-Cavallaro *et al*., 2013; Duarte *et al*., 2014a; 2014b; Duarte & Lecci, 2016; Almeida & Duarte, 2017). O Centro Oeste, por sua vez, apresenta os trabalhos de Bispo *et al*. (2005, 2014), Bispo & Froehlich (2004, 2007), Froehlich (2007), Righi-Cavallaro & Lecci (2010) e Miguel *et al*. (2022). O Sudeste foi até o momento, a região mais estudada, principalmente o Estado de São Paulo, graças aos trabalhos

publicados pelo Prof. C. G. Froehlich e colaboradores (ver Froehlich, 2011b), além de trabalhos desenvolvidos no Estado do Espírito Santo (ver Gonçalves *et al*., 2019) e no Estado do Rio de Janeiro (Avelino-Capistrano *et al*., 2013; 2014; 2016). A região Sul foi pouco estudada, apresentando os trabalhos de Jewett (1959), Froehlich (2002), De Ribeiro & Froehlich (2007), Novaes & Bispo (2014a, 2014b) e Duarte *et al*. (2019). É provável que o número de espécies esteja subestimado, principalmente devido à ausência de trabalhos e registros para a ordem em alguns estados da região Norte e Nordeste.

Perlidae Latreille, 1802

Os perlídeos são representados por mais de 1100 espécies (Fochetti & Tierno de Figueroa, 2008; DeWalt & Ower, 2019), são encontrados em todas as regiões do mundo, com exceção da Australásia (DeWalt & Ower, 2019) e representam aproximadamente 30% de todas as espécies de Plecoptera (Stark & Gaufin, 1976). A família Perlidae faz parte da superfamília Perloidea, e segundo estudos recentes (Chen *et al*., 2018; Ding *et al*., 2019; South *et al*., 2021; Letsch *et al*., 2021), é grupo irmão de Chloroperlidae + Perlodidae. Este grupo é sustentado por apresentar imaturos predadores, de palpos longos, mandíbulas e lacínia delgadas, lacínia fortemente dentada e paraglossa maior que a glossa (Zwick, 2000).

Segundo Zwick (2000), dentre algumas sinapomorfias da família Perlidae estão: fusões em ambas as extremidades do cordão nervoso abdominal e apenas seis gânglios livres, ninfas com escleritos pilosos na base das brânquias, mento alargado cobrindo a parte inferior da maxila, gena expandida cobrindo a base das mandíbulas, paraglossa inflada e cabeça com gena cobrindo a base das mandíbulas.

Atualmente, a família Perlidae é dividida em duas subfamílias (Perlinae e Acroneuriinae) e seis tribos viventes. Perlinae é composta por Claasseniini, Neoperlini e Perlini, enquanto Acroneuriinae é dividida em Acroneuriini, Anacroneuriini e Kiotinini (DeWalt *et al*., 2022. No Brasil, todos os gêneros registrados estão alocados na subfamília Acroneuriinae Klapálek, 1914, tribo Anacroneuriini Stak & Gaufin, 1976. Acroneuriinae reúne 30 gêneros viventes, os quais são agrupados de acordo com os seguintes caracteres: epiprocto geralmente ausente, paraproctos dos machos esclerosados e curvados sobre o tergo 10, nono esterno geralmente apresentando um martelo nos machos e linha de espinhos occipitais incompleta, irregular ou ausente nas ninfas (Stark & Gaufin, 1976). A tribo Anacroneuriini, por sua vez, é representada por seis gêneros, todos registrados na América do Sul. Os indivíduos dessa tribo são marcados pelo martelo normalmente elevado nos machos (ausente em alguns casos) e armadura penial geralmente com um par de ganchos proeminentes (Stark & Gaufin, 1976).

Perlidae no Brasil e os déficits em seu conhecimento

No Brasil, a família é representada por 152 espécies (Froehlich, 2011c; 2012; Pessacq *et al*., 2019) distribuídas em quatro gêneros: 1) *Anacroneuria* Klapálek, 1909 possui 97espécies no país e ampla distribuição (Pessacq *et al.*, 2019; Rippel *et al*., 2019; Carvalho *et al*., 2020; Menezes *et al*., 2020; Miguel *et al*., 2022), sendo também o gênero com a maior diversidade na região Neotropical (Froehlich, 2012; Pessacq *et al*., 2019); 2) *Enderleina* Jewett, 1960 possui quatro espécies descritas para o Brasil com distribuição restrita à região amazônica (Ribeiro & Gorayeb, 2016; Hamada *et al*., 2016; Hamada & Silva, 2019); 3) *Kempnyia* Klapálek, 1914 é o único dos gêneros de Perlidae endêmico do Brasil, possuindo 36 espécies válidas (Avelino-

Capistrano *et al*., 2016; Almeida & Bispo, 2020) e outras seis ainda não formalmente descritas, mas que constam descritas na tese de doutoramento de Lucas S. Lecci (Lecci, 2013); e 4) *Macrogynoplax* Enderlein, 1909 ocorre principalmente na região norte do Brasil e possui oito espécies registradas no país (Ribeiro & Gorayeb, 2016; Menezes *et al*., 2020).

Anacroneuria foi proposto por Klapálek (1909) sem designação de espécie tipo (Stark *et al.*, 2009). Posteriormente, nove espécies foram descritas por Klapálek (1921), sendo *A. albimacula* a primeira a ser descrita (Stark *et al.*, 2009). Atualmente, algumas espécies do gênero *Perla* Geoffroy fazem parte do gênero *Anacroneuria*. Além disso, os gêneros *Forquilla* Navás, 1924 e *Coeloperla* Navás, 1936 são sinônimos de *Anacroneuria* (Illies, 1966; Froehlich, 2004; Stark *et al*., 2009). *Anacroneuria* se distribui em quase toda a Região Neotropical, desde o Uruguai e norte da Argentina até o norte do México, com registros isolados de duas espécies no sul dos Estados Unidos. No Brasil, o gênero pode ser encontrado em todas as regiões, sendo o gênero com distribuição mais ampla no país (Pessacq *et al*., 2019).

O gênero *Enderleina* foi proposto por Jewett (1960) com *E. preclara* como espécie tipo (Stark *et al.*, 2009). *Enderleina* é considerado um gênero raro de se encontrar em coleções (Stark *et al*., 2009), provavelmente um dos mais raros de se encontrar e coletar na natureza. O gênero se distribui principalmente na região amazônica, ocorrendo, ao que se sabe até o presente momento, na Venezuela e nos estados brasileiros do Amazonas, Amapá e Roraima (Pessacq *et al*., 2019).

Kempnyia foi proposto por Klapálek (1914), sendo *Kempnyia tenebrosa* Klapálek, 1916 a espécie tipo (Stark *et al.*, 2009). Atualmente, os gêneros *Eutactophebia* Klapálek, 1914, *Collampla* Navás, 1929, *Diperla* Navás, 1936, *Forca* Navás, 1925, *Laessia* Navás, 1934 e *Nedanta* Navás, 1932 são sinônimos de *Kempnyia* (Illies, 1966; Jewett, 1960; Zwick, 1983; Froehlich, 1988; Stark, 2001; Stark *et al*., 2009). *Kempnyia* se distribui principalmente associado

ao clima subtropical (principalmente na Mata Atlântica Costeira e em enclaves isolados em regiões montanhosas de Cerrado), ocorrendo desde as regiões Sul e Sudeste do país até áreas montanhosas isoladas no Brasil central e regiões costeiras do Estado da Bahia (Pessacq *et al*., 2019; Rippel *et al*., 2019).

Macrogynoplax foi proposto por Enderlein (1909) com *M. guayanensis* como espécie tipo, a qual foi redescrita por Zwick (1973). Apesar do gênero ter sido descrito no início do século XX, ele permaneceu pouco estudado e com apenas uma espécie até o trabalho de Froehlich (1984), no qual descreve *M. veneranda* para o sudeste do Brasil. A partir de então, outros trabalhos foram realizados, melhorando o conhecimento sobre o gênero, o qual atinge maior diversidade na região amazônica (Froehlich, 2010; Ribeiro & Santos, 2018). *Macrogynoplax* se distribui principalmente associado a região da Floresta Amazônica, com pontos no Peru, Colômbia, Venezuela, Guiana, Suriname e nos estados brasileiros do Amazonas, Amapá, Pará, Roraima e Tocantins. Além disso, pode ser encontrado também nos estados do Espírito Santo, Mato Grosso e São Paulo (Pessacq *et al*., 2019; Gonçalves *et al*., 2019; Rippel *et al*., 2019; Menezes *et al*., 2020).

Como mencionado anteriormente, Perlidae é a família mais diversa dentro de Plecoptera, sendo também a que possui mais representantes na América do Sul e no Brasil. Porém, apesar do relativo bom conhecimento sobre a família no Brasil, os perlídeos começaram a ser regularmente estudados apenas após a década de 1980 com os trabalhos do Professor Claudio G. Froehlich. Por tratar-se de uma taxonomia recente, é esperado que ainda exista muito a ser estudado sobre os perlídeos brasileiros. Neste contexto, é possível observar para essa fauna a presença dos déficits no conhecimento sobre a biodiversidade listados por Hortal *et al*. (2015) e Faria *et al*. (2020). Dentre os quais, os déficits Linneano (limitação do conhecimento sobre as espécies existentes),

Wallaceano (limitação do conhecimento sobre a distribuição geográfica das espécies) e o Haeckeliano (limitação do conhecimento sobre os estágios de vida), os quais são os mais tradicionais e também frequentemente presentes em estudos taxonômicos de grupos com histórico recente de estudos, como é o caso dos perlídeos brasileiros. Diante disso, aqui, nós estudamos os perlídeos brasileiros com os objetivos de descrever novas espécies, realizar novos registros geográficos, discutir as variações morfológicas observadas e catalogar e organizar o conhecimento acerca de todas as espécies ocorrentes no Brasil.

A presente tese foi organizada em quatro capítulos, a saber:

Capitulo 1: New species and records of *Anacroneuria* Klapálek, 1909 (Plecoptera: Perlidae) from Brazil;

Capítulo 2: New species and records of *Kempnyia* Klapálek, 1914 (Plecoptera: Perlidae) from Brazil;

Capítulo 3: A new species of *Enderleina* Jewett, 1960 and new records of *Macrogynoplax* Enderlein, 1909 (Plecoptera: Perlidae) from Brazil;

Capítulo 4: Perlidae (Plecoptera) from Brazil: diversity, sampling effort, research status, challenges, and perspectives.

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Capítulo 1

New species and records of *Anacroneuria* Klapálek, 1909 (Plecoptera: Perlidae) from Brazil

New species and records of *Anacroneuria* Klapálek, 1909 (Plecoptera: Perlidae) from Brazil

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Abstract

In this paper, we studied Brazilian *Anacroneuria* from Aquatic Biology Laboratory (UNESP, Assis) and Entomology Museum of the Federal University of Viçosa (UFVB, Viçosa) collections. We presented species occurrence maps highlighting the new records, discussed important morphological variations of some species, and described two new species *A. duarte* sp. nov. and *A. sallesi* sp. nov. The new species described and the new geographic records presented here reduce the Linnean and Wallacean deficits for Brazilian *Anacroneuria* (Plecoptera: Perlidae).

Keywords: Biodiversity; aquatic insects; freshwater; stoneflies; distribution.

Introduction

Plecoptera (stoneflies) has about 3700 species described into 17 families that are distributed in all regions of the world, except Antarctica (DeWalt & Ower, 2019; South *et al*., 2021). Perlidae Latreille, 1802 is the most diverse family among the stoneflies, about 30% of all species, and can be found in the Neartic, Paleartic, Afrotropical, and Neotropical regions (DeWalt & Ower, 2019). Currently, about 400 species of Perlidae are known in the Neotropical region (Pessacq *et al.*, 2019). Despite the high number of species already described, the rate of species descriptions remains growing year by year in the Neotropical region (Pessacq *et al.*, 2019; DeWalt & Ower, 2019). In Brazil, the perlids has been consistently studied only since the 1980s (e.g. Froehlich, 1984a; 1984b; 1988; 1996; Dorvillé & Froehlich, 1997; 2001). However, only in the early 2000s, the genus *Anacroneuria* Klapálek, 1909, the most specious among the genera of Perlidae, began to be constantly studied (e.g. Ribeiro-Ferreira & Froehlich, 2001; Froehlich, 2002; 2003; 2004; 2007; 2008; 2010a; Bispo & Froehlich, 2004a; 2004b; Avelino–Capistrano *et al*., 2014; Novaes & Bispo, 2014a; 2014b; 2014c; Gonçalves *et al*., 2017; 2019; Almeida *et al*., 2018; 2019; Almeida & Bispo, 2020; Miguel *et al*., 2022).

Anacroneuria is distributed from the southern United States of America to northern Argentina and has more than 330 species described in Neotropical region, at least 97 occurring in Brazil (Pessacq *et al.*, 2019; Rippel *et al*., 2019; Carvalho *et al*., 2020; Menezes *et al*., 2020; Miguel *et al*., 2022). Although *Anacroneuria* from southeastern Brazil have been constantly studied (e.g. Froehlich, 2004; Bispo & Froehlich, 2004a; Froelhich, 2010a; Avelino–Capistrano *et al*., 2014; Novaes & Bispo, 2014a; 2014b; 2014c; Gonçalves *et al*., 2017; 2019; Almeida *et al*., 2018; 2019), efforts have been made to sample and study *Anacroneuria* from other regions, such

as northern (e.g. Ribeiro-Ferreira & Froehlich, 2001; Froehlich, 2003; Ribeiro & Rafael, 2009; Ribeiro & Gorayeb, 2014; Rippel *et al*., 2019; and Menezes *et al*., 2020) and center-western regions (e.g Bispo *et al*., 2005; 2014; Froehlich, 2007; Righi-Cavallaro & Lecci, 2010; Miguel *et al*., 2022).

Streams are systems heavily impacted by human activities, so it is urgent that the aquatic insect fauna of these environments be described and recorded. This is especially important for Plecoptera since these insects generally reach greater diversity in preserved streams with well-oxygenated clean waters. In Brazil, the genus *Anacroneuria* is distributed throughout the country, but its study is recent (Froehlich, 2002) and much taxonomic and distributional information remains unknown. Reducing the traditional Linnean (ignorance of the totality of existing species; Brown & Lomolino, 1998) and Wallacean (lack of knowledge of the geographic distribution of the species; Lomolino, 2004) shortfalls for this specious genus is fundamental and can provide a solid foundations for studies on semaphoronts, biology, phylogeny, biogeography, and conservation of these organisms.

In this paper, Brazilian *Anacroneuria* from some important collections were studied. Herein, we discuss important morphological variations of some species, document several new records, and describe two new species.

Material e methods

Collection of specimens. Using white sheet and light pan trap with UV and white lamps (Calor & Mariano, 2012), the specimens were collected in several localities of Brazil. The materials collected before 2014 were conditioned in 80% ethanol in an air-conditioned room

(17ºC). The material collected from 2014 onwards was preserved in absolute ethanol in a freezer (-20ºC). The materials studied are deposited in the Aquatic Biology Laboratory Collection (CLBA - UNESP, Assis), Entomology Museum of the Federal University of Viçosa (UFVB, Viçosa), and Museum of Zoology of the University of São Paulo (MZUSP, São Paulo). Holotypes of new species will be deposited in the MZUSP and UFVB.

Identification. For each male adult studied, the abdomen was severed between segments seven and eight and treated with 10% KOH overnight. Then, to neutralize the reaction, the abdomen was placed in acetic acid and washed with 80% ethanol. We extracted the penial armature and identified the species by comparing the morphology of male penial armature with those described in the literature. After the dissecation, the penial armature was illustrated using lucida camera mounted on a Leica DM1000 microscope and rendered in Adobe Illustrator CS6® editor. The images were taken using a digital camera on a Leica M205A stereomicroscope and edited in Adobe Photoshop CS3® editor. The species distribution maps were made using QGIS Bucur 3.14.15 software (QGIS Development Team, 2020).

Collectors. Almeida – Lucas Henrique de Almeida; Amorim – Dalton Amorim; Barreto – Hamilton Barreto; Blahnik – Roger Blahnik; Bravo – Freddy Bravo; Calor – Adolfo Calor; Campos – Rogério Campos; Dias – Everton Santos Dias; França – Diogo França; Gonçalves – Maísa de Carvalho Gonçalves; Guillermo–Ferreira – Rhainer Guillermo–Ferreira; Holzenthal – Ralph Wayne Holzenthal; Lecci – Lucas Lecci; Mariano – Rodolfo Mariano; Mendes – Humberto Fonseca Mendes; Lamas – Carlos José Einicker Lamas; Menezes – Eliomar Cruz Menezes; Moretto – Rafael Moretto; Nihei – Silvio Shigueo Nihei; Novaes – Marcos Carneiro Novaes; Oliveira – Jader Oliveira; Paprocki – Henrique Paprocki; Pinho – Luiz Carlos de Pinho; Polegatto – Cleber Macedo Polegatto; Prather – Aysha Prather; Quinteiro – Fábio Batagini

Quinteiro; Roque – Fabio de Oliveira Roque; Salles – Frederico Falcão Salles; Silva–Neto – Alberto Moreira Silva–Neto; Vilarino – Albane Vilarino.

Results and discussion

Anacroneuria bahiensis Righi–Cavallaro & Lecci, 2013 (Fig. 1)

Anacroneuria bahiensis Righi–Cavallaro & Lecci, 2013 (in Righi–Cavallaro *et al*., 2013): 3, male description; Duarte & Lecci, 2016: 293, record.

Material examined. BR, BA: Igatu, Rio Coisa Boa, 16.v.2015, Calor *et al*. col., 1 male.

Measurement data. Male (n=1) forewing length: 10.3 mm.

Remarks. The studied specimen presented variation only in the body size when compared to the original description. Our specimen is greater than those studied by Righi–Cavallaro *et al*. (2013) (7.5–8.6 mm). Until the present moment, this species has been recorded only in the Bahia state (Righi–Cavallaro *et al*., 2013; Duarte & Lecci, 2016) (Fig. 1).

Anacroneuria boraceiensis Froehlich, 2004 (Fig. 1)

Anacroneuria boraceiensis Froehlich, 2004: 54, description; Bispo & Froehlich, 2004a: 103, record; Froehlich, 2010a: 149, catalog; Novaes & Bispo, 2014c: 434, record; Almeida & Bispo, 2020: 10, picture.

Material examined. BR, SP: São Luiz do Paraitinga, Parque Estadual da Serra do Mar, Núcleo Santa Virgínia, Ribeirão Barro Branco, 20.i.2006, Bispo *et al*., col., 1 male.

Measurement data. Male (n=1) forewing length: 10.9 mm.

Remarks. This species has been recorded only in the Minas Gerais and São Paulo states (Froehlich, 2004; Bispo & Froehlich, 2004a; Novaes & Bispo, 2014c). Herein, we expanded the species distribution in the São Paulo state (Fig. 1).

***Anacroneuria cathia* Froehlich, 2002** (Fig. 1)

Anacroneuria cathia Froehlich, 2002: 91, description; De Ribeiro & Froehlich, 2007: 55, record; Froehlich, 2010a: 151, catalog; Novaes & Bispo, 2014a: 459, record and illustrations; Romero, 2017: 46, record.

Material examined. BR, PR: Juvinópolis, Sítio Rodrigues, 21.iv.2006, 1 male.

Measurement data. Male (n=1) forewing length: 12 mm.

Remarks. The studied specimen showed light spots on the head and pronotum greater than the holotype (Froehlich, 2002). On the other hand, the medium brown spots on the head are similar, mainly below the M–line. In lateral view, the apex of the penial armature is totally vertical when compared to the holotype, which is inclined (Froehlich, 2002). This species has been recorded in Argentina and in Brazil, in Rio Grande do Sul and Santa Catarina states (Froehlich, 2002; De Ribeiro & Froehlich, 2007; Novaes & Bispo, 2014c) and now in the Paraná state (Fig. 1).

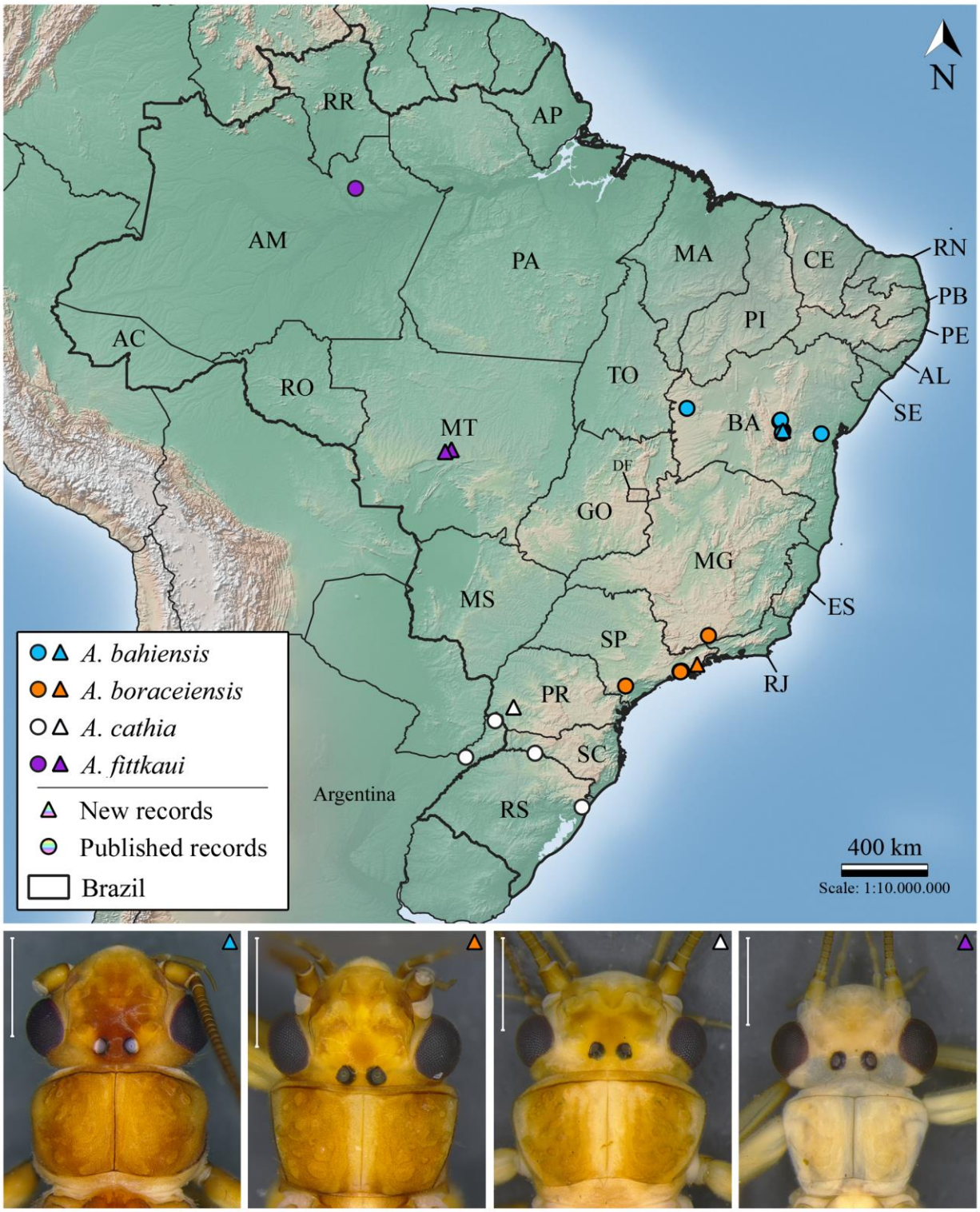

Figure 1. Occurrence map and specimens of *Anacroneuria bahiensis*, *A. boraceiensis*, *A. cathia*, *A. fittkaui*. Scale: 1 mm. Brazilian states acronyms: Acre - AC; Alagoas - AL; Amazonas - AM; Amapá - AP; Bahia - BA; Ceará - CE; Distrito Federal - DF; Espírito Santo - ES; Goiás - GO; Maranhão - MA; Mato Grosso - MT; Mato Grosso do Sul - MS; Minas Gerais - MG; Paraíba - PB; Paraná - PR;

Pernambuco - PE; Piauí - PI; Rondônia - RO; Rio de Janeiro - RJ; Rio Grande do Norte - RN; Rio Grande do Sul - RS; Roraima - RR; Sergipe - SE; Santa Catarina - SC; São Paulo - SP; Tocantins - TO.

***Anacroneuria debilis* (Pictet, 1841)** (Fig. 2)

Perla (*Perla*) *debilis* Pictet, 1841: 255, description; *Anacroneuria debilis* Zwick, 1972: 1155, new combination and holotype illustration; Zwick, 1973: 486, illustration; Froehlich, 2002: 76, illustration; De Ribeiro & Froehlich, 2007: 55, record; Froehlich, 2010a: 154, catalog; Froehlich, 2010b: 56, record; Avelino–Capistrano *et al*., 2011, nymph description; Baldin *et al*., 2013: 392, illustration; Bispo *et al*., 2014, illustration and picture; Novaes & Bispo, 2014a: 459, illustration and picture; Novaes & Bispo, 2014c: 434, record; Duarte & Lecci, 2016: 293, record; Novaes *et al*., 2016: 95, record; Almeida & Duarte, 2017: 481, picture; Gonçalves *et al*., 2017: 146, record; Romero, 2017: 46, record; Rippel *et al*., 2019: 359, record; Almeida & Bispo, 2020: 11, male illustration, COI sequences, and pictures.

Material examined. BR, AL: Quebrangulo, Bica da Juliana, 10.xi.2014, 1 male. **BA**: Andaraí, Chapada Diamantina, Vale do Patí, Ponte p/ Dona Linda, 12º47’46.7”S, 41º25’04”W, 681 m, 17.vii.2015, Calor col., 1 male; Entroncamento p/ Dona Raquel, 12º46’46.3”S, 41º27’27”W, 919 m, 17.vii.2015, Calor col., 4 males. Camacan, RPPN Serra Bonita, iv.2009, 1 male; Fazenda Waldemar da Farmácia, 28.iii.2011, Calor, Quinteiro, França & Barreto col., 4 males. Igatu, Rio Coisa Boa, 13.v.2010, 2 males. Lençóis, Parque Nacional da Chapada Diamantina, Rio Ribeirão, 12º35’17”S, 41º22’56.3”W, Calor & Mariano col., 1 male. Palmeiras, Conceição dos Gatos, Cachoeira do Zezão, 08.vi.2019, 1 male. Uruçuca, Distrito de Serra Grande, Parque Estadual da Serra do Conduru, 19.i.2014, Dias col., 4 males. **ES**: Santa Marta, Parque Nacional do Caparaó,

17–18.ii.2016, 1 male. Santa Teresa, REBIO Augusto Ruschi, Córrego da Estrada, 20–21.ii.2018, Salles & Gonçalves col., 2 males. Mucugê, Parque Municipal de Mucugê, Córrego Boiadeiro, 12°59'46.5"S, 41°19'39.9"W, 10.i.2015, Dias & Campos col., 1 male. Wenceslau Guimarães, Estação Ecológica Wenceslau Guimarães, Riacho Patioba, 13º34’43.9”S, 39º42’09.3”W, 07.xi.2013, Calor col., 2 males. **MG**: Cabo Verde, Fazenda da Cata, clareira, 21º27’11”S, 46º20’52”W, 17.ii–13.v.2014, Amorim col., 2 males. Ipoema, Serra do Cipó, Fazenda Cachoeira Alta, Córrego abaixo Cachoeira Alta, 19º34’46”S, 43º29’34”W, 838 m, 11.viii.2008, Calor, Lecci, Pinho & Moretto, 3 males. Nova Lima, Mata, 24.i–07.ii.2016, Vilarino col., 1 male. Uberlândia, Mata Rancho amigo, 21.x.2018, Guillermo–Ferreira col., 6 machos. **PR**: Foz do Iguaçu, Parque Nacional de Foz do Iguaçu, Trilha do Macuco, 11.x.2017, 5 males. **RJ**: Itatiaia, Parque Nacional do Itatiaia, Córrego Taquaral, 22º27’07”S, 44º36’34”W, 06.x.2017, 810 m, Dias & Campos col., 1 male; Córrego Campo Belo, 22º25’36.8”S, 44º37’06.8”W, 798 m, 06.xi.2017, Dias & Campos col., 2 males. **SP**: São Luis do Paraitinga, Parque Estadual da Serra do Mar Núcleo Santa Virgínia, Ribeirão Barro Branco, 20.i.2006, Bispo *et al*. col., 1 male. Pedregulho, Ribeirão São Pedro, 20°09'13.5"S, 47°30'38.3"W, 620 m, 17.ix.2019, Almeida col., 1 male. Ribeirão Preto, Reserva Santa Teresa, 2–x–03.xi.2006, Polegatto col., 3 males.

Measurement data. Male (n=49) forewing length: 8.9–14.5 mm (mean=11.28 mm).

Remarks. It is the first time that *A. debilis* was extensively studied. We found five morphotypes of penial armature from specimens identified as *A. debilis*. In dorsal view, the keel was the most variant morphological characteristic in the penial armature (Fig. 2). We found keels rounded (green group), transverse pointed (blue group), transverse being weak or strongly double pointed (red group), rounded with V-shaped invagination (yellow group), and a small transverse almost square keel (pink group) (Fig. 2). In addition, the pink group specimens presented an additional

and important variation in the apex of penial armature, which is longer and inclined in lateral view. The shape of the apex of the penial armature also varied, being thin in the green group, very similar between the blue and red groups, with a broad base in the yellow group and with medial constriction in the pink group (Fig. 2). The pattern of spots on the head and pronotum of the species also showed some cohesion in relation to the groups. In the green, red and pink groups, the M line is well delimited, differing from each other by the presence of a light spot in the center of the pronotum of specimens in the green and pink groups, being wider in the pink group. The blue group presented a light spot in the shape of a tip in the center of the M line. The yellow group presented a visible M line, but it was slightly delimited (Fig. 2). In fact, we concluded that *A. debilis* may be a complex of cryptic species. Our observations are a first step towards understanding the delimitation of this species. It is necessary to study the species using an integrative approach, including, mainly, molecular and nymph data in order to better understanding the variability of the specimens currently identified as *A. debilis*.

***Anacroneuria fittkaui* Froehlich, 2003** (Fig. 1)

Anacroneuria fittkaui Froehlich, 2003: 131, description; Froehlich, 2010a: 155, catalog.

Material examined. BR, MT: Nova Mutum, Fazenda Buritis, 07.xii.1997, Mendes col., 1 male; 13.xii.1997, Mendes col., 1 male; Rio Arinos, 12.i.2000, Mendes col., 4 males.

Measurement data. Male (n=6) forewing length: 9.2–10.1 mm (mean=9.83 mm).

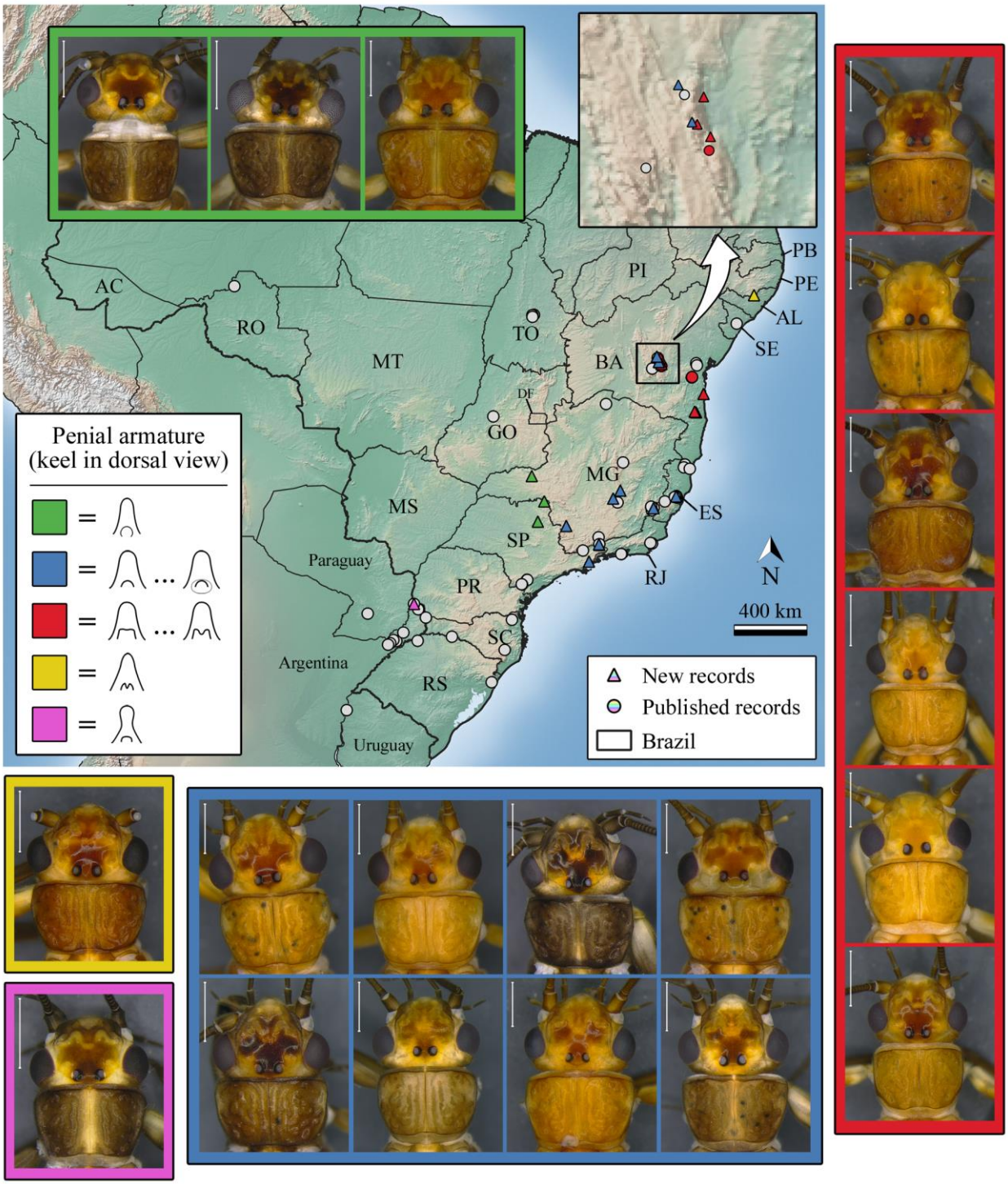

Figure 2. Occurrence map and specimen variations of *Anacroneuria debilis*. Scale: 1 mm. Grey circles: geographic records based on literature.

Remarks. The studied specimens are in agreement with the holotypes of *A. fittkaui* and *A. terere* Righi–Cavallaro & Lecci, including the keel variation of the penial armature in lateral view

(Froehlich, 2003; Righi–Cavallaro *et al*., 2013). We decided to consider the studied specimens belonging to *A. fittkaui*. This species has been recorded in the Amazonas state (Froehlich, 2003) and now in the Mato Grosso state (Fig. 1).

***Anacroneuria flintorum* Froehlich, 2002** (Fig. 3)

Anacroneuria flintorum Froehlich, 2002: 93, description; Bispo & Froehlich, 2004a: 99, female description and male illustration; De Ribeiro & Froehlich, 2007: 55, record; Froehlich, 2010a: 156, catalog; Froehlich, 2010b: 56, illustration; Baldin *et al*., 2013: 394, record; Gonçalves *et al*., 2017: 146, record; Almeida *et al*., 2018: 413, distribution, nymph description, pictures and COI sequences; Almeida & Bispo, 2020: 12, record.

Material examined. BR, MG: Cabo Verde, Fazenda da Cata, clareira, 17.ii–13.v.2014, Amorim col., 1 male. **SP**: Parque Nacional da Serra da Bocaina, Cachoeira dos Posses, 22°46.437'S, 44°36.250'W, 1250 m, 03.ii.2002, Holzenthal, Blahnik, Paprocki & Prather col., 5 males.

Measurement data. Male (n=6) forewing length: 9.8–13 mm (mean=11.96 mm).

Remarks. The color and spots on the head of the studied specimens are in agreement with the holotype description (Froehlich, 2002) and variations described for other populations (Almeida *et al*., 2018). The specimen from Cabo Verde (Minas Gerais state) is smaller than the other ones (9.8 mm). The penial armature of the specimens from Parque Nacional da Serra da Bocaina (São Paulo state) is robust and morphologically similar to those of the specimens from north coast of São Paulo state (Froehlich, 2010b) and populations from Espírito Santo and Rio de Janeiro states (Baldin *et al*., 2013; Gonçalves *et al*., 2017). Although penial armatures can be divided between

robust and thin, molecular information and other morphological characters support *A. flintorum* as a single species (Almeida *et al*., 2018). Herein, we expanded the species distribution (Fig. 3).

***Anacroneuria itajaimirim* Bispo & Froehlich, 2004** (Figs 4A–F and 5)

Anacroneuria itajaimirim Bispo & Froehlich, 2004a: 105, description; Froehlich, 2010a: 159, catalog; Almeida & Bispo, 2020: 15, pictures and COI sequences.

Material examined. BR, SP: Cananéia, Córrego da Gruta, ii.2003, Roque col., 1 male.

Measurement data. Male (n=1) forewing length: 10 mm.

Remarks. The studied specimen is in agreement with the holotype described by Bispo & Froehlich (2004), presenting only a keel of the penial armature pointing downwards in lateral view and a pattern of three concentric lines in dorsal view (Fig. 4). This is the first record of the species outside the Serra de Paranapiacaba (Bispo & Froehlich, 2004a; Almeida & Bispo, 2020) (Fig. 5).

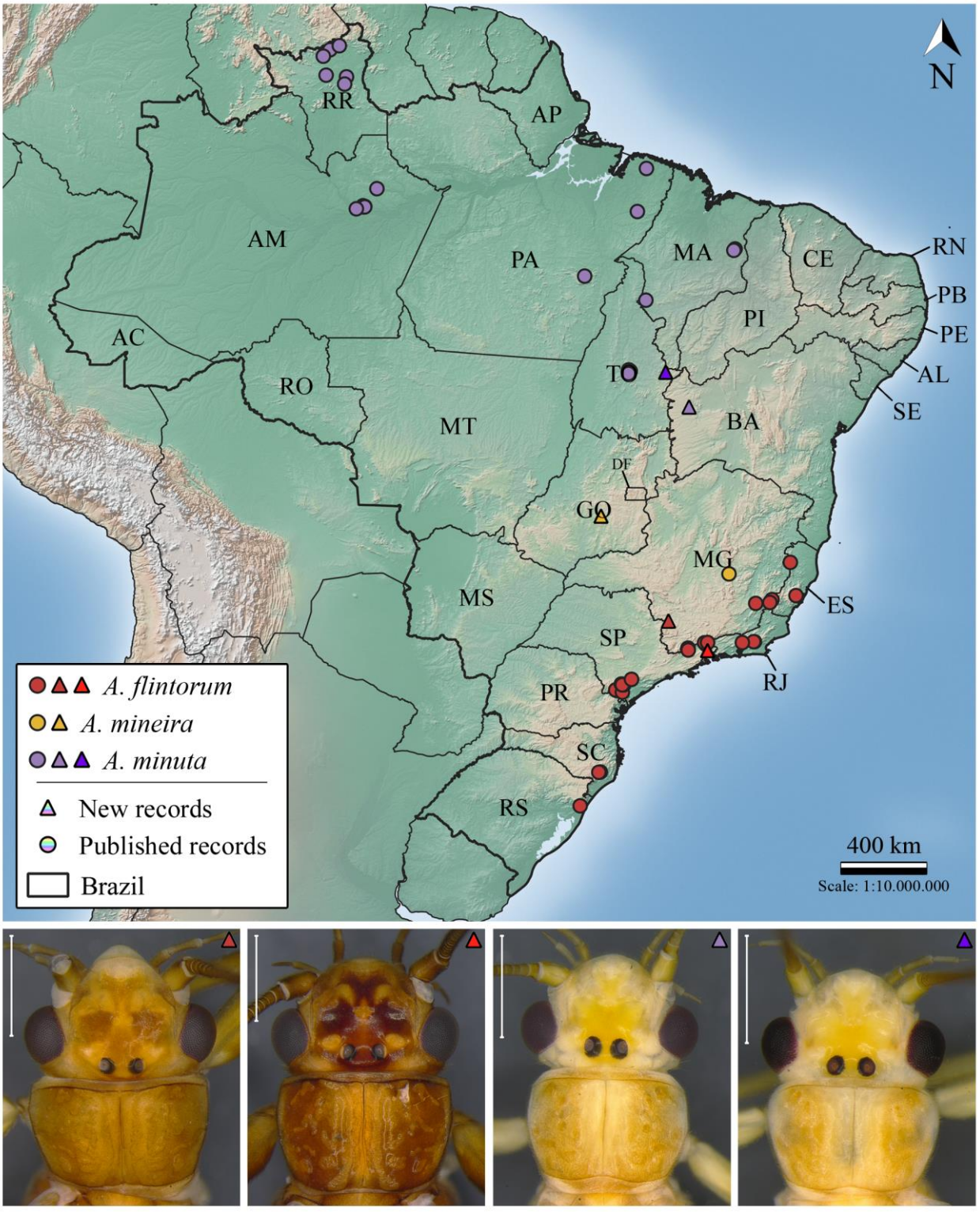

Figure 3. Occurrence map and specimens of *Anacroneuria flintorum*, *A. mineira*, and *A. minuta*. Scale: 1 mm.

***Anacroneuria marlieri* Froehlich, 2001** (Fig. 6)

Anacroneuria marlieri Froehlich, 2001 (in Ribeiro–Ferreira & Froehlich, 2001): 191, description; Froehlich, 2003: 130, record; Ribeiro & Rafael, 2009: 7, redescription; Froehlich, 2010a: 161, catalog; Ribeiro & Gorayeb, 2014: 19, nymph description; Rippel *et al*., 2019: 360, record; Menezes *et al*., 2020: 468, record; Carvalho *et al*., 2020: 439, illustration.

Material examined. BR, MT: Nova Xavantina, Fazenda Buriti, 07.i. 2000, Mendes col., 1 male.

Measurement data. Male (n=1) forewing length: 9.7 mm.

Remarks. The specimen is in agreement with the neotype (Ribeiro & Rafael, 2009) in terms of body size, general color, pattern of spots on the head and pronotum, and color pattern of the veins in the forewings. Stark (1995) created a complex of species formed by *A. cushueme* Stark & Gill, 2012 (in Stark *et al*., 2012), *A. pakitza* Stark & Sivec, 1998, *A. pinza* Stark, 1995, *A. vistosa* Stark, 1995, *A. yameo* Stark & Sivec, 1998 and *A. zunigae* Stark, 2001 as a way to discuss the delimitation of these species. All these species have penial armature strongly similar to that of *A. marlieri* and *A. pictipes* Klapálek, 1923. In this context, we included the previously mentioned species and *A. marlieri* and *A. pictipes* in the discussion. Despite the morphological characteristics of penial armature used by Stark (1995) to separate the species that he described, we are sure that it is necessary to study them in detail in order to understand their delimitation, mainly using molecular tools, including A. marlieri, and *A. pictipes*. This is evident when we compare the disjunct or concentrated distribution of some of these species (Fig. 6). *A. marlieri* has been recorded in the Amazonas, Amapá, Maranhão, Mato Grosso, Pará, Roraima and Tocantins states (Ribeiro & Santos, 2018; Rippel *et al*., 2019; Carvalho *et al*., 2020; Menezes *et*

al., 2020) (Fig. 6). On the other hand, the other species forming the complex have been recorded in the Amazon Forest of Bolivia (*A. yameo*), Colombia (*A. yameo*), Ecuador (*A. cushueme*, *A. zunigae*), Peru (*A. pakitza*, *A. yameo*), and Venezuela (*A. pinza*, *A. vistosa*) (Pessacq *et al*., 2019). Herein, we expanded the distribution of *A. marlieri* in the Mato Grosso state (Fig. 6).

***Anacroneuria mineira* Novaes & Bispo, 2014** (Fig. 3)

Anacroneuria mineira Novaes & Bispo, 2014c: 433, description.

Material examined. BR, GO: Campinas (current Goiânia), 1925, R. Spitz col., 1 male (MZUSP/#117).

Measurement data. Male (n=1) forewing length: 15.3 mm.

Remarks. The studied specimen is in agreement with the original description, showing no variation in the penial armature. This species has been recorded only in the Goiás and Minas Gerais states (Novaes & Bispo, 2014c; Miguel *et al*., 2022). Here, we expanded the species distribution in the Goiás state (Fig. 3).

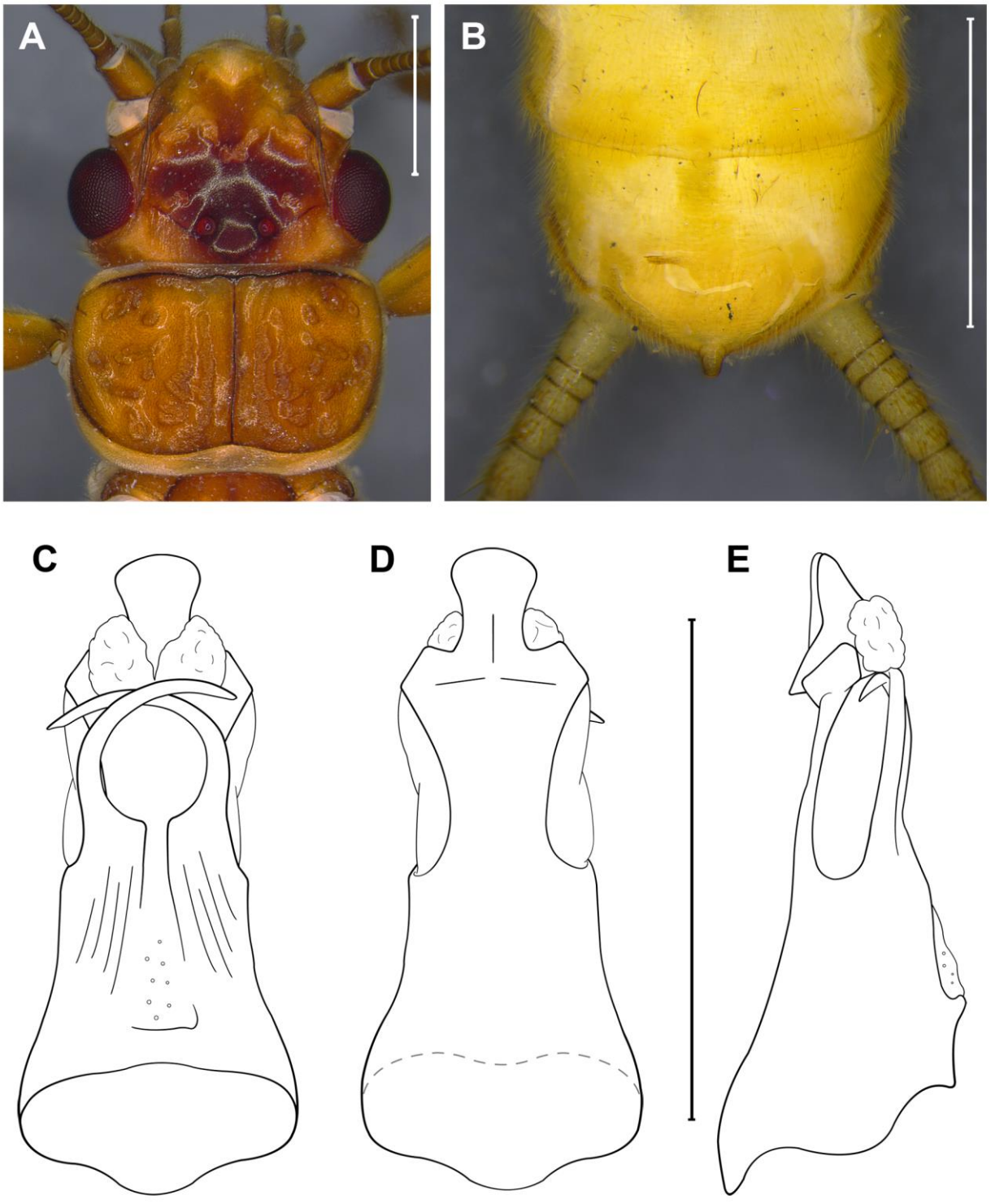

Figure 4. *Anacroneuria itajaimirim*. Adult male, head and pronotum (A) and hammer (B). Penial armature in dorsal (C), ventral (D), and lateral views (E). Scale: 1 mm.

***Anacroneuria minuta* Klapálek, 1922** (Fig. 3)

Anacroneuria minuta Klapálek, 1922: 89, description; Ribeiro–Ferreira & Froehlich, 2001: 188, redescription; Froehlich, 2002: 80, illustration; Ribeiro & Rafael, 2009: 2, redescription; Froehlich, 2010a: 162, catalog; Ribeiro & Gorayeb, 2014: 23, nymph description; Rippel *et al*., 2019: 360, record and picture; Menezes *et al*., 2020: 468, record; Carvalho *et al*., 2020: 439, record.

Material examined. BR, BA: Barreiras, Cachoeira do Redondo, 11º53’S, 45º25’W, 573 m, 05.vi.2008, Bravo, Menezes & Silva–Neto col., 1 male. **TO**: Mateiros, Parque Estadual do Jalapão, Cachoeira da Formiga, 10º20’05.8”S, 46º28’23.1”W, 461 m, 17.x.2008, Calor & Mariano col., 1 male.

Measurement data. Male (n=2) forewing length: 8.8–9.4 mm (mean=9.1 mm).

Remarks. The studied specimens are in agreement with the redescribed lectotype (Ribeiro–Ferreira & Froehlich, 2001) in color and pattern of spots on the head and pronotum. However, in the specimens studied, the depression below the keel of the penial armature, in lateral view, is less abrupt and profound than in the specimens redescribed (Ribeiro–Ferreira & Froehlich, 2001), with present a slight variation in this caracter. This species has been recorded in the Amazonas, Maranhão, Pará, Roraima, and Tocantins states (Ribeiro & Santos, 2018; Rippel *et al*., 2019; Carvalho *et al*., 2020; Menezes *et al*., 2020) and now in the Bahia state (Fig. 3).

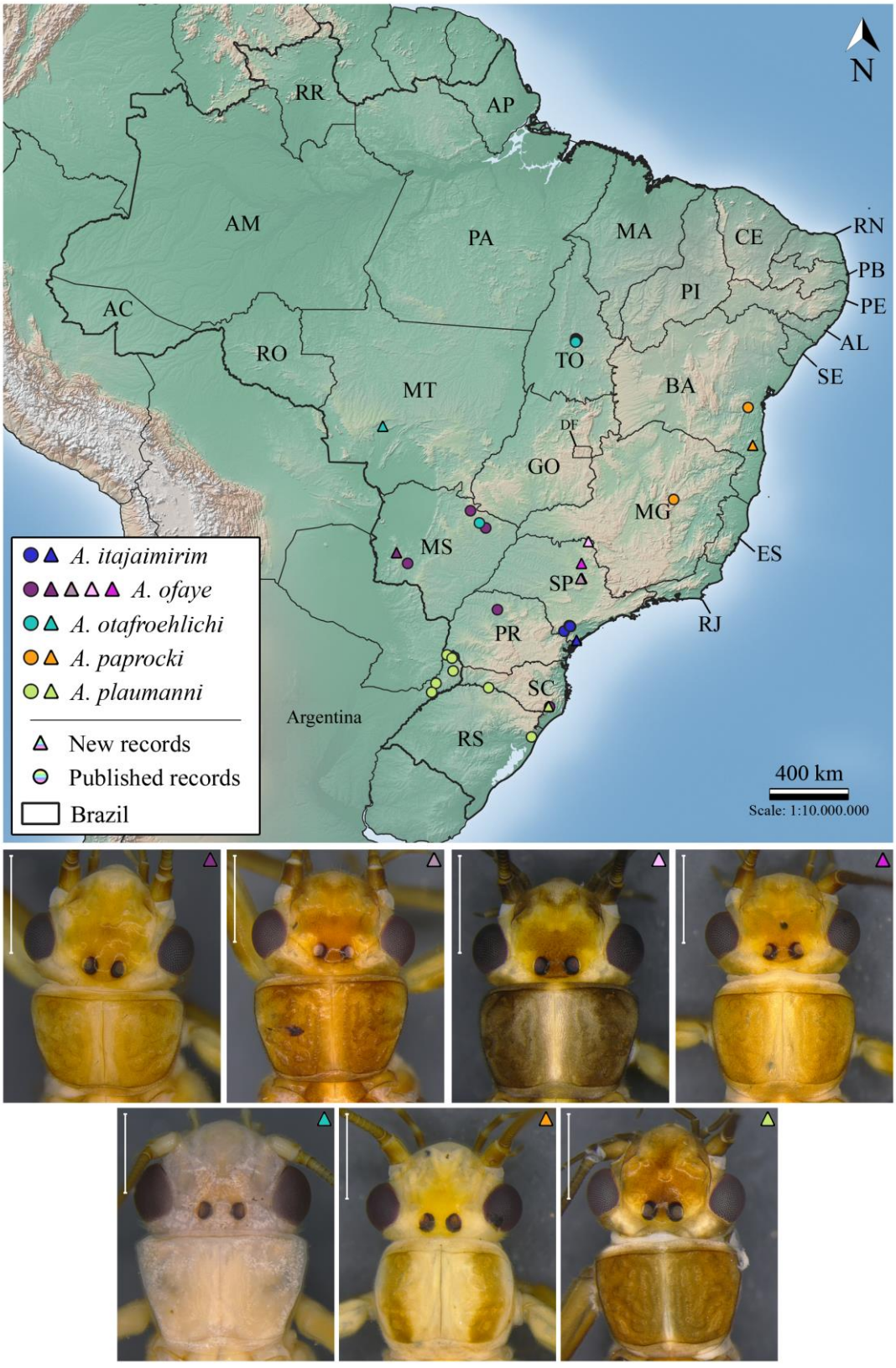

Figure 5. Occurrence map and specimens of *Anacroneuria itajaimirim*, *A. ofaye*, *A. otafroehlichi*, *A. paprocki*, and *A. plaumanni*. Scale: 1 mm.

***Anacroneuria ofaye* Froehlich, 2007** (Fig. 5)

Anacroneuria ofaye Froehlich, 2007: 16, description; Froehlich, 2010a: 164, catalog; Righi–Cavallaro & Lecci, 2010: 36, record; Novaes *et al*., 2012: 73, nymph description and illustrations; Novaes & Bispo, 2014b: 278, record and illustrations.

Material examined. BR, MS: Bodoquena, Fazenda Califórnia – Ciliar, 20º41'49.9"S, 56º52'54"W, Lamas & Nihei col., 5 males. **SP**: Pedregulho, Ribeirão São Pedro, 620 m, 17.ix.2019, Almeida col., 1 male. Ribeirão Preto, Reserva Santa Teresa, 27.x–08.xi.2006, Polegatto col., 1 male; 20.xi–19.12.2006, Polegatto col., 1 male. São Carlos, Fazenda Embrapa, Córrego Canchim, 21º57'07"S, 47º50'12"W, 20.ii.2007, Roque col., 1 male; 15.ix.2007, Roque col., 1 male; 08.xi.2007, Roque col., 2 males; UFSCAR, Córrego Fazzari, 21º58'07"S, 47º53'08"W, 20.xii.2007, Roque col., 1 male.

Measurement data. Male (n=12) forewing length: 8.2–10 mm (mean=9.38 mm).

Remarks. Among the specimes studied here, the specimens from São Paulo state are darker than those from Mato Grosso do Sul state. They are also darker than the holotype, also from Mato Grosso do Sul state (Froehlich, 2007), and the specimens from Paraná state (Novaes *et al*., 2012). Apparently, the difference observed in color was caused by variations in the preservation of the material in 80% ethanol. Despite this, the pattern of spots on the head and pronotum are in agreement. The M–line presents a characteristic pattern of spots (Fig. 5). Herein, we expanded the species distribution to São Paulo state (Fig. 5).

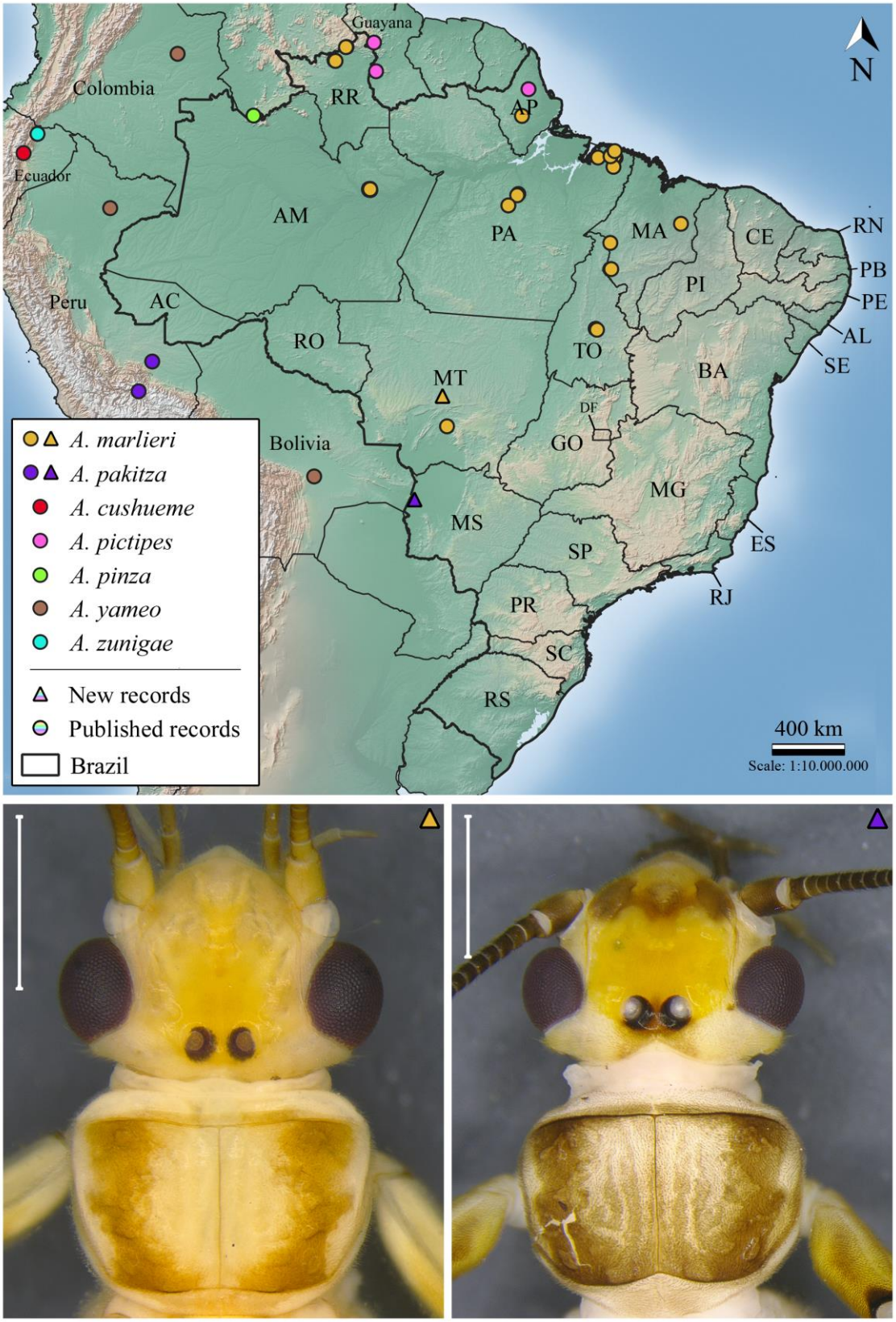

Figure 6. Occurrence map of *Anacroneuria marlieri*, *A. pakitza*, *A. cushueme*, *A. pictipes*, *A. pinza*, *A. yameo*, and *A. zunigae*, including specimens *A. marlieri* and *A. pakitza*. Scale: 1 mm.

***Anacroneuria otafroehlichi* Righi–Cavallaro & Lecci, 2010** (Fig. 5)

Anacroneuria otafroehlichi Righi–Cavallaro & Lecci, 2010: 37, description; Rippel *et al*., 2019: 362, record and female description.

Material examined. BR, MT: Tangará da Serra, Fazenda Calcário Tangará, Rio Sepotuba, 02.i.2003, Calor col., 1 male.

Measurement data. Male (n=1) forewing length: 11.5 mm.

Remarks. The studied specimen is almost uniform whitish, probably a teneral specimen (Fig. 5). This species has been recorded in the Mato Grosso do Sul and Tocantins states (Righi–Cavallaro & Lecci, 2010; Rippel *et al*., 2019) and now in the Mato Grosso state (Fig. 5).

***Anacroneuria pakitza* Stark & Sevic, 1998** (Figs 6 and 7A–E)

Anacroneuria pakitza Stark & Sivec, 1998: 45, description; Stark, 2004: 80, checklist; Froehlich, 2010a: 165. catalog.

Material examined. BR, MS: Corumbá, Morro Urucum, Pantanal, 19º08.698'S, 57º34.749'W, 18–23.vii.2013, Oliveira col., 3 males.

Measurement data. Male (n=3) forewing length: 10.8–11 mm (mean=10.9 mm).

Remarks. This is the first record of *A. pakitza* in Brazil. As mentioned above, *A. pakitza* belongs to a species complex now formed by other eight species. The studied specimens are strongly similar to that described by Stark & Sevic (1998) for Peru. This species has penial armature similar to that of *A. marlieri*, but the keel is different in dorsal and ventral views and the color of

the body and wings is darker (Fig. 7). All species belonging to this species complex have slightly differences in the penial armature and pattern of spots on head, pronotum and wings. We highlighted the need to study these species in detail, since researchers noticed morphological variations in the penial armature and color pattern in other species of *Anacroneuria* (Almeida *et al*., 2018, Almeida & Bispo, 2020). Another important argument is the approximated distribution of these species, as shown in Figure 6.

***Anacroneuria paprockii* Novaes & Bispo, 2014** (Fig. 5)

Anacroneuria paprockii Novaes & Bispo, 2014c: 436, description; Almeida & Duarte, 2017: 483, record and illustrations.

Material examined. BR, BA: Camacan, Fazenda Waldemar da Farmácia, 28.iii.2011, Calor, Quinteiro, França & Barreto col., 5 males.

Measurement data. Male (n=5) forewing length: 10.5–11.8 mm (mean=11.2 mm).

Remarks. The studied specimens are in agreement with the holotype (Novaes & Bispo, 2014c) and the specimens found in Wenceslau Guimarães, Bahia state (Almeida & Duarte, 2017). Herein, we expanded the species distribution (Fig. 5).

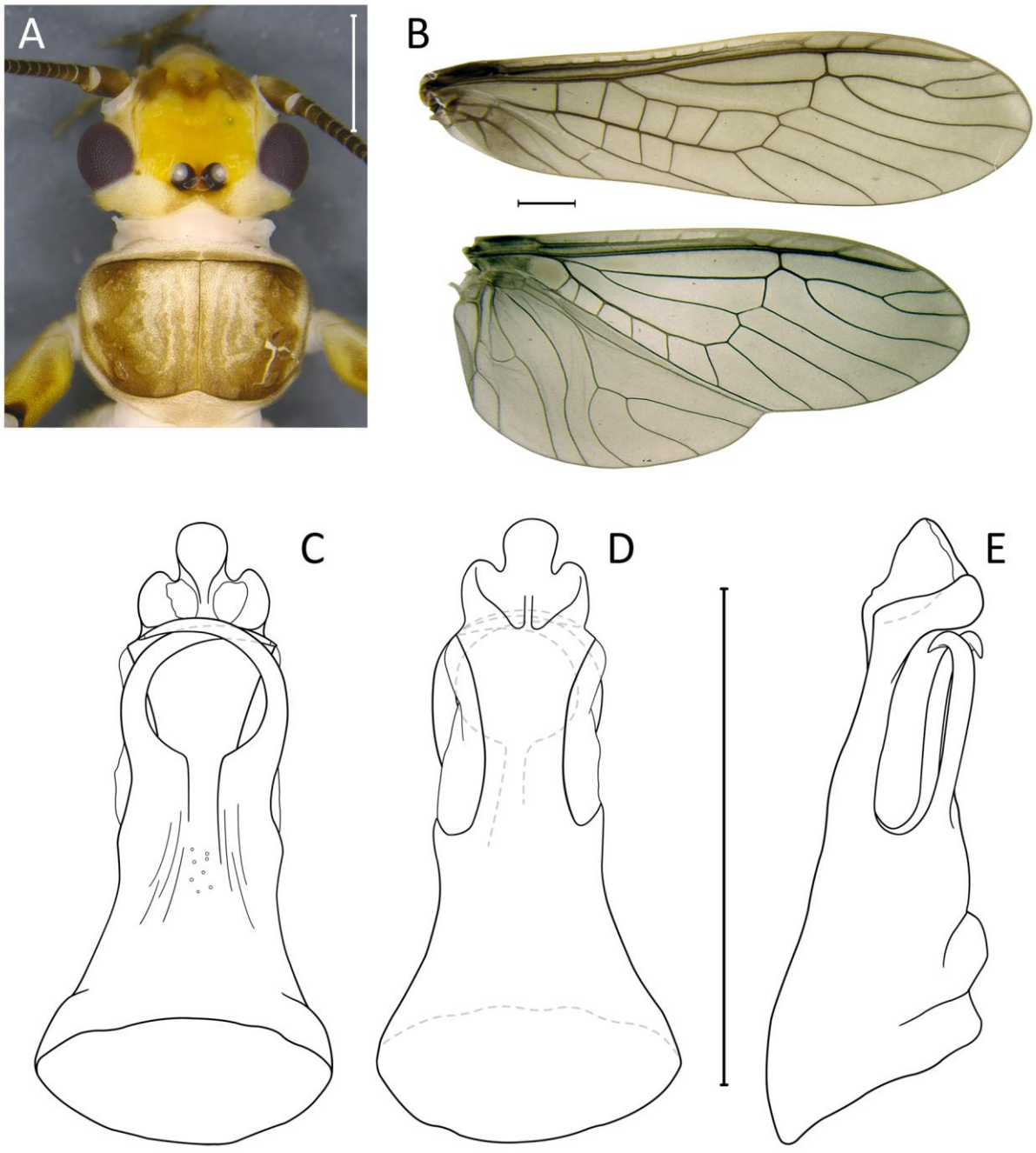

Figure 7. *Anacroneuria pakitza*. Adult male, head and pronotum (A) and wings (B). Penial armature in ventral (C), dorsal (D) and lateral views (E). Scale: 1 mm.

***Anacroneuria plaumanni* Jewett, 1959** (Fig. 5)

Anacroneuria plaumanni, Jewett, 1959: 154, description; Froehlich, 2002: 115, illustration; De Ribeiro & Froehlich, 2007: 56, record; Froehlich, 2010a: 167, catalog; Stark, 2013: 98, checklist; Romero, 2017: 49, record.

Material examined. BR, SC: Orleans, Rio da Serra, 28º12'42"S, 49º27'21"W, 07.iv-7.v.2013, Pinho col., 1 male.

Measurement data. Male (n=1) forewing length: 9.4 mm.

Remarks. This species has been recorded in Argentina and in the Rio Grande do Sul and Santa Catarina states (Froehlich, 2002; De Ribeiro & Froehlich, 2007; Romero, 2017). Herein, expanded the species distribution (Fig. 5).

***Anacroneuria polita* (Burmeister, 1839)** (Fig. 8)

Perla polita Burmeister, 1839: 879, description; *Anacroneuria polita*, Zwick, 1972: 1163, new combination and illustration; Froehlich, 2002: 85, illustration; Froehlich, 2004: 58, record; Bispo & Froehlich, 2004a: 98, illustration; Froehlich, 2010a: 168, catalog; Novaes & Bispo, 2014a: 459, illustration and picture; Novaes & Bispo, 2014c: 435, record; Romero, 2017: 49, record; Almeida & Bispo, 2020: 15, COI sequence and pictures.

Material examined. BR, PR: Morretes, Mata Atlântica Park Hotel, 12.i.2012, Novaes col., 3 males. **RJ**: Itatiaia, Parque Nacional do Itatiaia, Córrego Taquaral, 810 m, 06.x.2017, Dias & Campos col., 1 male.

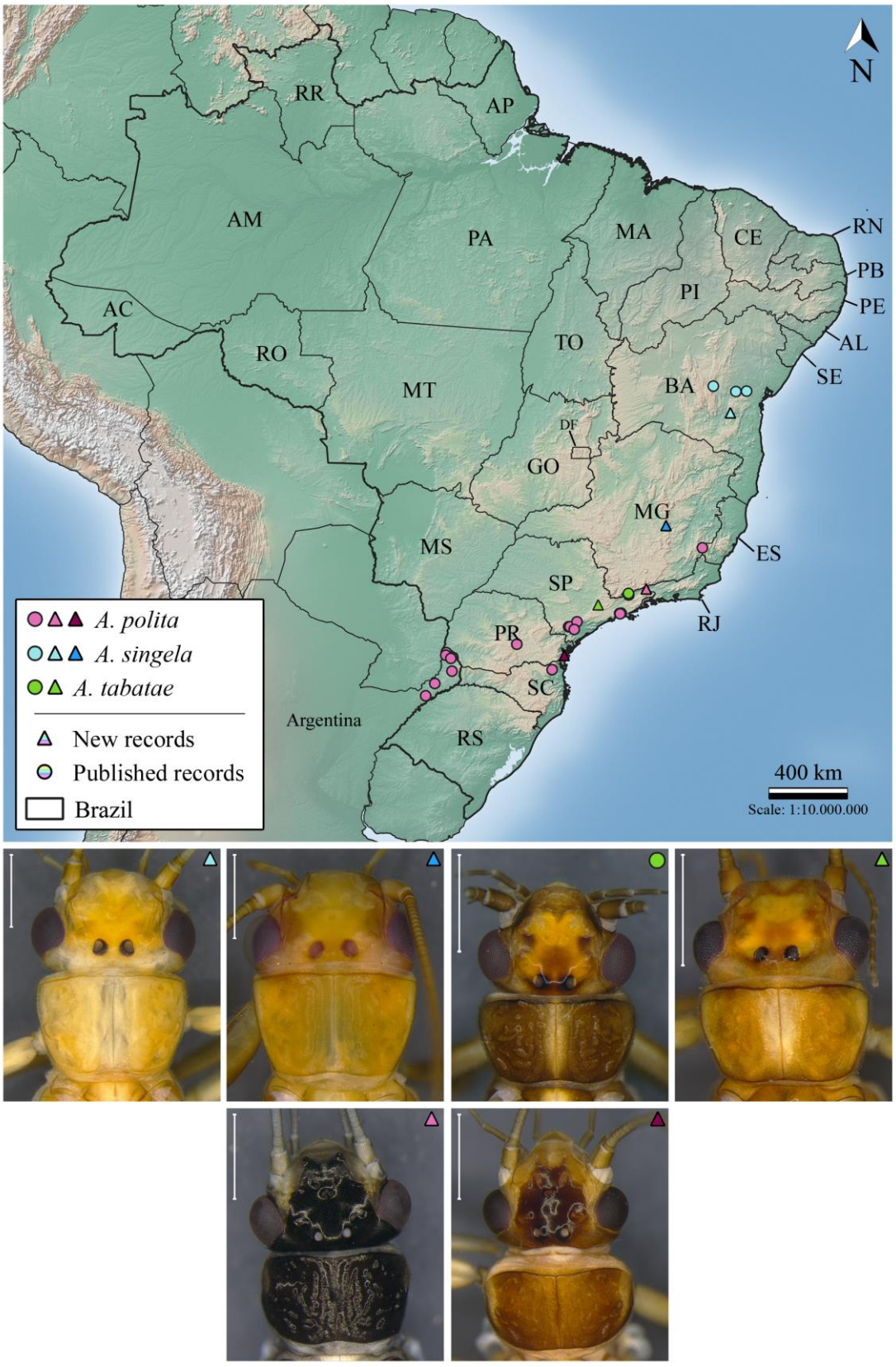

Figure 8. Occurrence map and specimens of *Anacroneuria polita*, *A. singela*, and *A. tabatae*. Scale: 1 mm.

Measurement data. Male (n=3) forewing length: 9–9.3 mm (mean=9.12 mm).

Remarks. All studied specimens are teneral and have spots on the head and pronotum (Fig. 8) similar to that presented by Almeida & Bispo (2020). The penial armature is in agreement with the specimen illustrated by Bispo & Froehlich (2004a). Here, we expanded the species distribution, including a new record for the Rio de Janeiro state (Fig. 8).

***Anacroneuria singela* Duarte & Lecci, 2016** (Fig. 8)

Anacroneuria singela Duarte & Lecci, 2016: 297, description.

Material examined. BR, BA: Piatã, Rio das Contas, Cachoeira das Deuzas, Fazenda Oshoki, no date, Menezes col., 1 male. **MG**: Serra do Cipó, Rio Cipó in Cardeal Mota (Cachoeira Baixo), 10.xi.2001, Holzenthal, Paprocki & Blahnik col., 1 male.

Measurement data. Male (n=2) forewing length: 11–12.5 mm (mean=11.75 mm).

Remarks. The studied specimens are in agreement with the holotype described by Duarte & Lecci (2016). In the original description, the authors said that the penial armature of the *A. singela* is similar to that of *A. debilis*, in lateral view. However, despite the great difference in the pattern of colors and spots on the head and pronotum, we noted that the penial armature of *A. singela* is more similar to that of *A. rotunda* Gonçalves, Novaes & Salles. Here, we expanded the species distribution, including a new record for the Minas Gerais state (Fig. 8).

***Anacroneuria singularis* Righi–Cavallaro & Lecci, 2010** (Fig. 9)

Anacroneuria singularis Righi–Cavallaro & Lecci, 2010: 43, description; Novaes & Bispo, 2014c: 435, record; Ribeiro *et al*., 2015: 296. record and nymph description; Firmino *et al*., 2019: 447, record; Rippel *et al*., 2019: 361, record; Carvalho *et al*., 2020: 439, record.

Material examined. BR, BA: Barreiras, Cachoeira do Redondo, 05.vi.2008, Bravo, Menezes & Silva–Neto col., 1 male. **MS**: Campo Grande, Fazenda Duphol, Rio Engano, à montante da cachoeira, 18.xii.2015, 1 male. **TO**: Mateiros, Parque Estadual do Jalapão, Cachoeira da Formiga, 17.x.2008, Calor & Mariano col., 1 male.

Measurement data. Male (n=2) forewing length: 8.9–10 mm (mean=9.46 mm).

Remarks. There is no variation between the studied specimens and the holotype (Righi–Cavallaro & Lecci, 2010). The species is easily identified in reason of its characteristic penial armature (Righi–Cavallaro & Lecci, 2010). *A. singularis* is widely distributed, being found in the Maranhão, Mato Grosso do Sul, Minas Gerais, Pará, São Paulo, Rondônia, and Tocantins states (Righi–Cavallaro & Lecci, 2010; Novaes & Bispo, 2014c; Ribeiro *et al*., 2015; Firmino *et al*., 2019; Rippel *et al*., 2019; Carvalho *et al*., 2020), and now in the Bahia state (Fig. 9).

***Anacroneuria stanjewetti* Froehlich, 2002** (Fig. 9)

Anacroneuria stanjewetti Froehlich, 2002: 96, description; De Ribeiro & Froehlich, 2007: 56, Record; Froehlich, 2008: 130, record and illustration; Froehlich, 2010a: 171, catalog; Novaes & Bispo, 2014c: 435, record; Romero, 2017: 49, record.

Material examined. BR, PR: Foz do Iguaçu, Parque Nacional de Foz do Iguaçu, Trilha do Macuco, 11.x.2017, Dias & Campos col., 1 male. **RJ**: Itatiaia, Parque Nacional do Itatiaia, Córrego Campo Belo, 798 m, 06.xi.2017, Dias & Campos col., 1 male.

Measurement data. Male (n=2) forewing length: 9.9–10 mm (mean=9.95 mm).

Remarks. The studied specimens are in agreement with the holotype. *A. stanjewetti* have been found in Argentina and in Brazil, in Minas Gerais, Rio Grande do Sul, Santa Catarina, and São Paulo states (Froehlich, 2002; De Ribeiro & Froehlich, 2007; Froehlich, 2008; Novaes & Bispo, 2014c; Romero, 2017), and now in Paraná and Rio de Janeiro states (Fig. 9).

***Anacroneuria tabatae* Froehlich, 2010** (Fig. 8)

Anacroneuria tabatae Froehlich, 2010b: 63, description.

Material examined. BR, SP: Jundiaí, REBIO Serra do Japi, Córrego Togobã, 23°13’54.3”S, 46°57’51.7”W, 08.v.2008, Moretto col., 1 male.

Measurement data. Male (n=1) forewing length: 9.1 mm.

Remarks. The penial armature of the studied specimen is in agreement with the holotype described by Froehlich (2010b). This is the first record of the species outside the Mantiqueirae Mountains (Fig. 8).

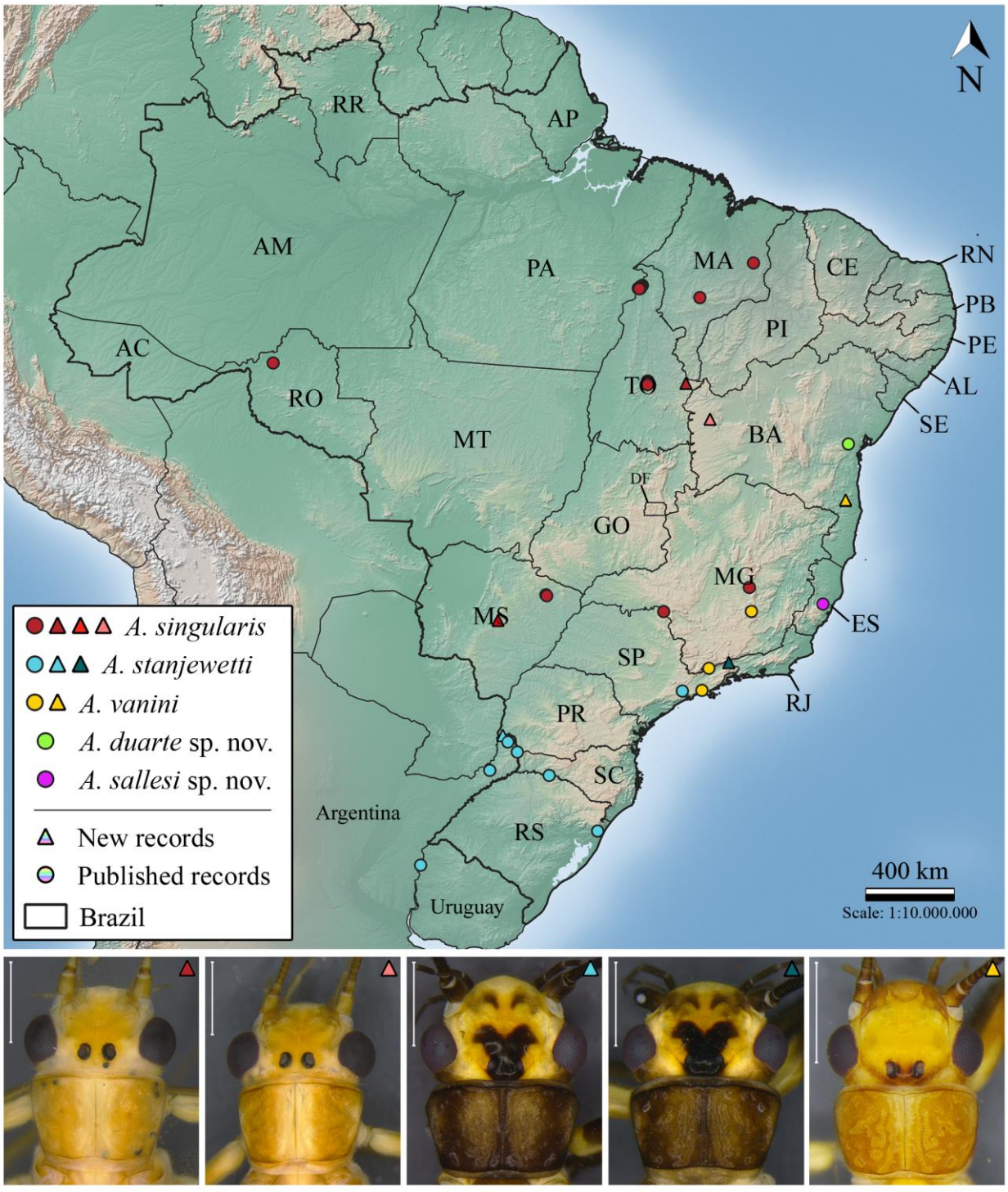

Figure 9. Occurrence map and specimens of *Anacroneuria duarte* sp. nov., *A. sallesi* sp. nov., *singularis*, *A. stanjewetti*, and *A. vanini*. Scale: 1 mm.

***Anacroneuria vanini* Froehlich, 2004** (Fig. 9)

Anacroneuria vanini Froehlich, 2004: 61, description; Froehlich, 2010a: 174, catalog; Froehlich, 2010b: 68, record; Novaes & Bispo, 2014c: 436, record.

Material examined. BR, BA: Camacan, RPPN Serra Bonita, iv.2009, 1 male; vii.2011, 1 male; Córrego Fechadinho, 15º23'9.1"S, 39º34'3.1"W, 27.xi.2011, 1 male.

Measurement data. Male (n=3) forewing length: 8.8–8.9 mm (mean=9.86 mm).

Remarks. The studied specimens are in agreement with the holotype in color and pattern of spots on the head and pronotum (Fig. 9). However, only two specimens presented the window on the wings, as described by Froehlich (2004). The species has been recorded in Minas Gerais and São Paulo states (Froehlich, 2004; 2010b; Novaes & Bispo, 2014c). This is the first record for theBahia state (Fig. 9).

***Anacroneuria duarte* sp. nov. Almeida, Gonçalves & Bispo** (Figs 9 and 10A–E)

Material examined. BR, BA: Varzedo, Fazenda Sr. Getúlio, Córrego Cai Camarão, vi.2016, malaise, Dias & Campos col., 1 male (Holotype/ MZUSP).

Measurement data. Male (n=1) forewing length: 9 mm.

Description. A small sized species. General color pale yellowish. Head and pronotum yellowish, head with inconspicuous M–line, central frons yellowish, frontoclypeus area and occiput pale yellowish (Fig. 10A). Antennae pale yellowish, scape with dark spot laterally. Palpi pale yellowish. Pronotum with two dark stripes laterally, posterior corners rounded (Fig. 10A). Wing

membrane and veins light yellow with the presence of a distal less pigmented circular area forming a barely apparent window, base of the subcostal posterior and radial anterior veins darker than the others (Fig. 10B). Legs pale yellowish with dark stripes in the distal part of the femur. Cerci pale yellowish. Sternum IX with small bristles on posterior area.

Male. Hammer simple, truncated cone. Penial armature (Figs. 10C–E) with a pair of large distal membranous vesicles. In ventral view, the penial armature tapering progressively to tip and the hooks are regularly curved and acute apically. (Fig. 10C). The keel of the penial armature, in dorsal view, appears as broadly curved lines, converging medially (Fig. 10D). In lateral view, rounded and protruding keel, growing abruptly at the bottom and ending gradually from the middle to the top, the terminal tube is high and pointed (Fig. 10E).

Female and nymph. Unknown.

Remarks. *Anacroneuria duarte* sp. nov. has color and pattern of spots on the head and pronotum similar to most species from Bahia state (Almeida & Duarte, 2017). This coloration is common in species in the Midwest, North and Northeast of Brazil (Almeida *et al*., 2018). The shape of the penial armature of the species resembles that of *A. tinga* Bispo & Froehlich, but the keel of *A. duarte* sp. nov. is different, since it is rounded in the dorsal view and very prominent and thin in dorsal and lateral views. This type of keel has not been observed in Brazilian species until now.

Etymology. Named in honor of Dr. Tácio Duarte, the first researcher born in Bahia state who devoted his effort to study Plecoptera. Treat as a noun in apposition.

***Anacroneuria sallesi* sp. nov. Gonçalves, Almeida & Bispo** (Figs 9 and 11A–E)

Material examined. BR, ES: Santa Teresa, REBIO Augusto Ruschi, Córrego Bragacho, 27–28.xii.2017, Salles & Gonçalves col., 2 males; 20–21.ii.2018, Salles & Gonçalves col., 1 male (Holotype/ UFVB).

Measurement data. Male (n=3) forewing length: 7.5–8.0 mm (mean=7.8 mm).

Description. A small sized species. General color light brown. Head and pronotum light brown, head with inconspicuous M–line, central frons brown near ocelli and yellowish near M-line, frontoclypeus area yellow and occiput brown (Fig. 11A). Antennae brown, scape and pedicel brownwish. Palpi brown. Pronotum with non-rounded corners and homogeneous color (Fig. 11A). Wing membrane light brown and veins dark brown, costal margin and apex darker with an unpigmented distal circular area forming a window, forewing with the base of the subcostal posterior and radial anterior veins darker (Fig. 11B). Legs almost completely brown with yellow stripe in proximal part of the femur, tarsi dark brown, almost black. Cerci brown. Sternum IX with small bristles on posterior area.

Male. Hammer simple, truncated cone. Penial armature (Figs. 11C–E) with a pair of large distal membranous vesicles. In ventral view, the penial armature tapering sharply at the tip and the hooks are regularly curved and acute apically (Fig. 11C). In dorsal view, there are two prominent structures laterally to the terminal tube and the keel is projected (Fig. 11D). In lateral view, keel and shoulders robust, becoming rounded and prominent as they approach the keel, high and pointed terminal tube forming a design of a triangle (Fig. 11E). The keel of the penial armature, in lateral view, has a portion of rounded spines on the tip (Fig. 11E).

Female and nymph. Unknown.

Remarks. The color and pattern of spots on the head and pronotum presented by *A. sallesi* sp. nov. does not immediately resemble any species described for Brazil. The yellow and brown spots on the head are well delimited and form a unique pattern. The shape of the penial armature also does not immediately resemble that of any species. In lateral view, the penial armature resembles that of *A. itajaimirim*, but, in dorsal view, the projected keel and the two prominent structures laterally to the terminal tube are unique of the species.

Etymology. The name honors Dr. Frederico Falcão Salles, a researcher who has dedicated to study aquatic insects, mainly Ephemeroptera.

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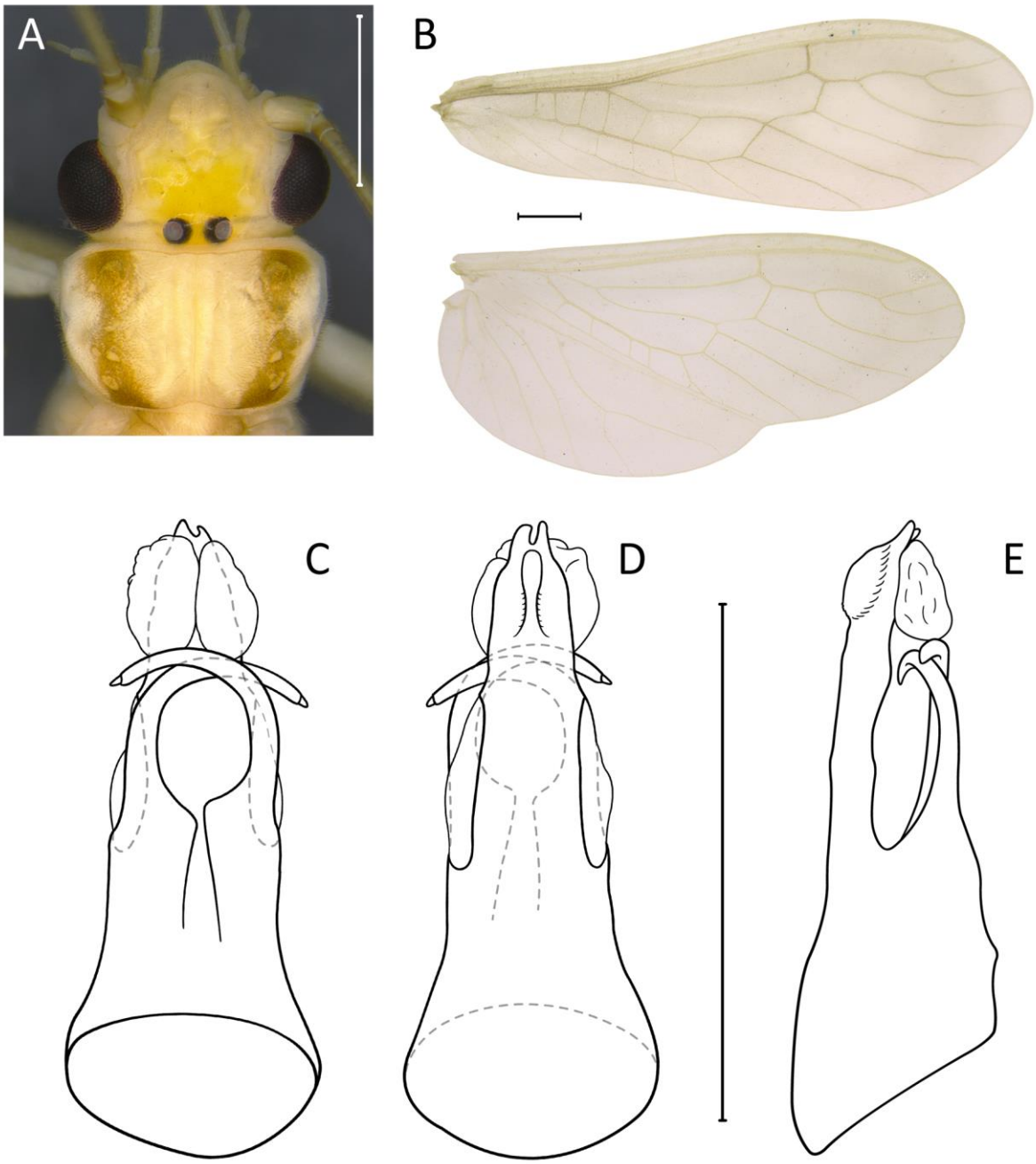

Figure 10. *Anacroneuria duarte* sp. nov. Adult male, head and pronotum (A) and wings (B). Penial armature in ventral (C), dorsal (D) and lateral views (E). Scale: 1 mm.

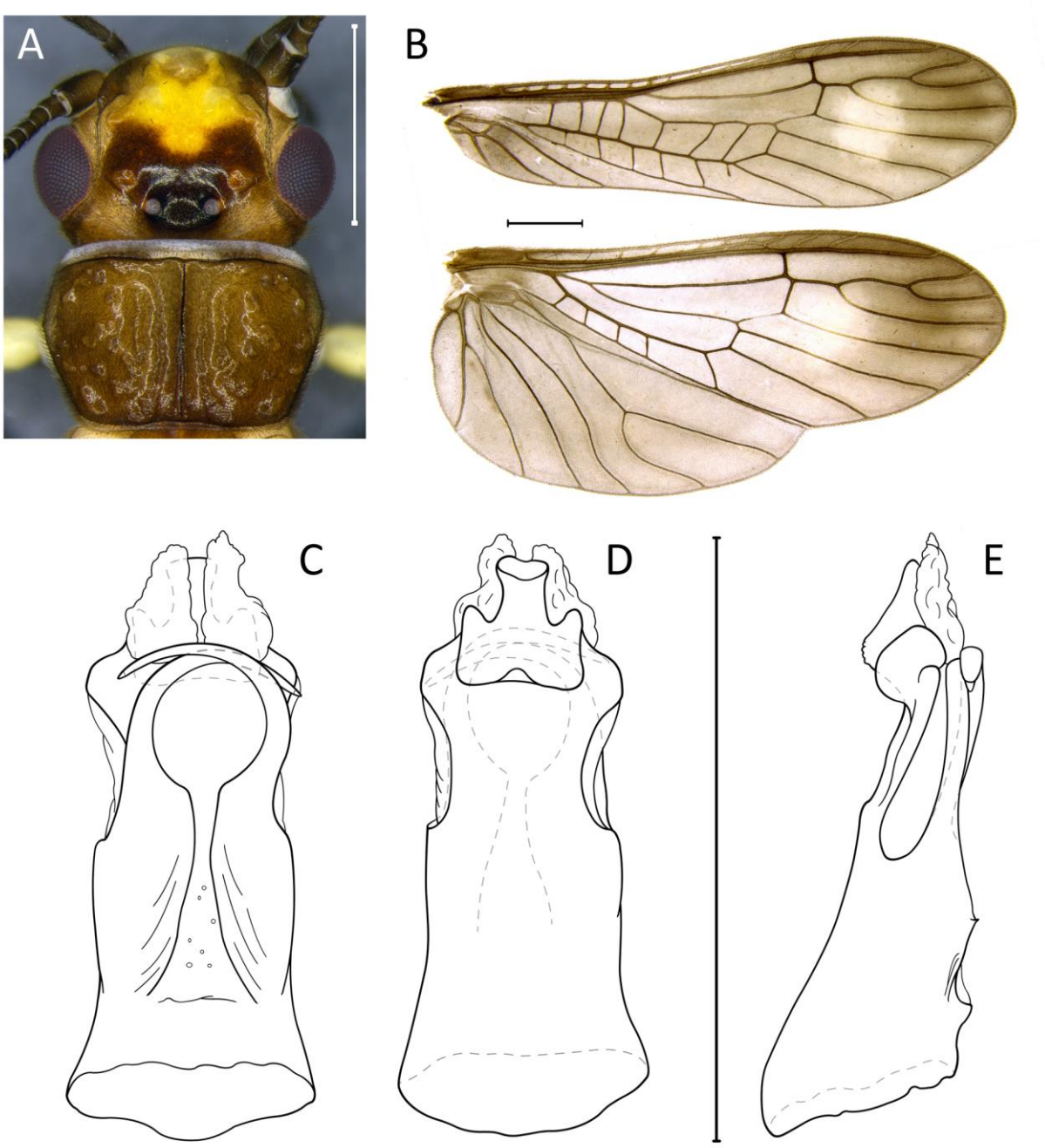

Figure 11. *Anacroneuria sallesi* sp. nov. Adult male, head and pronotum (A) and wings (B). Penial armature in ventral (C), dorsal (D) and lateral views (E). Scale: 1 mm.

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Capítulo 2

New species and records of *Kempnyia* Klapálek, 1914 (Plecoptera: Perlidae) from Brazil

New species and records of *Kempnyia* Klapálek, 1914 (Plecoptera: Perlidae) from Brazil

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Abstract

In this paper, we studied the genus *Kempnyia* (Plecoptera: Perlidae) deposited in the Aquatic Biology Laboratory (UNESP, Assis) and Entomology Museum of the Federal University of Viçosa (UFVB, Viçosa) collections. Herein, we described three new species *K. tupiniquim* sp. nov., *K. zwickii* sp. nov., and *K. guarani* sp. nov.; highlighted the urgent need to study the specimens identified as *K. varipes*; discussed morphological variations; and presented occurrence maps for all species, highlighting the new records. We also proposed the synonym of *K. obtusa* and *K. sordida* based on both head and pronotum pattern of spots.

Keywords: Aquatic insects; biodiversity; Brazilian stoneflies; freshwater; distribution.

Introduction

Plecoptera is an order of aquatic insects with more than 3700 species and 17 families found around the world with exception of Antarctica (DeWalt & Ower, 2019; South *et al*., 2021). Perlidae Latreille, 1802 has at least 1100 described species and is recorded in all suitable regions of the world, except Australasia region (DeWalt & Ower, 2019). In the Neotropical region, Pessacq *et al*. (2019) counted about 400 valid species. Among these, about 140 are recorded in Brazil, belonging to the genera *Anacroneuria* Klapálek 1909, *Enderleina* Jewett, 1960, *Kempnyia* Klapálek, 1914, and *Macrogynoplax* Enderlein, 1909.

Klapálek (1914) proposed the genus *Kempnyia* describing *K. tenebrosa* Klapálek, 1916 as the type species (Stark *et al*., 2009). Currently, *Kempnyia* has 36 valid species (Pessacq *et al*., 2019; Almeida & Bispo, 2020) and encompass the old genera *Eutactophebia* Klapálek, 1914, *Collampla* Navás, 1929, *Diperla* Navás, 1936, *Forca* Navás, 1925, *Laessia* Navás, 1934, and *Nedanta* Navás, 1932 as synonymous (Illies, 1966; Jewett, 1960; Zwick, 1983; Froehlich, 1988; Stark, 2001). The genus is found mainly in the coastal Atlantic Forest and mountainous regions of Central Brazil (Pessacq *et al*., 2019).

Zwick (1972a; 1972b; 1983), Froehlich (1984; 1988; 1996), and Dorvillé & Froehlich (1997) studied several of the older species of *Kempnyia*, and new species have been described more recently by Bispo & Froehlich (2004a), Froehlich (2011b; 2011c), and Avelino-Capistrano *et al*. (2013; 2014; 2016). Despite this, the genus presents several taxonomic problems, mainly involving species with old and insufficient descriptions. Among the 36 species described for the genus, 23 were described based on males and females, eight only on males, five only on females and only nine of them have nymphs associated and described. In addition, at least nine species

have problematic descriptions and holotypes deposited in museums outside Brazil (Froehlich, 2010).

In the present study, we studied *Kempnyia* specimens from different localities with the aim of describing new species, expanding the species distribution, and adding comments on important morphological variations observed in some species.

Material e methods

Collection of specimens. A part of the material studied was collected using a light pan trap and white sheet with UV and white lamps (Calor & Mariano, 2012). All specimens collected by Almeida were sampled using the same light traps, but using blue, green and UV LEDs (Price & Baker, 2016). The specimens collected before 2014 were preserved in 80% ethanol in air-conditioned temperature (17ºC). When collected from 2014 onwards the individuals were conditioned in absolute ethanol in a freezer (-20ºC). The specimens studied are part of the Aquatic Biology Laboratory (CLBA - UNESP, Assis), Entomology Museum of the Federal University of Viçosa (UFVB, Viçosa), and Museum of Zoology of the University of São Paulo (MZUSP, São Paulo) collections. The holotypes of the three new species will be deposited in the MZUSP and UFVB.

Identification. We severed the abdomen of the males between segments seven and eight and for overnight we treated them using 10% KOH. In order to neutralize the reaction, we placed the abdomens in acetic acid and washed using 80% ethanol. We carried out the extraction of penial armature to identify the species based on the morphological comparison of the penial armature already described in the literature. After the dissecation, we made the illustrations under

a lucida camera mounted on a Leica DM1000 microscope and rendered using Adobe Illustrator CS6® editor. To take and improve the pictures, we used a digital camera on a Leica M205A stereomicroscope and Adobe Photoshop CS3® editor, respectively. Using QGIS Bucur 3.14.15 software (QGIS Development Team, 2020) we made the species distribution maps.

Collectors. Almeida – Lucas Henrique de Almeida; Avelino-Capistrano: Fernanda Avelino-Capistrano; Bispo – Pitágoras da Conceição Bispo; Blahnik – Roger Blahnik; Cabette – Helena Soares Ramos Cabette; Calor – Adolfo Calor; Campos – Rogério Campos; Dias – Everton Santos Dias; Froehlich – Claudio Gilberto Froehlich; Fusari – Lívia Maria Fusari; Melo – Adriano Sanches Melo; Lecci – Lucas Lecci; Paprocki – Henrique Paprocki; Miguel – Marina Miguel; Pinho – Luiz Carlos de Pinho; Roque – Fabio de Oliveira Roque; Salles – Frederico Salles.

Results and discussion

Kempnyia Klapálek, 1914

Kempnyia flava Klapálek, 1916 (Fig. 1)

Kempnyia flava Klapálek, 1916: 53, 72, description; Jewett, 1960: 176, illustration; Illies, 1966: 340, catalog; Zwick, 1972: 1167, illustration; Zwick, 1973b: 276, catalog; Froehlich, 1988: 153, illustration; Stark, 2001: 415, checklist; Bispo & Froehlich, 2004b: 109, record; Stark *et al*., 2009: 124, checklist; Nessimian *et al*., 2009: 316, record; Froehlich, 2010: 180, catalog;

Froehlich, 2011a: 03, checklist; Froehlich, 2011c: 21, record; Gonçalves *et al*., 2017: 147, record; Gonçalves *et al*., 2019: 105, checklist; Almeida & Bispo, 2020: 19, COI sequence.

Material examined. BR, ES: Santa Teresa, i.2013, Salles & Lecci col., 1 male; REBIO Augusto Ruschi, Cachoeira da Estrada, 11.i.2016, Salles *et al*. col., 1 male; 20-21.ii.2018, Salles *et al*. col., 1 male; Córrego Bragacho, 21-22.x.2017, Salles *et al*. col., 1 male. **MG**: Ouro Preto, Vale do Tropeiro, Cachoeira do Abacaxi, 20°12.270'S, 43°38.163'W, 1120 m, 7.xi.2001, Paprocki col., 2 males and 2 females. Alto Caparaó, Parque Nacional do Caparaó, Rio Caparaó at Vale Verde, 20°25.029'S, 41°50.767'W, 1350 m, 12-13.iii.2002, Paprocki col., 1 male. **SP**: São Luiz do Paraitinga, Parque Estadual da Serra do Mar, Núcleo Santa Virgínia, Ribeirão Barro Branco, 20.i.2006, Bispo *et al*. col., 2 males. Iporanga, Parque Estadual Intervales, Rio do Carmo, bridge, 24°18'15"S, 48°24'31"W, 28.x.1999, 1 male; 20.ii.2000, 1 male. São Miguel Arcanjo, Parque Estadual Carlos Botelho, Rio Bonito, bridge, 24°08'31.6"S, 47°59'41.3"W, 06.ii.2017, Almeida *et al*. col., 2 females. Jundiaí, REBIO Serra do Japi, Córrego do Paraíso, 23°14'29.3"S, 46°57'17.6"W, 17.xi.2019, Almeida *et al*. col., 1 male and 1 female; 17.xi.2019, Almeida *et al*. col., 1 male; 01-06.xii.2019, Almeida *et al*. col., 1 male; Cachoeira das Bromélias, 23°14'18.4"S, 46°57'51.2"W, 17.xi.2019, Almeida *et al*. col., 3 males and 2 females.

Measurement data. Male (n=14) forewing length: 13.5–16 mm (mean=14.77 mm). Female (n=8) forewing length: 19–20.5 mm (mean=19.62 mm).

Remarks. The species is easily identified due to its characteristic penial armature and general yellowish color. However, the specimens from Espírito Santo state are darker than the others (Fig. 1). This species has been recorded in the Espírito Santo, Rio de Janeiro and São Paulo states

(Froehlich, 1988; 2011c; Bispo & Froehlich, 2004b; Nessimian *et al*., 2009; Gonçalves *et al*., 2017) and now in the Minas Gerais state (Fig. 1).

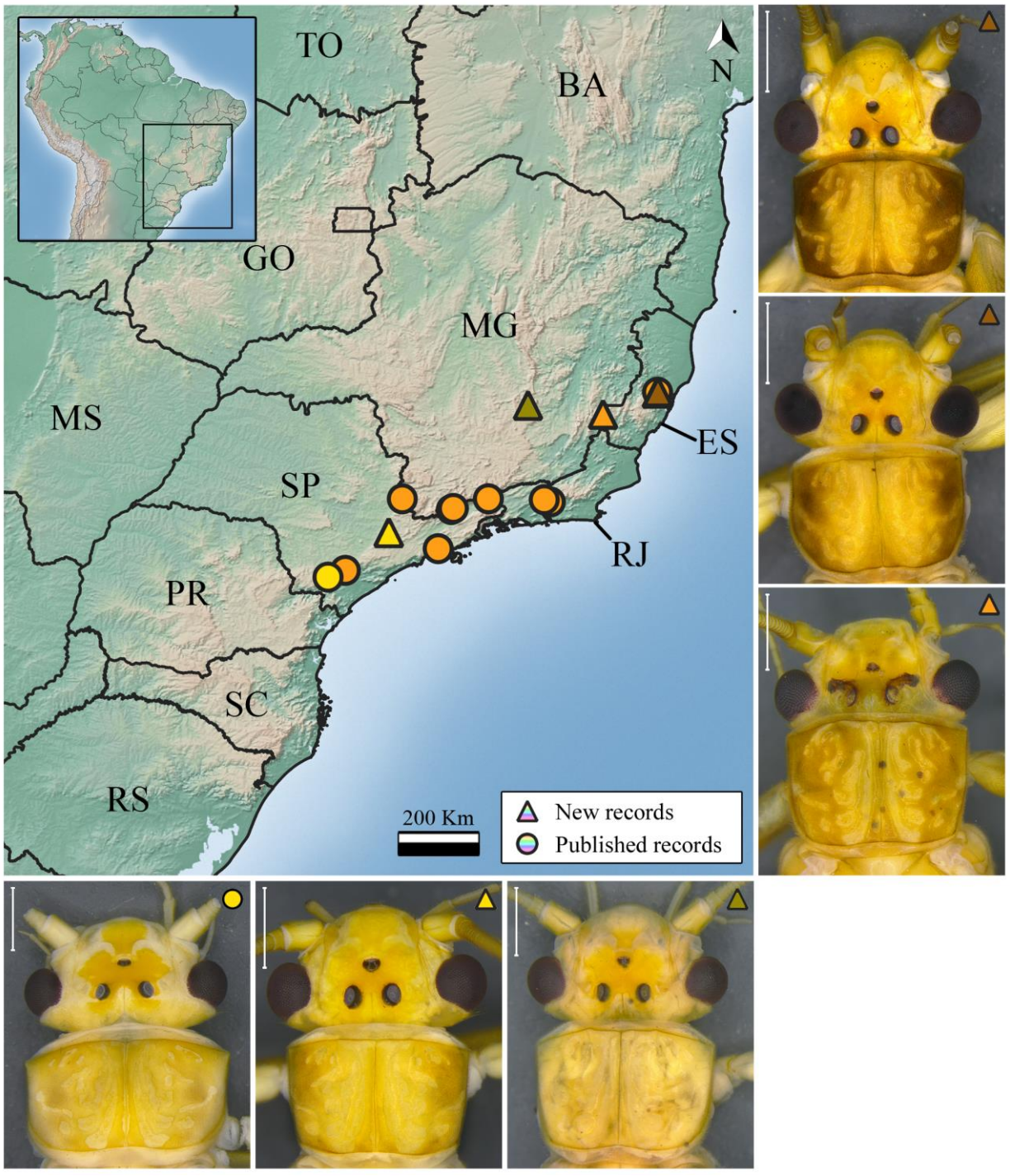

Figure 1. Occurrence map and specimens of *Kempnyia flava*. Scale: 1 mm. Brazilian states acronyms: Bahia - BA; Espírito Santo - ES; Goiás - GO; Mato Grosso - MT; Mato Grosso do Sul - MS; Minas

Gerais - MG; Paraná - PR; Rondônia - RO; Rio de Janeiro - RJ; Rio Grande do Sul - RS; Santa Catarina - SC; São Paulo - SP; Tocantins - TO.

***Kempnyia gracilenta* (Enderlein, 1909)** (Fig. 2)

Acroneuria gracilenta Enderlein, 1909: 397, description; *Eutactophlebia gracilenta* Klapálek, 1916: 67, new combination; Jewett, 1960: 175, illustrations; Illies, 1966: 332, catalog; Zwick, 1973a: 490, illustrations; *Kempnyia gracilenta* Zwick, 1983: 179, new combination; Froehlich, 1984: 137, illustration; Stark, 2001: 415, Checklist; Stark *et al*., 2009: 124, checklist; Froehlich, 2010: 180, catalog; Froehlich, 2011a: 03, checklist; Avelino-Capistrano *et al*., 2011: 143, nymph and complementary description; Avelino-Capistrano & Nessimian, 2014: 11, checklist; Avelino-Capistrano *et al*., 2014: 330, record; Duarte *et al*., 2014: 89, record; Gonçalves *et al*., 2017: 147, record; Gonçalves *et al*., 2019: 105, checklist.

Material examined. BR, ES: Santa Teresa, REBIO Augusto Ruschi, Córrego da Estrada, 27-28.xii.2017, Salles *et al*. col., 1 male; Córrego Bragacho, 18.xii.2017-17.i.2018, Salles *et al*. col., 2 males; 20-21.ii.2018, Salles *et al*. col., 1 male. **RJ**: Itatiaia, Parque Nacional do Itatiaia, Sítio da Acácias, 22º26.315'S, 44º36.625'W, 1300 m, 23-24.xi.2001, Paprocki col., 1 male.

Measurement data. Male (n=5) forewing length: 13–14.1 mm (mean=13.32 mm).

Remarks. The specimens studied are in agreement with the description. The specimen from Itatiaia (Rio de Janeiro state) has a lighter central spot longitudinal on the pronotum, while specimens from the Espírito Santo state have a homogeneously pigmented pronotum (Fig. 2). The specimens from Espírito Santo state presented penial armature greater than that of the specimen

from Itatiaia, Rio de Janeiro state. However, the observed variations are normal within the species of this genus (Almeida & Bispo, 2020). Herein, we expanded the species distribution (Fig. 2).

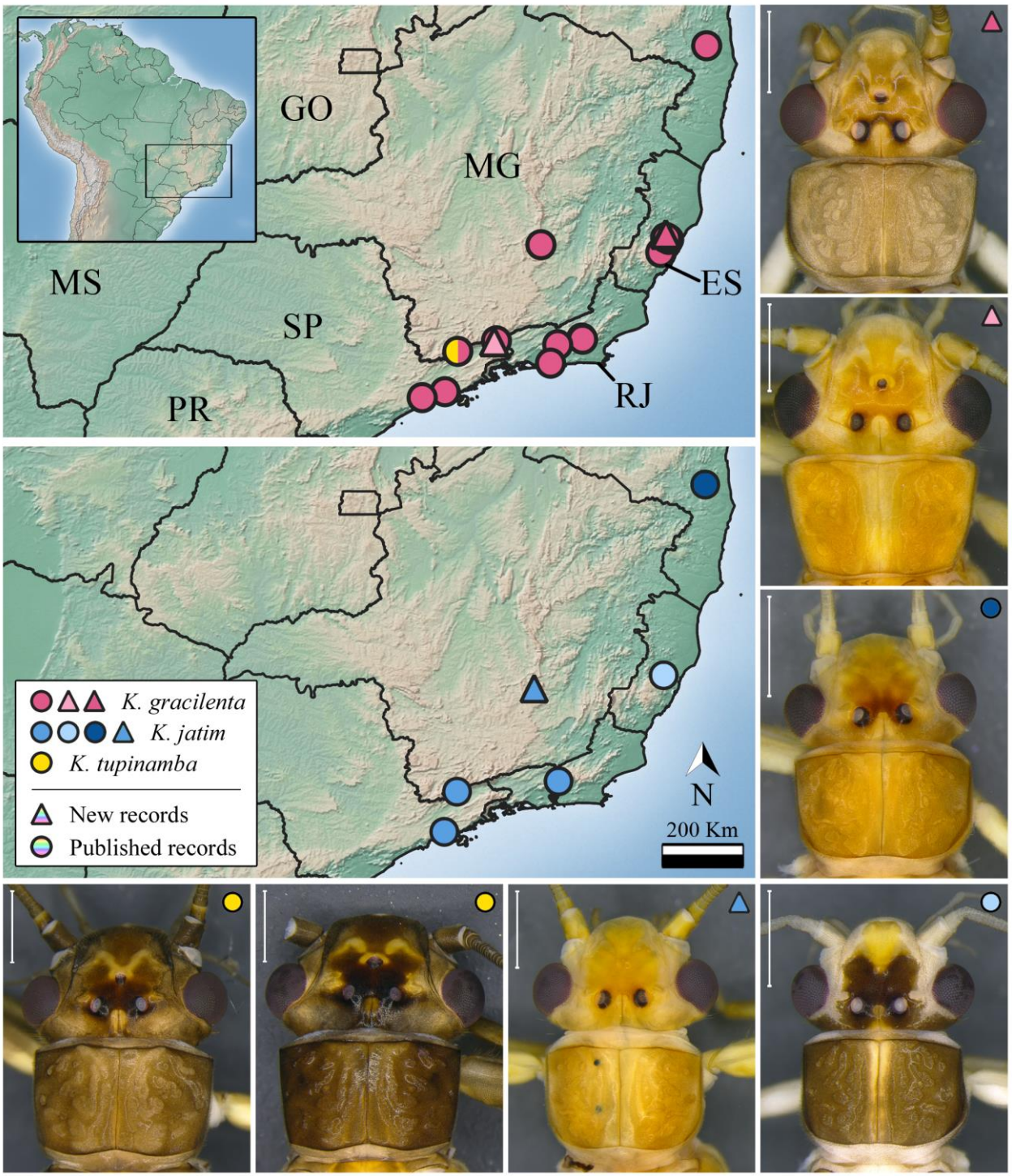

Figure 2. Occurrence map and specimens of *Kempnyia gracilenta*, *K. jatim*, and *K. tupinamba*. Scale: 1 mm. Light pink triangle: Specimens from Itatiaia, Rio de Janeiro state.

***Kempnyia jatim* Froehlich, 1988** (Fig. 2)

Kempnyia jatim Froehlich, 1988: 169, description; Stark, 2001: 415, checklist; Stark *et al*., 2009: 124, checklist; Froehlich, 2010: 181, catalog; Froehlich, 2011a: 03, checklist; Froehlich, 2011c: 22, record; Duarte *et al*., 2014: 89, record; Gonçalves *et al*., 2017: 147, record; Gonçalves *et al*., 2019: 105, checklist.

Material examined. BR, BA: Camacan, RPPN Serra Bonita, 2ª Cachoeira trilha, 04.xi.2009, Calor *et al*. col., 2 males. **ES**: Santa Teresa, REBIO Augusto Ruschi, Cachoeira da Estrada, 19.xi.2015, Salles *et al*. col., 5 males; Córrego da Estrada, 30.xi-01.x.2017, Salles *et al*. col., 1 male; 27-28.xii.2017, Salles *et al*. col., 1 male; 17-18.i.2018, Salles *et al*. col., 1 male; 20-21.iii.2018, Salles *et al*. col., 1 male. **MG**: Ouro Preto, Vale do Tropeiro, Cachoeira do Abacaxi, 7.xi.2001, Paprocki col., 4 males.

Measurement data. Male (n=15) forewing length: 9.5–13.5 mm (mean=10.51 mm).

Remarks. Although almost all the studied specimens are teneral, the shape of the penial armature is in agreement with that of the holotype (Froehlich, 1988). The color variations observed were due to the specimens of Espírito Santo state being teneral and the other specimens studied being preserved in 80% ethanol (Fig. 2), losing pigmentation over time (Almeida & Bispo, 2020). This species has been recorded in the Bahia, Espírito Santo, Rio de Janeiro, and São Paulo states (Froehlich, 1988; Nessimian *et al*., 2009; Duarte *et al*., 2014; Gonçalves *et al*., 2017) and now in the Minas Gerais state (Fig. 2).

***Kempnyia mirim* Froehlich, 1984** (Fig. 3)

Kempnyia mirim Froehlich, 1984: 140, description]; Stark, 2001: 415, checklist; Stark *et al*., 2009: 124, checklist; Froehlich, 2010: 181, catalog; Froehlich, 2011a: 03, checklist; Novaes & Bispo, 2014b: 283, record.

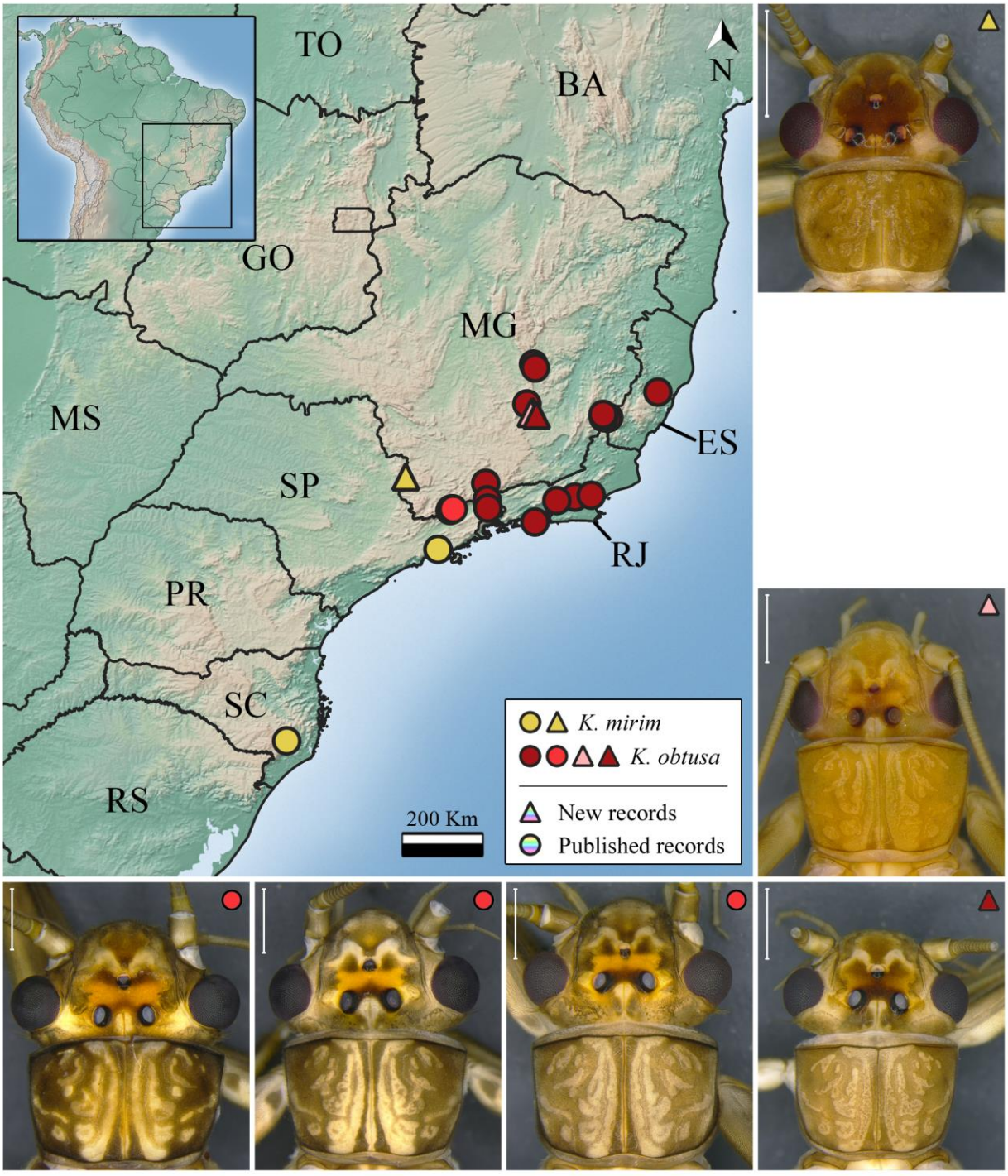

Figure 3. Occurrence map and specimens of *Kempnyia mirim* and *K. obtusa*. Scale: 1 mm.

Material examined. BR, MG: Fazenda de Eucalipto, 21°53’31.68”S, 46°32’57”W, 08.iii.2019, Fusari *et al*. col., 20 males and 4 females.

Measurement data. Male (n=20) forewing length: 9–10.1 mm (mean=9.67 mm). Female (n=4) forewing length: 11–12.1 mm (mean=11.7 mm).

Remarks. *Kempnyia mirim* is one of the smallest species of *Kempnyia*. The species is similar to *K. pinhoi* Froehlich, 2011, mainly in the size and shape of the penial armature. Froehlich (2011b), when describing *K. pinhoi*, observed some differences in the general color and penial armature, separating both species. However, now, we know about the differences in the color pattern between teneral and non-teneral specimens, and the effects on color caused by preservation in 80% ethanol (Almeida & Bispo, 2020). It is possible that both species are actually one. For this reason, both species should be restudied. *Kempnyia mirim* has been recorded in São Paulo and Santa Catarina states (Froehlich, 1984; Novaes & Bispo, 2014b) and now in Minas Gerais state (Fig. 3).

***Kempnyia neotropica* (Jacobson & Bianchi, 1905)** (Fig. 4)

Perla (*Perla*) *obscura* Pictet, 1841: 28, description; *Perla neotropica* Jacobson & Bianchi, 1905: 617, new name; *Macrogynoplax aterrima* Klapálek, 1916: 73, description; *Kempnyia neotropica*, Zwick, 1972: 1168, new combination and illustrations; Zwick, 1973a: 276, record; Bispo & Froehlich, 2004a: 2, record; Bispo & Froehlich, 2004b: 107, record; Bispo & Froehlich, 2008; 62, nymph and complementary description; Stark, 2001: 415, checklist; Stark *et al*., 2009: 124, checklist; Froehlich, 2010: 181, catalog; Froehlich, 2011b: 133, illustration; Froehlich, 2011c: 22, record; Duarte *et al*., 2014: 89, record; Novaes & Bispo, 2014a: 464, illustration and picture;

Novaes & Bispo, 2014b: 283, illustration and picture; Novaes & Bispo, 2014c: 439, record; Novaes *et al*., 2016: 98, record; Gonçalves *et al*., 2017: 147, record; Gonçalves *et al*., 2019: 107, checklist; Almeida & Duarte, 2020: 19, COI sequence.

Kempnyia petersorum Froehlich, 1996: 119, description; Froehlich, 2011b: 134, illustration.

Material examined. BR, BA: Wenceslau Guimarães, Estação Ecológica de Wenceslau Guimarães, Riacho Semo Grande, Cachoeira em cima, 10.x.2010, Calor *et al*. col., 1 male. **ES**: Santa Teresa, REBIO Augusto Ruschi, Cachoeirinha da Estrada, 19.xi.2015, Salles *et al*. col., 1 male; Cachoeirinha à Montante, 19.xi.2015, Salles *et al*. col., 1 male. Parque Nacional do Caparaó, Santa Marta, Rio Santa Marta, Sede, 18-19.ii.2016, Salles *et al*. col., 3 males. **MG**: Ouro Preto, Vale do Tropeiro, Cachoeira do Abacaxi, 07.xi.2001, Paprocki col., 3 males. Alto Caparaó, Rio Caparaó, Hotel Parque Caparaó, 20º25.498'S, 41º51.500'W, 830 m, 11-14.iii.2002, Paprocki col., 1 male. Poços de Caldas, Fazenda de Eucalipto, 08.iii.2019, Fusari *et al*. col., 1 male. **RJ**: Itatiaia, Parque Nacional do Itatiaia, Córrego Campo Belo, 798 m, 06.ix.2017, 7 males; 08.x.2017, Dias & Campos col., 1 male. **SP**: Iporanga, Parque Estadual Intervales, 7.ii.1989, 1 male (*K. petersorum* Holotype/MZUSP); Parque Estadual Intervales, Rio do Carmo, 27.ii.1997, Melo col., 1 male; 14.xii.2014, Bispo col., 7 females; 08.ii.2017, Almeida *et al*. col., 1 male and; 09.ii.2017, Almeida *et al*. col., 3 males; Ribeirão Água Comprida, 24°17'38"S, 48°25'04"W, 29.xi.2000, 1 male. São Miguel Arcanjo, Parque Estadual Carlos Botelho, Ribeirão de Pedras, bridge, 24°03'40"S, 47°59'51"W, 06.ii.2017, Almeida *et al*. col., 2 males. Campos do Jordão, Parque Estadual Campos do Jordão, Córrego Serrote, 22°39'30"S, 45°26'32"W, 18.xii.2018-15.i.2019, Almeida *et al*. col., 1 female; Córrego Galharada, 22°41'29"S, 45°27'58"W, 13.ii.2019, Almeida *et al*. col., 1 female; 08.xii.2019, Almeida *et al*. col., 1 male. Jundiaí, REBIO

Serra do Japi, Córrego do Paraíso, 15-20.xi.2019, Almeida *et al*. col., 3 male; 17.xi.2019, Almeida *et al*. col., 2 males; 20.xii.2019-07.i.2020, Almeida *et al*. col., 1 male.

Measurement data. Male (n=33) forewing length: 11.5–14.2 mm (mean=12.55 mm). Female (n=9) forewing length: 16–17.5 mm (mean=16.83 mm).

Remarks. Almeida & Bispo (2020) observed several variations in the pattern of spots on the head and pronotum of *K. neotropica*. The studied specimens presented all these variations (Fig. 4). The penial armature of the species showed variation, mainly presenting hooks ranging from cylindrical to flat. Herein, we expanded the species distribution (Fig. 4).

***Kempnyia obtusa* Klapálek, 1916** (Figs 3)

Kempnyia obtusa Klapálek, 1916: 51, description; Illies, 1966: 340, catalog; Zwick, 1972: 1171, illustrations; Froehlich, 1988: 153, illustrations; Stark, 2001: 415, checklist; Stark *et al*., 2009: 124, checklist; Froehlich, 2010: 181, catalog; Froehlich, 2011a: 03, checklist; Froehlich, 2011c: 22, illustration; Avelino-Capistrano *et al*., 2014: 330, nymph description; Novaes & Bispo, 2014c: 439, record; Gonçalves *et al*., 2017: 148, record; Gonçalves *et al*., 2019: 107, checklist.

Material examined. BR, MG: Ouro Preto, Parque Estadual do Itacolomi, trib. to Rio Belchior, 20º25.302’S, 43º25.697’W, 700 m, 06.xi.2001, Paprocki col., 4 males. Estação Ecológica de Tripuí, Córrego Botafogo, 20º22.908’S, 43º33.615’W, 1100 m, 25.xi.2001, Paprocki col., 3 males; Rio dos Velhos, Cachoeira Catarina Mendes, 09.i.2017, 1 male. **SP**: Campos do Jordão, Parque Estadual Campos do Jordão, Córrego Serrote, 18.xii.2018-15.i.2019, Fusari *et al*. col., 2

males; Córrego Galharada, 05.xii.2019, Almeida *et al*. col., 4 males; 07.xii.2019, Almeida *et al*. col., 3 males; 08.xii.2019, Almeida *et al*. col., 1 male; 09.xii.2019, Almeida *et al*. col., 3 males.

Measurement data. Male (n=21) forewing length: 15.1–20 mm (mean=17.27 mm).

Remarks. The species is easily identified as it has a characteristic penial armature (Zwick, 1972). The nymph of the species is one of the most easily identified due to the unique pattern of spots on the head and pronotum (Avelino-Capistrano *et al*., 2014). We observed consistency in the pattern of spots on the head and pronotum of the studied specimens, with some variation in the tone of the spots (Fig. 3). Herein, we expanded the species distribution (Fig. 3).

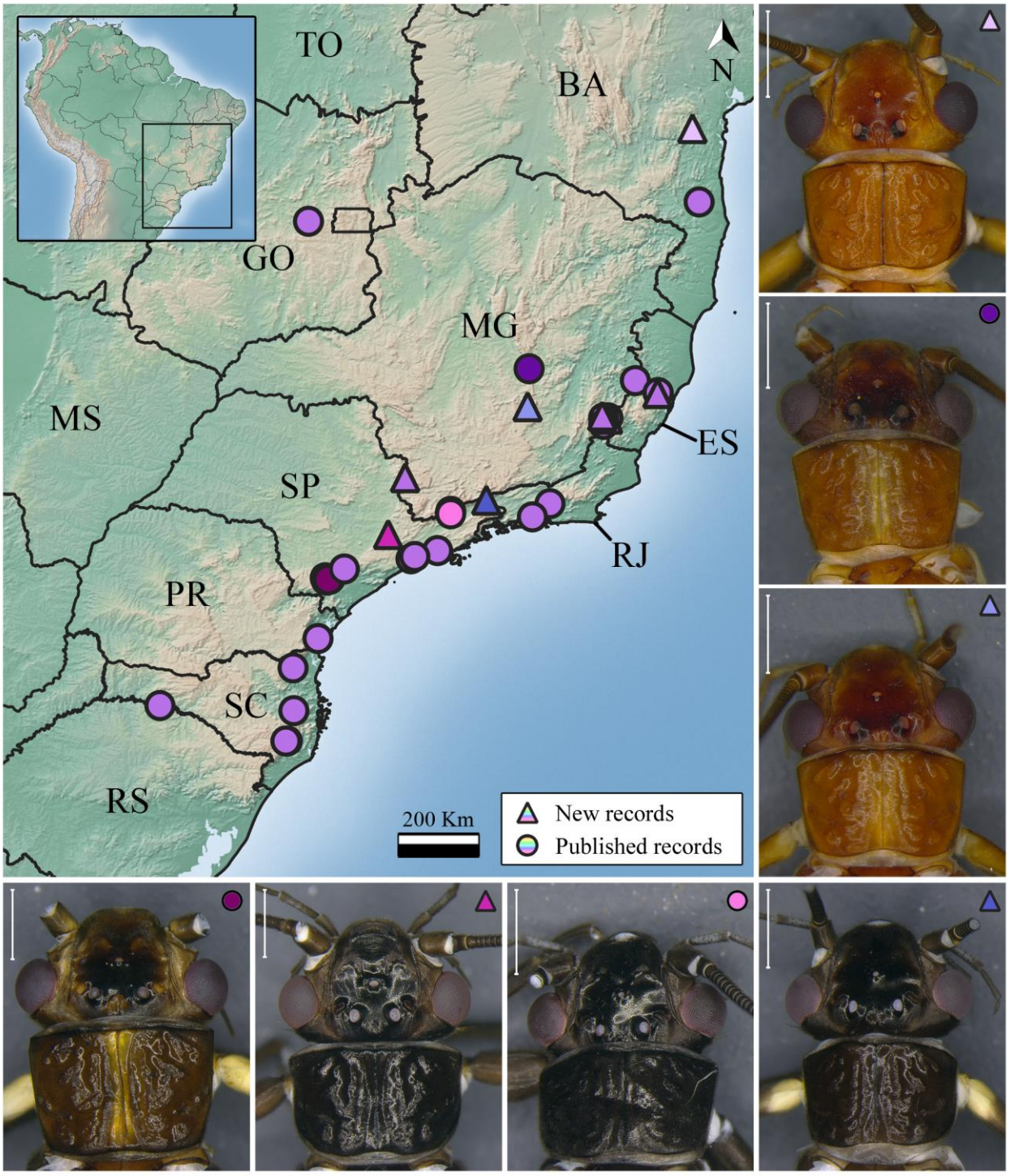

Figure 4. Occurrence map and specimens of *Kempnyia neotropica*. Scale: 1 mm.

***Kempnyia pirata* Froehlich, 2011** (Fig. 5)

Kempnyia pirata Froehlich, 2011c: 23, description.

Material examined. BR, ES: Santa Teresa, REBIO Augusto Ruschi, Córrego à Montante, 09.xii.2015, Salles *et al*. col., 1 female; Córrego Bragacho, 28.iv-27.v.20117, Salles *et al*. col., 1 female; Córrego da Estrada, 18.xii.2017-17.i.2018, Salles *et al*. col., 1 female; Roda D'agua, 18.xii.2017-17.i.2018, Salles *et al*. col., 1 female. **SP**: Jundiaí, REBIO Serra do Japi, Córrego do Paraíso, 20.xii.2019-07.i.2020, Almeida *et al*. col., 1 male.

Measurement data. Male (n=1) forewing length: 8 mm, Female (n=4) forewing length: 10–11.8 mm (mean=10.87 mm).

Remarks. The species is easily identified due to its characteristic penial armature, black general color, and wings with a colorless window (Froehlich, 2011c). This species has been recorded in the Mantiqueira Mountains, São Paulo state (Froehlich, 2011c), and now in another location in São Paulo state and in Espírito Santo state (Fig. 5).

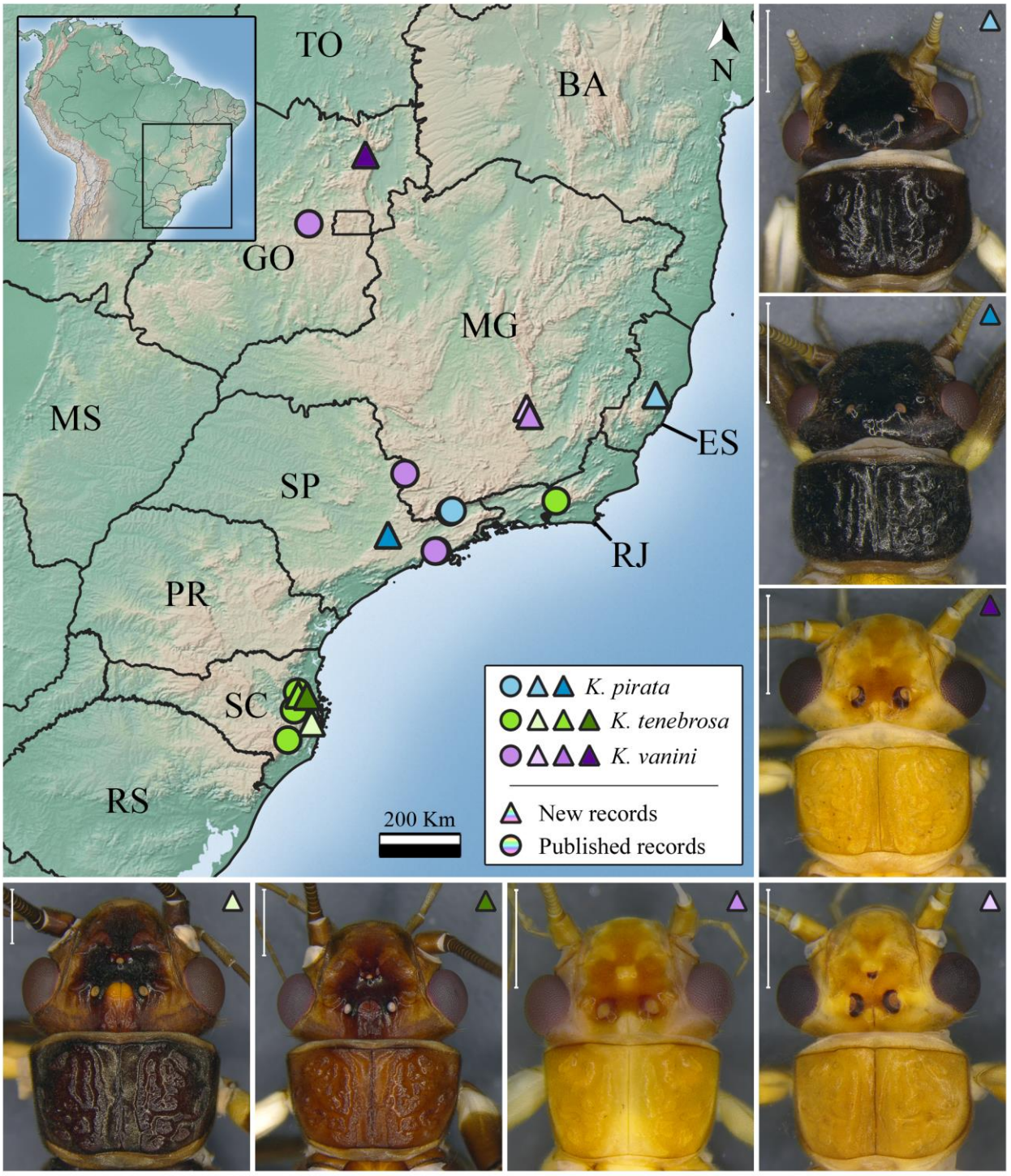

Figure 5. Occurrence map and specimens of *Kempnyia pirata*, *K. tenebrosa*, and *K. vanini*. Scale: 1 mm. Light blue circle: Mantiqueira Mountains. Light green triangle: Specimen from Tabuleiro Mountains; Dark green triangle: Specimen from Brusque.

***Kempnyia reichardti* Froehlich, 1984** (Fig. 6)

Kempnyia reichardti Froehlich, 1984: 143, description; Stark, 2001: 415, checklist; Stark *et al*., 2009: 124, checklist; Froehlich, 2010: 182, catalog; Froehlich, 2011a: 03, checklist; Froehlich, 2011c: 26, illustration.

Material examined. BR, MG: Parque Estadual do Ibitipoca, spring trib. Near director's house, 21°42.695'S, 43°53.760'W, 1357 m, 19-20.xi.2001, Paprocki & Blahnik col., 1 male. **SP**: Campos do Jordão, Parque Estadual Campos do Jordão, Córrego Galharada, 12-13.ii.2019, Almeida *et al*. col., 1 male; 14.ii.2019, Almeida *et al*. col., 1 male; 05.xii.2019, Almeida *et al*. col., 1 male; 07.xii.2019, Almeida *et al*. col., 1 male.

Measurement data. Male (n=5) forewing length: 13.8–15 mm (mean=14.36 mm).

Remarks. The studied specimens are in agreement with the holotype (Froehlich, 1984). However, we observed some variations in the body size, and in the pattern of spots on the head since some specimens have brown or yellow spots on the frons (Fig. 6). These variations approximate the limits between *Kempnyia reichardti* and *K. tamoya* Froehlich, 1984 (Froehlich, 1984). Perhaps, a molecular approach could contribute to solve any possible questions in the identification of the two species. Herein, we expanded the species distribution (Fig. 6).

***Kempnyia reticulata* (Klapálek, 1916)** (Fig. 6)

Eutactophlebia reticulata Klapálek, 1916: 46, description; Illies, 1966: 333, catalog; *Kempnyia reticulata* Zwick, 1983: 177, new combination and illustrations; Stark, 2001: 415, checklist; Stark *et al*., 2009: 124, checklist; Froehlich, 2010: 182, catalog; Avelino-Capistrano *et al*., 2011: 144, nymph and complementary description; Avelino-Capistrano & Nessimian, 2014: 12, record; Gonçalves *et al*., 2019: 107, checklist.

Material examined. BR, ES: Santa Teresa, REBIO Augusto Ruschi, Córrego Bragacho, 21-22.vi.2017, Salles *et al*. col., 1 male; 21.vi-26.vii.2017, Salles *et al*. col., 1 male; 26-27.vii.2017, Salles *et al*. col., 1 male; 26vii-23.viii.2017, Salles *et al*. col., 1 male; Córrego entre estradas, 21.vi-26.vii.2017, Salles *et al*. col., 1 male; Roda D'agua, 26.vii-23.viii.2017, Salles *et al*. col., 2 males.

Measurement data. Male (n=7) forewing length: 21–25.1 mm (mean=23.14 mm).

Remarks. The species is easily identified due to its characteristic penial armature (Zwick, 1983). This species has been recorded only in the Espírito Santo state (Klapálek, 1916; Zwick, 1983; Avelino-Capistrano *et al*., 2011), and herein, we included three new occurrence points (Fig. 6).

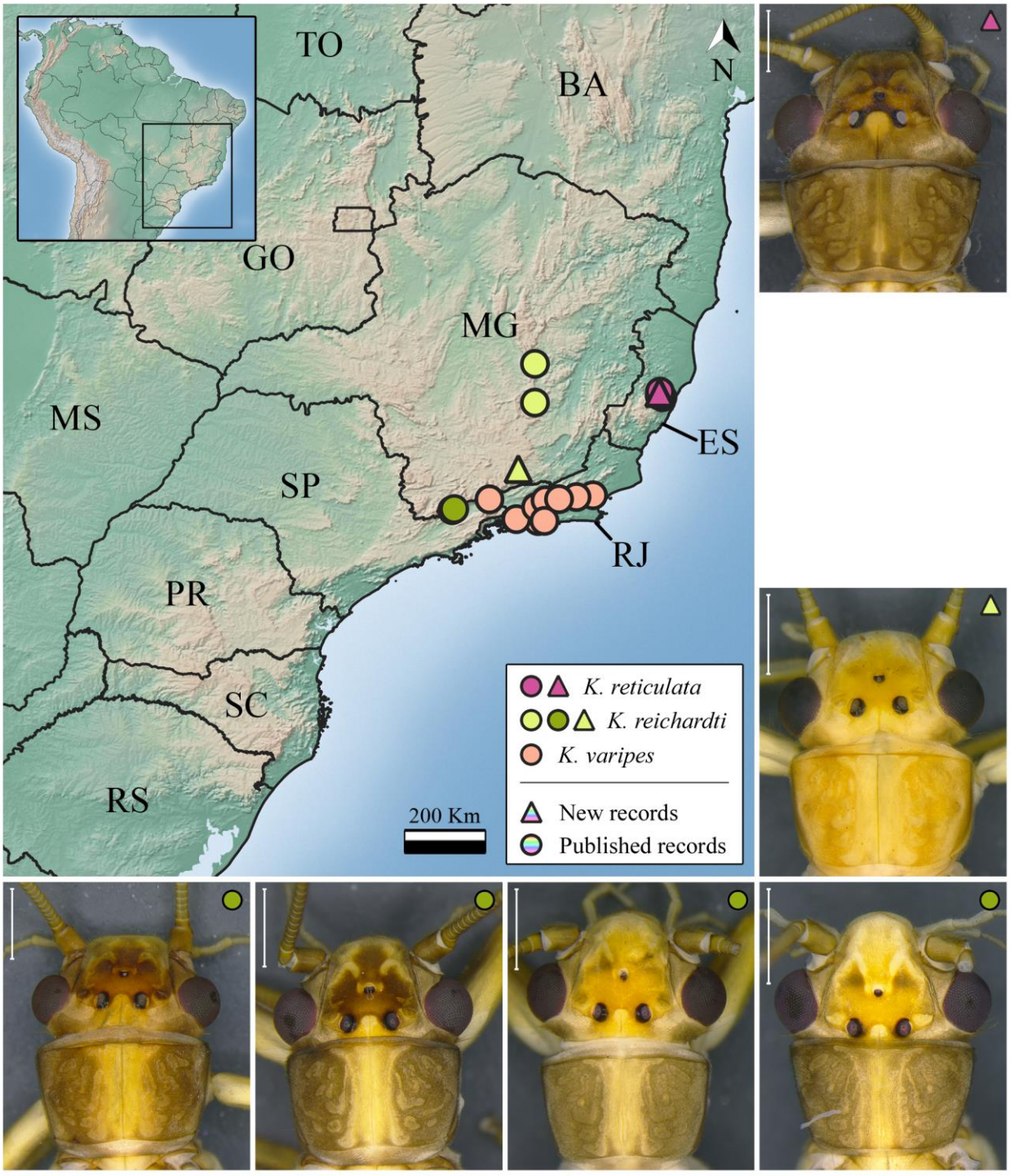

Figure 6. Occurrence map and specimens of *Kempnyia reichardti*, *K. reticulata*, and *K. varipes*. Scale 1 mm.

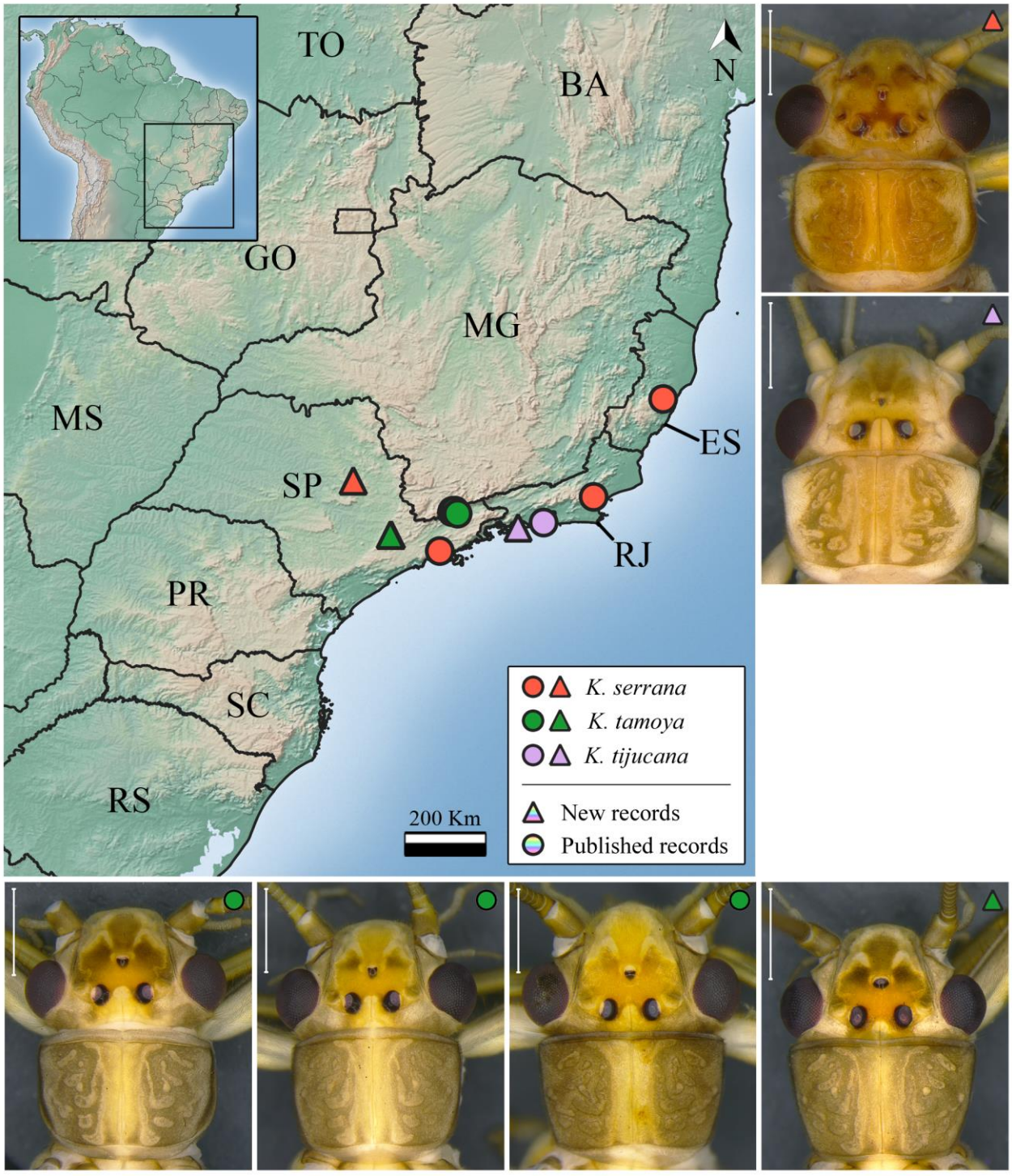

Figure 7. Occurrence map and specimens of *Kempnyia serrana*, *K. tamoya*, and *K. tijucana*. Scale: 1 mm.

***Kempnyia serrana* (Navás, 1936)** (Fig. 7)

Diperla serrana Navás, 1936: 729, description; Aubert, 1956: 439, catalog; Illies, 1966: 476, catalog; *Eutactophlebia gracilenta* Zwick, 1973b: 20, illustration; *Eutactophlebia serrana* Froehlich, 1979: 70, new combination; *Kempnyia serrana* Zwick, 1983: 179, new combination and illustrations; Froehlich, 1984: 139, complementary description; Stark, 2001: 415, checklist; Stark *et al*., 2009: 124, checklist; Froehlich, 2010: 182, catalog; Froehlich, 2011a: 03, checklist; Gonçalves *et al*., 2019: 109, checklist.

Material examined. BR, SP: São Carlos, Fazenda Embrapa, Córrego Canchim, 21°57’07”S, 47°50’12”W, 15.ix.2007, Roque col., 2 males and 1 female.

Measurement data. Male (n=2) forewing length: 12.9–13.5 mm (mean=13.2 mm). Female (n=1) forewing length: 14.9 mm.

Remarks. Herein, we expanded the species distribution (Fig. 7).

***Kempnyia tamoya* Froehlich, 1984** (Fig. 7)

Kempnyia tamoya Froehlich, 1984: 145, description; Stark, 2001: 415, checklist; Stark *et al*., 2009: 124, checklist; Froehlich, 2010: 182, catalog; Froehlich, 2011a: 03, checklist; Froehlich, 2011c: 28, illustrations.

Material examined. BR, SP: Jundiaí, REBIO Serra do Japi, Cachoeira do Chá II, 23°14’34.7”S, 46°56’06.7”W, 16.xi.2019, Almeida *et al*. col., 2 males; Biquinha, 23°14’22.2”S, 46°56’07.2”W,

16.xi.2019, Almeida *et al*. col., 2 males; Córrego do Paraíso, 17.xi.2019, Almeida *et al*. col., 3 males; 17.xi.2019, Almeida *et al*. col., 3 males; 18.xi.2019, Almeida *et al*. col., 1 male; Cachoeira do Chá I, 23°14'17.3"S, 46°56'00.7"W, 18.xi.2019, Almeida *et al*. col., 4 males. Campos do Jordão, Parque Estadual Campos do Jodrão, Córrego Galharada, 05.xii.2019, Almeida *et al*. col., 4 males; 06.xii.2019, Almeida *et al*. col., 1 male; 07.xii.2019, Almeida *et al*. col., 5 males; 08.xii.2019, Almeida *et al*. col., 1 male; 09.xii.2019, Almeida *et al*. col., 2 males.

Measurement data. Male (n=29) forewing length: 12–15.5 mm (mean=14.39 mm).

Remarks. The studied specimens are in agreement with the holotype (Froehlich, 1984). However, there are specimens with wider penial armature in dorsal view and with darker spots on the head (Fig. 7). Herein, we expanded the species distribution (Fig. 7).

***Kempnyia tenebrosa* Klapálek, 1916** (Fig. 5)

Kempnyia tenebrosa Klapálek, 1916: 50, description; Jewett, 1960: 177, notes; Illies, 1966: 340, catalog; Zwick, 1972: 1172, illustrations; Stark, 2001: 415, checklist; Stark *et al*., 2009: 124, checklist; Froehlich, 2010: 182, catalog; Froehlich, 2011b: 136, illustration; Novaes & Bispo, 2014b: 283, record and illustrations.

Material examined. BR, SC: Santo Amaro Imperatriz, Serra do Tabuleiro, 13.ii-2.viii.2014, Pinho col., 1 male. Brusque, RPPN Chácara Edith, 21.vii.2018, Pinho col., 1 female; 04.v-3.vi.2019, Pinho col., 2 males; i-ii.2019, Pinho col., 3 male and 1 female. Blumenau, Parque Nacional da Serra do Itajaí, Parque das Nascentes, Trilha do Morro do Sapo, Córrego da Placa, 05.xi.2019, Almeida & Miguel col., 1 female.

Measurement data. Male (n=6) forewing length: 12.2–17 mm (mean=13.81 mm). Female (n=3) forewing length: 15.5–16.5 mm (mean=16.16 mm).

Remarks. The studied male specimens are similar to the holotype from Rio de Janeiro state (Zwick, 1972). However, the specimens presented variations in body size, pattern of spots on the head and pronotum (Fig. 5), and in the shape of the penial armature. The specimen from the Tabuleiro Mountains (Santa Catarina state) is larger (17.5 mm) and has differences in the shape of the penial armature, mainly in lateral view, compared to the specimens from Brusque (Santa Catarina state) (12.2–13.5 mm). The penial armature of the specimen from the Tabuleiro Mountains is more similar to that illustrated by Novaes & Bispo (2014b), while that of the specimens from Brusque are similar to that illustrated by Froehlich (2011b). A molecular approach would be beneficial to better understand these variations. Herein, we expanded the species distribution (Fig. 5).

***Kempnyia tijucana* Dorvillé & Froehlich, 1997** (Fig. 7)

Kempnyia tijucana Dorvillé & Froehlich, 1997: 178, description; Dorvillé & Froehlich, 2001: 385, nymph description; Stark, 2001: 415, checklist; Stark *et al*., 2009: 124, checklist; Froehlich, 2010: 182, catalog.

Material examined. BR, RJ: Mangaratiba, Ilha da Marambaia, Rio Marambaia, 23°04’12.1”S, 43°55’06.8”W, 20.x-24.xi.2018, Avelino-Capistrano col., 1 female; 15.vii-18.viii.2018, Avelino-Capistrano col., 1 male.

Measurement data. Male (n=1) forewing length: 12.1 mm, Female (n=1) forewing length: 13 mm.

Remarks. The studied male specimen presented no asymmetrical arrangement of the penial armature, as illustrated by Dorvillé & Froehlich (1997). Herein, we expanded the species distribution (Fig. 7).

***Kempnyia tupinamba* Froehlich, 2011** (Fig. 2)

Kempnyia tupinamba Froehlich, 2011c: 28, description.

Material examined. BR, SP: Campos do Jordão, Parque Estadual Campos do Jordão, Córrego Galharada, 07.xii.2019, Almeida *et al*. col., 3 males.

Measurement data. Male (n=3) forewing length: 14.2–16 mm (mean=15.06 mm).

Remarks. One of the studied specimens presented variation in the spots on the head, being slightly different from the description (Froehlich, 2011c). This specimen showed a fragmented M-line forming three yellow spots (Fig. 2).

***Kempnyia vanini* Froehlich, 1988** (Fig. 5)

Kempnyia vanini Froehlich, 1988: 164, description; Bispo & Froehlich, 2004a: 02, record; Stark, 2001: 415, checklist; Stark *et al*., 2009: 124, checklist; Froehlich, 2010: 182, catalog; Froehlich, 2011a: 03, checklist.

Material examined. BR, GO: Alto Paraíso de Goiás, Chapada dos Veadeiros, Córrego Loquinhas, 15.xii.2006, Bispo col., 1 male. **MG**: Ouro Preto, Vale do Tropeiro, Cachoeira do Abacaxi, 7.xi.2001, Paprocki col., 3 males. Estação Ecológica de Tripuí, Córrego Botafogo, 25.xi.2001, Paprocki col., 2 males. **SP**: Salesópolis, Estação Biológica de Boracéia, 22.xii.1987, Froehlich col., 1 male.

Measurement data. Male (n=7) forewing length: 10.8–13 mm (mean=11.77 mm).

Remarks. The studied specimens presented variation in general color, pattern of spots on the head, and number of ocelli. Probably, the general color variation was due the preservation in 80% ethanol (Almeida & Bispo, 2020). The number of ocelli ranged from two, in populations from Goiás and Minas Gerais states, to three, in the population from São Paulo state and a population from Minas Gerais state (Estação Ecológica Tripuí) (Fig. 5). The penial armature of *Kempnyia vanini* can be confused with that of species such as *K. tupinamba*, *K. sazimai* Froehlich, and *K. umbrina* Froehlich. For all the reasons presented, we suggest that a molecular study including all populations of these species be carried out in order to better understand the limits among species. Despite that, we expanded the species distribution (Fig. 5).

***Kempnyia varipes* Klapálek, 1916** (Figs 6, 8–9)

Kempnyia varipes Klapálek, 1916: 52, description; Jewett, 1960: 178, misidentification; Illies, 1966: 341, catalog; Zwick, 1972: 1174, lectotype and paralectotype designations; Zwick, 1973: 277; Stark, 2001: 415, checklist; Stark *et al*., 2009: 124, checklist; Froehlich, 2010: 183, catalog; Avelino-Capistrano *et al*., 2014: 332, nymph and complementary description.

Material examined. BR: Lectotype male and paralectotype female (Naturhistorische Museum Wien, Austria).

Figure 8. Lectotype of *Kempnyia varipes*. Head and pronotum (A), information tag (B), and hammer (C).

Remarks. *Kempnyia varipes* is a taxonomically problematic species. Described based on a female (Klapálek, 1916), the species had males mistakenly associated by Jewett (1960) (Zwick, 1972). Zwick (1972) also designated a male lectotype (Fig. 8) and a female paralectotype (Fig. 9). Despite this, the male lectotype did not have his penial armature illustrated by Zwick. Apparently,

the lectotype does not have its abdomen dissected, but it is not possible to see the penial armature through the specimen's abdomen (Fig. 8). This indicates that the specimen needs to be carefully studied. In addition, both the lectotype and the paralectotype (Figs 8A, 9A) present a pattern of spots on the head and pronotum different from those observed by Avelino-Capistrano *et al*. (2014) in specimens from Rio de Janeiro state. This may indicate that the type specimens and those studied by Avelino-Capistrano *et al*. (2014) are different. In this case, the specimen described by Avelino-Capistrano *et al*. (2014) would be a new species. To solve this taxonomic situation, the male lectotype must have at least the penial armature studied, illustrated, and compared to that of the specimen studied by Avelino-Capistrano *et al*. (2014). The species is recorded only in Rio de Janeiro state (Fig. 6).

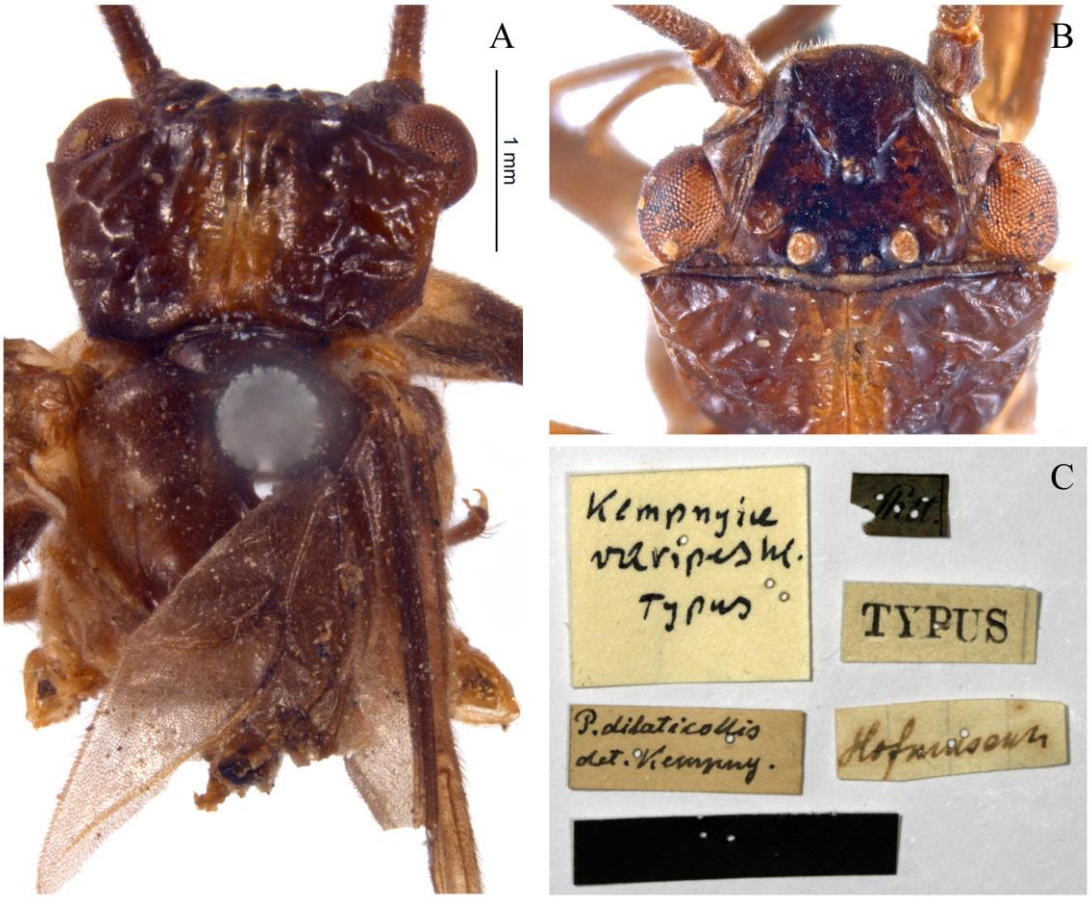

Figure 9. Paralectotype of *Kempnyia varipes*. Pronotum (A), head (B), and information tag (C).

***Kempnyia tupiniquim* sp. nov. Almeida, Gonçalves & Bispo** (Figs 10A–G)

Material examined. BR, ES: Santa Teresa, REBIO Augusto Ruschi, Córrego da Estrada, 27-28.xii.2017, Salles *et al*. col., 1 male (Holotype/UFVB); Córrego Bragacho, 21-22.x.2017, Salles *et al*. col., 1 male (Paratype/CLBA); 18-19.xi.2017, Salles *et al*. col., 1 male (Paratype/MZUSP).

Measurement data. Male (n=3) forewing length: 10.1–11 mm (mean=10.63 mm).

Description. General color whitish. Anterior ocellus absent. Frons dark brown, whitish along sides of the frons and genae; M-line light brown; lappets whitish, frontoclypeus, in front of M line, predominantly dark brown with whitish tip; parietalia dark brown with a barely whitish longitudinal line (Fig. 10A). Antennae whitish. Pronotum dark brown with rounded corners (Fig. 10A). Milky wings, general color gray, whitish proximally (Fig. 10B). Legs whitish, tibia darker. Cercomeres whitish.

Male. Tergum X pale medially and at bases of cerci, most sensilla basiconica carrot-shaped. Projection of the subgenital plate as broad as long; hammer whitish, triangle-shaped; from the base to the tip of the hammer there is a light ocher stripe; subgenital plate mostly whitish (Figs 10C–D). Paraprocts finger-like, with a subapical denticle; apical sensillae with sparse minute hairs. Basal ring of the penial armature broad, hooks making an almost completely round curve in the same plane (Figs 10E–G). The membranous structures of the penial armature are large, mainly in lateral view (Fig. 10G).

Female and nymph. Unknown.

Remarks. It is important to note that we described this species based on teneral individuals, this means that the colors shown here may vary. *Kempnyia tupiniquim* sp. nov. has a penial armature similar to that presented by *K. vanini*. However, the penial armature of *K. tupiniquim* sp. nov. is

more robust, being proportionally wider. In addition, the membranous structures of the penile armature, mainly in lateral view, are larger in *K. tupiniquim* sp. nov. than in *K. vanini*.

Etymology. The name honors the Tupiniquim people of Brazil, which still lives in indigenous areas in Espírito Santo state.

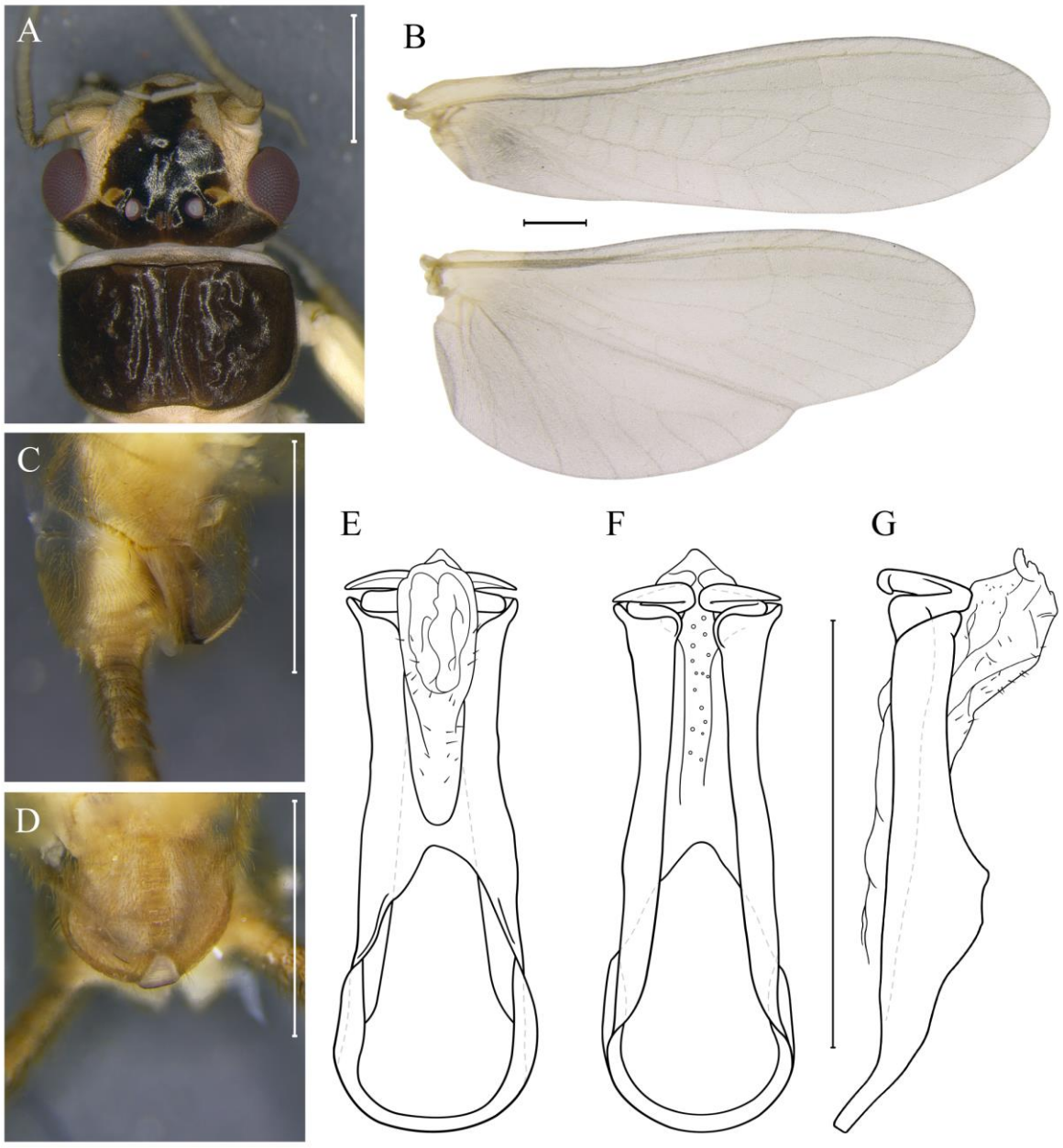

Figure 10. *Kempnyia tupiniquim* sp. nov. Adult male, head and pronotum (A), wings (B) and hammer in lateral (C) and ventral views (D). Penial armature in dorsal (E), ventral (F) and lateral views (G). Scale: 1 mm.

***Kempnyia zwicki* sp. nov. Almeida, Gonçalves & Bispo** (Fig. 11)

Material examined. BR, ES: Santa Teresa, REBIO Augusto Ruschi, Córrego Bragacho, 18.xii.2017-17.i.2018, Salles *et al*., 1 male (Holotype/UFVB).

Measurement data. Male (n=1) forewing length: 11.5 mm.

Description. General color dark brown. Anterior ocellus absent. Frons dark brown, often lighter along sides of the frons and genae; M-line brown and incomplete; lappets dark brown, frontoclypeus, in front of M line, dark brown; parietalia dark brown to brown, lighter laterally and darker in the middle near the coronal suture (Fig. 11A). Scape of antennae brown, pedicel brown, flagellum mostly brown. Pronotum square and dark brown (Fig. 11A). Membrane and veins of forewings brown and dark brown respectively; membrane of hindwings dark brown in the apex and lighter in anal lobe, veins dark brown (Fig. 11B). Legs white to dark brown; coxae, trochanters, and base of femora and tibia white; apex of femora and tibia dark brown; tarsus dark brown. Cercomeres brown with darker apices.

Male. Tergum X pale medially and at bases of cerci, most sensilla basiconica carrot-shaped. Projection of the subgenital plate about twice as broad as long; T-shaped hammer white; from the base to the tip of the hammer, there is a light ocher stripe; subgenital plate mostly whitish (Figs 11C–D). Paraprocts finger-like, with a subapical denticle; apical sensillae with sparse minute hairs. Basal ring of the penial armature broad, long hooks making a wide curve (Figs 11E–G).

Female and nymph. Unknown.

Remarks. *Kempnyia zwicki* sp. nov. has a pattern of spots on the head and pronotum that resemble that of *K. neotropica*. Both species are almost homogeneously dark, but the shapes of

penial armature are different. In lateral view, the penial armature of *K. zwicki* sp. nov. does not resemble that of any other species described to date.

Etymology. The name honors Peter Zwick, a researcher who has given an important contribution to the study of stoneflies Plecoptera.

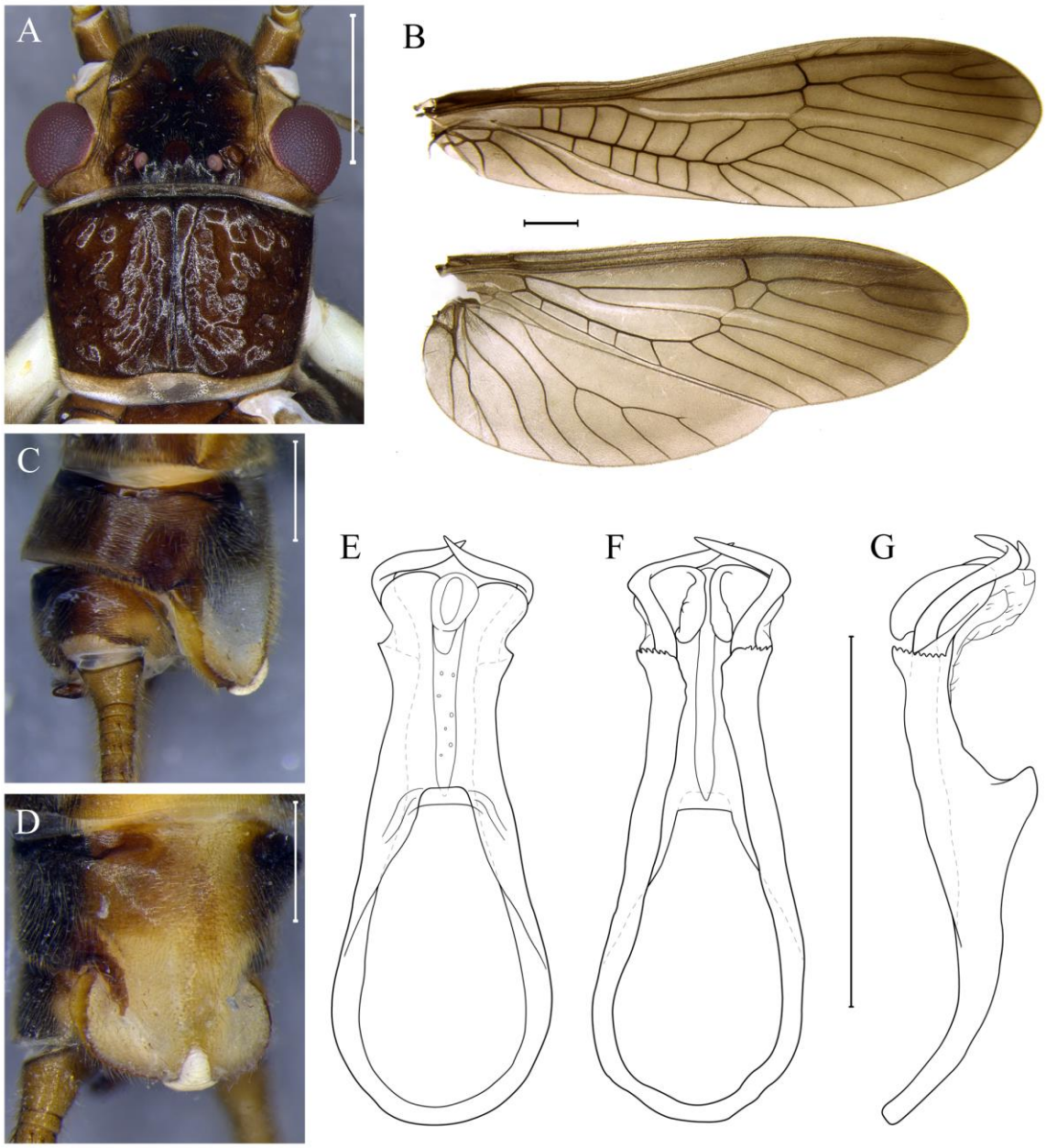

Figure 11. *Kempnyia zwicki* sp. nov. Adult male, head and pronotum (A), wings (B) and hammer in lateral (C) and ventral views (D). Penial armature in dorsal (E), ventral (F) and lateral views (G). Scale: 1 mm.

***Kempnyia guarani* sp. nov. Almeida, Gonçalves & Bispo** (Fig. 12)

Material examined. BR, SC: Blumenau, Parque Nacional da Serra do Itajaí, Parque das Nascentes, Encontro das águas, 03.xi.2019, Almeida & Miguel col., 1 male (Holotype/MZUSP), 1 male (Paratype/CLBA).

Measurement data. Male (n=2) forewing length: 16–17 mm (mean=16.5 mm).

Description. General color brown to yellowish. Anterior ocellus present. Frons brown, often lighter along sides of frons and genae; M-line yellowish; lappets brown, frontoclypeus, in front of M line, ochraceous; parietalia brown, yellow in the middle, near the coronal suture (Fig. 12A). Antennae brown. Pronotum square, brown with yellowish longitudinal and centralized spot (Fig. 12A). Membrane and veins of wings brown and dark brown, respectively (Fig. 12B). Legs yellow to brown; coxae, trochanters, and base of femora yellow; apex of femora dark brown; tibia and tarsus dark brown. Cercomeres yellowish.

Male. Tergum X pale medially and at bases of cerci, most sensilla basiconica carrot-shaped. Projection of the subgenital plate about twice as broad as long; T-shaped hammer white, prominent in lateral view; from the base to the tip of the hammer there is a light ocher stripe; subgenital plate mostly whitish (Figs 12C–D). Paraprocts finger-like, without a subapical denticle; apical sensillae with sparse minute hairs. Basal ring of armature narrow, long and flat hooks making an upward curve (Figs 12E–G).

Female and nymph. Unknown.

Remarks. The *K. guarani* sp. nov. species resembles *K. neotropica* in the shape of penial armature (Froehlich, 1996; Bispo & Froehlich, 2004b). However, *K. guarani* sp. nov. has a larger body (16–17 mm) than *K. neotropica* (11.5–14.2 mm) and presents well-defined dark and light

spots on the head and pronotum, different from *K. neotropica* (Almeida & Bispo, 2020). Other species resemble *K. guarani* sp. nov. in some respect, such as that of *K. alterosarum* Froehlich, 1996 and *K. ocellata* Froehlich, 2011 (Froehlich, 1996; 2011b). However, none of them has sufficiently congruent characters with *K. guarani* sp. nov., so we considered it as a new species.

Etymology. The name honors the Guarani people of Brazil, which still lives in indigenous areas in Santa Catarina state and in nearby states.

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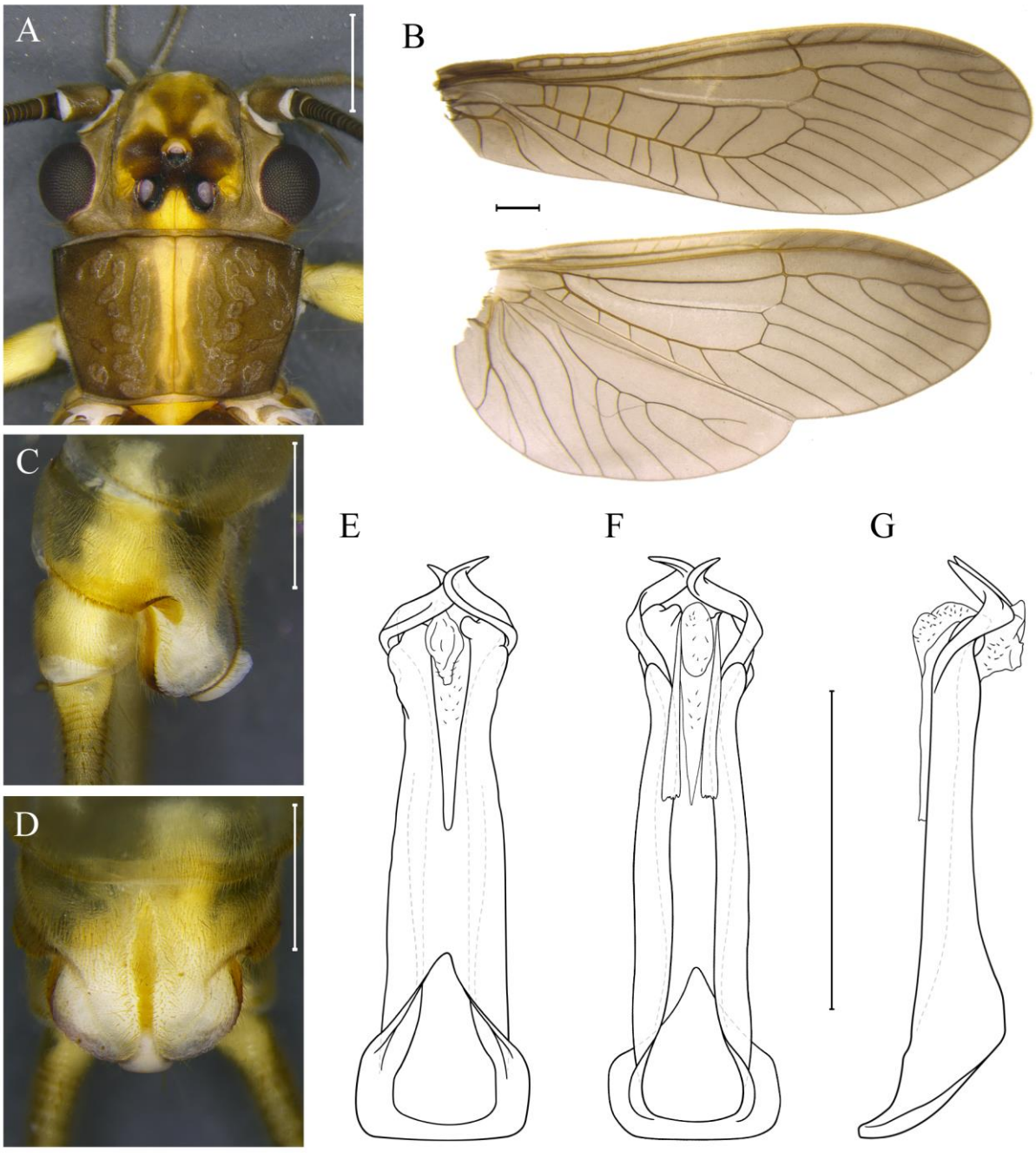

Figure 12. *Kempnyia guarani* sp. nov. Adult male, head and pronotum (A), wings (B) and hammer in lateral (C) and ventral views (D). Penial armature in dorsal (E), ventral (F) and lateral views (G). Scale: 1 mm.

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Capítulo 3

A new species of *Enderleina* Jewett, 1960 and new records of *Macrogynoplax* Enderlein, 1909 (Plecoptera: Perlidae) from Brazil

A new species of *Enderleina* Jewett, 1960 and new records of *Macrogynoplax* Enderlein, 1909 (Plecoptera: Perlidae) from Brazil

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Abstract

Herein, we describe a new species of *Enderleina* and update the geographic distribution of *Macrogynoplax*, including new records of *M. veneranda* Froehlich, 1984, the first record of *M. matogrossensis* in the Goiás state, the first record of a *Macrogynoplax* in the Bahia state, and the first report of *M. yupanqui* Stark, 1996 in Brazil. In addition, we provide comments on the morphological variations, mainly pattern of spots, in these species.

Keywords: Stoneflies; Aquatic Insects; Amazon Forest.

Introduction

Perlidae is the most specious family among the Plecoptera, representing more than 30% of all species (DeWalt & Ower, 2019). In the Neotropical region, stoneflies are represented for at least 400 valid species (Pessacq *et al.*, 2019). Among these, about 140 are recorded in Brazil and mainly distributed to the genera *Anacroneuria* Klapálek 1909, *Enderleina* Jewett, 1960, *Kempnyia* Klapálek, 1914, and *Macrogynoplax* Enderlein, 1909.

Jewett (1960) proposed the genus *Enderleina* describing *E. preclara* Jewett, 1960 as type species. The specimens of the genus have three ocelli, modified male genitalia forming a small button-shaped structure or hammer near its posterior border, and hind wing venation with a reduced anal area (Jewett, 1960). There are nine species described for the genus, which are recorded in the Brazilian (4) and Venezuelan (5) Amazon Forest (Derka *et al.*, 2019; Hamada & Silva, 2019).

Enderlein (1909) proposed the genus *Macrogynoplax* with *M. guayanensis* as the type species. Currently, the genus has 16 valid species (eight in Brazil) and is found mainly in the Amazon Forest from Brazil, Colombia, Guyana, Peru, Suriname, and Venezuela (Pessacq *et al*., 2019). In Brazil, there are only two non-Amazonian species, *M. matogrossensis* Bispo & Neves, 2005 (Bispo *et al*., 2005) and *M. veneranda* Froehlich, 1984 with some isolated records in mountainous regions of the Central Brazil and coastal Atlantic Forest respectively.

Herein, we describe a new species of *Enderleina*. We also update the geographic distribution of *Macrogynoplax matogrossensis* and *M. veneranda*, and report for the first time *M. yupanqui* Stark, 1996 in Brazil. In addition, we provide comments on the morphological variations in these species.

Material and methods

Fieldworks were carried out November 2013, 2019, and 2021. The new species of *Enderleina* and *Macrogynoplax yupanqui* were collected in Nova Xavantina – MT, *M. matogrossensis* at the Chapada dos Veadeiros National Park, Alto Paraíso de Goiás – GO, and *M. veneranda* at the Biological Reserve of Serra do Japi, Jundiaí – SP. The specimens of *Enderleina* sp. nov., *Macrogynoplax matogrossensis* and *M. veneranda* were collected using a LED light pan trap, using blue, green and UV LEDs (Price & Baker, 2016).

To extract the penial armature, we severed the abdomen between segments seven and eight and treated the severed segments using 10% KOH for overnight. To neutralize the reaction, we dipped the segments in acetic acid for a few seconds and then washed them using ethanol. We performed the extraction of penial armature and identified the species based on the comparison of male penial armature morphology with those described in the literature (Froehlich, 1984; Stark, 1996; Bispo *et al*., 2005). Morphological terminologies of the penial armature followed those used by Hamada & Silva (2019). We illustrated the penial armature under a camera lucida mounted on a Leica DM1000 microscope and rendered it using Adobe Illustrator CS6® editor. Using a digital camera on a Leica M205A stereomicroscope and the Adobe Photoshop CS3® editor, we took and improved the pictures, respectively. We made the species distribution maps using QGIS Bucur 3.14.15 software (QGIS Development Team, 2020). Finally, holotype of the new species will be deposited in the Museum of Zoology of the University of São Paulo (MZUSP).

Results

Enderleina Jewett, 1960

***Enderleina castro* sp. nov. Almeida & Bispo, 2022** (Figs 1A–F)

Material examined. BR, MT: Nova Xavantina, Córrego da Mata (14º59’56”S, 52º28’44.6”W), 12.xi.2021, Almeida, L.H. *et al.* col. 1 male (Holotype/MZUSP); 2 males, same label (Paratypes/MZUSP).

Measurement data. Male (n=3) forewing length: 0.85– 0.91 mm (mean=0.886 mm).

Male description. General color black to dark brown dorsally with light yellow areas ventrally. Anterior ocellus present (Fig. 1A). Head almost entire black to dark brown, frontoclypeus distally yellow (Fig. 1A). Antennae brown. Pronotum black with square corners on the anterior margins and rounded on posterior margins (Fig. 1A). Wings membrane color dark gray, veins black; wings with a distal yellow transversal stripe, almost uniform on the forewings and tapering from anterior to posterior margin on the hindwings (Fig. 1C). Legs general color black; femora dorsally black with a yellow stripe near proximal region and ventrally almost entire yellow, black near distal region; tibiae and tarsi black. Tergum X white medially and dark brown at bases of cerci, most sensilla basiconica carrot-shaped. Sternum IX general color black to dark brown with two parallel white stripes (Fig. 1B); subgenital plate projection flattened on top; hammer brown, rounded, and slightly pronounced (Fig. 1B). Cercomeres black. Paraprocts finger-like, with some irregular subapical denticles; apical sensillae with sparse and minute hairs. Penial armature with a median sclerite and a pair of basolateral arms (Figs. 1D–E); median sclerite

elongated tapering toward about 2/3 of apex, apex extremely sharp and entire covered by spiniform projections (Figs. 1D–F); basolateral arms with two pairs (ventral and dorsal) of elongated projections (Figs. 1D–E), ventral projection shorter than dorsal one, both projections with a pair of asymmetrical needle-like structures on the distal margin (Fig. 1F).

Female and nymph. Unknown.

Distribution. Known only from the type locality in Córrego da Mata, Nova Xavantina–MT.

Remarks. Among the known *Enderleina* species, *E. castro* sp. nov. has the shape of the penial armature similar only to that of *E. plagata* Hamada & Silva, 2019, with substantial differences in all structures. In the penial armature of *E. castro* sp. nov. the median sclerite is narrower and completely covered by spiniform projections, the basolateral arms are more robust, the ventral projections of basolateral arms are considerably smaller, and both dorsal and ventral projections have two needle-like structures on the distal margin (Figs 1D–F). In addition, the maculation pattern on the head and pronotum of *E. castro* sp. nov. differs considerably from that of *E. plagata*. *Enderleina castro* sp. nov. is almost entirely dark (Figs. 1A–B), while *E. plagata* has an almost entirely yellow body with dark spots scattered throughout the body, such as on the head, legs, and cerci (Hamada & Silva, 2019). The wings of *E. plagata* have yellow spots in the proximal region (Hamada & Silva, 2019), while in *E. castro* sp. nov. the wings have only yellow spots in the distal region (Fig. 1C).

Etymology. Named in honor of Dr. Lourivaldo Amancio de Castro, laboratory assistant of the Mato Grosso state University (UNEMAT) and our guide during fieldwork in Nova Xavantina (Mato Grosso state). Dr. Castro is an entomologist who devoted a great deal of his life to the

study of aquatic insects, assisting in the formation of hundreds of aquatic entomologists and publishing important contributions to Brazilian Entomology. Treat as a noun in apposition.

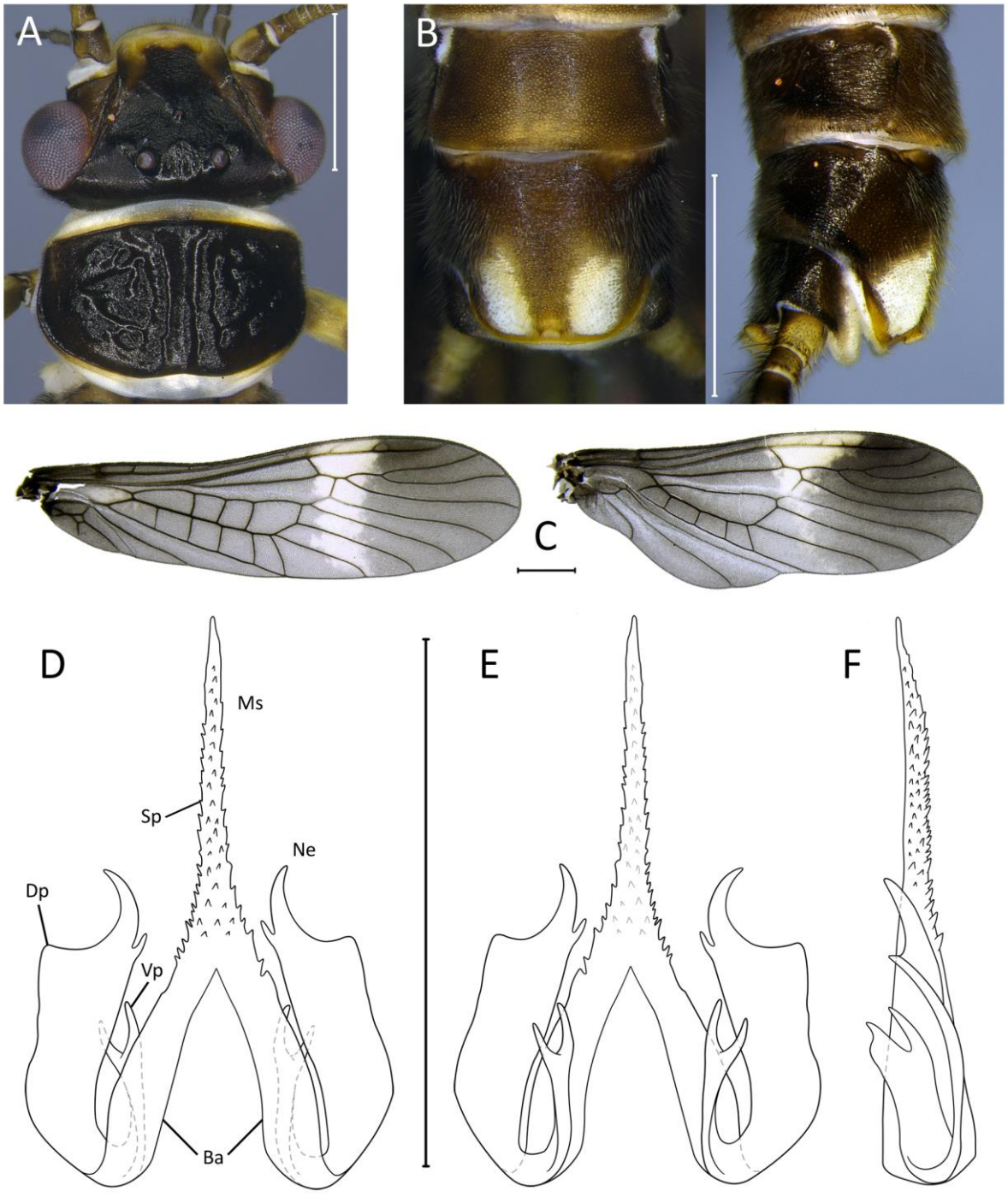

Figure 1. *Enderleina castro* sp. nov.. Adult male, head and pronotum (A), hammer in lateral and ventral views (B), wings (C) and penial armature in ventral (D), dorsal (E), and lateral views (F). Scale: 1 mm.

Ms: median sclerite; Ba: basolateral arms of median sclerite; Dp: dorsal projection of basolateral arms; Vp: ventral projection of basolateral arms; Ne: needle-like structure on the dorsal and ventral projections of basolateral arms; Sp: spiniform projections.

***Macrogynoplax* Enderlein, 1909**

***Macrogynoplax matogrossensis* Bispo & Neves, 2005** (Fig. 2)

Macrogynoplax matogrossensis Bispo & Neves, 2005 (in Bispo *et al*., 2005): 39, male description; Froehlich, 2010: 184, catalog; Ribeiro & Gorayeb, 2016: 434, checklist; Rippel *et al*., 2019: 473, record and nymph description.

Material examined. BR, GO: Alto Paraíso de Goiás, Chapada dos Veadeiros National Park, Cachoeira dos Cristais, Córrego Saltador (14º06'07.3"S, 47º29'58.3"W), 26.xii.2021, Almeida, L.H. *et al.* col., 1 male and 1 female.

Measurement data. Male (n=1) forewing length: 14.6 mm. Female (n=1) forewing length: 15.5 mm.

Remarks. Nymphs and a female of *Macrogynoplax* were previously recorded for Goiás state (Bispo *et al*., 2002a; 2002b), but without a nominal record. Herein, we performed the first nominal record of a *Macrogynoplax* species from Goiás. Therefore, *Macrogynoplax matogrossensis* has been recorded in the Mato Grosso and Tocantins states (Bispo *et al*., 2005; Rippel *et al*., 2019), and now in the Goiás state (Fig. 2).

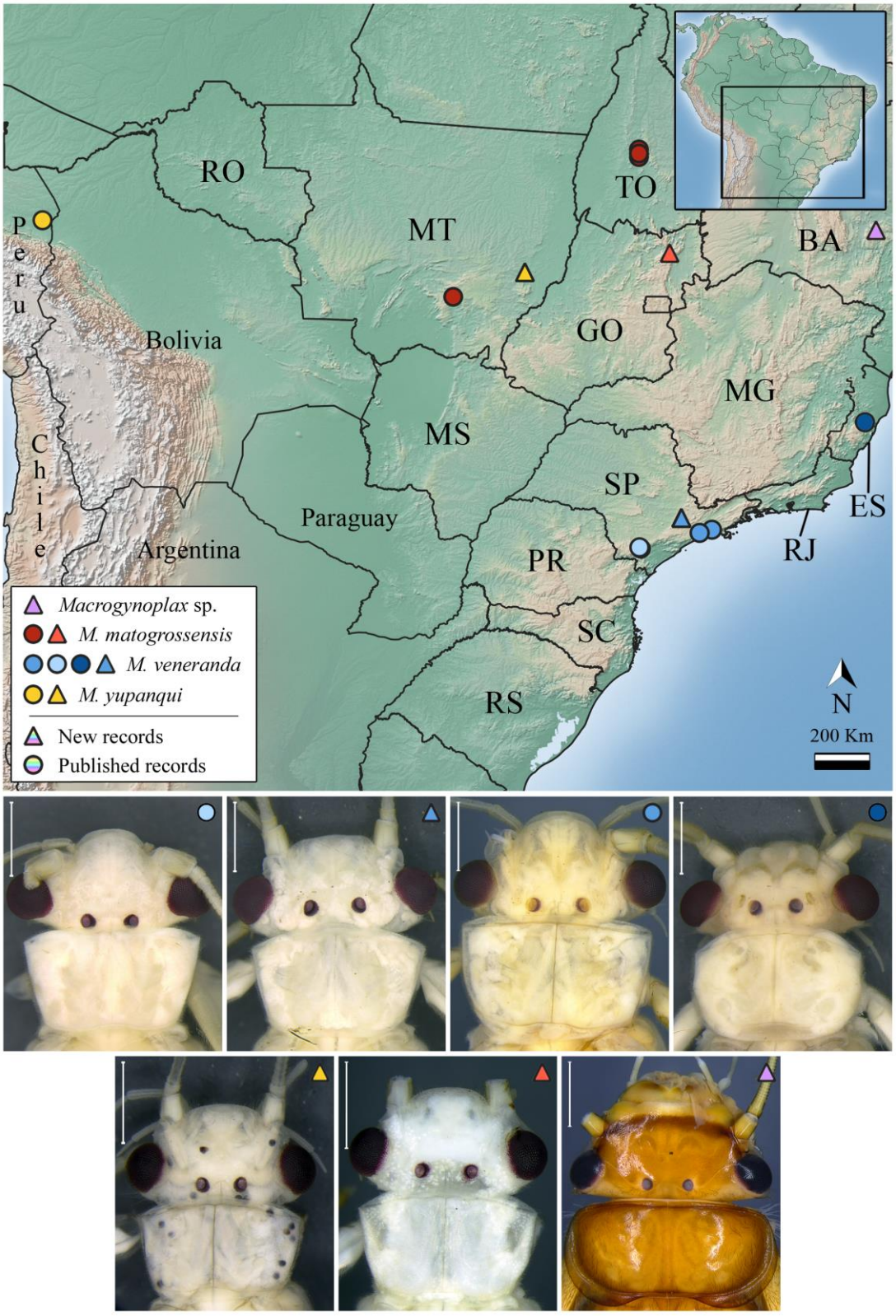

Figure 2. Occurrence map and specimens of *Macrogynoplax matogrossensis*, *M. veneranda* and *M. yupanqui*. Brazilian states acronyms: Acre – AC; Amazonas - AM; Amapá - AP; Goiás - GO; Maranhão - MA; Mato Grosso - MT; Pará - PA; Rondônia - RO; Roraima - RR; Tocantins - TO.

***Macrogynoplax veneranda* Froehlich, 1984** (Fig. 2)

Macrogynoplax veneranda Froehlich, 1984: 39, male, female and nymph descriptions; Bispo & Froehlich, 2004: 111, record; Froehlich, 2010: 185, catalog; Gonçalves *et al*., 2019: 109, record; Almeida & Bispo, 2020: 20, COI sequence.

Material examined. BR, SP: Jundiaí, Biological Reserve of Serra do Japi, Córrego do Paraíso (23º14'49.1"S, 46º56'36.9"W), 15.xi.2019, Almeida, L.H. *et al.* col., 1 male; 17.xi.2019, Almeida, L.H. *et al.* col., 3 males and 2 females.

Measurement data. Male (n=4) forewing length: 14.1–14.6 mm (mean=14.3 mm). Female (n=2) forewing length: 16.7–17.3 mm (mean=17.15 mm).

Remarks. The species is distributed in southeastern Brazil (Froehlich, 1984; Bispo & Froehlich, 2004; Gonçalves *et al.*, 2019). Herein, we expanded the species distribution (Fig. 2).

***Macrogynoplax yupanqui* Stark, 1996** (Fig. 2)

Macrogynoplax yupanqui Stark, 1996: 318, description; Froehlich, 2010: 185, catalog.

Material examined. BR, MT: Nova Xavantina, Córrego Sucuri, 27.xi.2013, Cabette, col., 2 males.

Measurement data. Male (n=2) forewing length: 11.9–12.3 mm (mean=12.1 mm).

Remarks. This species was recorded only in Peru (Stark, 1996), and herein, we included the first record in Brazil (Fig. 2).

***Macrogynoplax* sp.** (Fig. 2)

Material examined. BR, BA: Wenceslau Guimarães, Riacho Serra Grande (13º35’43”S, 39º43’12”W), 27.xi.2013, França, col., 1 nymph.

Measurement data. Nymph (n=1) body length: 15.2 mm.

Remarks. The nymph studied here is very similar to the nymph of *Macrogynoplax veneranda* found by Gonçalves *et al*. (2019) in the Espírito Santo state. However, the nymph studied here presents a subtle variation in the pattern of spots on the head in relation to the pattern observed in the original description of the nymph of *M. veneranda* (Froehlich, 1984). It is necessary to study the morphology of the adult male for the nominal confirmation of the species. As adult males have not yet been collected at the site, we performed here the first record of a *Macrogynoplax* specimen for the Bahia state (northeastern Brazil) (Fig. 2).

Discussion

Biological records of *Enderleina* species are considered rare (Stark *et al*., 2009) and all previous geographic records were based on studies of Amazonian species (Derka *et al.*, 2019; Hamada & Silva, 2019). Here, we described *Enderleina castro* sp. nov., increasing the number of species described for the genus to ten (i.e., five for Venezuela and five in Brazil) (Derka *et al.*, 2019; Hamada & Silva, 2019). In addition, even in a region close to the Amazon Forest, it is the first time that an *Enderleina* species has been recorded in the Brazilian Savanna. The record of *E. castro* sp. nov. characterizes the southernmost occurrence point of an *Enderleina* species and highlight an important spatial gap between our record and those of other species. Therefore,

future studies on *Enderleina* species should focus on the transition area between Brazilian Savanna and Amazon Forest, including areas along the spatial gap revealed between the records of the species after the description of *E. castro* sp. nov..

The greatest diversity of *Macrogynoplax* known is found in the Amazon Forest (Ribeiro & Santos, 2018). Only *M. matogrossensis* (i.e., in Brazilian Savanna) and *M. veneranda* (Atlantic Forest) were recorded outside the Amazon Forest (Froehlich, 1984; Bispo *et al*., 2005). Here, we reported the first Brazilian record of *M. yupanqui* for the Brazilian Savanna, the first record of a *Macrogynoplax* specimen for northeastern Atlantic Forest (Bahia state), and we expanded the distribution of *M. matogrossensis* in the Brazilian Savanna. For many years, until the record of the nymphs and a female in Goiás state and the description of *M. matogrossensis* for the Mato Grosso state (Bispo *et al*., 2002a; 2002b; 2005), the recorded distribution of the genus was disjunct between the Amazon and Atlantic Forests. With the previous records and the new geographic occurrences of *M. matogrossensis* and *M. yupanqui* recorded in our study, it is possible to infer that the genus probably also occurs in mountainous regions of other Brazilian states such as Minas Gerais (i.e., southeastern Brazil). Finally, we suggest that further studies on *Macrogynoplax* species in Brazilian Savanna areas are important to reduce apparent spatial gaps and approximate the real distribution of the genus.

Here, we studied the taxonomy of stoneflies mainly from the Brazilian Amazon Forest and Brazilian Savanna. Lastly, we encourage that future research should have as center point the stoneflies in transition zone between Brazilian Savanna and Amazon Forest, providing new taxonomic resolutions and ecological data, which could contribute to future biogeographic and phylogeographic studies.

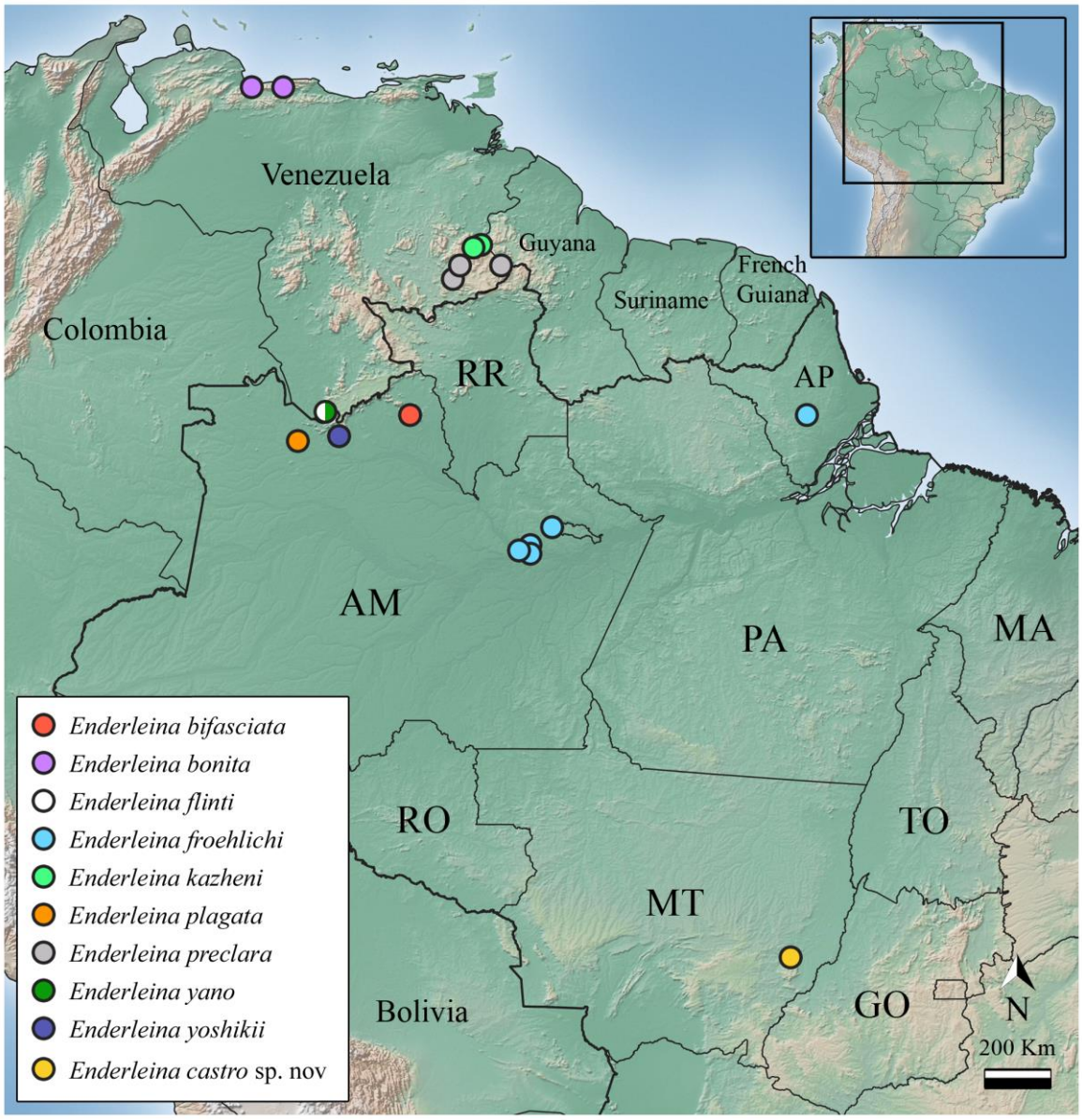

Figure 3. Occurrence map of the genus *Enderleina*, including *E. castro* sp. nov.. Circles with two colors represent occurrence of two species. Brazilian states acronyms: Goiás - GO; Minas Gerais - MG; Mato Grosso do Sul - MS; Mato Grosso - MT; Paraná - PR; Rio de Janeiro; Rondônia - RO; Rio Grande do Sul – RS; Santa Catarina - SC; Tocantins - TO.

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Capítulo 4

Perlidae (Plecoptera) from Brazil: diversity, sampling effort, research status, challenges, and perspectives

Perlidae (Plecoptera) from Brazil: diversity, sampling effort, research status, challenges, and perspectives

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Abstract

Perlidae represents at least 30% of all Plecoptera species. According to the last checklist, there were 451 valid species in Neotropical region and 139 in Brazil. Herein, we presented an updated checklist focused on Brazil, including information about life stages described and all the records organized by states. We counted 153 valid species, including species with "species inquirenda" and "nomen dubium" status. We also compiled all the published occurrence points and discussed about the sampling effort in a political and phytogeographic geographic delimitations. Based on our surveys, we highlighted the main research results obtained in the Perlidae taxonomy focusing on the beginning of the 21st century. We also discussed about problematic species, species delimitation, nymph impediment, and the lack of phylogenetic, biogeographic, and ecological

studies. Finally, we presented our perspectives on what may come from studies on Perlidae in Brazil.

Keywords: Aquatic insects, stoneflies, perlids, lotic environments.

Introduction

Perlidae Latreille, 1802 is the most diverse family among Plecoptera, representing around 30% of all species (DeWalt & Ower, 2019). Perlidae belongs to the suborder Arctoperlaria, infraorder Systelognatha, and superfamily Perloidea (Zwick, 2000). Its phylogenetic position within the superfamily has been controversial for some time (Zwick, 2000). However, recent studies have suggested Perlidae as a sister group of Chloroperlidae + Perlodidae (Chen *et al*., 2018; South *et al*., 2021; Letsch *et al*., 2021).

There are more than 1100 species of Perlidae described (DeWalt & Ower, 2019), which are distributed in two subfamilies, six extant tribes, and 50 genera (DeWalt *et al*., 2022). They are found around the world, with exception of the Antarctic and Australasia regions, mainly in the Asia-Temperate and in the Central and South America (DeWalt & Ower, 2019). For Neotropical region, Froehlich (2010a) carried out the first catalogue and counted 508 valid species of Plecoptera. Among them, 385 Perlidae species were counted (Froehlich, 2010a). Almost ten years later, Pessacq *et al*. (2019), the last checklist published, counted 451 valid species only for Perlidae and ten genera until October 2019. For Brazil, Pessacq *et al*. (2019) found four genera and 139 valid species. Since then, different authors in Brazil carried out several new records and descriptions of new species. Herein, we provided an updated list of species recorded in Brazil and discussed about challenges, gaps and perspectives for new studies.

Material and methods

Based on cross data from published catalogues (Froehlich, 2010a; Pessacq *et al*., 2019) and papers until July 2022, we carried out a checklist of Perlidae species recorded in Brazil. In this checklist, we included species with status "*species inquirenda*" or "*nomen dubium*", as done by Pessacq *et al*. (2019). This checklist has information about species descriptions, life stages descriptions, and all records. For each species, we indicated the life stages described and their respective authors. We compiled all known records for each species and organized them by states, indicating the studies that made the records. In order to facilitate understanding, we organized the compiled information in a table (Table 1).

All maps were carried out using QGIS Bucur 3.14.15 software (QGIS Development Team, 2021). For the development of the occurrence maps, we compiled all records of Brazilian Perlidae presented in the published papers until July 2022 (797 records). For each occurrence point, when the authors presented, the global coordinates were used. In cases that the coordinates were not presented, we inferred them based on information included by the authors in the "Material examined" sections. When the only information available was the country or state name, the coordinates were not inferred. To generate the species distribution maps in a phytogeographic perspective, we used the standard delimitation of Brazilian Phytogeographic Domains made by IBGE (Brazilian Institute of Geographic and Statistics). Data on species occurring in the phytogeographic domains were obtained using "Associate attributes by location" in QGIS. In order to maintain the accuracy of this data, the occurrence points that reported only the Brazilian state or the country name were excluded from this analysis.

The acronyms of the Brazilian states followed the usage in Brazil: AC – Acre; AM – Amazonas; AP – Amapá; BA – Bahia; CE – Ceará; ES – Espírito Santo; GO – Goiás; MA – Maranhão; MG – Minas Gerais; MS – Mato Grosso do Sul; MT – Mato Grosso; PA – Pará; PR – Paraná; RJ – Rio de Janeiro; RO – Rondônia; RR – Roraima; RS – Rio Grande do Sul; SE – Sergipe; SC – Santa Catarina; SP – São Paulo; and TO – Tocantins.

Results and discussion

Perlidae from Brazil

We found 152 species names, including *nomen dubium* as done by Pessacq *et al*. (2019). Since the checklist made by Pessacq *et al*. (2019) for Brazil, we counted 14 new species and 4 species considered *nomen dubium* (Table 1).

Table 1. Species of Perlidae from Brazil. **(M)**: Male, **(F)**: Female, **(N)**: Nymph, **(?)**: Unknown. ^: Reference illustration, *****: Redescription.

	Species information	Described life stages	Distribution
	Anacroneuria Klapálek, 1909		
1.	*A. amargosa* Righi-Cavallaro & Froehlich, 2013 [*in* Righi-Cavallaro *et al*., 2013] **(M)**	-	**Brazil**: BA (Righi-Cavallaro *et al*., 2013; Duarte & Lecci, 2016), ES (Gonçalves *et al*., 2019), GO (Miguel *et al*., 2022).
2.	*A. amazonica* Froehlich, 2003 **(F)**	Ribeiro & Froehlich (2009) **(F)**^	**Brazil**: AM (Froehlich, 2003; Ribeiro & Rafael, 2009).
3.	*A. annularis* (Pictet, 1841) **(?)** (Nomen dubium; Illies, 1966)	-	**Brazil**: (Pictet, 1841).
4.	*A. annulicauda* (Pictet, 1841) **(F)**	Zwick (1972a) **(F)**^* Zwick (1973a) **(M)**	**Brazil**: (Pictet, 1841; Costa & Lima, 1939), SC (Enderlein, 1909a), RJ (Needham & Broughton, 1927). **Guatemala**: (Zwick, 1973; Stark &

			Kondratieff, 2004). **Honduras**: (Stark & Kondratieff, 2004). **Mexico**: (Pictet, 1841; Zwick, 1972; Stark & Kondratieff, 2004).
5.	*A. atrifrons* Klapálek, 1922 **(M)**	Froehlich (2008) **(M)^**	**Brazil**: AM (Klapálek, 1922; Froehlich, 2008), BA (Stark, 2013), ES (Gonçalves *et al*., 2017), MA (Carvalho *et al*., 2020), MS (Righi-Cavallaro & Lecci, 2010), RR (Menezes *et al*., 2020). **Argentina**: (Stark, 2013). **Colombia**: **(**Stark *et al*., 2001). **Ecuador**: (Stark *et al*., 2012). **Paraguay**: (Stark *et al*., 2012). **Peru**: (Stark & Sivec, 1998; Stark *et al*., 2001; Stark, 2004).
6.	*A. aurata* Jewett, 1959 **(F)**	-	**Brazil**: SC (Jewett, 1959).
7.	*A. badilinea* Jewett, 1959 **(F)**	Froehlich (2002) **(F)^**	**Brazil**: SC (Jewett, 1959; Froehlich, 2002). **Argentina**: (Froehlich, 2002; Romero, 2017).
8.	*A. bahiensis* Righi-Cavallaro & Lecci, 2013 [*in* Righi-Cavallaro *et al*., 2013] **(M)**	Righi-Cavallaro *et al*. (2013) **(F)** De Carvalho *et al*. (2021) **(N)**	**Brazil**: BA (Righi-Cavallaro *et al*., 2013; Duarte & Lecci, 2016; De Carvalho *et al*., 2021; Almeida *et al*., in Chapter I).
9.	*A. blanca* Stark, 1995 **(M)**	-	**Brazil**: AM (Froehlich, 2003), RR (Menezes *et al*., 2020). **Venezuela**: (Stark, 1995).
10.	*A. boraceiensis* Froehlich, 2004 **(M)**	Froehlich (2004) **(F)**	**Brazil**: MG (Novaes & Bispo, 2014c), SP (Bispo & Froehlich, 2004b; Froehlich, 2004; Almeida & Bispo, 2020; Almeida *et al*., in Chapter I).
11.	*A. brandaoi* Bispo & Froehlich, 2004 **(M)**	Bispo & Froehlich (2004c) **(F)**	**Brazil**: GO (Bispo & Froehlich, 2004c; Miguel *et al*., 2022).
12.	*A. calori* Duarte & Lecci, 2016 **(M)**	Duarte & Lecci (2016) **(F)**	**Brazil**: CE (Duarte & Lecci, 2016), PI (Takiya *et al*., 2016).
13.	*A. caraa* De Ribeiro & Froehlich, 2007 **(M)**	-	**Brazil**: RS (De Ribeiro & Froehlich, 2007).
14.	*A. caraca* Stark, 1995 **(M)**	Stark (1995) **(F) (N)**	**Brazil**: RR (Menezes *et al*., 2020). **Colombia**: (Zúñiga & Stark, 2002; Zúñiga *et al*., 2006; Zúñiga *et al*., 2007). **Ecuador**: (Stark, 2001). **Venezuela**: (Stark, 1995; Stark, 1999).
15.	*A. caraja* Froehlich, 2002 **(M)**	-	**Brazil**: PA (Froehlich, 2002).

16.	*A. cathia* Froehlich, 2002 **(M)**	Froehlich (2002) **(F)**	**Brazil**: PR (Almeida *et al*., in Chapter I), RS (De Ribeiro & Froehlich, 2007); SC (Froehlich, 2002; Novaes & Bispo, 2014a). **Argentina**: (Romero, 2017).
17.	*A. collaris* (Navás, 1932) **(F)**	-	**Brazil**: RJ (Navás, 1932a).
18.	*A. corae* Bispo & Novaes, 2014 [*in* Bispo *et al*., 2014] **(M)**	Bispo *et al*. (2014) **(F)**	**Brazil**: GO (Bispo *et al*., 2014; Miguel *et al*., 2022), TO (Rippel *et al*., 2019a).
19.	*A. costalis* (Pictet, 1841) **(?)** (Nomen dubium; Stark & Kondratieff, 2004)	-	**Brazil**: SC (Pictet, 1841), RS (Pictet, 1841). **Mexico**: (Pictet, 1841).
20.	*A. cruza* Stark, 1995 **(M)**	Stark (1995) **(N)** Stark & Zúñiga (2014) **(F)**	**Brazil**: RR (Menezes *et al*., 2020). **Colombia**: (Stark & Zúñiga, 2014). **Guyana**: (Stark, 1999). **Venezuela**: (Stark, 1995; Stark, 1999).
21.	*A. debilis* (Pictet, 1841) **(M)**	Zwick (1972a) **(F) (M)^** Avelino-Capistrano *et al*. (2011a) **(N)**	**Brazil**: AL (Almeida *et al*., in Chapter I), BA (Duarte & Lecci, 2016; Almeida & Duarte, 2017; De Carvalho *et al*., 2021; Almeida *et al*., in Chapter I), ES (Froehlich, 2002; Gonçalves *et al*., 2017; Almeida *et al*., in Chapter I), GO (Bispo *et al*., 2014; Miguel *et al*., 2022), MG (Novaes & Bispo, 2014c; Almeida *et al*., in Chapter I), PR (Froehlich, 2002; Novaes & Bispo, 2014a; Almeida *et al*., in Chapter I), RJ (Avelino-Capistrano *et al*., 2011a; Baldin *et al*., 2013; Almeida *et al*., in Chapter I), RS (Zwick, 1973; De Ribeiro & Froehlich, 2007), RO (Banks, 1913), SC (Enderlein, 1909a; Zwick, 1973; Froehlich, 2002; Novaes & Bispo, 2014a), SP (Froehlich, 2010b; Almeida & Bispo, 2020; Almeida *et al*., in Chapter I), SE (Duarte & Lecci, 2016), TO (Rippel *et al*., 2019a). **Argentina**: (Froehlch, 2002; Romero, 2017). **Paraguay**: (Froehlch, 2002).
22.	*A. diaphana* Klapálek, 1921 **(F)**	-	**Brazil**: RS (Klapálek, 1921).
23.	*A. dilaticollis* (Burmeister, 1839) **(F)**	Needham & Broughton (1927) **(M)^** Froehlich (2002) **(F)^***	**Brazil**: (Burmeister, 1839; Pictet, 1841), ES (Enderlein, 1909b; Freohlich, 2002), MT (Needham & Broughton, 1927). **Bolivia**:

			(Enderlein, 1909b). **Colombia**: (Pictet, 1841). **Guatemala**: (Needham & Broughton, 1927). **Mexico**: (Pictet, 1841; Banks, 1901; Needham & Broughton, 1927, Jewett, 1958). **Panamá**: (Needham & Broughton, 1927).
24.	*A. dourada* Jewett, 1960 **(F)**	Jewett (1960b) **(F)**	**Brazil**: GO (Jewett, 1960b; Miguel *et al*., 2022).
25.	*A. duarte* Almeida, Gonçalves & Bispo [*in* Almeida *et al*., Chapter I] **(M)**	-	**Brazil**: BA (Almeida *et al*., in Chapter I).
26.	*A. egena* (Navás, 1916) **(?)** (Nomen dubium; Illies, 1966)	-	**Brazil**: RJ (Navás, 1916).
27.	*A. fiorentini* De Ribeiro & Froehlich, 2007 **(M)**	De Ribeiro & Froehlich (2007) **(F)**	**Brazil**: RS (De Ribeiro & Froehlich, 2007), SC (Novaes & Bispo, 2014b), SP (Almeida & Bispo, 2020).
28.	*A. fittkaui* Froehlich, 2003 **(M)**	Froehlich (2003) **(F)**	**Brazil**: AM (Froehlich, 2003); MT (Almeida *et al*., in Chapter I).
29.	*A. flintorum* Froehlich, 2002 **(M)**	Bispo & Froehlich (2004b) **(F)** Almeida *et al*. (2018) **(N)**	**Brazil**: ES (Gonçalves *et al*., 2017; Almeida *et al*., 2018), MG (Almeida *et al*., 2018; Almeida *et al*., in Chapter I), RJ (Froehlich, 2002; Baldin *et al*., 2013), RS (De Ribeiro & Froehlich, 2007), SC (Almeida *et al*., 2018), SP (Bispo & Froehlich, 2004b; Froehlich, 2010b; Almeida *et al*., 2018; Almeida & Bispo, 2020; Almeida *et al*., in Chapter I).
30.	*A. froehlichi* Miguel, Almeida & Bispo, 2022 [*in* Miguel *et al*., 2022] **(M)**	-	**Brazil**: GO (Miguel *et al*., 2022).
31.	*A. fumigata* Klapálek, 1922 **(?)**	Stark (2013) **(M)^***	**Brazil**: ES (Klapálek, 1922; Stark, 2013).
32.	*A. furfurosa* Jewett, 1960 **(F)**	-	**Brazil**: RJ (Jewett, 1960b).
33.	*A. fuscicosta* (Enderlein, 1909) **(M)**	Enderlein (1909a) **(F)** Froehlich (2002) **(M)^**	**Brazil**: ES (Jewett, 1960b; Avelino-Capistrano & Nessimian, 2014), PR (Froehlich, 2002), RJ (Jewett, 1960b), RS (De Ribeiro & Froehlich, 2007), SC (Enderlein, 1909a; Jewett, 1959; Froehlich, 2002). **Argentina**: (Froehlich, 2002; Romero, 2017).
34.	*A. galba* Jewett, 1960 **(F)**	Jewett (1960a) **(M)**	**Brazil**: GO (Jewett, 1960a; Miguel *et al*., 2022).
35.	*A. genualis* (Navás, 1932) **(M)**	-	**Brazil**: MS (Navás, 1932a; Froehlich, 2007).

36.	*A. guaikuru* Froehlich, 2007 **(M)**	-	**Brazil**: MS (Froehlich, 2007).
37.	*A. hemiphaea* (Navás, 1936) **(F)** (Species inquirenda; Illies, 1966)	-	**Brazil**: SC (Navás, 1936).
38.	*A. hyalina* (Pictet, 1841) **(?)**	Zwick (1972a) **(F)^***	**Brazil**: (Pictet, 1841), RS (Enderlein, 1909a).
39.	*A. iquazu* Novaes & Bispo, 2014 **(M)**	-	**Brazil**: PR (Novaes & Bispo, 2014a).
40.	*A. impensa* Jewett, 1959 **(F)**	Froehlich (2002) **(M) (F)^**	**Brazil**: SC (Jewett, 1959; Froehlich, 2002). **Argentina**: (Froehlich, 2002; Romero, 2017).
41.	*A. iporanga* Bispo & Froehlich, 2004 **(M)**	Almeida *et al*. (2019) **(N)**	**Brazil**: SP (Bispo & Froehlich, 2004b; Almeida *et al*., 2019; Almeida & Bispo, 2020).
42.	*A. itajaimirim* Bispo & Froehlich, 2004 **(M)**	Bispo & Froehlich, (2004b) **(F)**	**Brazil**: SP (Bispo & Froehlich, 2004b; Almeida & Bispo, 2020; Almeida *et al*., in Chapter I).
43.	*A. itatiaiensis* Baldin, Bispo & Novaes, 2013 **(M)**	-	**Brazil**: MG (Novaes & Bispo, 2014c), RJ (Baldin *et al*., 2013).
44.	*A. jaciara* Bispo & Neves, 2005 [*in* Bispo *et al*., 2005] **(M)**	Bispo *et al*. (2005) **(F)**	**Brazil**: MT (Bispo *et al*., 2005).
45.	*A. kariri* Righi-Cavallaro & Lecci, 2013 [*in* Righi-Cavallaro *et al*., 2013] **(M)**	-	**Brazil**: BA (Righi-Cavallaro *et al*., 2013; Duarte & Lecci, 2016; Almeida & Duarte, 2017), ES (Gonçalves *et al*., 2017).
46.	*A. lacunosa* (Navás, 1926) **(F)** (Species inquirenda; Illies, 1966)	-	**Brazil**: BA (Navás, 1926).
47.	*A. laminata* Klapálek, 1923 **(?)**	-	**Brazil**: RS (Klapálek, 1923).
48.	*A. leccii* Carvalho, Almeida & Lima, 2020 [*in* Carvalho *et al*., 2020] **(M)**	-	**Brazil**: MA (Carvalho *et al*., 2020).
49.	*A. lencoensis* Righi-Cavallaro & Lecci, 2013 [*in* Righi-Cavallaro *et al*., 2013] **(M)**	-	**Brazil**: BA (Righi-Cavallaro *et al*., 2013).
50.	*A. lepida* Klapálek, 1922 **(?)**	-	**Brazil**: MT (Klapálek, 1922).
51.	*A. longicauda* (Pictet, 1841) **(F)**	Zwick (1972a) **(F)^**	**Brazil** (Pictet, 1841). **Ecuador**: (Enderlein, 1909a).
52.	*A. manauensis* Ribeiro-Ferreira, 2001 [*in* Ribeiro-Ferreira & Froehlich, 2001] **(M)**	Ribeiro-Ferreira & Froehlich (2001) **(F)** Ribeiro & Gorayeb (2014) **(N)**	**Brazil**: AC (Ribeiro & Rafael, 2009), AM (Ribeiro-Ferreira & Froehlich, 2001; Froehlich, 2003; Ribeiro & Rafael, 2009; Ribeiro & Gorayeb, 2014), MA (Ribeiro & Rafael, 2009; Ribeiro & Gorayeb, 2014), PA (Ribeiro & Rafael, 2009; Ribeiro & Gorayeb, 2014).
53.	*A. mantiqueirae* Froehlich, 2010 **(M)**	Froehlich (2010b) **(F)**	**Brazil**: SP (Froehlich, 2010b).

54.	*A. marlieri* Froehlich, 2001 [*in* Ribeiro-Ferreira & Froehlich, 2001] **(M)**	Froehlich (2003) **(F)** Ribeiro & Gorayeb (2014) **(N)**	**Brazil**: AM (Ribeiro-Ferreira & Froehlich, 2001; Froehlich, 2003; Ribeiro & Rafael, 2009; Ribeiro & Gorayeb, 2014), AP (Ribeiro & Gorayeb, 2014), MA (Ribeiro & Rafael, 2009; Ribeiro & Gorayeb, 2014; Carvalho *et al*., 2020), MT (Ribeiro & Rafael, 2009; Ribeiro & Gorayeb, 2014; Almeida *et al*., in Chapter I), PA (Ribeiro & Rafael, 2009; Ribeiro & Gorayeb, 2014), RR (Ribeiro & Gorayeb, 2014, Menezes *et al*., 2020), TO (Rippel *et al*., 2019a).
55.	*A. meloi* Bispo & Novaes, 2014 [*in* Bispo *et al*., 2014] **(M)**	-	**Brazil**: GO (Bispo & Novaes, 2014).
56.	*A. melzeri* (Navás, 1932) **(F)**	Froehlich (2007) **(F)^**	**Brazil**: MS (Navás, 1932a; Froehlich, 2007).
57.	*A. mineira* Novaes & Bispo, 2014 **(M)**	-	**Brazil**: GO (Miguel *et al*., 2022; Almeida *et al*., in Chapter I), MG (Novaes & Bispo, 2014c).
58.	*A. minuta* Klapálek, 1922 **(M)**	Ribeiro-Ferreira & Froehlich (2001) **(F) (M)*** Ribeiro & Gorayeb (2014) **(N)**	**Brazil**: AM (Ribeiro-Ferreira & Froehlich, 2001; Froehlich, 2002; Ribeiro & Rafael, 2009, Ribeiro & Gorayeb, 2014), BA (Almeida *et al*., in Chapter I); GO (Miguel *et al*., 2022). MA (Carvalho *et al*., 2020), PA (Ribeiro-Ferreira & Froehlich, 2001; Ribeiro & Gorayeb, 2014), RR (Menezes *et al*., 2020), TO (Rippel *et al*., 2019a; Almeida *et al*., in Chapter I).
59.	*A. novateutonia* Jewett, 1959 **(F)**	Froehlich (2002) **(M) (F)^**	**Brazil**: PR (Froehlich, 2002), SC (Jewett, 1959; Froehlich, 2002). **Argentina**: (Froehlich, 2002; Romero, 2017).
60.	*A. oculatila* Jewett, 1959 **(F)**	Froehlich (2002) **(M) (F)^**	**Brazil**: SC (Jewett, 1959; Froehlich, 2002). **Argentina**: (Froehlich, 2002; Romero, 2017).
61.	*A. ofaye* Froehlich, 2007 **(M)**	Froehlich (2007) **(F)** Novaes *et al*. (2012) **(N)**	**Brazil**: GO (Miguel *et al*., 2022), MS (Froehlich, 2007; Righi-Cavallaro & Lecci, 2010; Almeida *et al*., in Chapter I), PR (Novaes *et al*., 2012), SC (Novaes & Bispo, 2014b), SP (Almeida *et al*., in Chapter I).

62.	*A. otafroehlichi* Righi-Cavallaro & Lecci, 2010 **(M)**	Righi-Cavallaro & Lecci (2010) **(N)** Rippel *et al*. (2019a) **(F)**	**Brazil**: MS (Righi-Cavallaro & Lecci, 2010), MT (Almeida *et al*., in Chapter I), TO (Rippel *et al*., 2019a).
63.	*A. pakitza* Stark & Sivec, 1998 **(M)**	-	**Brazil**: MS (Almeida *et al*., in Chapter I). **Peru**: (Stark & Sivec, 1998).
64.	*A. paprockii* Novaes & Bispo, 2014 **(M)**	-	**Brazil**: BA (Almeida & Duarte, 2017; Almeida *et al*., in Chapter I), MG (Novaes & Bispo, 2014c).
65.	*A. parilobata* Klapálek, 1922 **(F)**	-	**Brazil**: BA (Klapálek, 1922).
66.	*A. pastaza* Stark, 2001 **(M)**	Stark (2001a) **(F)**	**Brazil**: AM (Froehlich, 2003), GO (Bispo & Froehlich, 2004c), MS (Righi-Cavallaro & Lecci, 2010). **Ecuador**: (Stark, 2001).
67.	*A. patioba* Almeida & Duarte, 2017 **(M)**	-	**Brazil**: BA (Almeida & Duarte, 2017).
68.	*A. paulina* (Navás, 1936) **(F)**	Froehlich (2004) **(M)**	**Brazil**: SP (Froehlich, 2004).
69.	*A. payagua* Froehlich, 2007 **(M)**	Froehlich (2007) **(F)** Righi-Cavallaro & Froehlich (2013) **(N)**	**Brazil**: GO (Miguel *et al*., 2022), MS (Froehlich, 2007; Righi-Cavallaro & Lecci, 2010; Righi-Cavallaro & Froehlich, 2013).
70.	*A. petersi* Froehlich, 2002 **(M)**	Froehlich (2002) **(F)**	**Brazil**: PR (Froehlich, 2002), RJ (Baldin *et al*., 2013), SP (Froehlich, 2002; 2004).
71.	*A. pictipes* Klapálek, 1923 **(F)**	Stark (1999) **(M) (F)^**	**Brazil**: AP (Klapálek, 1923), RR (Menezes *et al*., 2020). **Guyana**: (Stark, 1999).
72.	*A. piti* Gonçalves, Novaes & Salles, 2017 **(M)**	Gonçalves *et al*. (2017) **(N)**	**Brazil**: ES (Gonçalves *et al*., 2017).
73.	*A. plaumanni* Jewett, 1959 **(F)**	Froehlich (2002) **(M) (F)^**	**Brazil**: RS (De Ribeiro & Froehlich, 2007), SC (Jewett, 1959; Froehlich, 2002; Almeida *et al*., in Chapter I). **Argentina**: (Froehlich, 2002; Romero, 2017).
74.	*A. polita* (Burmeister, 1839) **(M)**	Zwick (1972a) **(M)^** Froehlich (2002) **(F)**	**Brazil**: MG (Novaes & Bispo, 2014c), PR (Froehlich, 2002), SC (Froehlich, 2002; Novaes & Bispo, 2014a; Almeida *et al*., in Chapter I), RJ (Almeida *et al*., in Chapter I), SP (Bispo & Froehlich, 2004b; Froehlich, 2004; Almeida & Bispo, 2020). **Argentina**: (Froehlich, 2002; Romero, 2017).

75.	*A. posticata* (Banks, 1913) **(?)** (Species inquirenda; Illies, 1966)	-	**Brazil**: RO (Banks, 1913).
76.	*A. quilombola* Righi-Cavallaro & Froehlich, 2013 [*in* Righi-Cavallaro *et al*., 2013] **(M)**	Righi-Cavallaro *et al*. (2013) **(F)**	**Brazil**: BA (Righi-Cavallaro *et al*., 2013; Duarte & Lecci, 2016), ES (Gonçalves *et al*., 2017), PE (Duarte & Lecci, 2016).
77.	*A. rondoniae* Froehlich, 2002 **(M)**	Froehlich (2002) **(F)**	**Brazil**: RO (Froehlich, 2002).
78.	*A. rotunda* Goncalves, Novaes & Salles, 2017 **(M)**	-	**Brazil**: ES (Gonçalves, *et al*., 2017).
79.	*A. ruschii* Novaes, Bispo & Goncalves, 2016 **(M)**	-	**Brazil**: ES (Novaes *et al*., 2016).
80.	*A. sallesi* Gonçalves, Almeida & Bispo [*in* Almeida *et al*., Chapter I] **(M)**	-	**Brazil**: ES (Almeida *et al*., in Chapter I).
81.	*A. saofrancisco* Novaes, Vilela, Lopez & Ferreira, 2018 **(M)**	Novaes *et al*. (2018) **(F) (N)**	**Brazil**: MG (Novaes *et al*., 2018).
82.	*A. simulans* Froehlich, 2010 **(M)**	Froehlich (2010b) **(F)**	**Brazil**: RJ (Baldin *et al*., 2013), SP (Froehlich, 2010b).
83.	*A. singela* Duarte & Lecci, 2016 **(M)**	Duarte & Lecci (2016) **(F)**	**Brazil**: BA (Duarte & Lecci, 2016; Almeida *et al*., in Chapter I), MG (Almeida *et al*., in Chapter I), GO (Miguel *et al*., 2022).
84.	*A. singularis* Righi-Cavallaro & Lecci, 2010 **(M)**	Righi-Cavallaro & Lecci (2010) **(F)** Ribeiro *et al*. (2015) **(N)**	**Brazil**: BA (Almeida *et al*., in Chapter I), GO (Miguel *et al*., 2022), MA (Carvalho *et al*., 2020), MS (Righi-Cavallaro & Lecci, 2010; Almeida *et al*., in Chapter I), MG (Novaes & Bispo, 2014c), PA (Ribeiro *et al*., 2015; Ribeiro & Gorayeb, 2016), SP (Righi-Cavallaro & Lecci, 2010), RO (Firmino *et al*., 2019), TO (Rippel *et al*., 2019a; Almeida *et al*., in Chapter I).
85.	*A. stanjewetti* Froehlich, 2002 **(M)**	Froehlich (2002) **(F)**	**Brazil**: MG (Novaes & Bispo, 2014c), PR (Almeida *et al*., in Chapter I), RJ (Almeida *et al*., in Chapter I), RS (De Ribeiro & Froehlich, 2007), SC (Froehlich, 2002), SP (Weidner, 1962, Froehlich, 2008). **Argentina**: (Froehlich, 2002; Romero, 2017).
86.	*A. subcostalis* Klapálek, 1921 **(F)**	Froehlich (2002) **(F)^** Froehlich (2004) **(M)**	**Brazil**: ES (Klapálek, 1921; Froehlich, 2002; Gonçalves *et al*., 2017), RJ (Jewett, 1960b; Froehlich, 2002; Baldin *et al*., 2013), SP (Bispo

			& Froehlich, 2004b; Froehlich, 2004; Almeida & Bispo, 2020). **Argentina**: (Romero, 2017).
87.	*A. tabatae* Froehlich, 2010 **(M)**	Froehlich (2010b) **(F)**	**Brazil**: SP (Froehlich, 2010b; Almeida *et al*., in Chapter I).
88.	*A. terere* Righi-Cavallaro & Lecci, 2010 **(M)**	Righi-Cavallaro & Lecci (2010) **(F)**	**Brazil**: BA (Duarte & Lecci, 2016), MS (Righi-Cavallaro & Lecci, 2010), MG (Novaes & Bispo, 2014c).
89.	*A. tinctilamella* Jewett, 1959 **(F)**	Froehlich (2002) **(F)^**	**Brazil**: PR (Froehlich, 2002), SC (Jewett, 1959; Froehlich, 2002). **Argentina**: (Froehlich, 2002; Romero, 2017).
90.	*A. tinga* Bispo & Froehlich, 2004 **(M)**	-	**Brazil**: GO (Bispo & Froehlich, 2004c).
91.	*A. toriba* Froehlich, 2002 **(M)**	Froehlich (2010b) **(F)**	**Brazil**: ES (Gonçalves *et al*., 2017), SP (Froehlich, 2002; Froehlich, 2010b).
92.	*A. trimacula* Jewett, 1959 **(F)**	Froehlich (2002) **(M) (F)^**	**Brazil**: RS (De Ribeiro & Froehlich, 2007), SC (Jewett, 1959; Froehlich, 2002; Novaes & Bispo, 2014a, 2014b). **Argentina**: (Froehlich, 2002; Romero, 2017). **Paraguay**: (Froehlich, 2002).
93.	*A. tupi* Bispo & Froehlich, 2004 **(M)**	Bispo & Froehlich (2004b) **(F)** Almeida & Bispo (2020) **(N)**	**Brazil**: SP (Bispo & Froehlich, 2004b; Almeida & Bispo, 2020).
94.	*A. uyara* Froehlich, 2002 **(M)**	Froehlich (2002) **(F)**	**Brazil**: PR (Froehlich, 2002), RS (De Ribeiro & Froehlich, 2007), SC (Froehlich, 2002), SP (Froehlich, 2004). **Argentina**: (Froehlich, 2002; Romero, 2017).
95.	*A. vanini* Froehlich, 2004 **(M)**	Froehlich (2004) **(F)**	**Brazil**: BA (Almeida *et al*., in Chapter I), MG (Novaes & Bispo, 2014c), SP (Froehlich, 2004; Froehlich, 2010b).
96.	*A. v-nigrum* (Navás, 1932) **(F)** (Nomen dubium; Petersen & Gaedike, 1968)	-	**Brazil**: RJ (Navás, 1932a)
97.	*A. xinguensis* Froehlich, 2002 **(M)**	Froehlich (2002) **(F)**	**Brazil**: PA (Froehlich, 2002).
98.	*A. xokleng* Novaes & Bispo, 2014 **(M)**	Novaes & Bispo (2014b) **(F)**	**Brazil**: SC (Novaes & Bispo, 2014b).
99.	*A. ypsilon* Klapálek, 1922 **(F)**	-	**Brazil**: RS (Klapálek, 1922).
100.	*A. zantedeschia* Rippel, Novaes & Krolow,	-	**Brazil**: TO (Rippel *et al*., 2019a).

	2019 **(M)**		
	Enderleina Jewett, 1960		
1.	*E. bifasciata* Hamada, Silva & Pedroza, 2016 **(M)**	-	**Brazil**: AM (Hamada *et al*., 2016).
2.	*E. castro* Almeida & Bispo [*in* Almeida *et al*., Chapter III] **(M)**	-	**Brazil**: MT (Almeida *et al*., in Chapter III).
3.	*E. froehlichi* Ribeiro-Ferreira, 1996 **(M)**	Ribeiro & Rafael (2005) **(F)** Ribeiro & Gorayeb (2015) **(N)**	**Brazil**: AP (Ribeiro & Rafael, 2005; Ribeiro & Gorayeb, 2015), AM (Ribeiro-Ferreira, 1996; Ribeiro & Rafael, 2005; Ribeiro & Gorayeb, 2015).
4.	*E. plagata* Hamada & Silva, 2019 **(M)**	-	**Brazil:** AM (Hamada & Silva, 2019).
5.	*E. yoshikii* Hamada & Silva, 2019 **(F)**	-	**Brazil:** AM (Hamada & Silva, 2019).
	Kempnyia Klapálek, 1914		
1.	*K. alterosarum* Froehlich, 1988 **(M)**	Froehlich (1988) **(F)**	**Brazil**: BA (Duarte *et al*., 2014), MG (Froehlich, 1988; Avelino-Capistrano *et al*., 2014), RJ (Avelino-Capistrano *et al*., 2014; 2016).
2.	*K. auberti* Froehlich, 1996 **(M)**	-	**Brazil**: PR (Froehlich, 1996), SP (Froehlich, 1996; Bispo & Froehlich, 2004b).
3.	*K. brasilica* (Navás, 1932) **(F)**	-	**Brazil**: RJ (Navás, 1932a).
4.	*K. brasiliensis* (Pictet, 1841) **(M)**	-	**Brazil**: (Pictet, 1841).
5.	*K. colossica* (Navás, 1934) **(F)**	Froehlich (1988) **(M) (F)^** Bispo *et al*. (2013) **(N)**	**Brazil**: MG (Avelino-Capistrano *et al*., 2014), PR (Froehlich, 1988; Novaes & Bispo, 2014a), RJ (Avelino-Capistrano *et al*., 2014; 2016), SC (Navás, 1934; Jewett, 1959; Froehlich, 1988; Almeida & Bispo, 2020), SP (Froehlich, 1988; Bispo & Froehlich, 2004b; Froehlich, 2011c; Bispo *et al*., 2013; Avelino-Capistrano *et al*., 2014; Almeida & Bispo, 2020).
6.	*K. couriae* Avelino-Capistrano, Barbosa & Takiya, 2016 **(M)**	Avelino-Capistrano *et al*. (2016) **(F) (N)**	**Brazil**: RJ (Avelino-Capistrano *et al*., 2016).
7.	*K. flava* Klapálek, 1916 **(F)**	Froehlich (1988) **(M)**	**Brazil**: ES (Klapálek, 1916; Gonçalves *et al*., 2017; Almeida *et al*., in Chapter II), MG (Almeida *et al*., in Chapter II), RJ (Jewett, 1960b; Froehlich, 1988), SP (Navás, 1925; Froehlich, 1988; Bispo & Froehlich,

			2004b; Froehlich, 2011c; Almeida & Bispo, 2020; Almeida *et al*., in Chapter II).
8.	*K. goiana* Bispo & Froehlich, 2004 **(M)**	Rippel *et al*. (2019b) **(F) (N)**	**Brazil**: GO (Bispo & Froehlich, 2004a), TO (Rippel *et al*., 2019b).
9.	*K. gracilenta* (Enderlein, 1909) **(M)**	Zwick (1973) **(M)^** Froehlich (1984a) **(F)** Avelino-Capistrano *et al*. (2011) **(N)**	**Brazil**: BA (Duarte *et al*., 2014), ES (Enderlein, 1909b; Avelino-Capistrano *et al*., 2011; Avelino-Capistrano & Nessimian, 2014; Avelino-Capistrano *et al*., 2014; 2016; Gonçalves *et al*., 2017; Almeida *et al*., in Chapter II), MG (Froehlich, 1984a), RJ (Jewett, 1960b; Nessimian *et al*., 2009; Avelino-Capistrano *et al*., 2014; Almeida *et al*., in Chapter II), SP (Froehlich, 1984a; Froehlich, 2011c).
10.	*K. guarani* Almeida, Gonçalves & Bispo [*in* Almeida *et al*., Chapter II] **(M)**	-	**Brazil**: SC (Almeida *et al*., in Chapter II).
11.	*K. guassu* Froehlich, 1988 **(M)**	Froehlich (1988) **(F)**	**Brazil**: RJ (Froehlich, 1988; Avelino-Capistrano *et al*., 2016).
12.	*K. jatim* Froehlich, 1988 **(M)**	Froehlich (1988) **(F)**	**Brazil**: BA (Duarte *et al*., 2014; Almeida *et al*., in Chapter II), ES (Gonçalves *et al*., 2017; Almeida *et al*., in Chapter II), MG (Almeida *et al*., in Chapter II), RJ (Froehlich, 1988), SP (Froehlich, 1988; Froehlich, 2011c).
13.	*K. kaingang* Froehlich, 2011 **(M)**	Froehlich (2011b) **(F)**	**Brazil**: SC (Froehlich, 2011b).
14.	*K. klugii* (Pictet, 1841) **(M)**	Zwick (1972a) **(M)^**	**Brazil** (Pictet, 1841).
15.	*K. mirim* Froehlich, 1984 **(M)**	Froehlich (1984a) **(F)**	**Brazil**: MG (Almeida *et al*., in Chapter II), SC (Novaes & Bispo, 2014b), SP (Froehlich, 1984a).
16.	*K. neotropica* (Jacobson & Bianchi, 1905) **(M)**	Zwick (1972a) **(F)** Bispo & Froehlich (2008) **(M)^ (N)**	**Brazil**: BA (Duarte *et al*., 2014; Almeida *et al*., in Chapter II), ES (Navás, 1932b; Jewett, 1959; Zwick, 1972; Novaes *et al*., 2016; Gonçalves *et al*., 2017; Almeida *et al*., in Chapter II), GO (Bispo & Froehlich, 2004a; Bispo & Froehlich, 2008; Froehlich, 2011b), MG (Bispo & Froehlich, 2008; Froehlich, 2011b; Novaes & Bispo, 2014c; Avelino-Capistrano *et al*., 2014; Almeida *et al*., in Chapter II), PR (Froehlich,

			1996; Novaes & Bispo, 2014a; Avelino-Capistrano *et al*., 2014), RJ (Klapálek, 1916; Zwick, 1972; Nessimian *et al*., 2009; Avelino-Capistrano *et al*., 2014; 2016; Almeida *et al*., in Chapter II), RS (Zwick, 1972), SC (Navás, 1932b; Jewett, 1959; Zwick, 1972; Novaes & Bispo, 2014b), SP (Froehlich, 1996; Bispo & Froehlich, 2004b; Bispo & Froehlich, 2008; Froehlich, 2011b; Froehlich, 2011c; Avelino-Capistrano *et al*., 2014; Almeida & Bispo, 2020; Almeida *et al*., in Chapter II).
17.	*K. obtusa* Klapálek, 1916 **(M)**	Zwick (1972a) **(M)^** Froehlich (1988) **(F)** Avelino-Capistrano *et al*. (2014) **(N)**	**Brazil**: ES (Jewett, 1960b; Avelino-Capistrano *et al*., 2014; Gonçalves *et al*., 2017), MG (Froehlich, 1988; Novaes & Bispo, 2014c; Avelino-Capistrano *et al*., 2014; Almeida *et al*., in Chapter II), RJ (Jewett, 1960; Froehlich, 1988; Nessimian *et al*., 2009; Avelino-Capistrano *et al*., 2014), SP (Froehlich, 1988; Froehlich, 2011c; Avelino-Capistrano *et al*., 2014; 2016; Almeida *et al*., in Chapter II).
18.	*K. ocellata* Froehlich, 2011 **(M)**	-	**Brazil**: RJ (Froehlich, 2011b).
19.	*K. oliveirai* Bispo & Froehlich, 2004 **(M)**	Bispo & Froehlich (2004a) **(F)**	**Brazil**: GO (Bispo & Froehlich, 2004a; Bispo & Froehlich, 2007).
20.	*K. petropolitana* (Navás, 1929) **(F)**	-	**Brazil**: RJ (Navás, 1929).
21.	*K. pinhoi* Froehlich, 2011 **(M)**	Froehlich (2011b) **(F)**	**Brazil**: SC (Froehlich, 2011b).
22.	*K. pirata* Froehlich, 2011 **(M)**	Froehlich (2011c) **(F)**	**Brazil**: SP (Froehlich, 2011c; Almeida *et al*., in Chapter II).
23.	*K. puri* Avelino-Capistrano, Souza & Nessiman, 2013 **(M)**	-	**Brazil**: RJ (Avelino-Capistrano *et al*., 2013).
24.	*K. reichardti* Froehlich, 1984 **(M)**	Froehlich (1984a) **(F)**	**Brazil**: MG (Froehlich, 1984a; Almeida *et al*., in Chapter II), SP (Froehlich, 2011c; Almeida *et al*., in Chapter II).
25.	*K. remota* (Banks, 1920) **(F)**	-	**Brazil**: RJ (Banks, 1920).
26.	*K. reticulata* (Klapálek, 1916) **(M)**	Zwick (1983) **(M)^*** Avelino-Capistrano *et al*. (2011) **(N)**	**Brazil**: ES (Klapálek, 1916; Zwick, 1983; Avelino-Capistrano *et al*., 2011; Avelino-Capistrano & Nessimian, 2014; Gonçalves *et al*.,

			2019; Almeida *et al*., in Chapter II).
27.	*K. sazimai* Froehlich, 1988 **(M)**	-	**Brazil**: MG (Froehlich, 1988).
28.	*K. serrana* Navás, 1936 **(M)**	Froehlich (1984a) **(M)^**	**Brazil**: ES (Zwick, 1973), RJ (Navás, 1936; Froehlich, 1984a), SP (Froehlich, 2011b; Almeida *et al*., in Chapter II).
29.	*K. tamoya* Froehlich, 1984 **(M)**	Froehlich (1984a) **(F)**	**Brazil**: SP (Froehlich, 1984a; Froehlich, 2011c; Almeida *et al*., in Chapter II).
30.	*K. taunayi* (Navás, 1936) **(F)** (Species inquirenda; Illies, 1966)	-	**Brazil**: RJ (Navás, 1936).
31.	*K. tenebrosa* Klapálek, 1916 **(M)**	Zwick (1972a) **(M)^ (F)**	**Brazil**: RJ (Klapálek, 1916; Zwick, 1972), RS (Klapálek, 1916), SC (Zwick, 1972; Froehlich, 2011b; Novaes & Bispo, 2014b; Almeida *et al*., in Chapter II).
32.	*K. tijucana* Dorvillé & Froehlich, 1997 **(M)**	Dorvillé & Froehlich (1997) **(F)** Dorvillé & Froehlich (2001) **(N)**	**Brazil**: RJ (Dorvillé & Froehlich, 1997; 2001; Almeida *et al*., in Chapter II).
33.	*K. tupiniquim* Almeida, Gonçalves & Bispo [*in* Almeida *et al*., Chapter II] **(M)**	-	**Brazil**: ES (Almeida *et al*., in Chapter II).
34.	*K. tupinamba* Froehlich, 2011 **(M)**	Froehlich (2011c) **(F)**	**Brazil**: SP (Froehlich, 2011c; Almeida *et al*., in Chapter II).
35.	*K. umbrina* Froehlich, 1988 **(M)**	Froehlich (1988) **(F)**	**Brazil**: MG (Froehlich, 1988), RJ (Froehlich, 1988; Nessimian *et al*., 2009), SP (Froehlich, 1988).
36.	*K. vanini* Froehlich, 1988 **(M)**	Froehlich (1988) **(F)**	**Brazil**: GO (Bispo & Froehlich, 2004a; Almeida *et al*., in Chapter II), MG (Froehlich, 1988; Almeida *et al*., in Chapter II), SP (Froehlich, 1988; Almeida *et al*., in Chapter II).
37.	*K. varipes* Klapálek, 1916 **(M)**	Avelino-Capistrano *et al*. (2014) **(M)^ (N)**	**Brazil**: RJ (Jewett, 1960b; Nessimian *et al*., 2009; Avelino-Capistrano *et al*., 2014; 2016; Almeida *et al*., in Chapter II).
38.	*K. zwicki* Almeida, Gonçalves & Bispo [*in* Almeida *et al*., Chapter II] **(M)**	-	**Brazil**: ES (Almeida *et al*., in Chapter II).
	Macrogynoplax Enderlein, 1909		
1.	*M. anae* Ribeiro & Rafael, 2007 **(M)**	-	**Brazil**: AM (Ribeiro & Rafael, 2007).
2.	*M. delicata* Ribeiro-Ferreira & Froehlich, 1999	Ribeiro-Ferreira &	**Brazil**: AP (Ribeiro & Gorayeb,

	(M)	Froehlich (1999) **(F)** Ribeiro & Rafael (2007) **(M)*** Ribeiro & Gorayeb (2015) **(N)**	2015), AM (Ribeiro-Ferreira & Froehlich, 1999; Froehlich, 2003; Ribeiro & Rafael, 2007; Ribeiro & Gorayeb, 2015), PA (Ribeiro & Rafael, 2007; Ribeiro & Gorayeb, 2015).
3.	*M. guayanensis* Enderlein, 1909 **(F)**	Zwick (1973a) **(M) (F)^**	**Brazil**: PA (Enderlein, 1909b; Zwick, 1973). **Guyana**: (Enderlein, 1909b).
4.	*M. matogrossensis* Bispo & Neves, 2005 [in Bispo *et al*., 2005] **(M)**	Bispo *et al*. (2005) **(F)** Rippel *et al*. (2019b) **(N)**	**Brazil**: GO (Almeida *et al*., in Chapter III), MT (Bispo *et al*., 2005), TO (Rippel *et al*., 2019b).
5.	*M. poranga* Ribeiro-Ferreira & Froehlich, 1999 **(M)**	Ribeiro & Rafael (2007) **(M)***	**Brazil**: AM (Ribeiro-Ferreira & Froehlich, 1999; Ribeiro & Rafael, 2007).
6.	*M. pulchra* Ribeiro-Ferreira & Froehlich, 1999 **(M)**	Ribeiro-Ferreira & Froehlich (1999) **(F)** Ribeiro & Rafael (2007) **(M)*** Ribeiro & Gorayeb (2015) **(N)**	**Brazil**: AP (Ribeiro & Gorayeb, 2015), AM (Ribeiro-Ferreira & Froehlich, 1999; Ribeiro & Rafael, 2007; Ribeiro & Gorayeb, 2015), PA (Ribeiro & Gorayeb, 2015).
7.	*M. quadrispina* Menezes, Boldrini & Novaes, 2020 **(M)**	Menezes *et al*. (2020) **(F)**	**Brazil**: RR (Menezes *et al*., 2020)
8.	*M. veneranda* Froehlich, 1984 **(M)**	Froehlich (1984a) **(F) (N)**	**Brazil**: ES (Gonçalves *et al*., 2019), SP (Froehlich, 1984b; Bispo & Froehlich, 2004b; Almeida & Bispo, 2020; Almeida *et al*., in Chapter III).
9.	*M. yupanqui* Stark, 1996	-	**Brazil**: MT (Almeida *et al*., in Chapter III). **Peru**: (Stark, 1996).

Main results of the early 21st century

Pictet (1841) developed the first substantial study on Brazilian stoneflies. Despite this, it was only from the 1980s that studies began to be carried out regularly. Studies such as those by Froehlich (1969, 1984a, 1984b, 1988, 1990, 1994, 1996, 1998) contributed considerably and improved knowledge about stoneflies in Brazil. Equally important was Professor Froehlich's contribution training new researchers from the beginning of the 21st century, who have

maintained this strategy, increasing the number of researchers who are dedicated to the study of the group.

Due to the increase in the number of published studies and researchers studying Plecoptera since the transition between the last centuries, scientific collections in Brazil have been benefited, becoming references. Among the Brazilian collections, we can highlight seven. The Museu de Zoologia da Universidade de São Paulo (MZUSP) presents the majority of Perlidae holotypes described from Brazil, most of them deposited by Professor Froehlich. The Instituto Nacional de Pesquisas da Amazônia (INPA) is reference for the fauna from Amazon Forest, presenting important collection of *Macrogynoplax* and *Enderleina*. The Coleção Entomológica Prof. José Alfredo Pinheiro Dutra (DZRJ) is a collection that had importance recently due to studies carried out by Avelino-Capistrano *et al*. (2011a, 2011b, 2013, 2014, 2016), presenting species recorded until now only in Rio de Janeiro state and several other species. The Museu de Entomologia da Universidade Federal de Viçosa (UFVB) has species from studies by Gonçalves *et al*. (2017, 2019) (developed in Espírito Santo state) and have grown due samples in Minas Gerais state. The Coleção do Laboratório de Biologia Aquática (CLBA) is a collection that has no specimens of type series, but has several species deposited in 80% ethanol and many other deposited specifically for molecular studies (100% ethanol in freezer -20ºC).

In terms of diversity, at the end of the 20th century, 78 species of Brazilian Perlidae were known. Since the beginning of the 21st century, many new species have been described. According to Froehlich (2010a), until 2009 it was possible to recognize 110 species, of which 108 are valid today. Moreover, according to our data, 152 species could be recognized until July 2022. As we can see, the number of described species has almost doubled since the beginning of the century. The increase in the number of researchers was fundamental to this growth. In addition,

the new researcher have contributed to the association and description of different life stages. For illustrative purposes, until the end of the year 2000, only three species had associated and described nymphs, and among species described based on males, only 18 species had described females. Currently, the numbers are considerably higher, with 29 nymphs and 65 females of the species described.

Sampled locations and records

In total, we compiled 410 different valid collection points, among them, 284, nine, 137, and 31 were occurrence points for *Anacroneuria*, *Enderleina*, *Kempnyia* and *Macrogynoplax* respectively.

Historical context and sampling by political divisions

Currently, it remains common to make new records of Brazilian Perlidae based on political divisions such as states. Although this strategy is not informative from a biological point of view, it has made it possible to understand poorly sampled regions and plan priority collection areas. In addition, political divisions were important in the historical context as they may reflect the number of papers published in relation to the concentration of researchers in research centers or regions with better financial resources for research. Lastly, different states demand different requirements to grant authorization to collect biological material in protected areas, so, the political divisions can be usesul for planning the sampling.

Historically, the Southeast region was initially studied due mainly to researchers from other countries and the traveling naturalists from National Museum in Rio de Janeiro state. Later, through the studies of Prof. Dr. Cláudio G. Froehch from USP (University of São Paulo). Since then, due to the emergence of other research institutions, the number of studies focused on Perlidae and the regions sampled have increaded considerably. Currently, we have Perlidae collections in almost all Brazilian states and the sampling effort has spread to regions that were under-sampled.

Among the 26 states and the Federal District, 24 states present Perlidae records. Until now, the Federal District and the states of Paraíba, and Rio Grande do Norte do not present records. All these states belong to the Northeast region, which started to be studied recently, mainly the Bahia state (Righi-Cavallaro *et al*., 2013; Duarte *et al*., 2014; Duarte & Lecci, 2016; Almeida & Duarte, 2017; De Carvalho *et al*., 2021). The other states from Northeast region have only sporadic sampling and few records (Fig. 1). The North region, was sampled mainly near Manaus (Amazonas state) and some locations in the Pará state (Ribeiro-Ferreira & Froehlich, 2001; Froehlich, 2003; Ribeiro & Rafael, 2007; Ribeiro & Gorayeb, 2015; 2016), and only a few papers have studied fauna from other locations (Hamada *et al*., 2016; Hamada & Silva, 2019; Rippel *et al*., 2019a; 2019b; Menezes *et al*., 2020). In the Midwest of Brazil, the fauna from all states was studied based on a few locations (Bispo *et al*., 2005; 2014; Bispo & Froehlich, 2004c; 2007; Froehlich, 2007; Righi-Cavallaro & Lecci, 2010; Miguel *et al*., 2022). Brazil is a continental country and in addition to this geographical difficulty, other limitations such as the number of research centers and researchers in these regions were responsible for the historical low sample coverage.

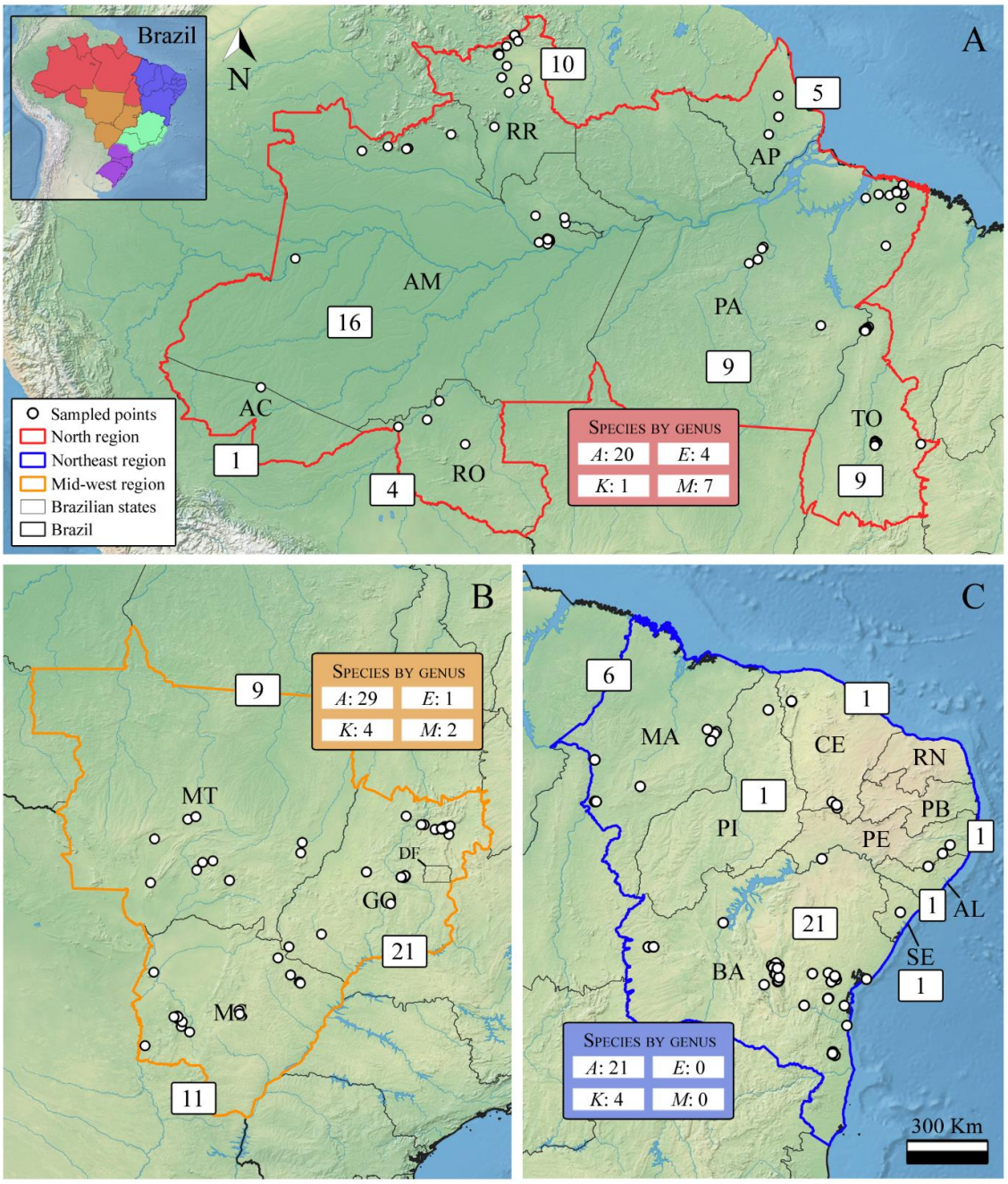

Figure 1. North (A), Mid-west (B), and Northeast regions of Brazil, highlighting sampled effort, number of species by state, and number of species found in each region, divided by genus. *A*: *Anacroneuria*; *E*: *Enderleina*; *K*: *Kempnyia*; *M*: *Macrogynoplax*.

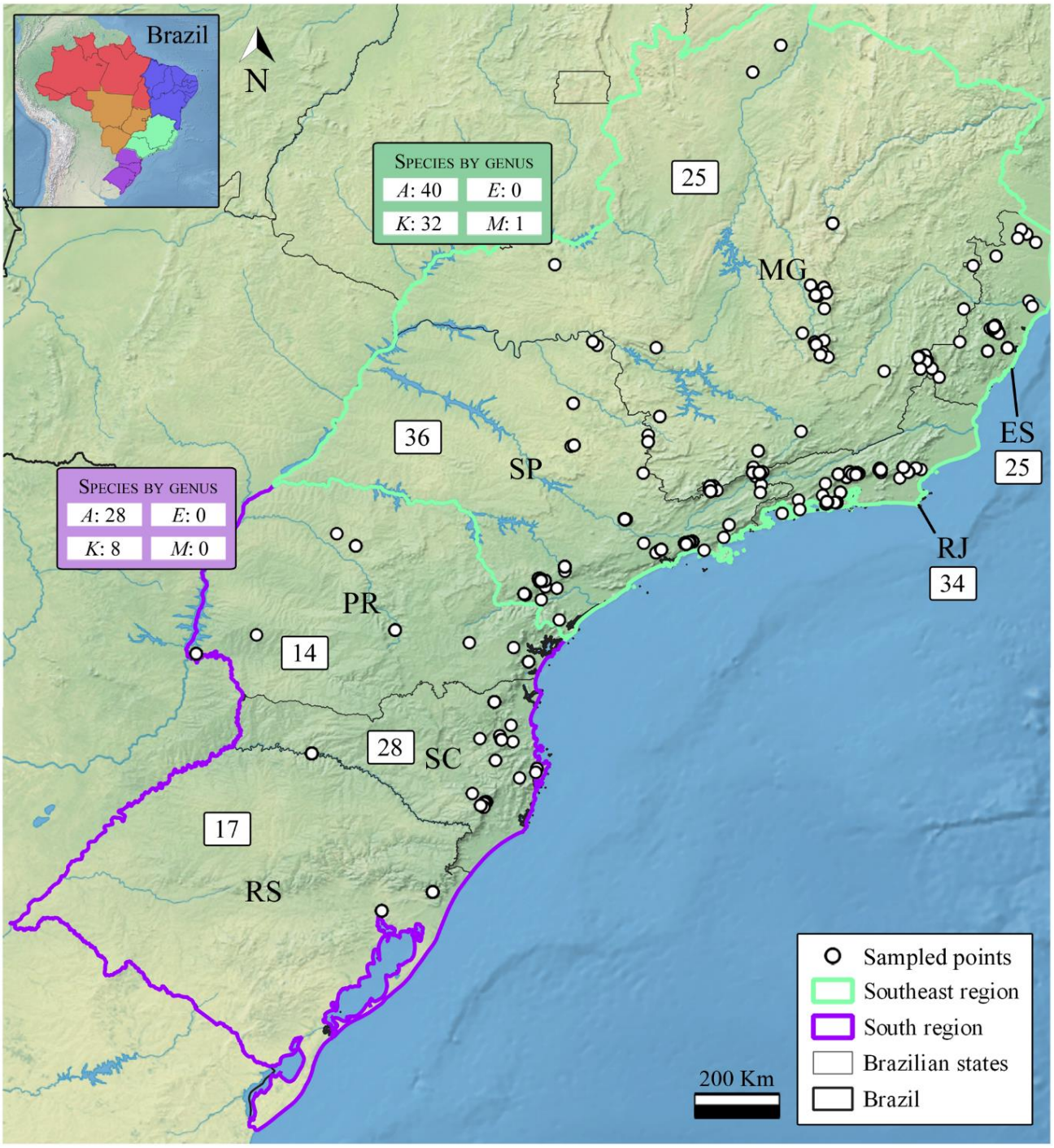

Figure 2. South and Southeast regions of Brazil, highlighting sampled effort, number of species by state, and number of species found in each region, divided by genus. *A*: *Anacroneuria*; *E*: *Enderleina*; *K*: *Kempnyia*; *M*: *Macrogynoplax*.

The South and Southeast regions have a larger number of species and studies published (Froehlich, 2002; 2010b; 2011a; 2011b; 2011c; Bispo & Froehlich, 2004b; De Ribeiro & Froehlich, 2007; Novaes *et al*., 2012; Baldin *et al*., 2013; Bispo *et al*., 2013; Novaes & Bispo, 2014a; 2014b; 2014c; Avelino-Capistrano *et al*., 2011a; 2011b; 2013; 2014; 2016; Almeida *et al*., 2018; 2019; Gonçalves *et al*., 2017; 2019; Almeida & Bispo, 2020) (Fig. 2). However, west areas of these regions remain under or not sampled.

Phytogeographic Domains perspective

Although initially, geographic records were made based on political divisions, it is important to seek geographic boundaries that have biological meaning (e.g. biomes, biogeographic regions, watersheds, etc.). Here, we based the geographic units based on Brazilian Phytogeographic Domains, named: Amazon Forest (49.3% of the Brazilian Territory), Brazilian Savanna (23.9%), Atlantic Forest (13%), Dry Forest (9.9%), Subtropical Grassland (2.1%) and Wetland (1.9%) (Fig. 3) (IBGE, 2022). Previously, due to the lack of a bibliographic survey of Perlidae records, we were not able to infer the number of species recorded in each domain. Based on our survey, we were able to conduct the first discussion about species occurrence in a phytogeographic context.

The Atlantic Forest, the third largest domain, has the largest number of sampled locations, there are 226 points, concentrated mainly in the coastal region. The second largest domain, the Brazilian Savanna, has 79 sampled points. The Amazon Forest, despite being the largest domain with wide advantage, is only the third in number of sampled locations, only 75. In fourth and fifth place are Dry Forest (27 points) and Wetland (three points). It is evident that the Atlantic Forest

has received more attention and, therefore, more sampling and that other domains need to be better sampled. The number of sampled locations reflects the total of recorded species (Fig. 3). Among Brazilian domains, only the Subtropical Grassland has no species recorded. The Atlantic Forest, Brazilian Savanna, Amazon Forest, Dry Forest, and Wetland domains presented 85, 47, 28, nine and three recorded species, respectively (Fig. 3).

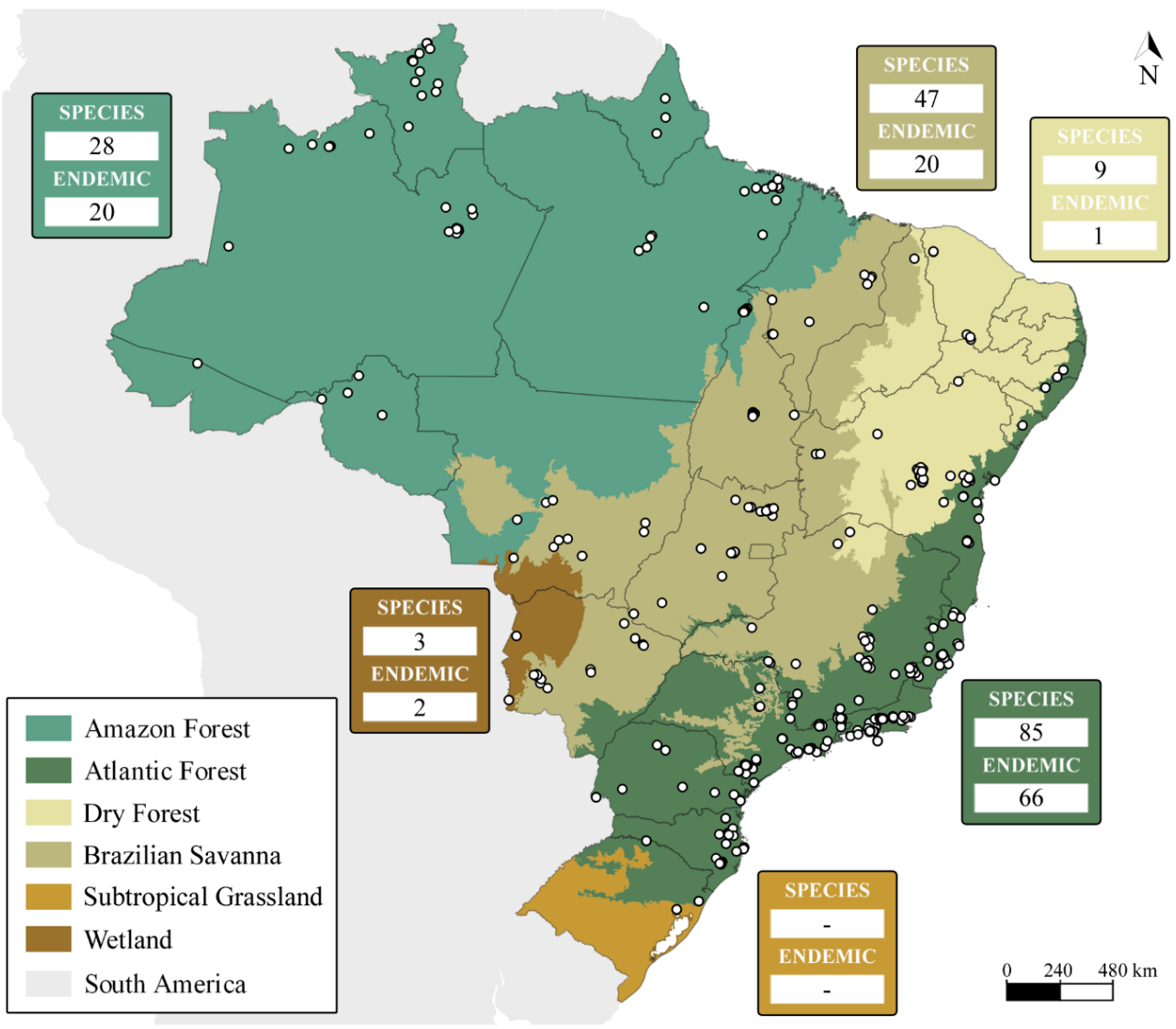

Figure 3. Sampling effort throughout the Brazilian Phytogeographic domains, highlighting the total of species and endemic species recorded in each domain.

Despite the positive correlation between sampled locations and the number of species observed, endemic species are recorded in different proportions. Among the 85 species recorded in the Atlantic Forest (54 species belonging to *Anacroneuria*, 30 to *Kempnyia*, and one to *Macrogynoplax*), 66 are endemic (~78%) (Table 4). This high number of species and endemism probably occurs mainly due to the cold water and the mountain ranges present in Atlantic Forest. Due to the political context afore discussed, the region of Atlantic Forest includes important research centers, consequently, it has been sampled continuously and has a more complete knowledge.

Table 3. Species by Brazilian Phytogeographic Domains, highlighting endemic species in bold.

Amazon Forest (20/28)
A. amazonica, *A. atrifrons*, ***A. blanca***, ***A. caraca***, ***A. caraja***, ***A. cruza***, *A. debilis*, ***A. fittkaui***, *A. manauensis*, *A. marlieri*, *A. minuta*, *A. otafroehlichi*, *A. pastaza*, ***A. pictipes***, ***A. posticata***, ***A. rondoniae***, *A. singularis*, ***A. xinguensis***. ***E. bifasciata***, ***E. froehlichi***, ***E. plagata***, ***E. yoshikii***. ***M. anae***, ***M. delicata***, ***M. guayanensis***, ***M. poranga***, ***M. pulchra***, ***M. quadrispina***.
Atlantic Forest (66/85)
A. amargosa, ***A. annulicauda***, *A. atrifrons*, ***A. aurata***, ***A. badilinea***, *A. bahiensis*, ***A. boraceiensis***, ***A. caraa***, ***A. cathia***, ***A. costalis***, *A. debilis*, *A. dilaticollis*, ***A. duarte***, ***A. egena***, ***A. fiorentini***, ***A. flintorum***, ***A. fumigata***, ***A. furfurosa***, ***A. fuscicosta***, ***A. hemiphaea***, ***A. iguazu***, ***A. impensa***, ***A. iporanga***, ***A. itajaimirim***, *A. itatiaiensis*, *A. kariri*, ***A. mantiqueirae***, ***A. novateutonia***, ***A. oculatila***, *A. ofaye*, *A. paprockii*, ***A. patioba***, ***A. paulina***, ***A. petersi***, ***A. piti***, ***A. plaumanni***, ***A. polita***, *A. quilombola*, ***A. rotunda***, ***A. ruschii***, ***A. sallesi***, ***A. simulans***, *A. singularis*, *A. stanjewetti*, ***A. subcostalis***, ***A. tabatae***, ***A. tinctilamella***, ***A. toriba***, ***A. trimacula***, ***A. tupi***, ***A. uyara***, ***A. vanini***, ***A. v-nigrum***, *A. xokleng*. *K. alterosarum*, ***K. auberti***, ***K. brasilica***, ***K. colossica***, ***K. couriae***, ***K. flava***, ***K. gracilenta***, ***K. guassu***, ***K. jatim***, ***K. kaingang***, ***K. mirim***, *K. neotropica*, *K. obtusa*, ***K. ocellata***, ***K. petropolitana***, ***K. pinhoi***, ***K. pirata***, ***K. puri***, *K. reichardti*, ***K. remota***, ***K. reticulata***, ***K. serrana***, ***K. tamoya***, ***K. taunayi***, ***K. tenebrosa***, ***K. tijucana***, ***K. tupinamba***, *K. umbrina*, *K. vanini*, ***K. varipes***. ***M. veneranda***.
Dry Forest (1/9)

A. atrifrons, A. bahiensis, A. calori, A. debilis, A. kariri, ***A. lencoensis****, A. quilombola, A. singela, A. terere*.
Brazilian Savanna (20/47)
A. amargosa, A. atrifrons, A. bahiensis, ***A. brandaoi****, A. calori,* ***A. corae****, A. debilis, A. dilaticollis,* ***A. dourada****,* ***A. froehlichi****,* ***A. galba****,* ***A. guaikuru****, A. itatiaiensis, A. jaciara,* ***A. leccii****,* ***A. lepida****, A. manauensis, A. marlieri,* ***A. meloi****,* ***A. mineira****, A. minuta, A. ofaye, A. otafroehlichi, A. paprockii, A. pastaza,* ***A. payagua****, A. quilombola,* ***A. saofrancisco****, A. singela, A. singularis, A. stanjewetti, A. terere,* ***A. tinga****, A. xokleng,* ***A. zantedeschia****.* ***E. castro****. K. alterosarum,* ***K. goiana****, K. neotropica, K. obtusa,* ***K. oliverai****, K. reichardti,* ***K. sazimai****, K. umbrina, K. vanini.* ***M. matogrossensis****,* ***M. yupanqui****.*
Wetland (2/3)
A. genualis*, A. jaciara,* ***A. pakitza****.*

There are 20 endemic species among 28 (~71%) in the Amazon Forest. It has 18 species belonging to *Anacroneuria*, four to *Enderleina*, and six to *Macrogynoplax* (Table 4). In addition, the genus *Enderleina* is mostly known for the Amazon Forest, with only *E. castro* for the Brazilian Savanna. Despite the high endemism, the Amazon Forest has certainly been poorly sampled, since most species have been recorded around Manaus, Amazonas state, and Belém, Pará state (Fig. 3).

Among the 47 recorded species, the Brazilian Savanna has 20 endemic species (~42%) (Table 4). The lower endemism can be explained by the presence of widely distributed species, some of which also occur in the Amazon Forest, Atlantic Forest and Dry Forest. It is possible that these species are more tolerant to the most severe conditions and, therefore, have reached a wide distribution. The Brazilian Savanna has 35 species belonging to *Anacroneuria*, one to *Enderleina*, nine to *Kempnyia*, and two to *Macrogynoplax* (Table 3).

Despite the diversity and endemism presented by the aforementioned domains, the Dry Forest and Wetland remain poorly studied or may not have as many Perlidae species as the other

domains. The reduced number of occurrence points recorded in both domains support these two hypotheses (Fig. 3). The Dry Forest has only one endemic species (~11%), whereas the Wetland recorded only three species (Table 3). If there is a scarcity of studies in these domains, including Subtropical Grassland, this occurred probably due to the historical context of research institutions and researchers located in other domains. In this case, it is expected that the number of species recorded in these domains will increase over the next few years.

Knowledge gaps, difficulties and challenges

Problematic species

Currently, there are 152 species of Perlidae in Brazil. However, at least 33 of these are now problematic species. We considered problematic that species described based only on females and/or before the 1960s and never collected again or re-studied. According to Almeida & Bispo (2020), specimens deposited for a long time in 80% ethanol may lose the pattern of spots on the head and pronotum. This is an important factor for species with old descriptions or species described based only on females, as it makes the association with new specimens from the type location a very hard task. This can occur even if we are using the holotype to base the association of the new specimens. In addition to the aforementioned problems, most types of these species are located in museums in other countries, not in Brazil. This makes the access to this material a difficult task. This often means that the comparison between specimens has to be carried out through photos and the original descriptions. Furthermore, even if we try to use holotypes for comparison, some of them may be poorly preserved or be lost. Therefore, taxonomists currently

describe the diversity they found and, when possible, associate some of the specimens with older species, as performed by Froehlich (2002). Possibly, some males or females of these problematic species have already been described as new species, causing species overlap and limiting our understanding of species distribution.

Species delimitation

The morphological comparison between males, mainly using penial armature, is the most used mechanism for delimit Brazilian Perlidae. However, the morphological variation of the penial armature presented by the different populations of *Anacroneuria debilis* (Baldin *et al*., 2013; Almeida & Bispo, 2020) has raised questions to researchers. What is the limit for considering a variation to be intraspecific? This has also occurred in other species, such as *A. flintorum* and *Kempnyia colossica*, for example (Almeida *et al*., 2018; Almeida & Bispo, 2020). Clearly, these species may be cryptic species complexes. However, few studies addressing other sources of information have been conducted using Brazilian species, with molecular markers being the most used (Avelino-Capistrano *et al*., 2014, 2016; Almeida *et al*., 2018; Almeida & Bispo, 2020). Among these, only Almeida *et al*. (2018) and Almeida & Bispo (2020) carried out some approach delimiting species or study their limits.

Historically, the taxonomy of Brazilian perlids has been carried out first on the Brazilian coast, in the eastern region of the country. On the other hand, most of Brazil's neighboring countries are in the western region of South America. This caused taxonomies to develop independently. However, with the advancement of sampling in the Amazon Forest and in the Brazilian Savanna, there should be greater interaction between Brazilian studies and those of

neighboring countries, mainly to delimit species. With the exception of Froehlich (2002), Bispo & Froehlich (2004c), and Menezes *et al*. (2020), other Brazilian authors hardly considered neighboring species. Probably, species described based on single specimens and the difficulty in accessing the type material described outside Brazil will be the main obstacles to be overcome. Until political barriers to access the type material and collect fresh specimens are overcome, research on the delimitation of similar species in different countries will be a problem. This scenario is worsened by the fact that the neotropical region includes one of the genera with the greatest diversity in Plecoptera, *Anacroneuria*, which represents approximately 10% of the described species of Plecoptera. Finally, it is important to note that although we discussed this issue from the perspective of Brazilian researchers, the difficulties presented by researchers from neighboring countries are the same in relation to Brazil.

Nymph impediment

Despite the increase in relevant knowledge about Brazilian Perlidae showed in "**Main results of the early 21st century**" section, in general, most studies have been restricted mainly to the morphology of adult males. As we presented here, only 30 species of Perlidae have nymphs described in Brazil, of which 16, one, nine, and four belong to *Anacroneuria*, *Enderleina*, *Kempnyia*, and *Macrogynoplax* respectively. The gap in knowledge about semaphoronts is quite common among insect groups and have been called Haeckelian shortfall (Faria *et al*., 2021). Herein, we focus the discussion on the nymphs and for the difficulty of advance in knowledge about nymphs of Brazilian perlids we are giving the name "Nymph impediment".

Why do we need to name the nymphs? Nymphs are important because they live in the water and suffer from environmental changes. They are great sources of data for studies on environmental monitoring. At the same time, they can provide taxonomic information that are relevant and different from that provided by adults because nymphs experience different selective pressures compared to adults. Morphological phylogenies made based only on adults could present different results, for example.

Rearing nymphs until adulthood, as many authors have reported, is often an arduous task in Brazil (Novaes *et al*., 2012; Almeida & Bispo, 2020). This is true both in the laboratory and in the streams. Normally, the sampled areas and laboratories are distant from each other. In this context, there are some very sensitive Brazilian species and many of them die until they reach the laboratory or even in a rearing apparatus mounted in the field. Therefore, knowing the nymphs by the specific name is a necessary and difficult task at the same time.

Recently, studies involving the molecular association of nymphs and adults of Perlidae have been carried out in Brazil (Avelino-Capistrano *et al*., 2014, 2016; Almeida *et al*., 2018; Almeida & Bispo, 2020). As these authors have reported, molecular association using COI (cytochrome c oxidase subunit I) may be an easier way to associate adults and nymphs. However, this method comes up against cost, which despite having decreased in recent years, is still expensive in Brazil. Therefore, "Nymph impediment" is one of the biggest challenges for Brazilian researchers.

Phylogenetic, Biogeographic, and Ecological studies

There are relevant gaps in taxonomic knowledge when it comes to Brazilian perlids. Taxonomic studies are the basis of the nominal knowledge about a species. However, there are no

published studies addressing phylogenetic and biogeographic hypotheses for the Brazilian perlid genera. The difficulty in accesing and studying holotypes outside Brazil to assemble morphological phylogenies may have contributed to this at the same time that studies with molecular markers are initial. Species with old or problematic descriptions, which have only type specimens, also make it difficult in cases of molecular phylogenies. Another factor that makes it difficult to carry out biogeographic studies is the lack of knowledge about the complete species distribution. Since the nymphs require preserved environments, most rivers and streams of possible occurrence of Perlidae species are often arduous to find or are in remote locations.

Our knowledge of biological and ecological aspects of Brazilian species is also scarce. There are few studies that address ecological aspects of nymphs, with information on distribution in the substrates (Bispo *et al*., 2002a; Bispo *et al*., 2006; Avelino-Capistrano *et al*., 2011c), development (Dorvillé & Froehlich, 2001), seasonal density (Bispo *et al*., 2002a), and information about diet and biological aspects (Bispo & Froehlich, 2008; Bispo *et al*., 2013; Almeida *et al*., 2019). Most of these studies, with the exception of Dorvillé & Froehlich (2001), Bispo & Froehlich (2008), Bispo *et al*. (2013) and Almeida *et al*. (2019), have studied nymphs at a generic level. Equally poorly studied are adults, with a study of their phenology (Bispo *et al*., 2002b) and size variation (Froehlich, 1990). Additionally, there are no records of studies addressing "drumming", a sound communication behavior between males and females that is extensively studied in the northern hemisphere.

Research perspectives for the next years

Taking into account the challenges presented, how can we overcome them? Advances in methods, mainly molecular, have improved the possibility of more complete studies on Perlidae. For example, in Brazil, due to the integrative studies including the use of the COI (cytochrome c oxidase subunit I) barcoding region (Hebert *et al.*, 2003), it was possible understanding the morphological characters that define a teneral specimen (Almeida *et al*., 2018; Almeida & Bispo, 2020), which possibly will result in the revision of some species. The use of molecular markers also enabled the researchers to associate immatures with adults (Avelino-Capistrano *et al*., 2014, 2016; Almeida *et al*., 2018; Almeida & Bispo, 2020) without the need of rearing specimens in the laboratory or in streams, a determining factor to decrease the Nymph impediment.

Although the aforementioned studies used molecular data to associate immatures and recognize teneral stages, only Almeida & Bispo (2020) carried out an integrative approach to test the COI potential to properly delimit Brazilian species. This study was carried out in a regional perspective with the inclusion of *Kempnyia colossica* specimens from other populations. The study showed the possibility of *K. colossica* being a species group with at least two species. This possibility highlights the need to study the delimitation of species, especially those that are morphologically problematic. Therefore, integrating the largest number of sources of information, mainly morphological and molecular, proves to be a fundamental way to study the limits of species, being widely desired. In addition, the use of an integrative approach may facilitate the proposition of phylogenies and biogeographic studies.

To make all of these possible, it is extremely necessary to continue increasing the sample efforts in areas that are still poorly known or unknown. Even from a political perspective, it is still necessary to increase the sampled area. Each region will need its own approaches, as they have different limitations. Thus, new species will be described and more specimens will be

collected, enabling better studies on species delimitation and biogeography, for example. Sampling fresh specimens is also important in a molecular studies perspective, since we can store them properly for optimal conservation of DNA. In addition, the increase in fieldwork may contribute to ecological studies, focusing on information on the nymph's life span, flight period, and adult reproduction, for example.

Therefore, we believe that in the coming years the sampling effort and the study of Brazilian perlids through an integrative approach should continue to increase. With that, we also expect to consolidate Perlidae collections that are specialized in the maintenance of specimens for DNA extraction. With that, gradually, we will overcome the current challenges of Brazilian Perlidae taxonomy and make room for new challenges.

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Considerações Finais

Nosso trabalho é o primeiro sobre Perlidae do Brasil a estudar espécies dos quatro gêneros brasileiros (*Anacroneuria*, *Enderleina*, *Kempnyia* e *Macrogynoplax*). Através do estudo de espécimes de diversas coleções científicas, o presente estudo nos permitiu ter uma visão mais ampla sobre a diversidade de Perlidae do Brasil. Esta visão amplificada nos possibilitou evidenciar problemas taxonômicos que necessitam de atenção, como por exemplo, a necessidade de se estudar os holótipos de *Anacroneuria fittkaui* e *A. terere*; ou de se estudar detalhadamente os limites das espécies que compõem o agrupamento criado por Stark (1995), ao qual pertencem *Anacroneuria cushueme*, *A. pakitza*, *A. pinza*, *A. vistosa*, *A. yameo* e *A. zunigae* além de *A. marlieri* e *A. pakitza*. Também trouxemos novos dados a discussão sobre as diferentes populações de *A. debilis* (Almeida & Bispo, 2020; Miguel *et al*., 2022) e *A. flintorum* (Almeida *et al*., 2018), e pudemos propor soluções para ruídos taxonomicos antigos, como a sinonímia de *Kempnyia sordida* com *K. obtusa*.

Nosso estudo contribuiu, portanto, para reduzir o déficit Linneano sobre a taxonomia de perlídeos brasileiros através da descrição de seis novas espécies (*Anacroneuria duarte* nov. sp., *A. sallesi* nov. sp., *Enderleina castro* nov. sp., *Kempnyia guarani* nov. sp., *K. tupiniquim* nov. sp. e *K. zwicki* nov. sp.) e para reduzir o déficit Wallaceano através da documentação de novos registros geográficos. É importante salientar que contribuímos para a taxonomia de Perlidae do Brasil não apenas com dados inéditos, mas também com a organização do conhecimento pré-existente. Apesar da existência de um catálogo das espécies neotropicais (Froehlich, 2010) e de uma recente lista de espécies da América do Sul (Pessacq *et al*., 2019), nosso trabalho atualizou e acrescentou informações sobre as espécies brasileiras, como por exemplo, a organização da

ocorrência das espécies por domínio fitogeográfico. Esta organização visou padronizar as informações sobre as espécies para auxiliar estudos futuros, facilitando o acesso às informações sobre as espécies. Além disso, o compilado de informações e pontos de ocorrência proporcionou que evidenciássemos as principais regiões que carecem de amostragem ou que o esforço amostral precisa ser expandido. Além disso, através da análise dos dados obtidos, foi possível evidenciar as dificuldades que precisam ser ultrapassadas para que possamos entender melhor a diversidade de Perlidae no Brasil. Esperamos que a conclusão e publicação dos estudos propostos aqui sirvam de alicerce para que a taxonomia dos perlídeos brasileiros se torne cada vez mais completa, robusta e padronizada.

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