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Streamlining Anacroneuriini (Plecoptera, Perlidae): a novel interactive tool for species identification

Rodrigo Braga Gastaldo
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Dissertation submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Frederico Falcao Salles

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ABSTRACT

GASTALDO, Rodrigo Braga, M.Sc., Universidade Federal de Viçosa, July, 2025. **Streamlining Anacroneuriini (Plecoptera, Perlidae): a novel interactive tool for species identification**. Adviser: Frederico Falcao Salles. Co-adviser: Lucas Henrique de Almeida.

Anacroneuria Klapálek, 1909 (Plecoptera, Perlidae) is the genus with the largest known species richness in the order Plecoptera. Over time, the importance of some characters and their use for taxonomy in the genus has been variable. In addition, the high rate of species descriptions, which are frequently incomplete or overly succinct, led to dubious and confusing species identity to many of its species. Finally, many of the existing dichotomous identification keys for the genus are incomplete and cumbersome. Currently, identifying species in Anacroneuria is a difficult task. We need, then, new tools to ease the identification of Anacroneuria, with plenty of illustrations and user-friendly characters. As a first step, given the lack of standardized morphological terminology, this work proposes a morphological atlas to include stable terminology in accordance with that of other insect groups for species description and character definition in Anacroneuriini stoneflies. This work then proposes electronic and interactive tools for the identification of known species of Anacroneuria. Based on the known species of Southeastern Brazil, we construct an interactive, multi-access key, which is a flexible, expansible and pictorial resource for species identification in Anacroneuria. The key currently includes almost half of the known species in Brazil and will continue to be in development to include the greatest number of species possible.

Keywords: stoneflies; interactive key; taxonomy; Neotropical Region; Xper3; cybertaxonomy

RESUMO

GASTALDO, Rodrigo Braga, M.Sc., Universidade Federal de Viçosa, julho de 2025. **Otimizando Anacroneuriini (Plecoptera, Perlidae): uma nova ferramenta interativa para identificação de espécies**. Orientador: Frederico Falcao Salles. Coorientador: Lucas Henrique de Almeida.

Anacroneuria Klapálek, 1909 (Plecoptera, Perlidae) é o gênero com a maior riqueza de espécies conhecida em toda a ordem Plecoptera. Ao longo de seu estudo, a importância de determinados caracteres e sua utilização para a taxonomia do gênero variou. Além disso, a alta taxa de descrição de espécies, frequentemente incompletas ou demasiadamente sucintas, levou a identidades dúbias e confusas de muitos de seus integrantes. Por fim, muitas das chaves existentes para o gênero são incompletas e trabalhosas. Atualmente, identificar as espécies de Anacroneuria é uma tarefa desafiadora. Assim, novas ferramentas de identificação de Anacroneuria com ilustrações e caracteres amigáveis se fazem necessárias. Assim, este trabalho propõe um atlas morfológico cujo intuito é incluir terminologia coesa com a de outros grupos de insetos e estável para a descrição de novas espécies e definição de caracteres taxonômicos em Plecoptera da tribo Anacroneuriini. Ainda, este trabalho propõe ferramentas eletrônicas, e interativas para a identificação das espécies conhecidas de Anacroneuria. Com base em material registrado para a Região Sudeste do Brasil, uma chave interativa e multi-acesso é construída, constituindo um recurso flexível, expansível e pictórico para a identificação das espécies de Anacroneuria. A chave atualmente inclui quase metade das espécies conhecidas no Brasil e continuará em desenvolvimento buscando incluir a maior quantidade de espécies possível.

Palavras-chave: Plecoptera; chave interativa; taxonomia; Região Neotropical; Xper3; cibertaxonomia

SUMÁRIO

GENERAL INTRODUCTION

Plecoptera is a small order of aquatic insects found globally (DeWalt et al., 2025, Speciesfile; DeWalt & Ower, 2019). Currently, the order includes around 3800 valid species spread throughout 17 different families (DeWalt et al., 2025). Originally, these insects were included in Neuroptera by Linnaeus (1758), alongside Trichoptera. Burmeister (1839) erected the order and separated them from Neuroptera and Trichoptera based on the pleated hindwings with enlarged anal lobes, a characteristic shared by many Polyneoptera, and the aspect of their mouthparts. Ever since 1839, many authors have occasionally studied the order and described its species, such as Pictet and Kempny still in the XIX century (Pictet, 1841; Kempny, 1898), or Klapálek, Enderlein, Navás and Illies in the XX century (Klapálek 1909, 1914, 1921; Enderlein, 1909; Navás, 1916, 1932, 1934; Illies, 1966), for example.

These authors worked with many insect groups, and when working with Plecoptera, described species in different families. Most of their works, however, included the description of Perlidae species - the most diverse family in the order. Since they are distributed around the world and are some of the most common stoneflies (DeWalt et al., 2025), Perlidae numbered many of the works involving Plecoptera in the XIX and XX centuries.

At that time, most of the taxonomy of the family focused on external appearance, color and female subgenital plate shape (Pictet, 1841; Jewett, 1959), while male genitalia were, for the most part, disregarded as a valuable taxonomic tool in describing species. Jewett (1959) even stated that “The male genitalia apparently have no specific characteristics.”. In contrast to the works of the authors from the early XX century, however, contemporary Perlidae taxonomy has a growing interest in male genitalia as the primary character-bearing structure for species description (Stark et al., 1999; Froehlich, 2002; Almeida & Duarte, 2017; Molineri et al., 2023; Zang, Huo & Du, 2025).

Male genitalia morphology has been taken as the most important structure for species description particularly for species in the Neotropical Region (sensu Morrone, 2014). The neotropical genera of Perlidae, especially those in the Anacroneuriini tribe, show large amounts of sclerotization and specialized structures in their male genitalia (Stark & Gaufin, 1976). However, while European and North American authors from the XIX and the first half of the XX century occasionally dealt with Neotropical specimens, extensive research on the Neotropical stonefly fauna only gained traction in the latter half of the XX century (Duarte &

Lecci, 2024). It was only in the 1960s when a South American researcher took upon the study of these insects. Over the latter half of the XX century and beginning of the XXI century, Dr. Cláudio G. Froehlich was the most prominent author studying the order in the Neotropical region, and particularly so in Brazil (Froehlich, 1969, 1984, 1988, 1999, 2002, 2004, 2007, 2010; Duarte & Lecci, 2024). Following professor Froehlich's legacy, research on Plecoptera in the Neotropical region has steadily seen an increase in papers, species and active researchers (Dorvillé & Froehlich, 2001; Ribeiro-Ferreira & Froehlich, 2001; Bispo & Froehlich, 2004; Bispo & Lecci, 2011; Duarte & Lecci, 2016; Almeida & Bispo, 2018).

Parallel to the studies in Brazil, other researchers have also worked on other regions of Latin America (Stark, 1994, 1998; Stark & Sivec, 1998; Stark & Kondratieff, 2004). The combined efforts of these researchers and their students led to South America having one of the highest species description rates in Plecoptera (DeWalt & Ower, 2019). Within the Anacroneuriini stoneflies, *Anacroneuria* Klapálek, 1909 currently includes around 400 species distributed in the Neotropical region and are one of the most frequently collected stonefly genera (Amaral et al., 2019). However, there are some problems in studying and identifying its species. The works of many authors in the XIX and early XX century, often in different languages, introduced many names for the same morphological structures (e.g. vertex and parietalia), while the sudden growth of study in the second half of the XX century, by different researchers in different countries, continued using different terminology in their descriptions (e.g. penis, aedeagus and penial armature). Choosing a specific vocabulary to follow may also present obstacles, as each terminology usually covers only a part of the morphological variation in Anacroneuriini.

Another problem in Anacroneuriini taxonomy arose from the way its many species were described. Many descriptions from the mid XX century, which didn't describe the male genitalia, became questionable as new, similar species were found. While these newer species descriptions included male genitalia, they were still too succinct, making species delimitation dubious. This problem has only increased over time, and different species share many morphological similarities with other species (e.g. *Anacroneuria polita* (Burmeister, 1839) and *Anacroneuria petersi* Froehlich, 2002; *Anacroneuria debilis* (Pictet, 1841) and *Anacroneuria ruschii* Novaes, Bispo & Gonçalves, 2016; *Anacroneuria singela* Duarte & Lecci, 2016 and *Anacroneuria rotunda* Gonçalves, Novaes & Salles, 2017). While the difficulty in identifying *Anacroneuria* species has been increasing over time, few studies have been published dedicated

to elucidating the identification troubles in the genus. Some works include dichotomous identification keys for the species in some geographical regions (Stark & Sivec, 1998; Stark, 1999; Duarte & Lecci, 2016). While these works are extremely important in providing tools for identifying the species of *Anacroneuria*, the dichotomous nature of analogical keys may be unsuited for the great number of species within the genus. With the advent of electronic multi-access keys and cybertaxonomy, new tools were developed that may be better suited for a general key for the identification of rich and morphologically homogeneous taxa such as *Anacroneuria*.

Considering this context, there is a twofold effort required for streamlining the study of *Anacroneuria*: stabilization of morphological terminology, and a reliable resource for identifying species. This work attempts to provide solutions for both problems, which are mostly interlinked. As such, this dissertation is structured in two sections, written in the format of academic papers. The first section proposes an updated and illustrated morphological glossary for the study of Anacroneuriini stoneflies, to be used as a standard in species descriptions and character definition for both systematic work and identification keys. The second section describes the structure of an interactive electronic multi-access identification key for the species of *Anacroneuria*, utilizing the terminology proposed in the first section. Each section is formatted according to the journal guidelines in which each paper will be submitted.

OBJECTIVES

The objective of this work is to enhance the taxonomic knowledge and facilitate the accurate identification of *Anacroneuria* Klapálek, 1909 (Plecoptera, Perlidae) species through the integration of standardized morphological terminology, detailed morphological documentation and the development of modern digital identification tools. This will be accomplished through:

- Standardization of the morphological terminology of *Anacroneuria* species.
- Detailed illustration of the general body plan and male genitalia representative of Anacroneuriini, highlighting diagnostic characters.
- Development of an electronic multi-access identification key to support accurate identification of *Anacroneuria* species from Southeastern Brazil.

- Documentation through high-quality photographs of the morphological diversity of *Anacroneuria* species found in the studied region.

SECTION 1 – ON ANACRONEURIINI (PLECOPTERA: PERLIDAE) MORPHOLOGY: STANDARDIZING VOCABULARY AND A MORPHOLOGICAL ATLAS

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On Anacroneuriini (Plecoptera: Perlidae) morphology: standardizing vocabulary and a morphological atlas

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Abstract

Anacroneuriini is a tribe of stoneflies within Perlidae which displays diagnostic characteristics in their male genitalia. As researchers are often focused on a few taxonomic characters of each particular genus, they rarely consider the morphological terminology of the tribe as a whole. As such, over time many different names for the same morphological structures have been coined, making it difficult to describe new species and semaphoronts accurately in a standardized way. Systematics may also struggle in determining primary homologies from the lack of comparable vocabulary between species and genera. The objective of this work is to provide a morphological atlas of Anacroneuriini, disclosing standardized names of each external morphological trait of the four different genera of the tribe. Specimens from Museu de Entomologia, Universidade Federal de Viçosa (UFVB) were analyzed, photographed and illustrated. Terminology primarily followed morphological accuracy based on homologies, while name recurrence in recent literature was used as secondary criteria. The resulting atlas is the first to gather detailed information on all body regions of all genera of Anacroneuriini. The atlas is useful as a resource both for beginners in Anacroneuriini research and experienced researchers alike, providing standard terminology with clear illustrations for species description, identification and general taxonomic work.

Keywords: Stoneflies; External morphology; *Anacroneuria*; *Enderleina*; *Kempnyia*; *Macrogynoplax*.

1 – Introduction

Plecoptera is a small order of aquatic insects. All of its 3800 known extant species (DeWalt et al., 2025) need aquatic environments during their development, and most spend their time as nymphs underwater in cold, well oxygenated low order streams (Hynes,1976; DeWalt et al., 2015; Bispo and Froehlich, 2024). While they are distributed globally, these insects have an antitropical distribution, in which higher diversities are found in subtropical and temperate regions (Letsch et al., 2021). They display varied feeding habits, being detritivores, predators or shredders as nymphs. Adults are usually weak flyers and often do not feed (Hynes, 1976).

Linnaeus (1758) classified these insects alongside Trichoptera under the order Neuroptera. Burmeister (1839) would be the one to give these insects their own order with its current name mainly due to the typical pronounced anal lobe in the hind wings – a characteristic shared with many other Polyneoptera orders (Wipfler et al., 2019). While the order has been considered valid and its monophyly is widely accepted, the inner relationship among stoneflies is still under debate. After proposing a subdivision of the order into two suborders (Zwick, 1969) - Arctoperlaria and Antarctoperlaria -, Zwick would spend the next few decades studying different aspects of phylogenetics, biogeography and ethology of stoneflies, corroborating with this division of the order (Zwick, 1973, 1990, 2000). While Antarctoperlaria likely originated in the Southern Hemisphere during the breakup of Pangaea, Arctoperlaria is thought to have a Northern origin and is divided into two clades – Euholognatha and Systellognatha – based on the functionality of their adult mouthparts (Zwick, 2000). Recently, some studies based on molecular data question the vicariance of this origin and propose new biogeographic hypotheses, while others corroborate the classical understanding of Plecoptera systematics (Letsch et al., 2021; García-Giron et al., 2024). Even still, the classification of Antarctoperlaria and two groups of Arctoperlaria - Euholognatha and Systellognatha - is still regarded today as the main interpretation of Plecoptera systematics (Letsch et al., 2021).

Systellognathans are characterized by the covering and atrophy of their mouthparts as adults, and thus they do not feed, although they do drink water (Hynes, 1976). Perlidae is one of the most studied and well-known Systellognatha families. Even though it is an Arctoperlaria family with most of its diversity in the Northern Hemisphere, it also occurs in South America, and its range in the continent is vast (Stark et al., 2009; Froehlich, 2010; AvelinoCapistrano et al., 2018; Pessacq et al., 2019).

The Perlidae genera found in South America all belong to the Acroneuriinae subfamily (Froehlich, 2010; DeWalt et al., 2025). Four of these genera compose the Anacroneuriini tribe,

those being: Anacroneuria Klap alek, 1909; Enderleina Jewett, 1960; Kempnyia Klap alek, 1914; and Macrogynoplax Enderlein, 1909. These genera are remarkably different to other Acroneurinae regarding the male genitalia. Anacroneuriini shows astounding sclerotization and specialization of this structure, with distinctive opposed large hooks, processes, and spines, in contrast to the most other genera in Perlidae, especially in the Americas (Stark and Gaufin, 1976). Currently, Anacroneuriini includes approximately 450 species, with 393 species belonging to Anacroneuria, 40 to Kempnyia, 16 to Macrogynoplax and 9 to Enderleina (DeWalt et al., 2025).

Since the works of Klapalek, Illies, and Froehlich, stone fly research has been a small but steadfast field in Neotropical and Nearctic aquatic entomology. New species are frequently described, new phylogenies are being proposed and molecular tools are rising in use (Duarte et al., 2022; Miguel et al., 2022; Almeida et al., 2023; Molineri et al., 2023). However, nomenclature and terminology haven't been regularly maintained, and authors often use terms based on similarity to other groups of stoneflies or other insects. As such, it can be confusing to navigate through Anacroneuriini morphology in the absence of standard terminology, both as a reader and as a researcher. Additionally, full specimen illustrations are few and far between, and morphological terms may be thrown around in species descriptions without regard for readers who have never seen said structure or heard that particular name for it.

Thus, in order to facilitate understanding among authors and lessen the lack of standard terminology in Anacroneuriini morphology, this study aims to propose a standard vocabulary for the tribe as well as illustrate and discuss particular morphological features and their variations, serving as a morphological atlas for Anacroneuriini.

2 – Methods

We analyzed both specimens kept in 80 % ethanol and dry ones present in the collection of the Museu de Entomologia da Universidade Federal de Viçosa (UFVB at the Department of Entomology, Federal University of Viçosa – UFV, Viçosa, MG, Brazil). For genitalia assessment, we dissected and clarified insects using 10 % potassium hydroxide solution (KOH 10 %). Different structures were dissected from multiple specimens to study varying aspects of morphology, including heads, abdomens, wings and legs. For genera lacking diversity in the UFVB collection (e.g Enderleina), additional material from the Aquatic Insect Collection “Prof. Dr. Cláudio Gilberto Froehlich” (CIACGF at Aquatic Biology Laboratory, State University of São Paulo – UNESP, Assis, SP, Brazil) and the Zoological Museum of the University of São Paulo (MZUSP at University of São Paulo – USP, São Paulo, SP, Brazil) was inspected and

used for illustration. A list of all species whose specimens were analyzed by the authors is present in Supplementary Material 1. Every Anacroneuriini species that was not analyzed in person had their description and illustrations checked to guarantee that the atlas still covers their morphology.

Photographs were taken using a Leica M205A stereomicroscope and a digital camera with auto montage image software (Leica MC170 HD), based on which digital illustrations were made. When necessary, corrections on the images were done using Adobe Bridge 2024 (Adobe Inc., 2019b). All illustrations were done in Adobe Illustrator 2024 (Adobe Inc., 2019a). Line drawings utilized the software's pen tool, while digital paintings were done using the software's native bristle brush library. Broader morphological terminology followed that proposed by Matsuda (1970, 1976) and Snodgrass (1935), while structures specific to stoneflies and Anacroneuriini were named based on morphological accuracy while favoring names that are already in use by researchers of the group.

3 – Results and Discussion

The general body shape of Anacroneuriini follows that of other Perlidae. They are mostly macropterous insects with flattened bodies and forewing size varying from around 8-50 mm. Their hind wings always show a distinct anal lobe, although its size in relation to the rest of the wing is variable. The cerci of these species are often long, and males have a simple projection at the end of the sternite IX, known as a hammer (Fig. 1). This hammer may be pronounced or barely visible, typically being longer and distinct in smaller species and being only visible through subtle rough rugosities in its place in larger species – e.g. Anacroneuria paulina Froehlich (2004). Nymphs vary in size according to their instar and are flattened insects with an abundance of branched gills with many terminal filaments in their thorax and occasionally at the end of the abdomen (Fig. 2). Some of these gills may be seen in teneral adults – those that have molted recently and have yet to finish sclerotization. Their color varies from yellow or pale to black, but a few species – particularly those in Enderleina and Macrogynoplax – may show more vibrant coloration in shades of green, red or purple.

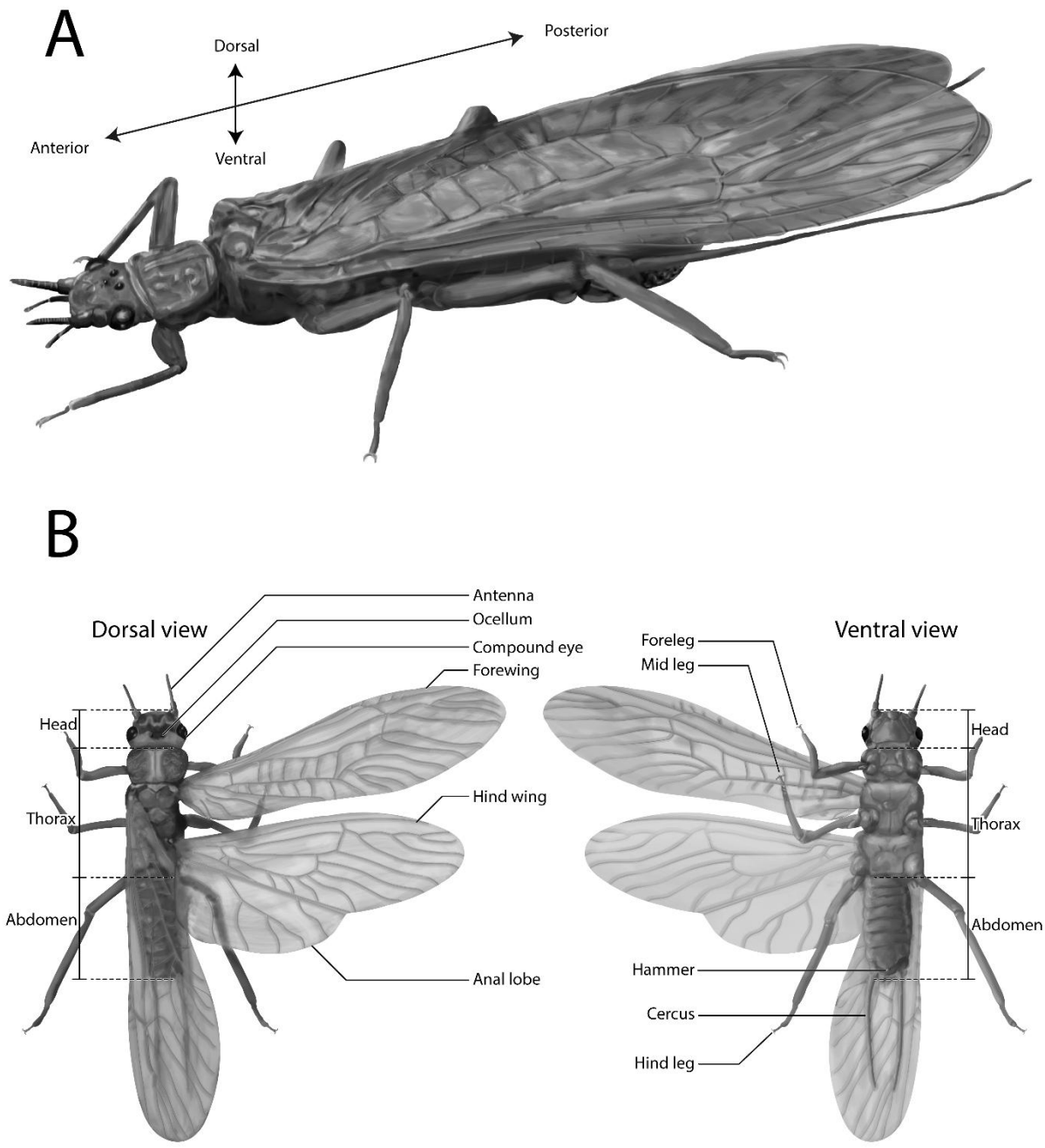

Fig. 1 – General habitus and disposition of an Anacroneuriini stonefly. A: Habitus of *Kempnyia* sp. with indication of body axes as they are considered in this study. B: General structure, tagmosis and notable features of *Anacroneuria* sp. morphology in dorsal and ventral views.

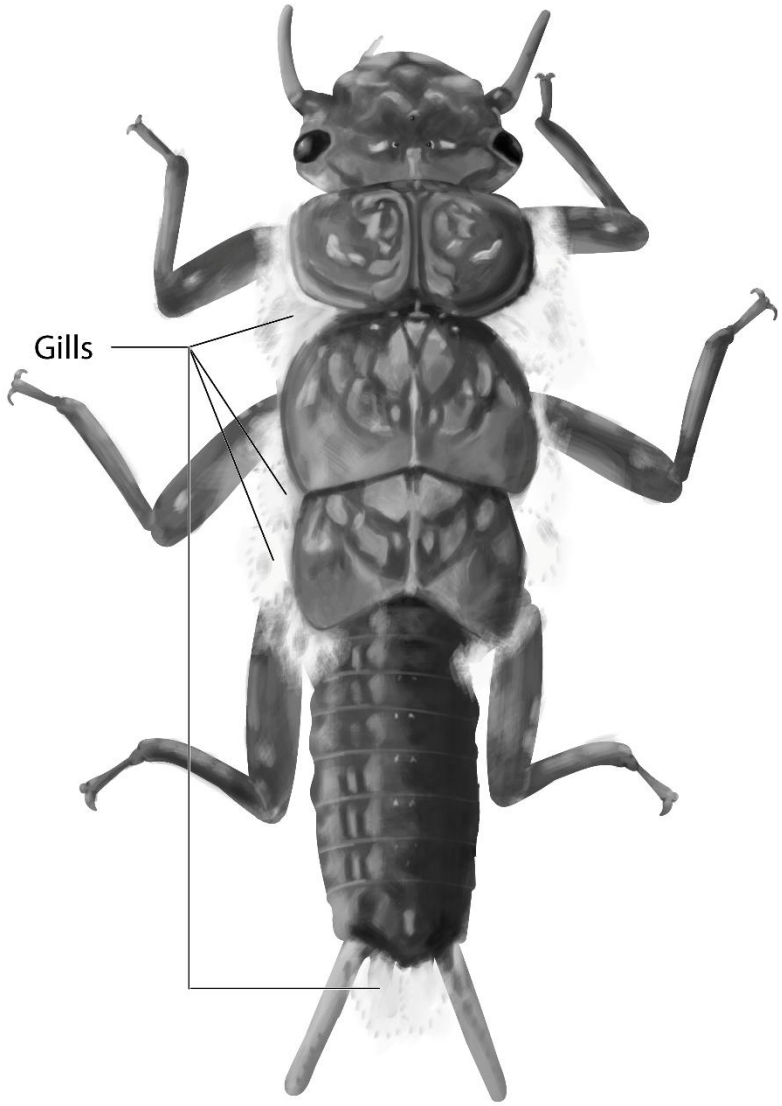

Fig. 2 – Digital painting showing the general habitus and disposition of an Anacroneuriini nymph.

3.1 Head

Anacroneuriini has the typical Perlidae head. In adults, most of the mouthparts are atrophied and not functional. The clypeus is fused to the **frons** forming a single plate in which two or three ocelli, the **M-line**, and the lappets are found. Thus, the frontal region of the head is delimited anteriorly by the **anterior clypeal margin** instead of the posterior clypeal margin. Laterally, it is delimited by the genae and posteriorly by the ecdysial suture. The **genae** are placed anteriorly to the eyes, in the lateral part of the head, while the **vertex** is placed posterior to the frons, delimited anteriorly by the ecdysial suture (Fig. 3A). The vertex has often been

called the parietalia by Brazilian researchers (e.g. Bispo and Froehlich, 2004; Froehlich, 2004; Righi-Cavallaro et al., 2013). However, this term has rarely been used outside Brazilian stonefly research, and to keep the same standard as other insect orders, we suggest the use of the term "vertex" for this region.

The **labrum** in nymphs is mobile, not fused to the frontoclypeus, and their mouthparts are specialized for predation. The **mandibles**, **galea**, and **lacinia** are sharpened and blade-like, particularly in older nymphs. The **glossae**, as in other Perlidae, are reduced, while the **paraglossa** are well developed. Both the glossa and paraglossa insert into the short **prementum**. The **mentum** is often a short rectangle or trapezoid plate, while the **submentum** covers most of the ventral face of the head (Fig. 3B).

The paired **ocelli** are found in all genera of Anacroneuriini in both nymphs and adults, while the median ocellus is only found in *Enderleina* and most *Kempnyia* species. The **M-line**, located in the frontal region of the head, is present in most species either in a distinctive color pattern or as an embossed texture in the frons. Similarly, a pair of embossed or discoloured **tentorial scars** can be found lateral to the ocelli. Both the M-line and the tentorial scars show muscle attachment points. Moulins (1968) described these muscles as the "oral angle retractors, the dorsal dilators of the precerebral pharynx and the extrinics of the labrum". Following the standardized nomenclature of head muscles in Dicondylia (Wipfler et al., 2011), these muscles appear to be musculus frontobuccalis anterior, musculus verticopharyngealis, and musculus frontolabralis, respectively, although further investigation into the precise identity of these muscles is needed.

The **lappets** are an anterolateral extension of the frontoclypeal plate. They cover part of the insertion of the antennae and are often different in color from the rest of the head, frequently appearing to be considerably darker. Their color is often noted as a taxonomic character used in species descriptions.

The **ecdysial suture** is used as a taxonomic character for generic identification both for nymphs and adults. While in *Enderleina*, *Kempnyia* and *Macrogynoplax* its median portion frequently advances in between the paired ocelli (Fig. 3), the ecdysial suture in *Anacroneuria* does not advance anteriorly in such a way, making a simple posteriorly curved suture along the head. It is worth noting, however, that the advance of the median portion of the ecdysial suture between the paired ocelli may be considerably less pronounced in the larger species of *Kempnyia* - e.g. *Kempnyia colossica* (Navás,1936) -, with the lateral arms of the ecdysial suture becoming almost straight. In general, the angle between the lateral arms and the median portion of the ecdysial suture is obtuse in *Anacroneuria* and varies from acute to a right angle in the

other genera according to their size. The ecdysial suture is frequently described as having the appearance of a “Y” in *Anacroneuria* and a “W” in other genera. Some researchers refer to it as the postfrontal suture or line (Lecci and Froehlich, 2007).

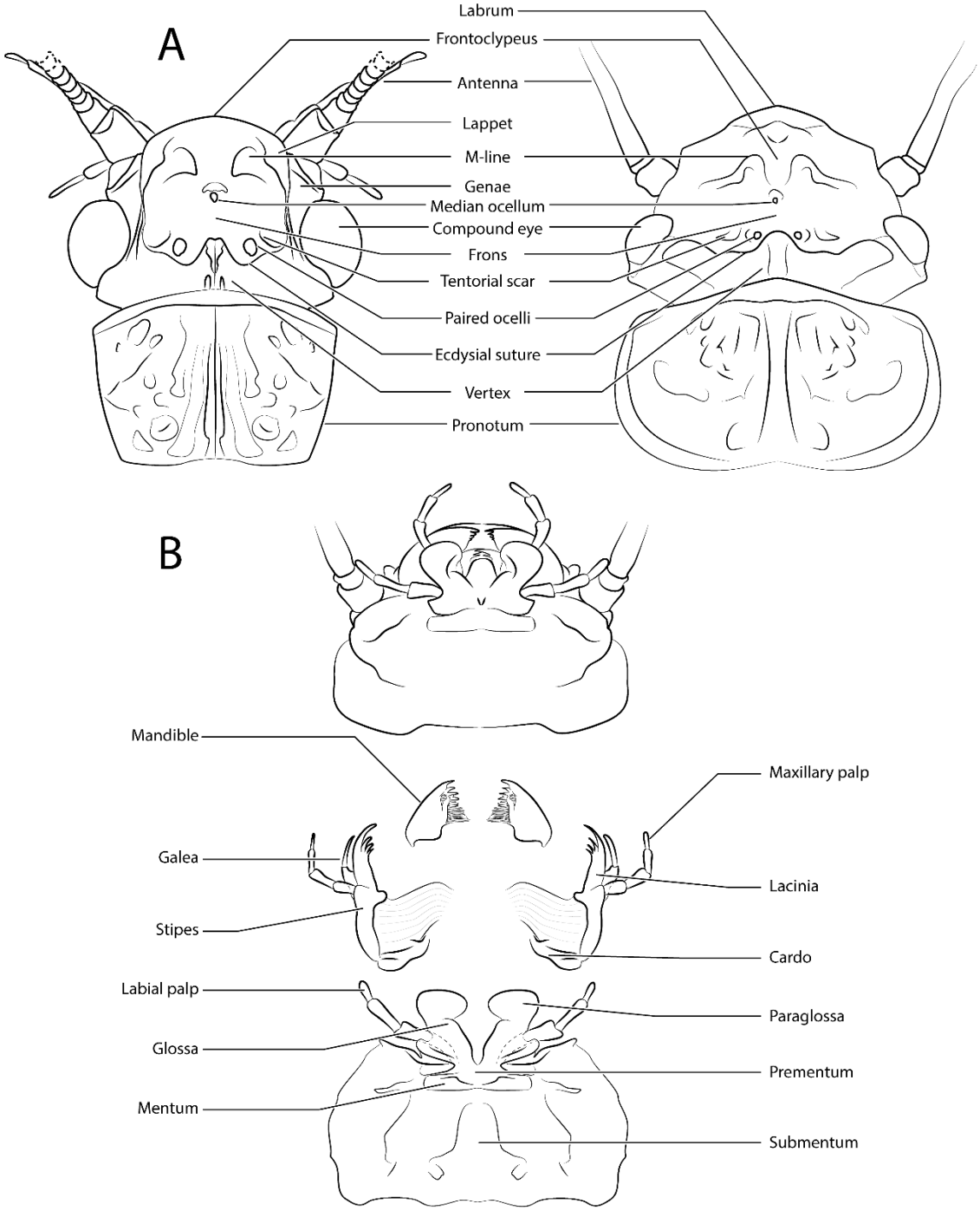

Fig. 3 – Structure and features of the Anacroneuriini head. A: head of an Anacroneuriini adult, on the left, and nymph, on the right, in dorsal view; B: mouthparts of Anacroneuriini nymph in ventral view.

3.2 Thorax

Most researchers don't use thoracic characters in their species descriptions or identification tools. As such, we find it necessary to point out and name the different structures found in this region. The pronotum in Anacroneuriini is a simple broad plate in the shape of either an oval – in nymphs or teneral adults –, or trapezoid – for most adults. During the sclerotization of adults, a posterolateral region of the pronotum is creased forming a **lateral pronotal fold**, turning the oval pronotum into a trapezoid (Almeida & Bispo, 2020). While the color of the pronotum is mostly uniform, some species have a median longitudinal band in a distinct color – usually lighter – when adults.

In adults, the meso- and metanotum are remarkably different from the pronotum but are close in appearance to each other. Both have a large **scutum** which is divided anteroposteriorly by the lateral parapsidal suture. On either side of the anterior part of the scutum, the prescutum can be found in the shape of a **prealar arm**. The prescutum is divided from the anterior part of the scutum by the parapsidal and prescutoscutal sutures. The scutum has a globed appearance on each of its sides. These globes are formed anteriorly by the lateral parapsidal suture and laterally by the tergal fissure. While a recurrent scutoscutellar suture is found in Anacroneuriini, the scutellum is absent. As such, the scutum leads directly to the **postnotum**, which is often wider and can be seen as a process in lateral view - what we are calling a **postnotal process** (Fig. 4A)

Ventrally, all three sterna are alike in configuration. The most anterior plate in each segment is the **presternum**, followed by a broad **basisternum**. Posteriorly, two large **furca** can be found in each segment, leading to internal arms of muscle insertion. In between the furca, the **furcasternum** can be found, which is often darker in color from the other sternal features. Posteriorly to the furcasternum, in some specimens two sutures form the Y-ridge, both of which lead to the **spina**, another invagination creating an internal arm for muscle insertion. The spina can be found only in the prosternum and mesosternum and is absent from the metasternum due to its fusion with the first abdominal sternite in adults (Fig. 4B).

The pleuron of adult Anacroneuriini follows the general neopteran pleuron. However, there are a couple of distinguishing features. In the mesopleuron and metapleuron, the **pleural**

suture is distinct and can be easily found. Posteriorly to the pleural suture, there is a single plate constituting the **epimeron**, which comes in contact with the postnotal process. Anteriorly to the pleural suture, the episternum is divided into a broad anterior **anepisternum**, a posterior triangular **katepisternum** and a ventral and rectangular **preepisternum**. In the posterior portion of each segment, a large **spiracle** belonging to the next segment is found in adults, while the base of the nymphal **gill filaments** can be found in nymphs. These gills may persist in teneral adults, but during sclerotization they are reduced or lost (Fig. 4C).

As for the propleuron, Snodgrass (1935) stated that Plecoptera display a unique configuration of pleural sclerites among Pterygota. According to him, Plecoptera retains a plesiomorphic condition, with an unfused anapleurite and coxopleurite, similar to what is observed in Zygentoma and Archaeognatha. In contrast, all other Pterygota exhibit the derived condition of fusion between these two sclerites. Although the arrangement of these sclerites in Anacroneuriini closely resembles Snodgrass's illustrations (p. 164), we chose a more parsimonious approach to naming them in relation to Pterygota evolution. If, as Snodgrass suggests, Plecoptera indeed retains a plesiomorphic separation of anapleurite and coxopleurite, while other pterygote do not, it would imply that Palaeoptera and other neopterans independently acquired a derived fusion state, with only Plecoptera preserving the ancestral condition. Thus, we consider that Plecoptera exhibits a derived condition relative to other Pterygota, which simply resembles the plesiomorphic state. Consequently, the propleuron of Anacroneuriini lacks an epimeron, and only two small supracoxal plates are present. As per Matsuda (1970), these would consist of a fused anepisternum and preepisternum, dorsally, and a fused katepisternum and trochantin, ventrally.

Nymphs may display complex patterns in light and dark colors in their pronotum which may be used for nymph discrimination. The meso- and metanotum are akin to the pronotum in nymphs, but it is worth noting that, in the last couple of instars, a cardioid shape takes the place of the usual oval shape, producing the wing pads (Fig. 2).

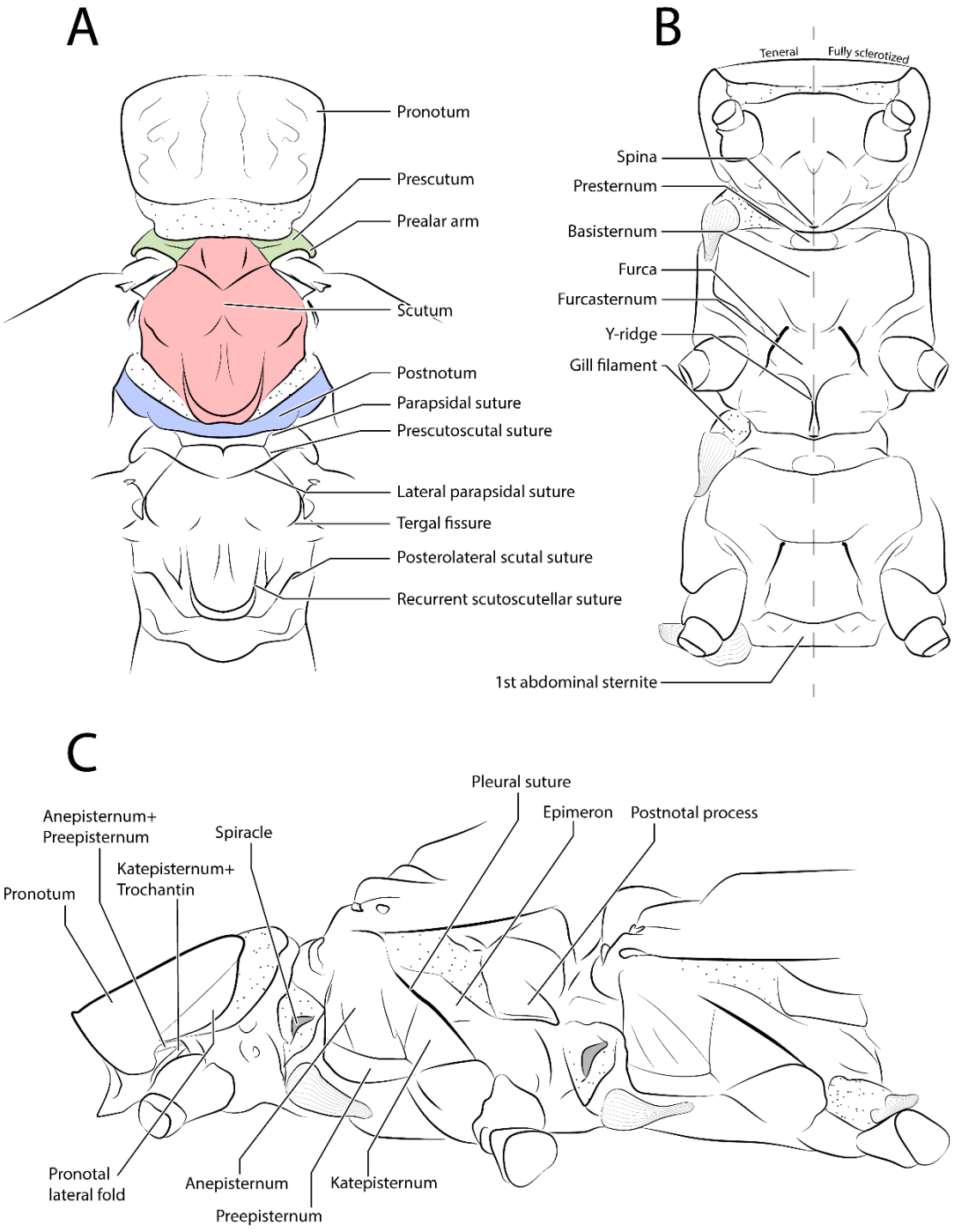

Fig. 4 – Structures and features of the adult Anacroneuriini thorax as exemplified by a *Kempnyia* specimen. A: dorsal view, with sclerite names on the mesonotum and respective suture names on the metanotum, given their similarity. Colors show the continuity of each sclerite in the mesonotum and are equivalent for the metanotum; B: ventral view, with a teneral specimen being represented on the left side of the image and a fully sclerotized specimen being

represented on the right, with special attention being paid to the presence of nymphal gill filaments in teneral adults; C: lateral view, with details on the origin of propleural sclerites.

3.3 Legs

Leg specialization in Anacroneuriini happens rarely. The legs are generally composed of well-developed coxae, trochanter, femur, tibia, and tarsus. The tibia of Anacroneuriini have two short apical **spurs**. The tarsus is composed of three tarsomeres and a pre-tarsus, as in other extant Plecoptera. This characteristic seems to have developed in Plecoptera independently from other Polyneopterans with trimerous tarsi, and as such is a defining feature of the order (Grimaldi and Engel, 2005). The third tarsomere is considerably longer than the first two. Additionally, in adults only the first two tarsomeres bear **euplantulae**, while the third tarsomere gives way to the pre-tarsum with a pair of **pretarsal claws** and an **arolium**. Ventrally, the **unguitractor** and its tendon may be seen through the surface of the third tarsomere (Fig. 5).

In nymphs, the legs follow the same disposition but lack the euplantulae and arolium. An exceptional specialized leg is found in nymphs of *Macrogynoplax*, where an enlarged femur and an inward-curved tibia in the front legs serve as raptorial limbs. Nymphal femora and tibiae often display dense rows of long bristles, giving them a fringed appearance. The femoral surface is also often covered in shorter, stouter bristles. These aspects are sometimes useful in nymph identification and description.

While the leg configuration is relatively straightforward, its position may be variable according to the development of these insects. As nymphs stay mostly in tight spaces between rocks and leaves, their bodies and positions are considerably dorsoventrally flattened. As such, the legs lay flat beside the body. In adults, who live in much more open environments, the legs are displayed almost vertically, although not completely. Thus, the face of the leg which in nymphs used to be dorsal, in adults may be positioned in a more anterior direction, while the face which used to be ventral is positioned posteriorly. Considering this, in order to propose a terminology applicable for both nymphs and adults in relation to leg position, we consider the former as the anterodorsal face of the leg, and the latter as the posteroventral face of the leg. Additionally, the leg edges may be categorized as an inner edge, anterior in nymphs and ventral in adults, and an outer edge, posterior in nymphs and dorsal in adults.

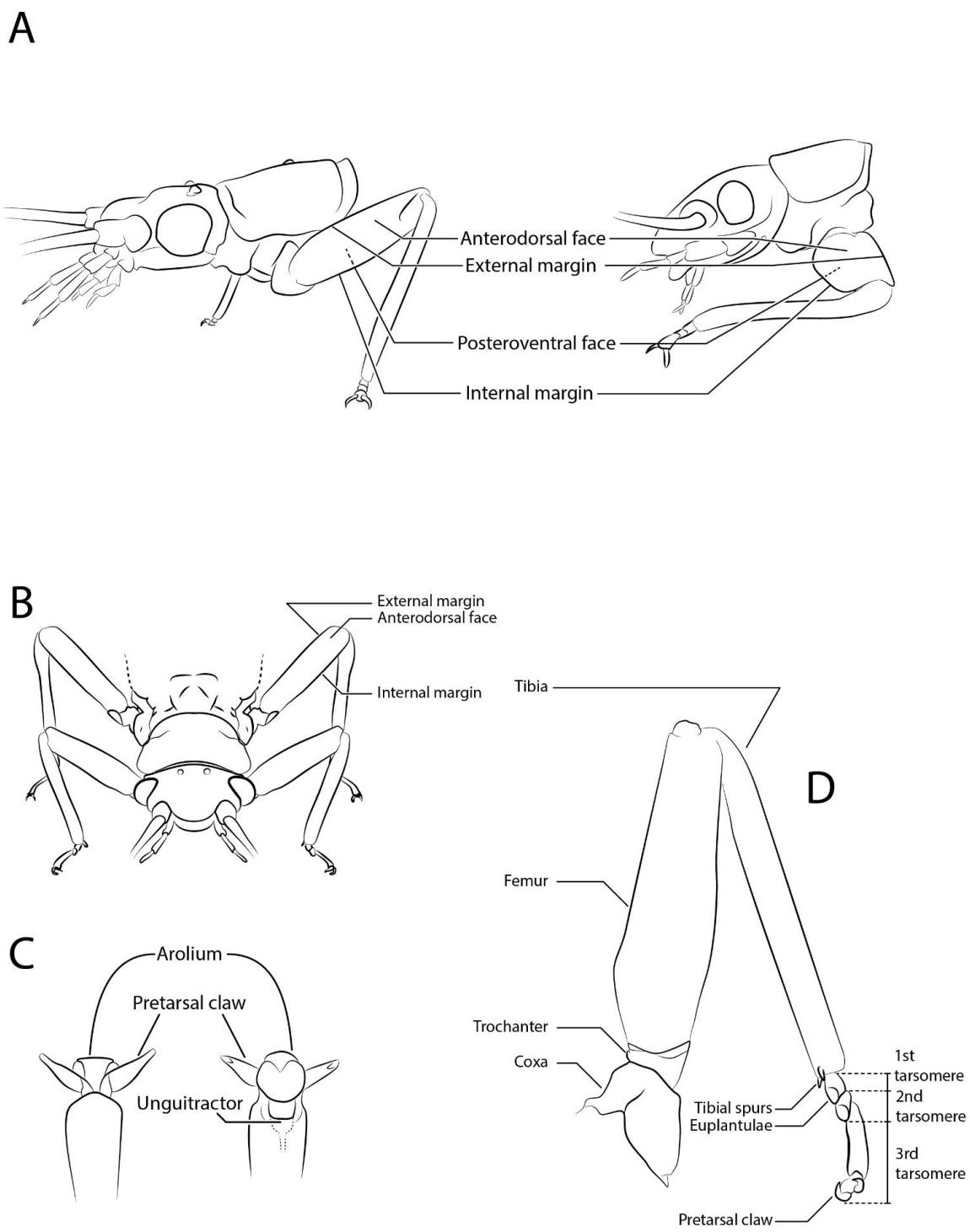

Fig. 5 – Leg structure of Anacroneuriini stonefly; A: leg position comparison in adult on the left and nymph on the right; B: leg position of adult in frontal view; C: pretarsal components of the adult Anacroneuriini leg; D: typical leg structure of Anacroneuriini stonefly.

3.4 Wings

The wings in adult Anacroneuriini are usually well developed and capable of flight in both sexes. Transverse crossveins in the apical portion of the wing generally do not occur and are mostly restricted to the M-CuA and CuA-CuP regions. While the posterior anal lobe typical of stoneflies is well developed in most genera of the tribe, *Enderleina* has a noticeably small anal lobe on its hind wings, a characteristic that was pointed out in the description of its genus (Jewett, 1960). While there may be individual differences between specimens, the wing venation follows a consistent pattern. We will use abbreviations based on Béthoux (2005). The ScP vein fuses with RA relatively early; RP is usually forked 2-4 times and is often fused basally with M in the hind wings; the M vein is not divided into MA and MP; an **arculus** - a distinctly sclerotized basal crossvein - may be present between M and CuA before the M-RP divergence (Bethoux, 2005); CuP and AA1 are unforked, while AA2 may show a large amount of subdivisions in the hind wings. The number of transverse CuA-CuP veins in the forewings is sometimes used as a taxonomic character in species descriptions (Fig. 6A).

The pigmentation of the wings may be of taxonomic importance in some species. The wings can be wholly pigmented or hyaline, as happens in most species. Some, however, may show striped wings (e.g. *Enderleina castro* Almeida et al., 2023) or a distinctly coloured round window in the wing surface (e.g. *Kempnyia pirata* Froehlich, 2011). The C, ScP and RA veins can be pigmented differently from the remaining veins in some species (e.g. *Anacroneuria subcostalis* Klapálek, 1921), being clearly lighter or darker than the rest of the wing. The RA-RP crossvein may also have distinct pigmentation, generally being darker than the other wing veins.

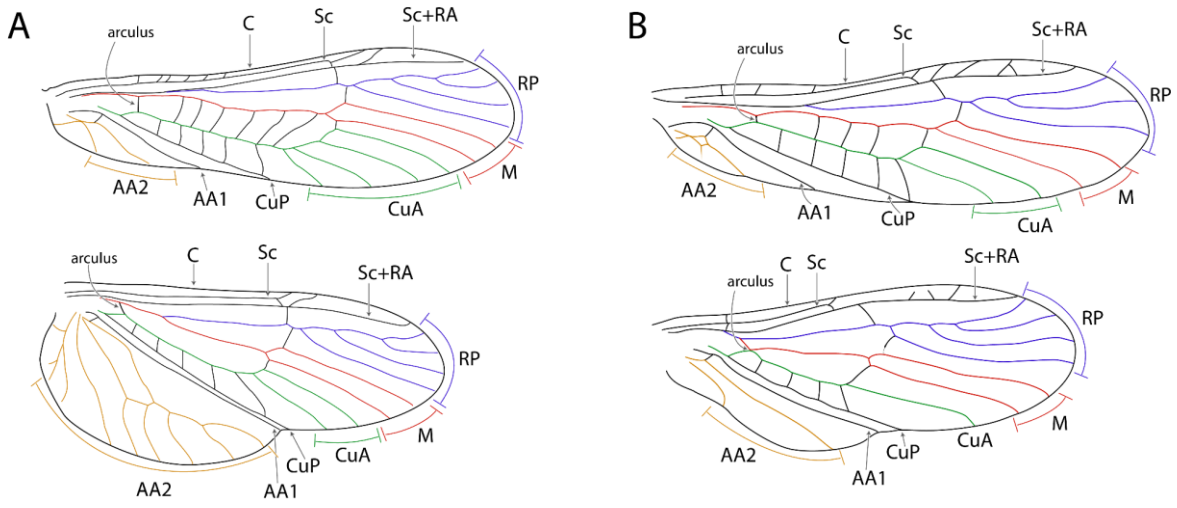

Fig. 6 – Typical venation patterns of Anacroneuriini adults. Forewings are found in the upper part of both images, while hind wings are found in the lower part of both images. A: wing

structure and venation of most Anacroneuriini stonefly; B: wing structure and venation of *Enderleina* sp.

The wing base of Anacroneuriini has a small **tegula** with sparse setae. The **first axillary sclerite** is long and sharp, while the **third axillary sclerite** has a three pointed appearance. The **flexor muscle** attached to the third axillary sclerite may be seen through the wing membrane (Fig. 7A). Laterally, the **basalare** is distinct from the episternum, and the **pleural wing process** widens considerably as it reaches into the wing base. The **subalare** may appear fused with the pleural wing process, but a thin membrane between them can be seen (Fig. 7B).

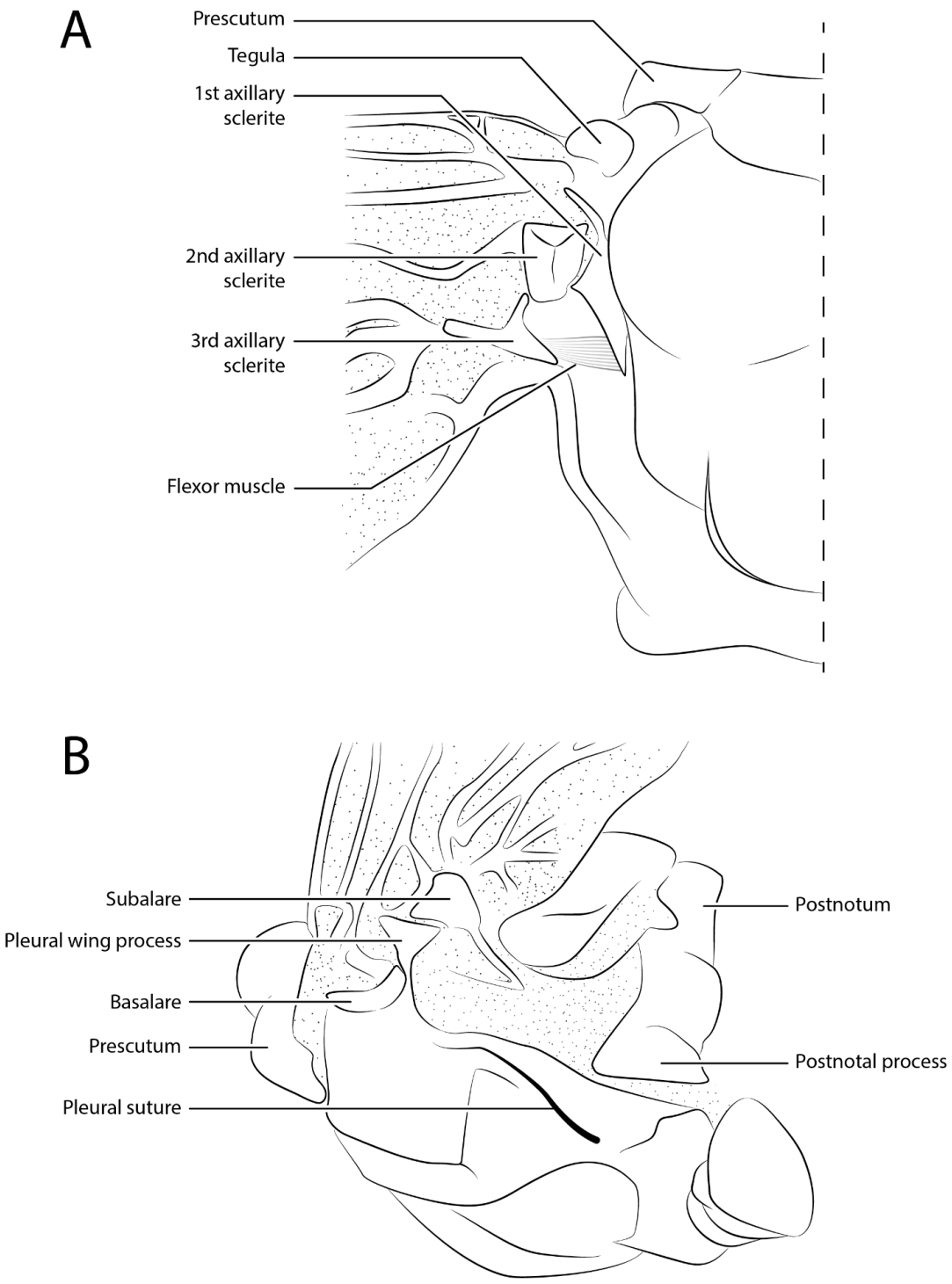

Fig. 7 – Wing base sclerites and structures in Anacroneuriini adult. A: dorsal view, with details on the flexor muscle attached to the third axillary sclerite; B: lateral view, showing the three processes ventrally involved in the wing base.

3.5 Abdomen

The abdomen in Anacroneuriini is mostly bare of notable structures. It is formed from 10 clearly discernible segments. The I sternite is fused to the metasternum, while all other segments are well separated from each other.

In nymphs, the tergites and sternites are fused forming abdominal rings. Apically, a pair of long **cerci** is found, with short **paraprocts** near their base. The paraprocts can bear paraproctal gills in some genera (e.g. *Kempnyia*, *Macrogynoplax*), or they can be bare.

In adults, the tergites are separate in all but the X segment. A pair of spiracles can be found lateroventrally in each of the tergites. Adults show ventral **subgenital plates** in the sternite IX, in males, or sternite VIII, in females. These plates are enlarged sternites that cover the reproductive organs and may have different shapes and features. The subgenital plates of females are usually broad with a bilobed or tetralobed appearance. In males, the presence of a **hammer** for drumming happens in almost all species, with few exceptions. The hammer can, however, be very low or only present as a slight corrugation, particularly in the larger species of Anacroneuria (e.g. *A. paulina* Froehlich, 2004). Adults retain a pair of long cerci and short paraprocts apically (Fig. 8). These paraprocts curve dorsally and frequently have a **mesoapical tooth** (Froehlich, 2011). Authors have described them as subanal lobes or subgenital hooks (Needham and Broughton, 1927; Matsuda, 1976 p. 54), although most researchers in recent decades have stayed consistent in naming them paraprocts (Froehlich, 2011). A median unpaired epiproct is missing.

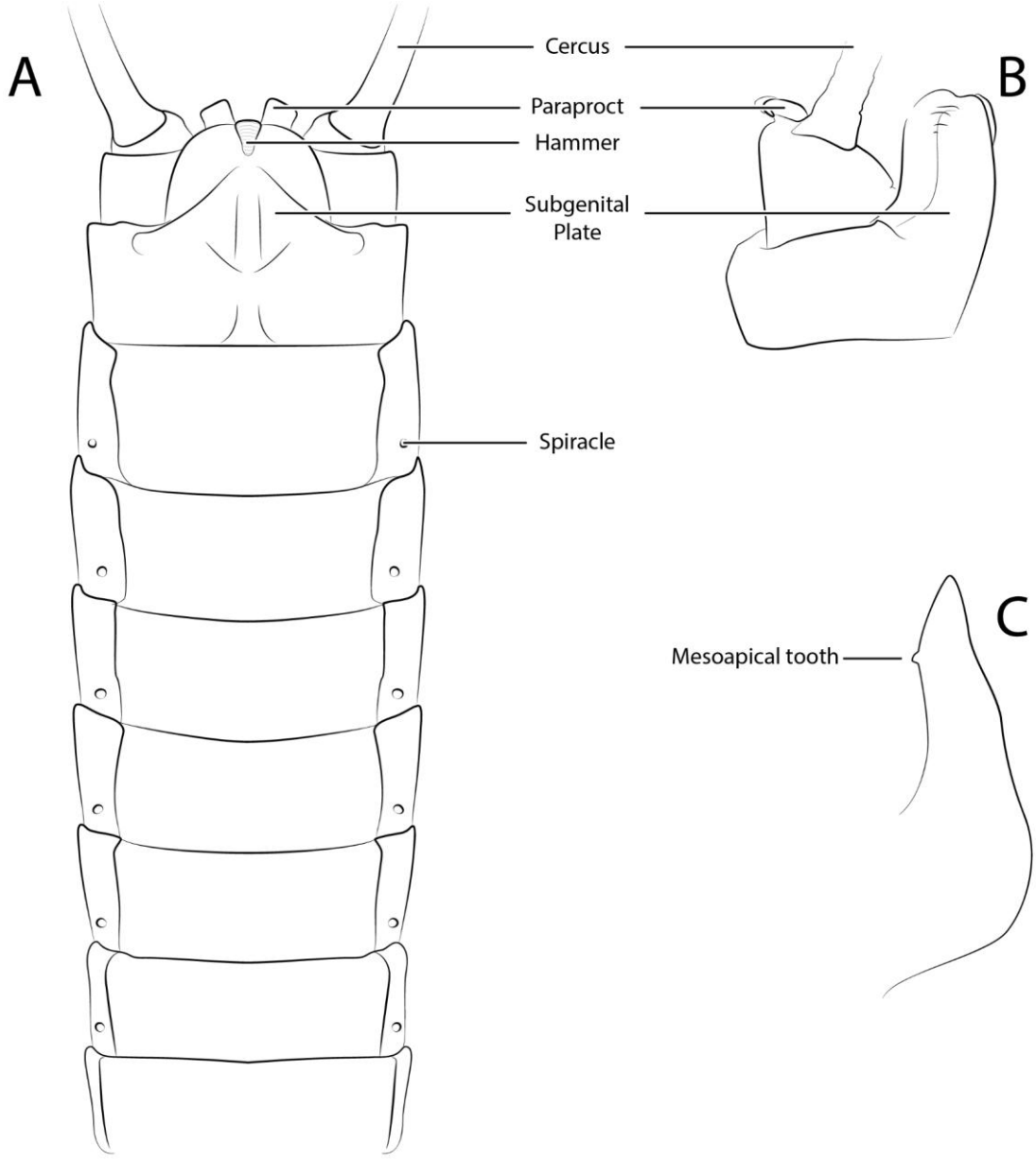

Fig 8 – Structure of a typical adult Anacroneuriini male abdomen A: ventral view of the abdomen; B: lateral view of the IX and X segments of the abdomen, showing the position of cerci and the subgenital plate; C: paraproct in lateral view, with details on the mesoapical tooth.

3.6 Male Genitalia

The particularity of the male genitalia of Anacroneuriini is the most remarkable feature of the tribe. According to Stark and Gaufin (1976), while in other Acroneurinae stoneflies the male genitalia is comprised of an endophallic eversible membrane with a few spines or supporting sclerites, the eversible genitalia of Anacroneuriini display large sclerites with

complex shapes, hooks and keels which form a distinct sclerotized penial structure in each species. This structure has been called by many different names with different meanings, such as penial armature, penial tube, penis or aedeagus (Stark and Gaufin, 1976; Stark, 1996; Froehlich, 2004; Ribeiro and Rafael, 2007). However, it is in fact endophallic in nature (Snodgrass, 1935; Matsuda, 1976). Given the absence of a typical aedeagus in most Plecoptera, all of the eversible tube found in Anacroneuriini would constitute the **endophallus**, with the penial armature as its sclerotized apex (Fig. 9).

Here, we will consider the **penial armature** as the whole sclerotized endophallic structure, while the rest of the eversible body will be considered the **endophallic membrane**. The penial armature is composed of an **endophallic capsule** – the main sclerotized body of the armature which surrounds the gonoduct – and varying accessory structures such as hooks, vesicles, denticles or keels. Each genus in Anacroneuriini may be easily identified by their penial armature, as they each have signature accessory structures or disposition of their penial armatures.

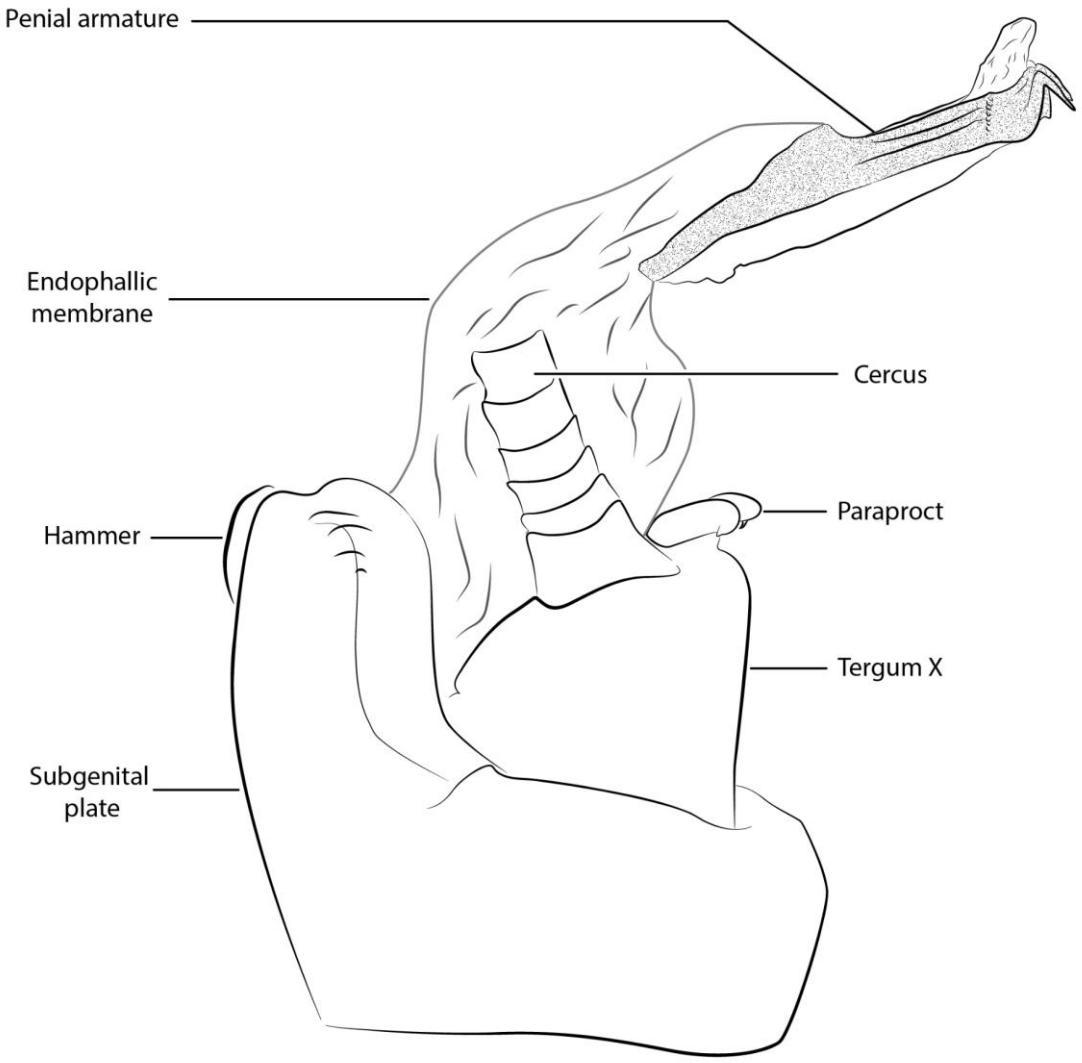

Fig. 9 – Schematic of posterior end of the abdomen and everted male genitalia in Anacroneuriini. Stippling is used in the everted endophallus to distinguish sclerotized structures from unsclerotized ones.

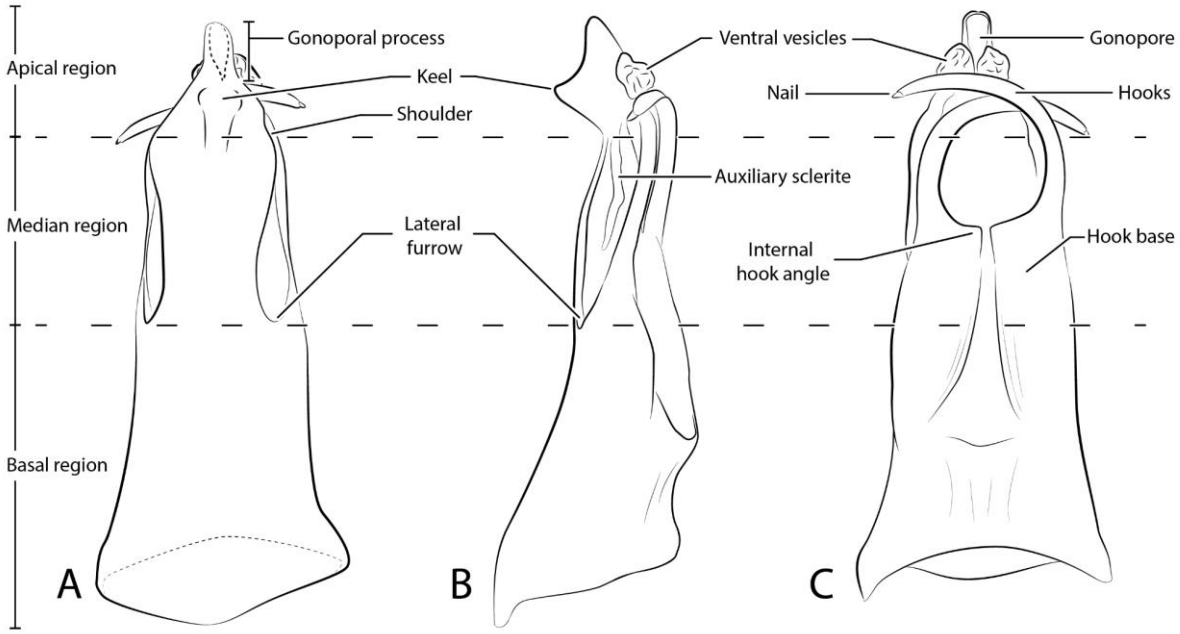

Fig. 10 – Typical configuration of *Anacroneuria* penial armature with details on the distinct regions of the armature. A: dorsal view of the armature; B: lateral view of the armature; C: ventral view of the armature.

The penial armature of *Anacroneuria* follows a structure to which most species adhere to. Even the species that vary the most to this basic structure can be easily identified as *Anacroneuria* through their armatures. The endophallic capsule is complete and surrounds the gonoduct dorsally through most of its extension, while laterally the capsule only surrounds the gonoduct in its basal region. Ventrally, the armature is only narrowly sclerotized, and a long membranous gap is found in most of the ventral side of the armature. Dorsally, the endophallic capsule may narrow abruptly as it reaches the apex of the armature leading to the **gonopore**, forming clear **shoulders** that distinguish a terminal **gonoporal process**. Thus, the armature can be divided into three parts, a basal region, going from the start of the sclerotized base up to the end of the lateral side of the endophallic capsule, a median region until the shoulders and an apical region above the shoulders constituting the gonoporal process. An **auxiliary sclerite** is found laterally, supporting the gonoduct in the transition from the median to the apical portion (Fig. 10).

A pair of large **hooks** is always found inserted ventrally in the basal region of the capsule. These hooks are often curved inwards and cross each other, but they can also curve dorsally or be relatively straight. The hook tips may be blunt, pointed or have a distinctive apical **nail** separate from the rest of the hook. While the hooks are frequently regular in width,

its possible irregularity is an interesting taxonomic characteristic. The hooks insert into a typically bloated ventral region of the endophallic capsule, the **hook bases**. Medially, in the transition of the hook bases to the hooks, an **internal hook angle** may be more or less pronounced. The long extension of the dorsal face of the endophallic capsule and the hook bases surround the shorter lateral faces of the capsule, creating a clear **lateral furrow** in the armature.

In the apical portion, **ventral vesicles** may be present. These may be paired, single or absent and of variable size. They sit beneath the gonopore and are membranous and globular. In some species (e.g. *Anacroneuria itatiaiensis* Baldin et al., 2013) these vesicles may be very small and their presence or absence should be noted carefully.

One final distinguishing feature in the *Anacroneuria* armature is the frequent presence of a **dorsal keel** in the gonoporal process. This keel is of variable size and is easily seen in lateral view when present. Its shape and size is of taxonomic importance, as well as its profile in dorsal view. On rare occasions, a seemingly double keel may be seen (e.g. *Anacroneuria vanini* Froehlich, 2004). In these cases, however, these double dorsal projections originate from the shoulders, and the actual median keel is absent.

Along with their description of Anacroneuriini, Stark & Gaufin (1976) described the *Anacroneuria* male genitalia as bearing a pair of opposed sclerotized grapples, also called tenacula by Needham and Broughton (1927). These grapples would correspond to the hooks described above. There is no mention of the penial armature in the original description of the genus (Klapalek, 1909), and it seems it wasn't used frequently for species delimitation until at least the 1970s (Zwick, 1971). By today's standards, however, careful dissection and examination of the penial armature constitutes the main determining morphological standard by which most *Anacroneuria* species descriptions abide to.

The subgenital plate in male *Anacroneuria* is a broad plate which covers most of the X sternite with a simple medial cylindrical or conical hammer distally, often called “button-shaped” or “thimble-shaped”. In very few species, this hammer may be very low and appear to be absent – e.g. *Anacroneuria paulina* Froehlich, 2004. Its proportions of height and width may be of taxonomic importance.

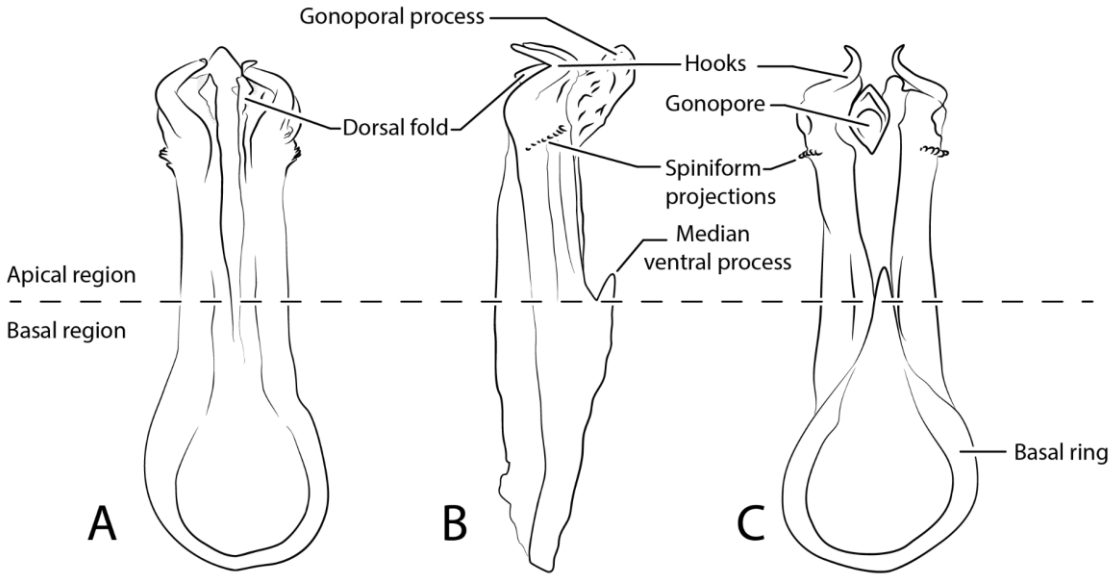

Fig. 11 – Typical configuration of *Kempnyia* penial armature; A: dorsal view; B: lateral view; C: ventral view.

In *Kempnyia*, the penial armature has a similarly structured but different disposition. The endophallic capsule surrounds the gonoduct laterally and has little sclerotization dorsally and ventrally. The gonopore is often curved ventrally. Basally, the endophallic capsule forms a complete or near complete ring around the gonoduct membrane, named the **basal ring** of the armature. It is frequently noted in species descriptions whether this ring is closed, open, rounded or squarish. A **median ventral process** may be found on the upper edge of the basal ring. While it is often in the shape of a spike, it may also present itself as a broad structure. The end of the basal ring may be used to distinguish the armature into an **apical region** and a **basal region**. Small **spiniform projections** may be present on the lateral surfaces of the endophallic capsule, often aligned in rows. Either one or two pairs of hooks are present in the apical region of the armature, frequently curved in a spiral. These hooks are generally shorter and stouter than those in *Anacroneuria*, and their position in the apical region may be variable.

The sclerotized apex of the endophallic capsule may have a curvature opposite to the direction of the gonoporal process. This curve may be pronounced enough for the sclerites to protrude into the dorsal face of the endophallic capsule as a pair of processes near its apex (e.g. *Kempnyia flava* Klapalek, 1916). We propose the name **dorsal fold** for these structures (Fig. 11), which may be sharp and tooth-like - e.g. *K. flava* Klapalek (1916) -, or broad and round - e.g. *Kempnyia zwicki* Almeida et al., 2024. Additionaly, a pair of long sclerites may originate

from the dorsal fold in some species - e.g. *Kempnyia guarany* Almeida et al., 2024. Finally, a pair of sclerotized **spiked pads** may be present in the endophallic membrane. While these spiked pads are only found in some species, their presence or absence is of taxonomic importance, and as such, dissection of *Kempnyia* armatures should be conducted with caution in order for these pads to not be lost.

Zwick (1971) described the *Kempnyia* penial armature as a basal skeletal ring expanded into two lateral braces, each with a basal sclerite and a distal sclerite. Upon seeing his illustrations, the basal skeletal ring refers to our basal ring, while the basal sclerite of the braces corresponds to the sclerotized part of the endophallic capsule. Finally, the distal sclerite of the braces correspond to what we have named the hooks.

The *Kempnyia* penial armature may be disposed in one of two main ways: slimmer, taller, and straight (e.g. *Kempnyia alterosarum* Froehlich, 1988) or shorter, stouter, and often with a degree of ventral curvature in the armature (e.g. *Kempnyia tijucana* Dorville and Froehlich, 1997). However, in either one of these dispositions, the same structures such as the basal ring, median ventral process or hook pairs may be present and easily recognized.

The subgenital plate in male *Kempnyia* is often large and pronounced. It has a clear projection distally, often spade-like or rounded. Its margins may be corrugated, and medially a pear-shaped hammer is present. This hammer may have transversal grooves or punctuation in its surface, which probably aids in the production of different sounds during courtship drumming.

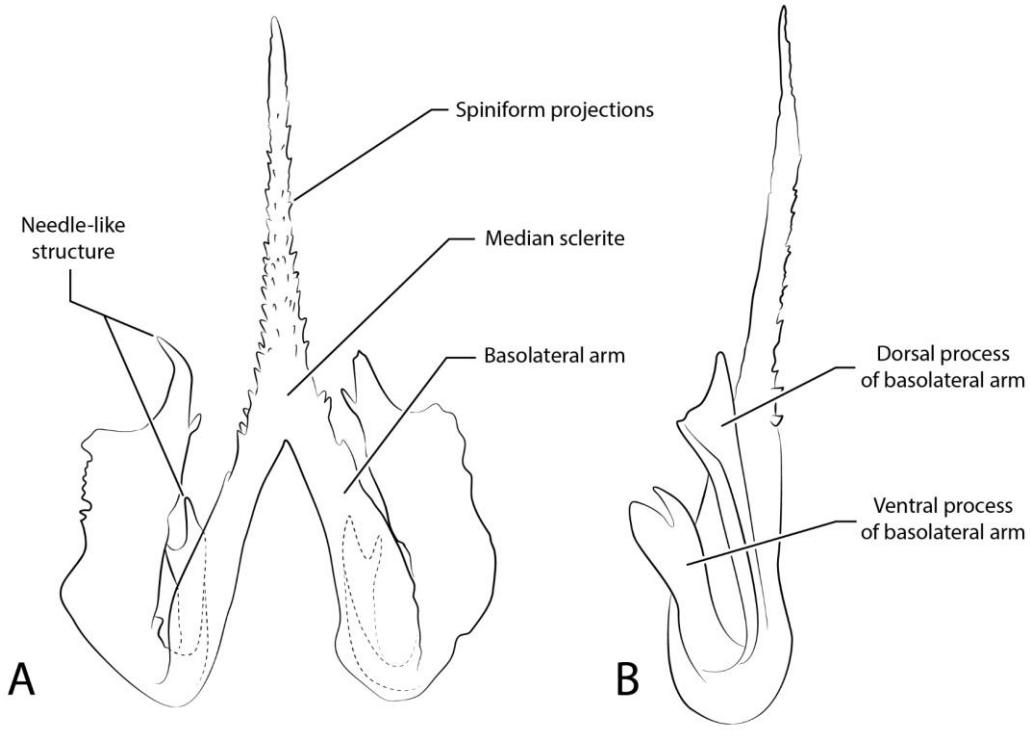

Fig. 12 – Typical configuration of *Enderleina* penial armature; A: dorsal view; B: lateral view.

Enderleina, perhaps for being a genus with few species, has a relatively stable nomenclature of its armature parts (Almeida et al., 2023; Hamada & Silva, 2019). The endophallic capsule is in the shape of a simple inverted Y, to which other structures may be attached to. The capsule is frequently called the **median sclerite** by researchers in the field. This median sclerite bears two **basolateral arms** which give the armature its characteristic appearance. On each of these basolateral arms, a dorsal and ventral **process** can usually be found. The dorsal process is generally larger than the ventral process, and both may have an apical acute process which is called a "needle-like structure". The median sclerite is long and ends in a sharp point. On its surface, a variable number of **spiniform projections** can be found (Fig. 12).

The male subgenital plate of *Enderleina* is relatively similar to that of *Anacroneuria*. It may have a simple distal projection, although it is not as pronounced as those in *Kempnyia*. The hammer is simple and nearly cylindrical.

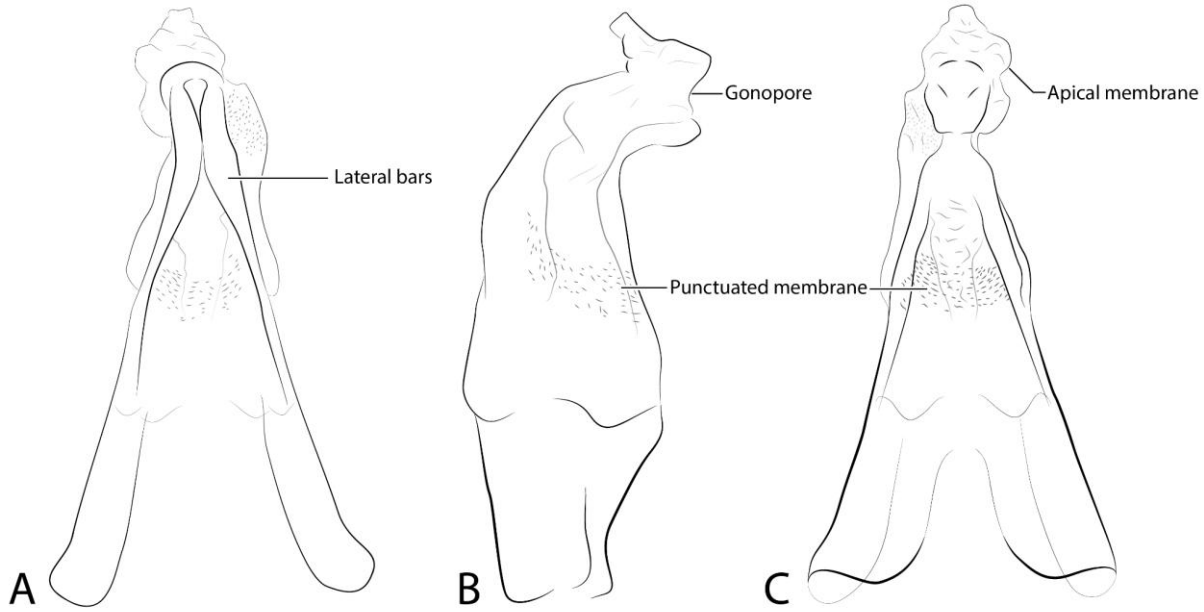

Fig. 13 – Typical structure of *Macrogynoplax* penial armature; A: dorsal view; B: lateral view; C: ventral view.

In *Macrogynoplax*, the penial armature is less consistent than in the other genera. Its typical structure includes a dorsally and ventrally unsclerotized endophallic capsule, giving the armature the appearance of a pair of sclerotized **lateral bars**. This disposition of the endophallic capsule has been named lateral bars, sclerotized ring, sclerotized sides and sclerotized lateral margins (Froehlich, 1984; Stark, 2011; Stark & Zwick, 1989). The membranous dorsal and ventral parts of the endophallic capsule are densely punctuated in median patches. In some species, a pair of hooks may be found in a configuration like those of *Anacroneuria* (e.g. *Macrogynoplax poranga* Ribeiro-Ferreira & Froehlich, 1999). These hooks, however, may be doubled (e.g. *Macrogynoplax quadrispina* Menezes et al., 2020).

The apex of the armature in *Macrogynoplax* is generally completely membranous, and is probably inflated during the eversion of the endophallus. In most descriptions, however, this **apical membrane** is limp and bunched up in what has been called a “mushroom shape” (Fig. 13). Seen as the apex is a flexible structure with a most likely different shape during mating, its status as a taxonomic character as is currently described may be questionable. Some species descriptions in *Macrogynoplax* also remark of a membranous sheath around the base of the

armature. It is unclear whether this structure is in fact a part of the armature or a remainder of the uneverted endophallic membrane surrounding the endophallic capsule.

Macrogynoplax males have subgenital plates that resemble those of *Kempnyia*, with a round or oval distal projection. The hammer is nearly round.

3.7 Female Terminalia

The female terminalia in Anacroneuriini has been noted by some authors as a species defining feature, particularly in older species descriptions (e.g. Navás, 1936). Nowadays, however, using the female terminalia as the only characters for a species description isn't usually considered valid. This may be due to the fact that several species in each genus share a common disposition of their subgenital plates and paraprocts, and species may be easily mistaken for each other if this is the only character in mind.

The subgenital plate in females is located in the VIII sternum, as opposed to the IX sternal male subgenital plate. It leads to a membranous cavity into which the male penial armature is inserted. Its internal structure has yet to be completely studied. Thus, the shape of the female subgenital plate, paraprocts, and perhaps the shape of the eggs, may be the only notable feature of female Anacroneuriini terminalia.

Anacroneuria has a simple, broad and usually tetralobed plate. While bilobed plates may occur in some species, they are less common. Authors frequently describe these plates only briefly, but the lobe shape may vary between species or specimens. Both *Kempnyia* and *Enderleina* show large plates with an usually single median notch, forming two broad lobes. In *Kempnyia*, it may have a projected appearance as in the male subgenital plate. Finally, *Macrogynoplax* has a large and broad subgenital plate, generally bare of notches or projections (Fig. 14).

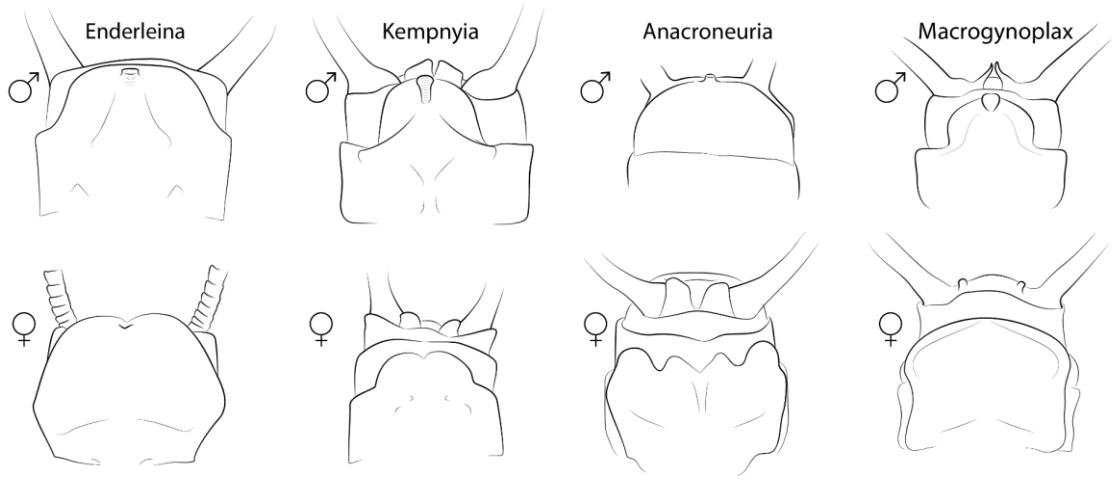

Fig. 14 – Male and female subgenital plates of each genus in Anacroneuriini

4 – Conclusion

Basic knowledge of insect morphology is the foundation on which all other fields can work upon. Without a good understanding of the body plan, its regions and characteristics, no species descriptions, morphological phylogenetics and systematics could take place. While Anacroneuriini are distinguished from all other perlid stoneflies due to their penial “opposed chitinized grapples” (Stark and Gaufin, 1976), other aspects of their morphology aren't discussed by researchers often. We hope that through this guide on the different morphological structures in the body plan of Anacroneuriini, the terminology and disposition of characters has been made clear. With standard terminology and a reference atlas, not only will species comparison and description be sped up, but future identification keys could be made to facilitate identification of species in ecological and conservation studies. Beside keys, future studies could include similar atlases for other perlid tribes or other South American stonefly families. Standard vocabulary is paramount in phylogenetic studies on Plecoptera evolution, where the identification of homologies and comparable structures is based on common terms and stable morphological concepts. Finally, we hope that standard terminology will facilitate the process of Anacroneuriini taxonomy, which proves to be a difficult task in Brazil. With a robust guide on morphology, confusion may be minimized once a referenceable standard is proposed.

CRediT authorship contribution statement

Rodrigo Braga Gastaldo: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Conceptualization. Lucas

Henrique de Almeida: Writing – review & editing, Resources, Investigation. Frederico Falcao Salles: Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

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Declaration of competing Interest

We confirm that all authors of this study have no conflicts of interest to declare for any financial or personal connection in relation to this work.

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5 – Glossary

anterior clypeal margin - anteriormost margin of the frontoclypeal plate in the head of adults.

apical membrane - membranous portion of the apex of the penial armature of *Macrogynoplax*.

arculus - first RP-M crossvein which is distinctly sclerotized in relation to the other crossveins.

auxiliary sclerite - lateral supporting free sclerite in the penial armature of *Anacroneuria.*

basal ring - basal portion of the penial armature of *Kempnyia*, which may form a closed or open ring.

basolateral arms - side processes adjacent to the endophallic capsule in the penial armature of *Enderleina.*

dorsal fold - curvature of the ventral surface of the endophallic capsule of *Kempnyia* which projects into its dorsal surface, creating a process which may vary in size and shape.

dorsal keel - median dorsal process of the penial armature of *Anacroneuria.*

endophallic capsule - main sclerotized body of the penial armature, to which accessory structures are attached.

endophallic membrane - unsclerotized membranous base of the endophallus, which is everted for copulation. The penial armature is found on its apex.

gill filaments - bundle of membranous gills found in the thorax of nymphs and teneral adults.

gonoporal process - terminal portion of the penial armature, which leads to the opening of the gonopore. It may be membranous as those in *Kempnyia* or sclerotized as those in *Anacroneuria*.

hammer - distinctly sclerotized and often projected region of the subgenital plate of males used for drumming and sexual courtship.

hooks - curved and sharp processes found in the penial armatures of *Anacroneuria*, *Kempnyia* and some *Macrogynoplax*.

hook bases - enlarged region basal to the hooks in the penial armatures of *Anacroneuria*.

internal hook angle - distinct angle formed in the transition from the hooks to the hook bases in the penial armature of *Anacroneuria*.

lateral bars - sclerotized regions of the endophallic capsule of *Macrogynoplax*.

lateral furrow - long lateral concavity in the penial armature of *Anacroneuria*, used for distinguishing the regions of the armature in this genus.

lateral pronotal fold - distinct crease formed during sclerotization of adults in their pronotum.

lappet - anterolateral expansion of the frontal plate in adults. It may be disticly coloured and cover the base of the scape.

M-line - embossed pattern found in the heads of both nymphs and adults. It has the shape of an "M" and is formed from muscle attachments in the head.

median sclerite - name used for the single sclerite of the endophallic capsule in *Enderleina*.

median ventral process - process found in the apex of the basal ring of the penial armature of *Kempnyia*. Its shape may vary.

mesoapical tooth - distinct tooth found subapically in the paraprocts of many species.

nail - apex of the hooks in the penial armature of *Anacroneuria*. It is found when the hook tips show a clear suture separating the nail from the hook body.

penial armature - Set of the endophallic capsule and its accessory structures such as hooks and processes.

postnotal process - lateral portion of the postnotum in the meso- and metanotum, which projects into the epimeron.

shoulders - structure originated from a lateral constriction of the gonoporal process in the penial armature of *Anacroneuria*.

spiked pads - a pair of spiked sclerites present in the endophallic membrane of somre *Kempnyia* species.

spiniform projections - small spikes present on the surface of the endophallic capsule of some species of *Kempnyia* and *Enderleina*.

subgenital plate - A projection of the VIIIth or IXth sternite in females and males, respectively. It covers the genital opening leading to the vagina in females and the endophallus in males.

tentorial scars - pair of embossed depressions lateral to the ocelli formed from muscle insertions in the tentorium.

ventral vesicles - pair of membranous vesicles in the penial armatures of *Anacroneuria*. They may be present or absent, distinctly paired or without clear separation.

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SECTION 2 – ANACROINDEX: THE FIRST STEP TOWARDS INTERACTIVE IDENTIFICATION FOR *ANACRONEURIA* KLAPÁLEK, 1909 (PLECOPTERA: PERLIDAE)

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AnacroIndex: the first step towards interactive identification for *Anacroneuria* Klapálek, 1909 (Plecoptera: Perlidae)

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Abstract

Anacroneuria is the genus of Plecoptera with the greatest known species richness, encompassing more than 10% of the order's known diversity. It is one of the most frequently collected genera in the Neotropical region, where all its species are found. While reliable identification of the species crucial for good taxonomic, ecological and biomonitoring work, the genus has many poor descriptions and many similarities between species. The dichotomic nature of traditional keys may pose challenges to identification in genera such as *Anacroneuria*, and new identification tools can attenuate these difficulties. Thus, this work aims to produce the first step of an online, interactive, multi-access and efficient identification key for the males of the species of *Anacroneuria*. In this work, the first iteration of the key is released, allowing the identification of the species found in Southeastern Brazil – almost half of the species found in Brazil. So far, the key covers 39 species by means of 15 characters, having one of the largest taxon coverages and being the most efficient key for this genus to date. The key is housed in Xper3, a cooperative database for interactive identification, and in collaborative efforts the project will continuously expand its species coverage.

Keywords: interactive identification; stoneflies; taxonomy; Xper3

1 – Introduction

Anacroneuria Klapálek, 1909 is the genus with the greatest number of known species in Plecoptera, and is widely distributed and abundant across the Neotropical region (DeWalt et al., 2025; Morrone et al., 2022). Including more than 10% of the valid extant species in the order, this genus has a remarkable distribution, abundance and species richness along the tropics, which is uncommon for the insects in this order (DeWalt et al., 2015; Hynes, 1976). Stoneflies in general are frequently used as bioindicators, and seen as *Anacroneuria* accounts for much of the stonefly abundance in the Neotropical region, identifying its species is crucial for biomonitoring and ecological work involving stream invertebrates.

Anacroneuria is characterized by their highly complex sclerotized endophallic apex, known as a penial armature (Stark & Gaufin, 1976). While the penial armatures of *Anacroneuria* are of similar composition and structure in all species, the specific shape and disposition of each component of the penial armature varies among them, making the penial armature shape the most useful structure in taxonomy and species descriptions (Froehlich, 2002; Froehlich, 2004; Gutiérrez-Fonseca, 2015; Stark et al., 1999).

Even if the penial armature is reliable in providing characters for describing species, considering the species richness in the genus, many species still resemble each other. Considering that many descriptions often only focus on either external appearance or penial armature disposition, confident identification is very hard for some species (Baldin et al., 2013; Froehlich, 2010). It is also worth noting that specimen color and specific markings on the head and pronotum – which are often used for species description and identification – can vary greatly depending on the tenerality and material preservation of the analyzed specimen (Almeida & Bispo, 2020). As such, reliable identification of *Anacroneuria* is a difficult task at best, often done by comparison of a specimen with multiple species descriptions and illustrations.

To aid in identifying the many species of *Anacroneuria*, some authors have produced dichotomic identification keys (e.g. Bispo & Froehlich, 2004; Jewett, 1959; Mayorga-Villalobos & Barba-Álvarez, 2019; Needham & Broughton, 1927; Stark, 1995, 1998, 2001, 2012; Stark & Zúñiga, 2014). These works pioneered identification in the genus and provided brand new tools for it. However, the nature of dichotomic keys poses some challenges in their structure and architecture. With the development of new methods, particularly in cybertaxonomy, some of these challenges can be solved through new identification tools (Chenthamarakshan & Rajmohana, 2012).

A possible alternative to dichotomic identification keys is that of electronic multi-access interactive keys (Kerner et al., 2021). The ability to fill character states according to the availability of the structures in the analyzed specimen provides these keys the necessary flexibility for dealing with species rich and morphologically similar taxa such as *Anacroneuria*. Over the last decade, different platforms such as Xper3 have made interactive key creation and usage simple and powerful, and this technology is seeing use across different taxa (Bodin et al., 2019; Jouveau et al., 2018; Kerner et al., 2021; Klimmek & Baur, 2018; Salles et al., 2015). An electronic identification key may also be continuously updated as new species are described or as the coverage of said key is increased, making it an ever-expanding and cooperative endeavor among researchers in a particular taxon. Ultimately, interactive multi-access keys provide users with a more flexible and often faster approach to identification.

For *Anacroneuria*, a species rich genus with very similar species, the interactivity of an electronic multi-access key could provide users with faster and more accurate identification. With that in mind, we introduce the AnacroIndex project, which aims to provide an illustrated interactive multi-access key for the males of *Anacroneuria*. The objective of this work is to detail and release the first iteration of this project, built under Xper3. While the objective of the project is to include as many *Anacroneuria* species as possible, that is quite a daunting task. Thus, we decided to release the first version of the key including the species present on a delimited geographical region. Considering most keys for *Anacroneuria* species do not cover Brazil, and keys on Brazilian species often cover small geographical areas or a small number of species (Bispo & Froehlich, 2004; Duarte & Lecci, 2016), in this first version, the key covers the *Anacroneuria* species of Southeastern Brazil. It is the most well-studied region in Brazil, where around 40 of the 90 Brazilian species occur (Froehlich, 2010b) and with the most well-preserved and abundant material, and also with the easiest access to type specimens and recently collected material.

2 – Materials and Methods

2.1 Examined Material

We analyzed reference material from the Museu de Entomologia da Universidade Federal de Viçosa (UFVB at the Department of Entomology, Federal University of Viçosa – UFV, Viçosa, MG, Brazil), the Aquatic Insect Collection "Prof. Dr. Cláudio Gilberto Froehlich" (CIACGF at Aquatic Biology Laboratory, State University of São Paulo – UNESP, Assis, SP, Brazil), the Museu de Zoologia da Universidade de São Paulo (MZUSP at the University of São Paulo – USP, São Paulo, SP, Brazil), and the Coleção de Entomologia da

Universidade Federal do Tocantins (CEUFT at the Laboratory of Entomology, Federal University of Tocantins – UFT, Porto Nacional, TO, Brazil). Preference was given to the type series of each species, when possible, and relatively recent material due to better condition of specimens.

Specimen preparations followed male genitalia extraction by severing the abdomen and clarifying the structures in potassium hydroxide (KOH 10%). Reactions were neutralized with acetic acid and the male penial armature was removed. Some specimens had their wings mounted on temporary slides for photography. Whenever possible, the holotype of each species was analyzed. Specimens were compared to their original descriptions or redescriptions when the holotype was unavailable to check for potential intraspecific morphological plasticity when filling the character state matrix. Morphological notes on the analyzed specimens were taken following the framework established in Gastaldo et al. (2025) for the same purpose. Examined material for each species is given in the species notes.

2.2 Key Coverage

Southeastern Brazil (Fig. 1) encompasses the states of São Paulo, Minas Gerais, Rio de Janeiro and Espírito Santo. It includes mainly two ecoregions: the Atlantic Forest, a tropical rainforest which spans all of the Brazilian coastline, and the Cerrado, a savannah formation which covers much of the interior of Brazil. The region covers approximately 10% of Brazil, spanning 924,620 km². While in the Atlantic Forest, frequent rainfall is common, in the Cerrado rain is mostly seasonal. The region is mostly mountainous and has great altitude variation near its coast to the east, but tapers into more plain terrains westward.

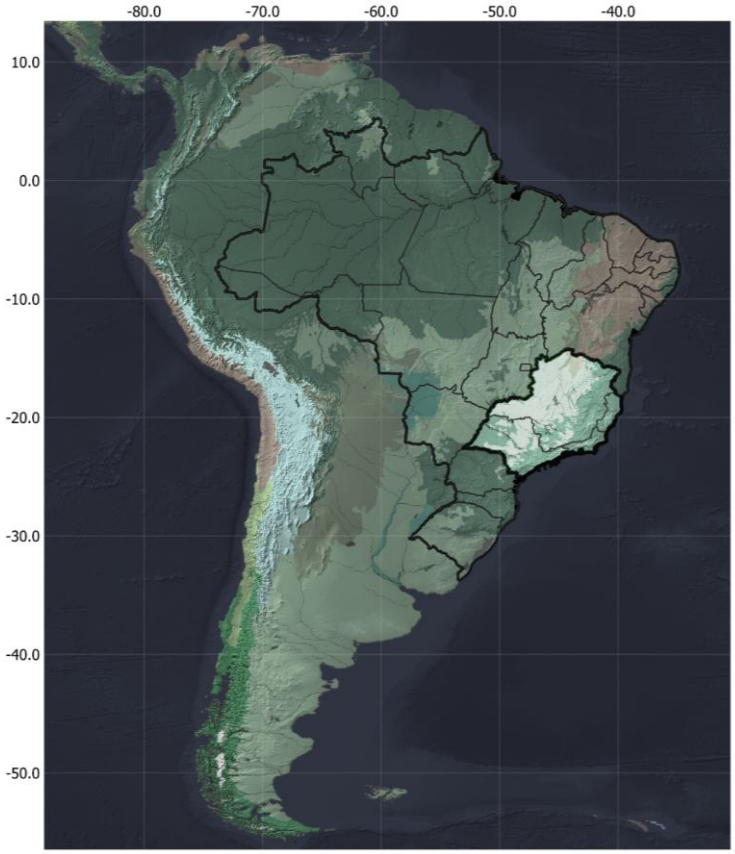

Figure 1 – Map of South American continent. The country of Brazil and its states are delimited by black lines, and the Southeastern Region of Brazil is highlighted in lighter colors.

We based species occurrence on Froehlich (2010b) and Pessacq et al. (2019), as well as more recent species descriptions for this region (Castillo-Velásquez et al., 2023). Currently, 37 *Anacroneuria* described species are considered valid and have known males from Southeastern Brazil.

2.3 Character choice and metrics

Characters were chosen by prioritizing features that are not easily lost due to specimen preservation and have little intraspecific variation. Considering the large amount of variation in maculation patterns due to tenerality of specimens or preservation, most of the characters refer to the penial armature of the species (Almeida & Bispo, 2020). Some external characters were also chosen, with priority given to those which are easily recognizable and well-preserved in older material. An efficiency metric was calculated based on the division of the number of characters by the number of covered species. This metric was also applied to other previously published identification keys for *Anacroneuria* for comparison. If a key has a low efficiency metric, it indicates that relatively more species can be identified using less characters, while

higher values of this metric point to many characters being needed to identify a smaller pool of species.

2.4 Image acquisition

Reference material for each species and character state was photographed using a Leica M205A stereomicroscope and a digital camera Leica MC170 HD with auto montage image software. Preference for photography was given to type specimens that had not been previously photographed, followed by well-preserved specimens from Southeastern Brazil, and finally teneral specimens or specimens collected outside of Southeastern Brazil, when no other material was available for photography. All available species had their head, pronotum, and penial armature in dorsal, ventral and lateral view photographed when possible. Images were treated using Adobe Photoshop 2024 and Adobe Illustrator 2024. Image treatment prioritized clarity and visibility of structures.

Images were also taken to illustrate every character state included in the key. In this manner, other miscellaneous pictures were used, such as wing slides and photographs of live specimens. Character state images were frequently drawn over with Adobe Illustrator 2024 to point out the relevant structures in the picture.

2.5 Morphological terminology

The morphological terminology used followed that established in Gastaldo et al. (2025) for the external morphology of Anacroneuriini and male reproductive structures in *Anacroneuria*. All characters are coupled with succinct descriptions and pictures for easier use without consulting the terminology.

2.6 Xper3 usage

Xper3 is an online platform for interactive identification. With a free account, users may access, create or edit existing databases made for varying taxa. It has been used for a wide variety of different taxa and is a staple in interactive identification across its other iterations such as Xper and Xper2 (Bodin et al., 2019; Jouveau et al., 2018; Kerner et al., 2021; Klimmek & Baur, 2018). Species are inserted as items, while characters are inserted as descriptors. A description consists of assigning the corresponding descriptor states – or character states – in each descriptor for each item – or species. In this way, a description matrix is formed. In this matrix, descriptor states may be changed easily and readily, and insertion of new items or new descriptors is straightforward. Every item, descriptor and descriptor state may have a picture and short explanation of its contents. More information on Xper can be retrieved on their

website <xper3.fr> or in Kerner et al. (2021), which details the platform's uses, history and future developments.

3 – Results

The key is housed in Xper3 and is available on the following link: < https://app.xper3.fr/xper3GeneratedFiles/publish/identification/-2023637698988978647/mkey.html>. Our key includes 35 of the 37 species with known males in Southeastern Brazil. The species included in the key can be found accompanied by identification remarks below. The excluded species – *A. dilaticollis* (Burmeister 1839) and *A. fumigata* Klapálek, 1922 – were not included due to doubtful species identity. Photographs are available for all but three species – *A. stanjewetti*, *A. quilombola* and *A. fuscicosta*, for which no material was able to be examined. The key uses 15 characters (descriptors) to identify the included species, which can be found in Table 1.

Table 1 – Defined characters (descriptors) and respective states used in the Xper3 AnacroIndex key

Characters	Definition	Character States	Example species
Ventral vesicles	The penial armatures of *Anacroneuria* frequently have ventral vesicles in their apical region. When present, these vesicles may be paired and distinct from each other or have their separation imperceptible, appearing as a single large vesicle.	Vesicles absent	*A. mantiqueirae*
		Vesicles fused to each other	*A. stanjewetti*
		Vesicles distinctly paired	*A. debilis*
Dorsal keel	*Anacroneuria* penial armatures often bear a dorsal keel in their apical region, particularly visible in lateral view. In some species, however, the keel is not dorsally developed beyond the rest of the dorsal sclerite.	Undeveloped	*A. terere*
		Developed	*A. debilis*
Keel shape in dorsal view	When seen in dorsal view, the dorsal keel may appear as a profile of lines in a variety of configurations. Most of the time, these lines appear to draw the shape of the configurations shown on the right column. Note that these shapes may be stretched or rotated, sometimes appearing as upside-down	U	*A. amargosa*
		V	*A. piranga*
		Y	*A. kariri*
		)(	*A. polita*
Base of apical and basal	A rough estimate of the proportions between the width of the base of the apical and basal region respectively.	Base of apical region narrower	*A. polita*

regions width proportions		than basal region	
		Base of apical region subequal or wider than basal region	*A. sallesi*
Basal region length proportions	A rough estimate of how long the basal region is in relation to the total armature length.	Basal region occupies less than half of total armature length	*A. vanini*
		Basal region occupies half or more than half of total armature length	*A. paulina*
Wing membrane color	In most species, the wing membrane is wholly hyaline or uniformly pigmented. In some species a clear hyaline area, or window, can be found amidst a pigmented wing.	Wing pigmentation homogeneous	*A. debilis*
		Hyaline window present	*A. sallesi*
Fore wing vein color	The coloration of the veins on the fore wings in *Anacroneuria* is variable. Some species may have all veins of a similar color, while others may display particular veins in a distinctly darker or lighter color. This is most often visible in the C, ScP and RA veins.	All veins similarly pigmented	*A. debilis*
		C and ScP lighter than remaining longitudinal veins	*A. fiorentini*
		C and ScP darker than remaining longitudinal veins	*A. subcostalis*
Armature dorsal plate lateral margins	In the penial armature of *Anacroneuria*, the basal and apical regions are dorsally connected by a bridge. The lateral margins of this bridge may be subparallel, convergent or divergent.	Divergent	*A. itatiaiensis*
		Subparallel	*A. paprockii*
Shoulder projection	When projected, the shoulders advance apically and surpass their origin point, forming lobes in the apical region.	Not projected	*A. toriba*
		Projected	*A. tabatae*
Lateral margins of		Parallel or subparallel	*A. paulina*

the gonoporal process base	The lateral margins of the gonoporal process, in dorsal view, may be either parallel, convergent or divergent.	Convergent	*A. itatiaiensis*
		Divergent	*A. iporanga*
Membranous expansion in the lateral furrow	The endophallic membrane is exposed by the lateral furrow. In some species, this membrane may be expanded laterally and clearly visible in dorsal view.	Not expanded	*A. paulina*
		Expanded	*A. debilis*
Gonoporal process notch	The gonopore may have a simple rounded shape or a longer, sharper shape. When long and sharp, the gonopore gives the gonoporal process a deeply notched appearance, often visible in dorsal view.	Without notch	*A. debilis*
		Notched	*A. toriba*
Gonoporal process apex	The apex of the gonoporal process may have a couple of distinct appearances. It may be broad, thin, round, truncated or sharp. When truncated, the very tip of the gonoporal process appears as if it were cut off, and can either be broad or thin. When not truncated, it will either be clearly round or sharp.	Either rounded or sharp	*A. itatiaiensis*
		Truncated	*A. kariri*
Hook width	The hooks of the penial armature may have the same thickness throughout their extension or have distinct wide and thin portions.	Uniform throughout extension	*A. debilis*
		With distinctly wide and thin regions	*A. atrifrons*
Hook curvature	The hooks of the penial armature may be straight, curved on a single plane or curved in more than one plane. It is more common for the hooks to be regularly curved following the frontal plane. When curved on more than one plane, the hooks often bend dorsally in a slight corkscrew.	Curved on a single plane	*A. debilis*
		Curved on more than one plane	*A. singularis*
		Mostly straight	*A. piranga*

When using the key in Xper3, users are first greeted by the characters as drop-down menus with their character states as the options on the left, and a list of remaining possible taxa on the right (Fig. 2). At any moment, users may click on any of the taxa on the right to see all of their character states, pictures of the species and a short comment on each. Users may choose the states for as many or as few characters as they like before entering “Submit” on the top.

When submitted, the key will be refreshed, keeping only the taxa which fulfill the character states entered (Fig. 3).

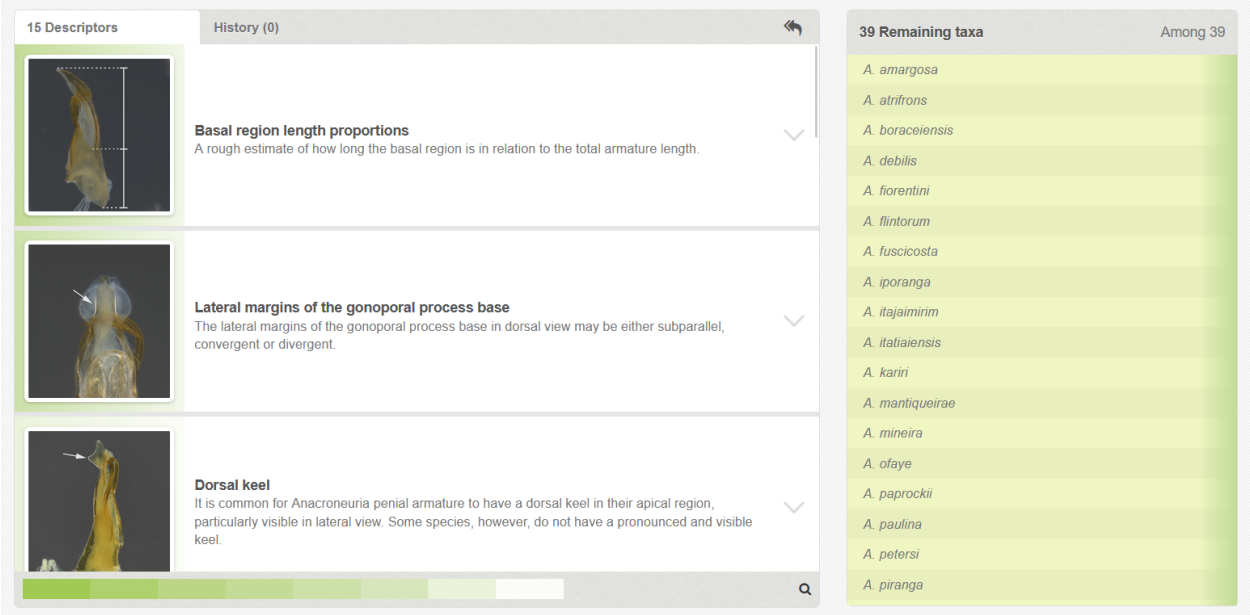

Figure 2 – Printscreen of the first page of the AnacroIndex key. No characters have been filled, and every species is still possible to be identified, as indicated by the column on the right.

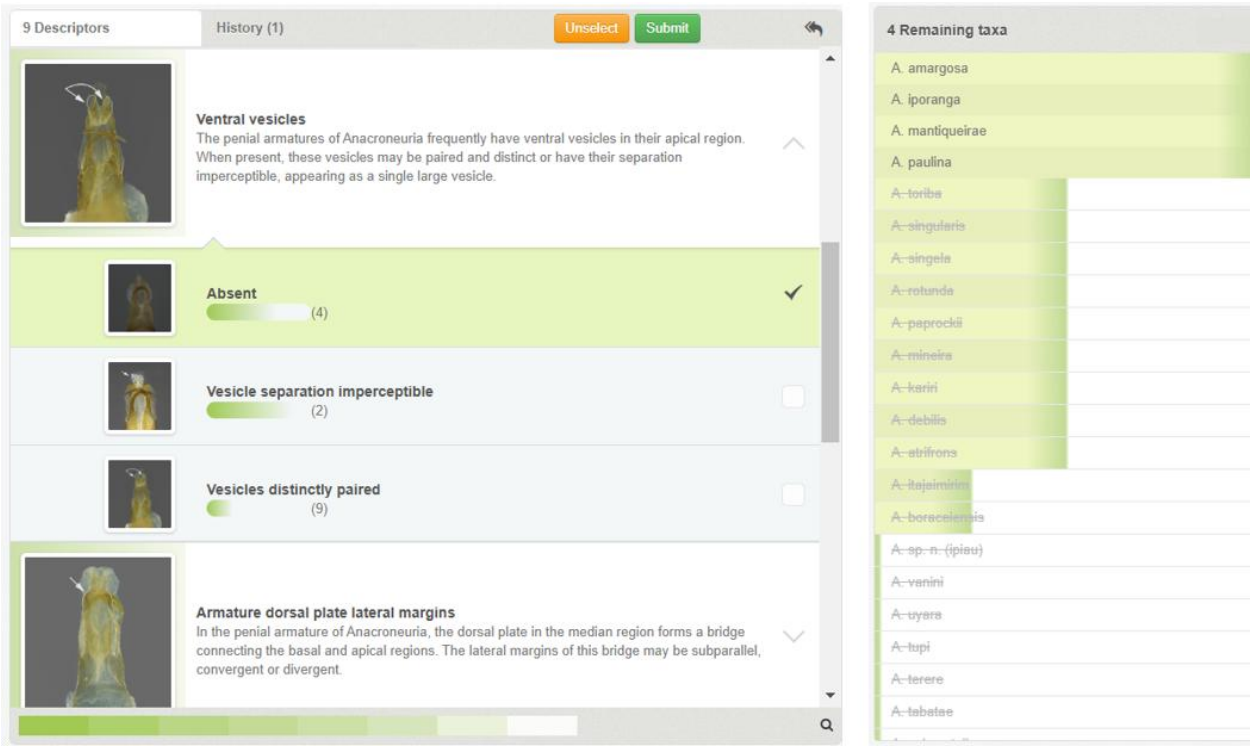

Figure 3 – Printscreen of the AnacroIndex key during its use. In this step, a single character has been chosen so far, indicated by the "History" tab, and four remaining species are possible, indicated in the column on the right.

The key performs smoothly during the initial stages of identification. However, as species are excluded and only a few possible species are left, identification may require filling more subtle characters. The species remarks, pictures and the recommendation section of this paper provide details to help in this harder stage of identification. The key can identify most species with approximately five characters. Remarks on identification for each of the species can be found below.

***Anacroneuria amargosa* Righi-Cavallaro & Froehlich, 2013** (Fig. 4A)

Examined material. Brazil, Espírito Santo: Santa Teresa, REBIO Augusto Ruschi, Córrego da Estrada, 2018.iii.20 – 2018.iii.21, light trap, 1 male, FF Salles leg. (CIACGF – DP57).

Remarks: This species occurs in northern Espírito Santo. The photographed specimen is teneral, so comparison with the photographs should be done with care, especially regarding the maculation pattern. Regarding the penial armature, this species is quite similar to *A. boraceiensis*, which occurs in Minas Gerais and São Paulo. They may be distinguished in the key due to differences in the ventral vesicles – *A. amargosa* does not show ventral vesicles while they may or may not be seen in *A. boraceiensis* –, keel shape in dorsal view – U-shaped in *A. amargosa* and U or)(shaped in *A. boraceiensis* –, lateral margins of the gonoporal process base – convergent in *A. amargosa* and subparallel in *A. boraceiensis* – and proportions between the apical and basal region widths – occupying half of the total armature length in *A. amargosa* and less than half in *A. boraceiensis*.

***Anacroneuria atrifrons* Klapálek, 1922** (Fig. 4B)

Examined material. Brazil, Minas Gerais: Aimorés, Rio Manhuaçu, 19°29'38"S 41°16'31"W, 2022.i.06 – 2022.i.07, light trap, 1 male, ADL Viana, P Bonfá, P Rodrigues & M Rothe-Neves leg. (UFVB – PL00452)

Remarks: This species occurs in most of Southeastern Brazil. This species may be considered similar to *A. mineira* due to the general shape of the armature. For identifying this species, careful observation of the width of the hooks in the penial armature is crucial, as they widen over their extension and constrict apically.

***Anacroneuria boraceiensis* Froehlich, 2004** (Fig. 4C)

Examined material. Brazil, São Paulo: Salesópolis, Estação Biológica de Boracéia, Córrego Coruja, 2022.xii.08, light sheet, 1 male, LH Almeida & PN Taniguti leg. (CIACGF – DP785).

Remarks: See the remarks for *A. amargosa*.

***Anacroneuria debilis* (Pictet, 1841)** (Fig. 4D)

Examined material. Brazil, Minas Gerais: Araponga, Parque Estadual da Serra do Brigadeiro, 2022.x.19 – 2022.xi.03, Malaise, 1 male FF Salles leg. (UFVB – PL193); 2023.iii.12 – 2023.iv.14, Malaise, 2 male, MLS Rippel leg. (UFVB – PL223, PL224); 2023.vii.23 – 2023.ix.08, Malaise, 1 male, FF Salles leg. (UFVB – PL241); Araponga, Pousada Fazenda do Remanso, 2023.ii.14 – 2023.ii.17, light trap, 1 male, MLS Rippel leg. (UFVB – PL368, PL395, PL398); 2021.xi.17, light trap, 1 male (UFVB – PL443); 2022.vi.29 – 2022.vii.29, Malaise, 1 male, FF Salles leg. (UFVB – PL505); Jaboticatubas, Parque Nacional da Serra do Cipó, Córrego das Pedras, Capão dos Palmitos, 2022.x.06, 1 male, light trap, MLS Rippel leg. (UFVB – PL206); Brumadinho, Córrego Laranjeira, 20° 7' 25,63" S 44° 8' 57,92" W, 2021.vi.23, Malaise, 6 males, Roxinol leg. (UFVB – PL455, PL456, PL457, PL459, PL506); **Espírito Santo:** Reserva Biológica Augusto Ruschii, Córrego da Estrada, light trap, 1 male (UFVB – PL449).

Remarks: This species occurs in most of Southeastern Brazil. This species has a very broad range of distribution and may exhibit some morphological plasticity regarding the keel shape in dorsal view and the gonoporal process apex. In Southeastern Brazil, it may be mistaken for *A. ruschii*, which occurs in Espírito Santo, and *A. uyara*, which occurs in São Paulo, and the differentiation between these species is difficult. These three species may be distinguished in the key due to differences in the basal region length proportion – occupying more than half of total armature length in *A. debilis* and *A. uyara*, and less than half in *A. ruschii* – and the gonoporal process shape – rounded in *A. debilis* and truncated in *A. uyara*.

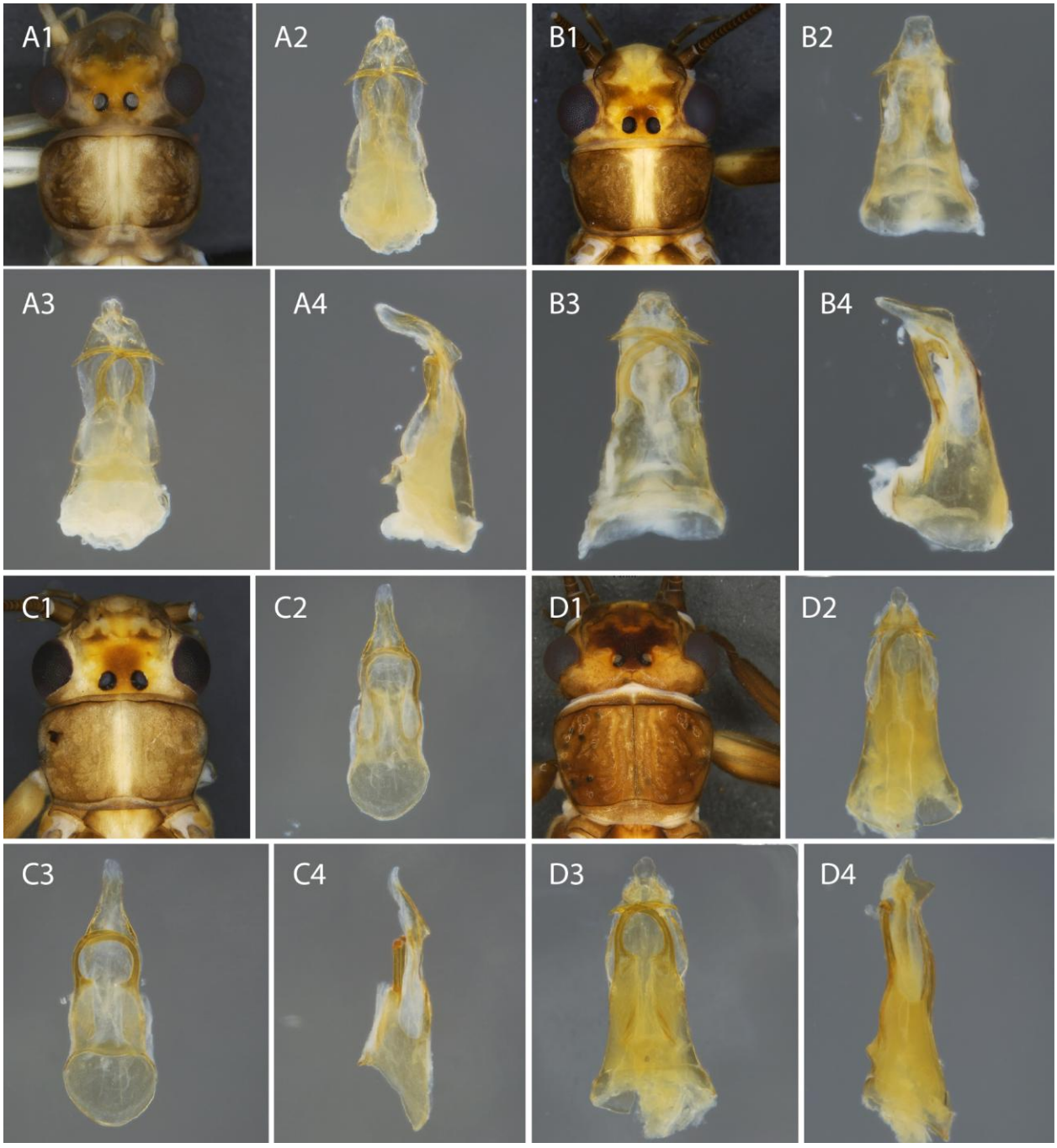

Figure 4 – Figures provided in the AnacroIndex key. Each figure shows (1) head and pronotum, penial armature in (2) dorsal, (3) ventral and (4) lateral views. (A) *Anacroneuria amargosa*; (B) *Anacroneuria atrifrons*; (C) *Anacroneuria boraceiensis*; (D) *Anacroneuria debilis*.

***Anacroneuria fiorentini* De Ribeiro & Froehlich, 2007** (Fig. 5A)

Examined material. Brazil, São Paulo: Iporanga, Parque Estadual de Intervales, Ribeirão do Carmo (Próx. Alecrim), 2010.viii.11, light sheet, LH Almeida leg. (CIACGF – LM15)

Remarks: This species occurs in Southern Brazil and southern São Paulo. The photographed specimen is teneral, so comparison with the photographs should be done with care. This species is quite unique regarding the penial armature and the combination of a number of characters distinguish it from other species, such as the shape of the lateral margins of the gonoporal process base – diverging – and the shape of the gonoporal process apex – rounded or sharp.

Anacroneuria flintorum Froehlich, 2002 (Fig. 5B)

Examined material. Brazil, Minas Gerais: Araponga, Parque Estadual da Serra do Brigadeiro, 20° 43' 52" S 42° 27' 50" W, 1110 m, 2022.x.03 - 2022.xii.07, Malaise, 1 male, FF Salles leg. (UFVB – PL210); 2022.vi.29, Malaise, 2 males, FF Salles leg. (UFVB – PL211); 2023.iii.12 – 2023.iv.14, Malaise, 1 male, MLS Rippel leg. (UFVB – PL222); Araponga, Pousada Fazenda do Remanso, 2023.iii.21 – 2023.iii.22, light trap, 5 males, (UFVB – PL196, PL200, PL231, PL232, PL242); 2023.ii.15 - 2023.ii.17, 20° 39' 24" S 42° 27' 08" W, light trap, 2 males, MLS Rippel leg. (UFVB – PL369, PL399). **Rio de Janeiro:** Itatiaia, Parque Nacional do Itatiaia, Véu da Noiva, 22° 25' 37" S 44° 37' 6" W, 1170 m, 2022.iii.15 – 2022.iii.16, light trap, 3 males, FF Sallles leg. (UFVB – PL333, PL334, PL335); Itatiaia, Parque Nacional do Itatiaia, Cachoeira da Maromba, 22° 25' 46" S 44° 37' 10" W, 1100 m, 2022.iii.14, light trap, 1 male, FF Sallles leg. (UFVB – PL332).

Remarks: This species occurs in most of Southeastern Brazil. This species may be confused with *A. polita*, as their penial armatures are relatively similar. They are mostly distinct in the keel shape in dorsal view, keel development – undeveloped in *A. flintorum* and developed in *A. polita* – and lateral margin of the dorsal plate. The maculation pattern on the head is distinct and may be used in cases of doubt in well sclerotized and well-preserved specimens by comparison with the photographed specimen.

Anacroneuria fuscicosta (Enderlein, 1909)

Examined material. No material could be examined for this species, as no material has been collected recently and the type series could not be located. Inclusion in the key followed the illustrations of Froehlich (2002), who examined 2 male specimens: Brazil, Santa Catarina, Nova Teutonia, F. Plaumann leg., Jan 1963 (CAS); Paraná, Rio dos Patos, 3 km E of Prudentópolis, 700m, 2 Mar 1969, W.L & J.G. Peters (NMNH).

Remarks: This species occurs in Southern Brazil and in Southern São Paulo. This species is distinguished from others included in the key due to the combination of the basal

region length proportions – occupying more than half of total armature length –, gonoporal process apex – rounded or sharp –, gonoporal process notch – without notch –, lateral margins of the gonoporal process base – convergent –, and keel shape in dorsal view – V-shaped.

***Anacroneuria iporanga* Bispo & Froehlich, 2004** (Fig. 5C)

Examined material. Paratype/MZUSP. Brazil, São Paulo: Iporanga, Parque Estadual de Intervales, Córrego Bocaina, 2000.xi.28 – 2000.xi.30, 1 male, PC Bispo leg.

Remarks: This species is known from Southern São Paulo. The photographed paratype has lost its entire maculation pattern, and this aspect should be disregarded when comparing specimens with the species photographs. It is a very large species, with a forewing size around 17 mm. As most other large *Anacroneuria*, the basal region length proportion is an important character – occupying more than half of total armature length. Its penial armature is relatively similar to that of *A. mantiqueirae* and *A. paulina*, but may be differentiated from them due to the lateral margin of the gonoporal process – divergent in *A. iporanga*, subparallel in *A. mantiqueirae* and *A. paulina* – and lateral expansion of the membrane – not expanded in *A. iporanga* and *A. mantiqueirae*, expanded in *A. paulina*. A final distinguishing feature not included in the key is the external appearance of the head and pronotum, as both *A. iporanga* and *A. paulina* are much darker species than *A. mantiqueirae*. As always, the head and pronotum color and maculation patterns may only be used as a last resort in particularly well sclerotized and well-preserved material.

***Anacroneuria itajaimirim* Bispo & Froehlich, 2004** (Fig. 5D)

Examined material. Brazil, São Paulo: Iporanga, Parque Estadual de Intervales, Rio do Carmo, 2017.ii.09, light trap, 1 male, LH Almeida leg. (CIACGF – DP144); Apiaí, Parque Estadual Turístico do Alto Ribeira, Núcleo Santana, Riacho Furnas, 2017.ii.14, light trap, LH Almeida leg. (CIACGF – DP145).

Remarks: The photographed specimen is teneral, so comparison with the photographs should be done with care in relation to the maculation pattern. This species differentiates itself from others in the key in hook width – *A. itajaimirim* may have distinct wide regions in its hooks, basal region length proportion – *A. itajaimirim* may have the basal region occupying half of the total armature length– and basal region width proportion – *A. itajaimirim* has the base of the apical region narrower than the base of the basal region. It is worth noting that *A. itajaimirim* is only known from the Paranapiacaba Mountains and Cananéia. While distribution

data is updated regularly, this may be used as auxiliary information in the identification of this species.

Figure 5 – Figures provided in the AnacroIndex key. Each figure shows (1) head and pronotum, penial armature in (2) dorsal, (3) ventral and (4) lateral views. (A) *Anacroneuria fiorentini*; (B) *Anacroneuria flintorum*; (C) *Anacroneuria iporanga* paratype; (D) *Anacroneuria itajaimirim*.

***Anacroneuria itatiaiensis* Baldin, Bispo & Novaes, 2013** (Fig. 6A)

Examined material. Brazil: Minas Gerais: Mariana, Rio Gualaxo do Norte, 20° 15' 09.81" S 43° 22' 24.56" W, 2022.iii.28, 1 male, light trap, TG Kloss leg. (UFVB – PL214); Mariana, Rio Gualaxo do Norte, 20° 16' 38.64" S 43° 26' 20.04" W, 2022.iii.2, 1 male, light trap, TG Kloss leg. (UFVB – PL218); Mariana, Rio Gualaxo do Norte, 20° 14' 52.00" S 43° 20' 55.66" W, 2022.iii.30, TG Kloss leg., 1 male, light trap (UFVB – PL219); Mariana, Rio Gualaxo do Norte, 20° 14' 26.23" S 43° 20' 37.13" W, 2022.iii.30, 1 male, light trap, TG Kloss leg. (UFVB – PL220); Mariana, Rio Gualaxo do Norte, 20° 14' 26.23" S 43° 20' 37.13" W, 2022.iii.30, 1 male, light trap, TG Kloss leg. (UFVB – PL225); Mariana, Rio Gualaxo do Norte, 20° 14' 21.61" S 43° 20' 06.91" W, 2022.iii.30, 1 male, light trap, TG Kloss leg. (UFVB – PL230); Mariana, Rio Gualaxo do Norte, 20° 17' 03.60" S 43° 27' 43.86" W, 2022.iii.27, 1 male, light trap, TG Kloss leg. (UFVB – PL234); Guaraciaba, Rio Piranga, 20° 32' 46.30" S 42° 59' 25.25" W, 2022.i.14, 2 male, light trap, ADL Viana leg. (UFVB – PL451, PL453).

Remarks: A common species with a wide distribution. It may be confused for *A. pitii* and *A. quilombola*. It may be distinguished from them due to the paired vesicles. This species may have more than one state in this character, however, and in some cases the species are indistinguishable. Their identity as separate species is questionable, and further studies should be conducted in order to uncover possible synonymy or currently unknown characters.

***Anacroneuria kariri* Righi-Cavallaro & Froehlich, 2013** (Fig. 6B)

Examined material. Brazil, BA: Mucugê, Parque Nacional da Chapada Diamantina, Rodovia BA242, Ponte Dr. Heitor M. Chamusca, 12º 59' 24" S 41º 21' 1" W, 2018.viii.19, light trap, 1 male, MC Gonçalves leg. (UFVB – PL00293); Mucugê, Parque Nacional da Chapada Diamantina, Cachoeira das Andorinhas, 13º 01' 02" S 41º 20' 30" W, 2018.vii.21 – 2018.vii.23, Pennsylvania, 1 male, Rodrigues & Prado leg. (UFVB – PL00294).

Remarks: A small species which occurs in Northeastern Brazil and the northern parts of Minas Gerais. It is quite distinct from the other species included in the key, with the keel shape in dorsal view – Y-shaped – and gonoporal process apex – truncated – being important characters for identifying this species.

***Anacroneuria mantiqueirae* Froehlich, 2010** (Fig. 6C)

Examined material. Brazil, Rio de Janeiro: Itatiaia, Parque Nacional do Itatiaia, Cachoeira Véu da Noiva, 2017.x.07, light trap, 1 male, R Campos leg. (CIACGF – DP02).

Remarks: See the remarks for *A. iporanga*.

***Anacroneuria mineira* Novaes & Bispo, 2014** (Fig. 6D)

Examined material. Holotype. Brazil, Minas Gerais: Santana do Riacho, Serra do Cipó, Rio Cipó, Cardeal Mota – Cachoeira Baixa, 19º 20' 55" S 43º 38' 53" W, 2001.xi.10, RW Holzenthal, H Paprocki leg. (MZUSP).

Remarks: A relatively large species that occurs in the Midwest of Brazil and western Minas Gerais. The photographed holotype has lost its entire maculation pattern, so this aspect should be disregarded when comparing specimens with the species photographs. The penial armature can be distinguished from other species by the basal region length proportion – occupying more than half of total armature length – and keel shape in dorsal view – forming a Y.

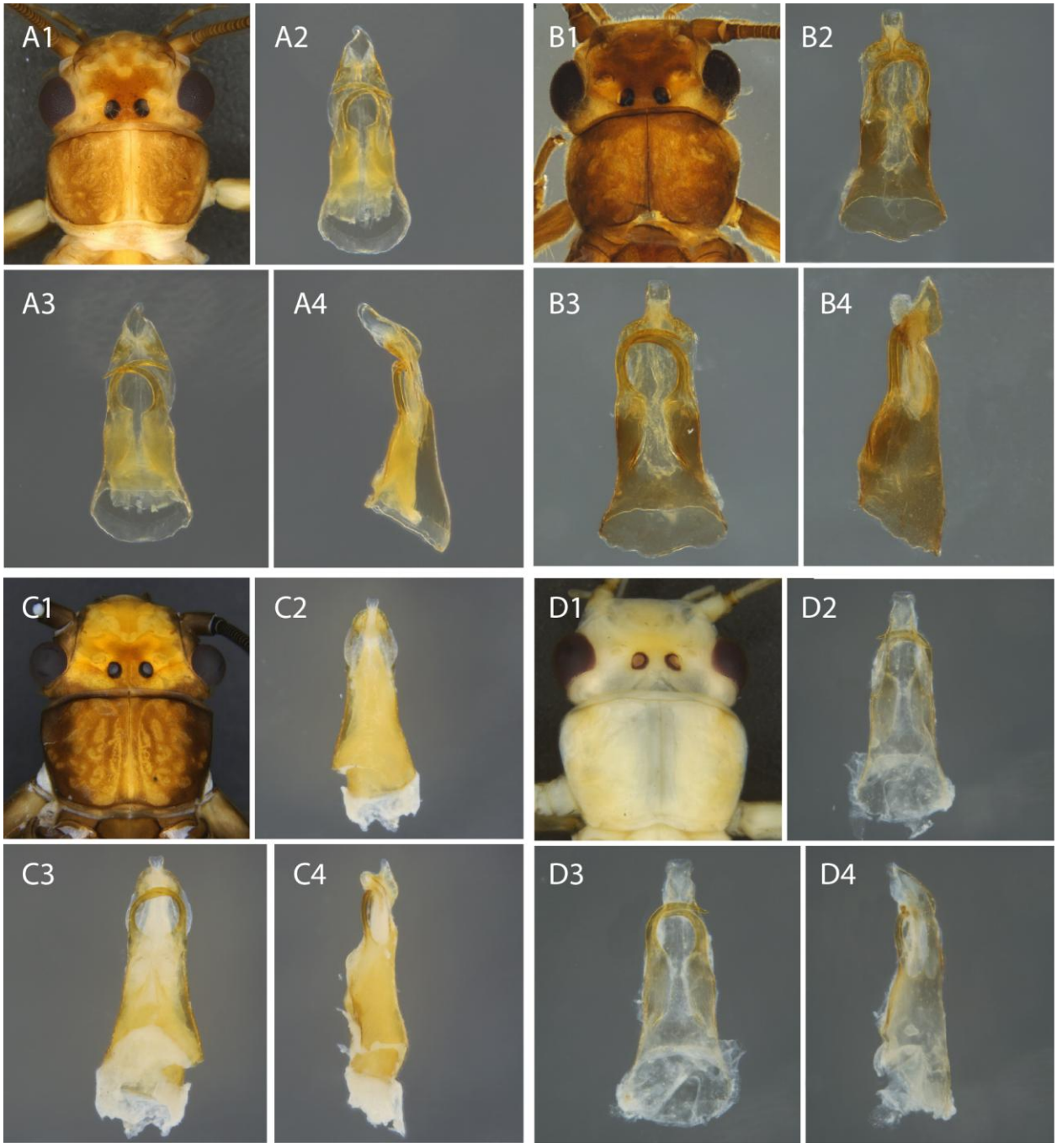

Figure 6 – Figures provided in the AnacroIndex key. Each figure shows (1) head and pronotum, penial armature in (2) dorsal, (3) ventral and (4) lateral views. (A) *Anacroneuria itatiaiensis*; (B) *Anacroneuria kariri*; (C) *Anacroneuria mantiqueirae*; (D) *Anacroneuria mineira* holotype.

***Anacroneuria ofaye* Froehlich, 2007** (Fig. 7A)

Examined material. Brazil, São Paulo: Assis, Estação Ecológica de Assis, Riacho Água do Xaxim, 2019.xi.18 – 2019.xii.18, Malaise, 1 male, LH Almeida leg. (CIACGF – F58).

Remarks: This species occurs mainly in São Paulo. It is quite distinct from others included in the key. The lateral margins of the gonoporal process base – divergent – and the acute yet truncated gonoporal process apex are important characters in its identification.

***Anacroneuria paprockii* Novaes & Bispo, 2014** (Fig. 7B)

Examined material. Holotype. Brazil, Minas Gerais: São Gonçalo do Rio Preto, Parque Estadual do Rio Preto, small stream near park entrance, 18º 05' 43" S 43º 20' 51" W, 2001.xi.14, H Paprocki leg. (MZUSP).

Remarks: This species occurs in Minas Gerais. The photographed holotype has lost its entire maculation pattern, so this aspect should be disregarded when comparing specimens with the species photographs. The combination of the lateral margins of the gonoporal process base – divergent –, the gonoporal process apex – truncated – and the basal region length proportions – occupying half or more than half of total armature length – are important characters in identifying this species.

***Anacroneuria paulina* (Navás, 1936)** (Fig. 7C)

Examined material. Brazil, São Paulo: Salesópolis, Estação Biológica de Boracéia, Rio Guaratuba, 2024.i.18, light trap, 1 male, PN Taniguti & B Sabino leg. (CIACGF – DP1179)

Remarks: See the remarks for *A. iporanga*.

***Anacroneuria petersi* Froehlich, 2002** (Fig. 7D)

Examined material. Brazil, São Paulo: Paratype Santo André, Estação Biológica de Paranapiacaba, 1963.x.15, 1 male, CG Froehlich leg. (MZUSP).

Remarks: This species occurs in São Paulo. The photographed paratype is a teneral specimen, so most of its maculation pattern has been lost due to the preservation techniques employed. This should be considered when comparing specimens with the species photographs. This species is similar to *A. polita*, and only the keel shape in dorsal view distinguishes them – V shaped in *A. petersi*,)(shaped in *A. polita*.

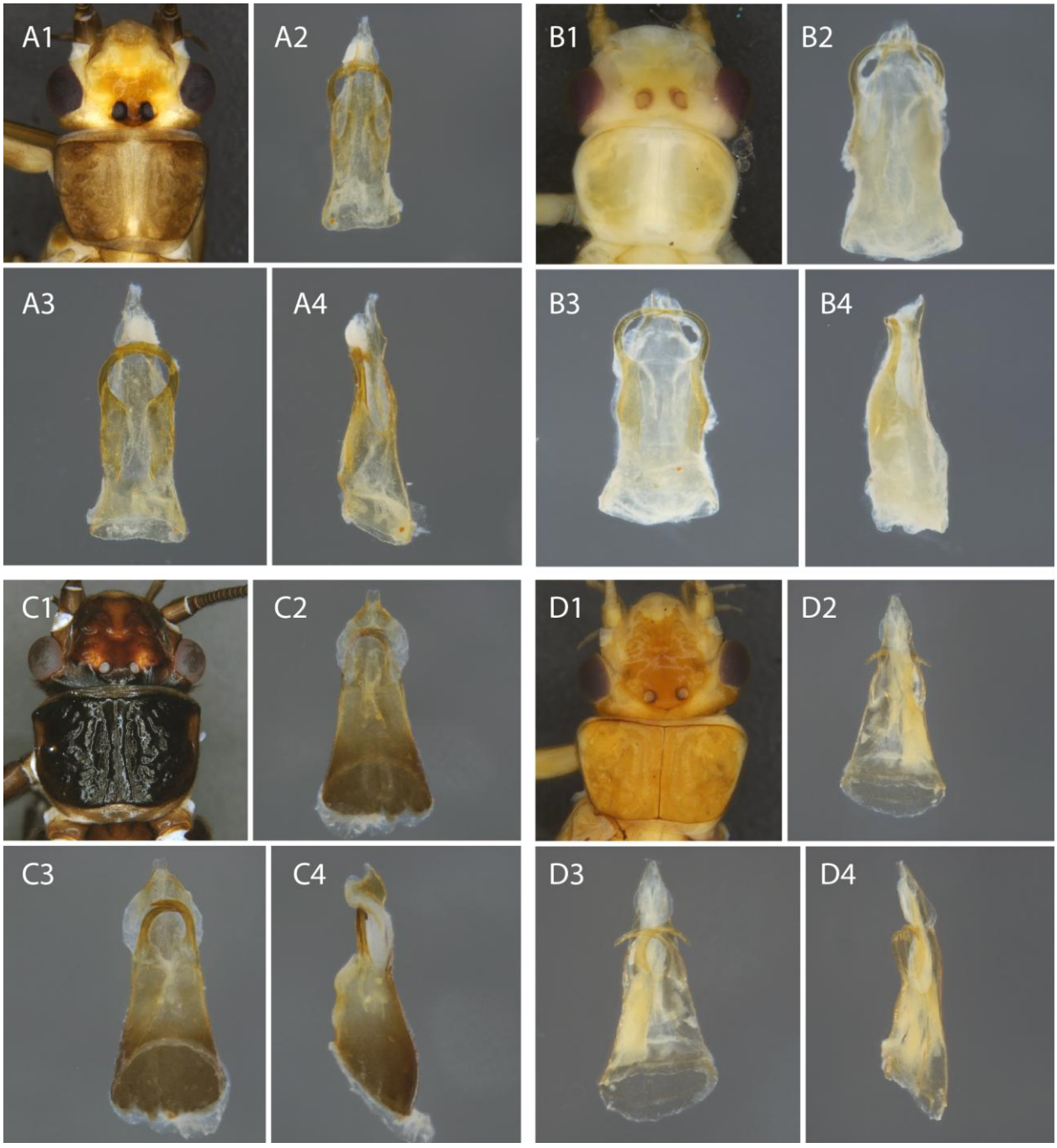

Figure 7 – Figures provided in the AnacroIndex key. Each figure shows (1) head and pronotum, penial armature in (2) dorsal, (3) ventral and (4) lateral views. (A) *Anacroneuria ofaye*; (B) *Anacroneuria paprockii* holotype; (C) *Anacroneuria paulina*; (D) *Anacroneuria petersi* paratype.

***Anacroneuria piranga* Castillo-Velásquez, Gonçalves & Salles, 2023** (Fig. 8A)

Examined material. Brazil, Minas Gerais: Holotype Guaraciaba, Rio Piranga, 20º 32' 46" S 42º 59' 25" W, 526 m, Pennsylvania, ADL Viana, P Bonfá & P Rodrigues leg. (UFVB); **Paratype** Guaraciaba, Rio Piranga, 20º 32' 46" S 42º 59' 25" W, 526 m, Pennsylvania,

1 male, ADL Viana, P Bonfá & P Rodrigues leg. (UFVB); Guaraciaba, Rio Piranga, 20º 32' 46" S 42º 59' 25" W, 2024.ix.30, light sheet, 1 male, FF Salles, C Nieto, T Senar-Serra & A Ruiz leg. (UFVB – PL00535).

Remarks: This species is only known from the type locality in Minas Gerais. The penial armature of this species is quite distinct from others included in the key. The main character used in identifying this species is the curvature of the hooks, which are mostly straight.

***Anacroneuria pitii* Gonçalves, Novaes & Salles, 2017** (Fig. 8B)

Examined material. Brazil, Espírito Santo: Holotype Alegre, Rio Norte, reared nymph collected 07.viii.2004, emerged 08.viii.2004, MC Gonçalves leg (UFVB - PL00597).

Remarks: See the remarks for *A. itatiaiensis*.

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

Examined material. Brazil, Rio de Janeiro: Itatiaia, Parque Nacional do Itatiaia, Córrego Taquaral – 810m, 06.x.2017, Malaise, 1 male, R Campos leg. (CIACGF – DP08).

Remarks: The photographed specimen is teneral, and comparison with the photographs should be done with care. See the remarks for *A. petersi*.

***Anacroneuria quilombola* Righi-Cavallaro & Froehlich, 2013**

Examined material. No material could be examined for this species, and we weren't able to access the type material, deposited at MZUSP, or any recently collected specimens. Inclusion in the key followed the description's illustration and photographs (Righi-Cavallaro & Froehlich, 2013).

Remarks: See the remarks for *A. itatiaiensis*.

***Anacroneuria rotunda* Gonçalves, Novaes & Salles, 2017** (Fig. 8D)

Examined material. Brazil, Espírito Santo: Holotype Alegre, 20º37'11"S 41º37'35"W, 04-05.iv.2016, light trap, Salles leg. (UFVB - PL00598); Alfredo Chaves, Mathilde, 20-21.i.2017, light sheet, 1 male, FF Salles leg. (CIACGF – DP58).

Remarks: This species is known from Espírito Santo and Minas Gerais. It has the same character states as *A. singela*, and the key is unable to differentiate them. This is due to the astounding similarity between the armatures of these species. The coloration of *A. rotunda* is characteristic, and even in teneral or badly preserved specimens, the external difference

between these two species is apparent. As such, when identifying either of these species, the external coloration should be considered.

Figure 8 – Figures provided in the AnacroIndex key. Each figure shows (1) head and pronotum, penial armature in (2) dorsal, (3) ventral and (4) lateral views. (A) *Anacroneuria piranga*; (B) *Anacroneuria pitii* holotype; (C) *Anacroneuria polita*; (D) *Anacroneuria rotunda* holotype.

***Anacroneuria ruschii* Novaes, Bispo & Gonçalves, 2016** (Fig. 9A)

Examined material. Brazil, Espírito Santo: Holotype Santa Teresa, 19º54'53"S 40º33'40"W, 08-09.ix.2015, light trap, FF Salles leg (UFVB - PL00599).

Remarks: See the remarks for *A. debilis*.

***Anacroneuria sallesi* Almeida, Gonçalves & Bispo, 2025** (Fig. 9B)

Examined material. Brazil, Espírito Santo: Holotype/UFVB Santa Teresa, Reserva Biológica Augusto Ruschi, Córrego Bragacho, 20-21.ii.2018, 1 male, FF Salles leg. (PL00617).

Remarks: This species is only known from Espírito Santo. It has some readily seen characters that may confuse it for *A. vanini*, such as the anterior wing membrane color – with a hyaline window present – or its pronounced shoulder projection. The species can be distinguished, however, due to shape of the gonoporal process – truncated in *A. sallesi* and rounded in *A. vanini* –, apical region width proportions – subequal in *A. sallesi* and narrower in *A. vanini* –, basal region length proportions – occupying half or more than half of total armature length in *A. sallesi* and less than half in *A. vanini* – and the dorsal keel – developed in *A. sallesi* and undeveloped in *A. vanini*.

***Anacroneuria saofrancisco* Novaes, Vilela, Lopez & Ferreira, 2018** (Fig. 9C)

Examined material. Brazil, Minas Gerais: Holotype Parque Nacional da Serra da Canastra, Rio do Peixe, -20.2569, -46.4097, 17–19.x.2017, GFT leg (MZUSP).

Remarks: This species occurs in Minas Gerais. This species may be distinguished from others in the key due to the dorsal keel – undeveloped –, armature dorsal plate lateral margins – divergent –, gonoporal process apex – truncated – and shoulder projection – not projected.

***Anacroneuria simulans* Froehlich, 2010** (Fig. 9D)

Examined material. Brazil, São Paulo: Campos do Jordão, Parque Estadual Campos do Jordão, Estrada Galharada, 15.ii.2019, light trap, 1 male, LH Almeida leg. (CIACGF – DP190); Campos do Jordão, Parque Estadual Campos do Jordão, Estrada Galharada, 15.ii.2019, light trap, 1 male, LH Almeida leg. (CIACGF – DP191).

Remarks: This species occurs in São Paulo and Rio de Janeiro. The penial armature of this species is similar to *A. subcostalis*. The fore wing vein coloration, however, distinguishes these two species – C and ScP darker than remaining longitudinal veins in *A. subcostalis* and similar or lighter than remaining longitudinal veins in *A. simulans*. As a final distinguishing

feature, although not included in the key, the head and pronotum colors and maculation patterns are distinct, which may be useful in identifying well sclerotized and well-preserved material. In *A. simulans*, the head is darker laterally, and the M-Line is distinctly light compared to the rest of the head, while in *A. subcostalis* the head is uniformly light in color.

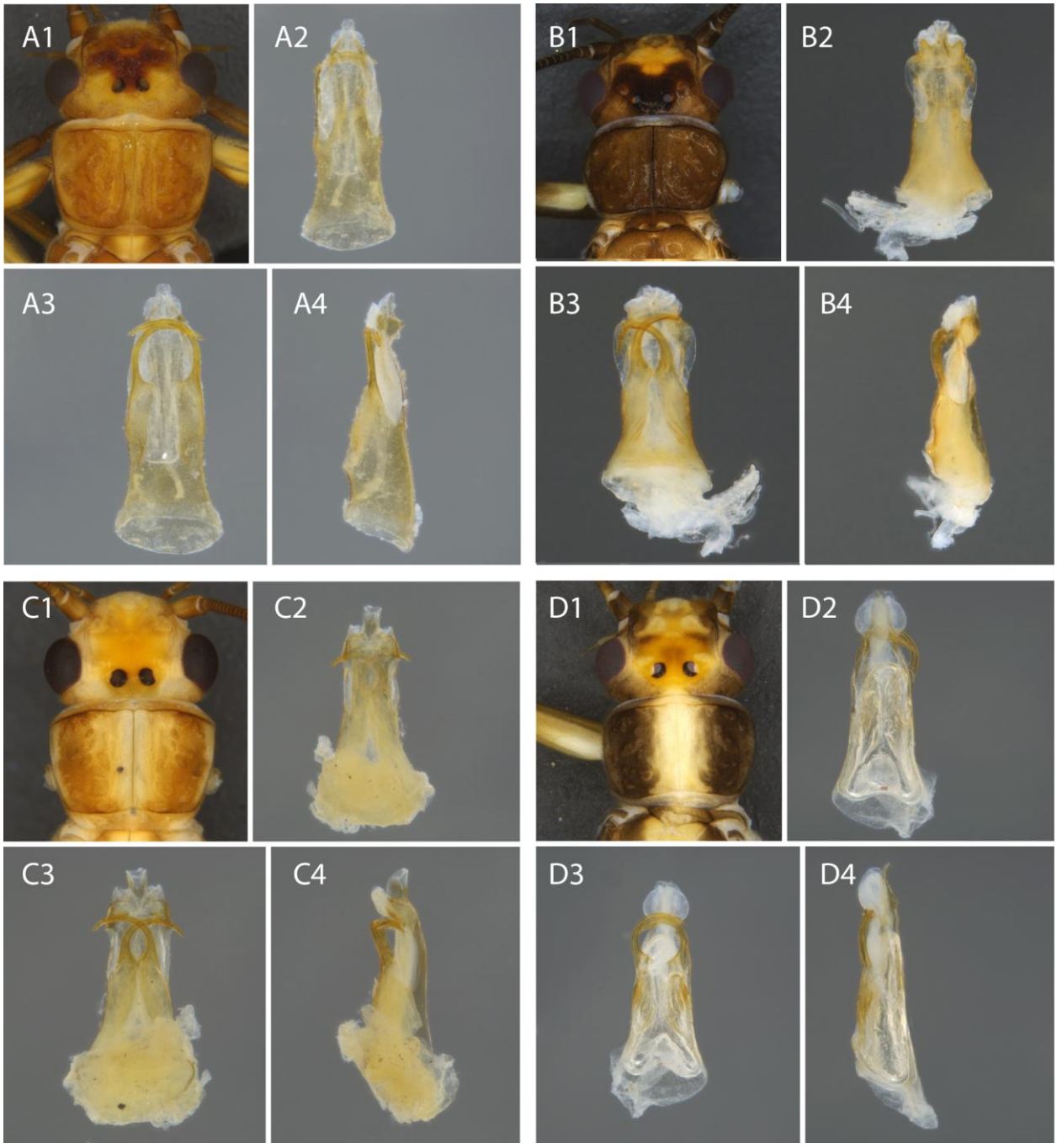

Figure 9 – Figures provided in the AnacroIndex key. Each figure shows (1) head and pronotum, penial armature in (2) dorsal, (3) ventral and (4) lateral views. (A) *Anacroneuria ruschii* holotype; (B) *Anacroneuria sallesi* holotype; (C) *Anacroneuria saofrancisco* holotype; (D) *Anacroneuria simulans*.

Anacroneuria singela **Duarte & Lecci, 2016** (Fig. 10A)

Examined material. Brazil, BA: Paratype Iaçu, Rio Paraguaçu, 24.iii.2012, by hand, 1 male, T Duarte & I Garcia leg. (MZUSP).

Remarks: See the remarks for *A. rotunda*.

Anacroneuria singularis **Righi-Cavallaro & Lecci, 2010** (Fig. 10B)

Examined material. Brazil, MT: Nova Xavantina, Córrego Antártico, 14.xi.2021, light sheet, 1 male, LH Almeida leg. (CIACFG – DP527); Nova Xavantina, Córrego Antártico, 14.xi.2021, light sheet, 1 male, LH Almeida leg. (CIACFG – DP529).

Remarks: This species occurs in northern Minas Gerais and Central Brazil. It has a unique penial armature due to the curvature of the hooks – curved in more than one plane.

Anacroneuria stanjewetti **Froehlich, 2002**

Examined material. No material could be confidently examined for this species. The type specimen, which is deposited at the California Academy of Sciences, was also not available for morphological study. Thus, we rely on the original description for inclusion in the key (Froehlich, 2002).

Remarks: This species occurs in Southern Brazil and all the states of Southeastern Brazil. It can be distinguished from others in the key based on the combination of the ventral vesicles – with an imperceptible separation between them –, lateral margin of the gonoporal process base – divergent –, gonoporal process apex – truncated – and basal region length proportions – occupying half or more than half of total armature length.

Anacroneuria subcostalis **Klapálek, 2021** (Fig. 10C)

Examined material. Brazil, Minas Gerais: Araponga, Parque Estadual da Serra do Brigadeiro, 20º43'52"S 42º27'50"W, 1110m, 25.v.2022, 1 male, GM Pantoja, TYS Orlando & ICH Cortes leg. (UFVB – PL00203); Araponga, Parque Estadual da Serra do Brigadeiro, 20º43'52"S 42º27'50"W, 1110m, 25.v.2022, 1 male, GM Pantoja, TYS Orlando & ICH Cortes leg. (UFVB – PL00204).

Remarks: See the remarks for *A. simulans*.

Anacroneuria tabatae **Froehlich, 2010** (Fig. 10D)

Examined material. Brazil, São Paulo: Campos do Jordão, Parque Estadual Campos do Jordão, Trilha Galharada, 12-13.ii.2019, light trap, 1 male, LH Almeida leg. (CIACGF – DP184).

Remarks: This species is only known from Campos do Jordão, in São Paulo. It has a unique penial armature among those included in the key. The shoulder projection – projected – and basal region length proportions – occupying half or more than half of total armature length – are important characters in identifying it. The head and pronotum color and maculation patterns are quite unique and are also informative in well sclerotized and well-preserved material.

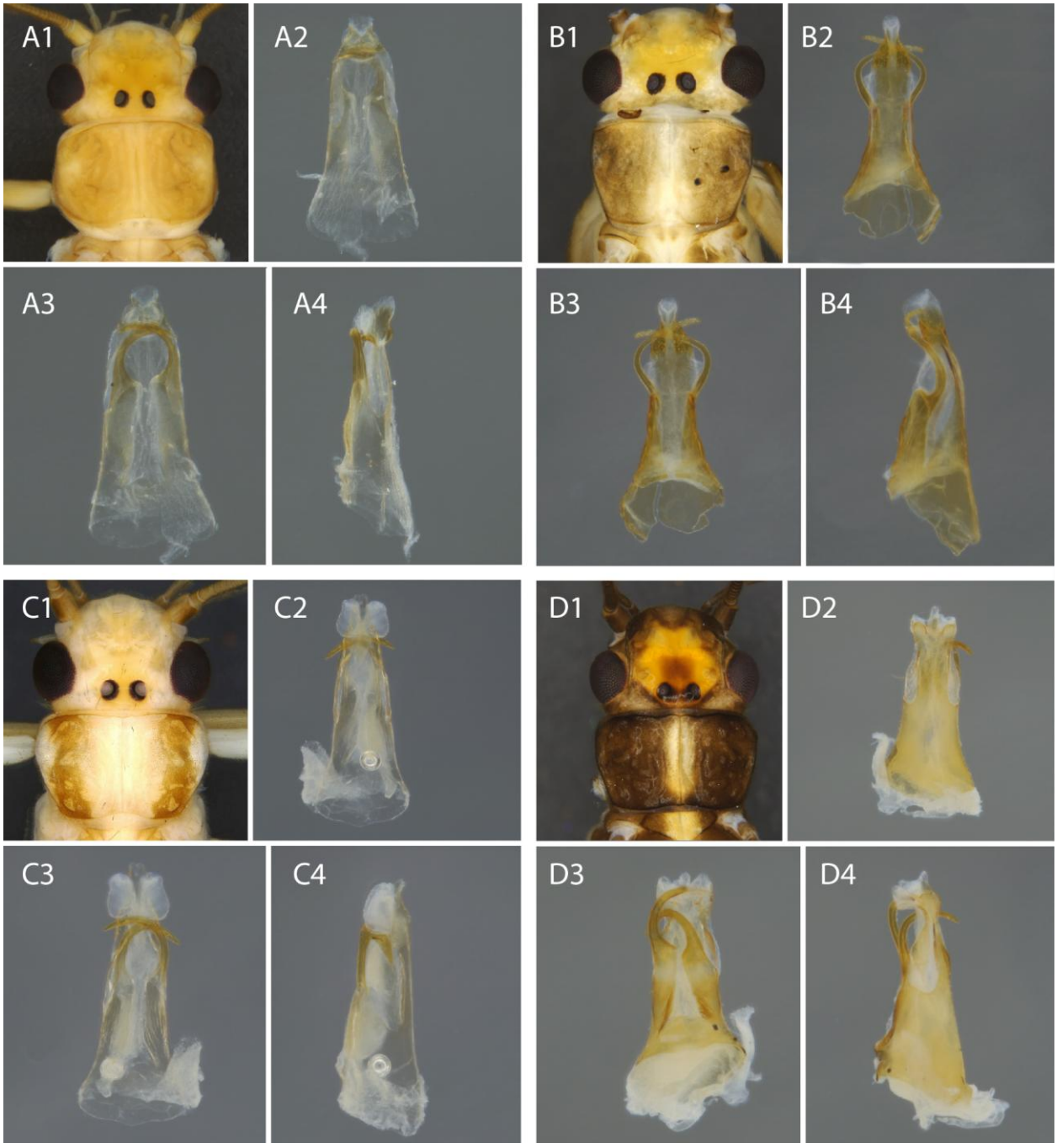

Figure 10 – Figures provided in the AnacroIndex key. Each figure shows (1) head and pronotum, penial armature in (2) dorsal, (3) ventral and (4) lateral views. (A) *Anacroneuria singela* paratype; (B) *Anacroneuria singularis*; (C) *Anacroneuria subcostalis*; (D) *Anacroneuria tabatae*;

Anacroneuria terere **Righi-Cavallaro & Lecci, 2010** (Fig. 11A)

Examined material. Brazil, Tocantins: Pedro Afonso municipality, Rio do Sono, -08.99590, -48.13934, 180 m, 11-12.x.2018, light sheet, TYS Orlando & T Krolow leg., 1 male. Only photographs of this specimen were examined, since we could not access the specimen itself.

Remarks: This species occurs in northern Minas Gerais, Central and Northern Brazil. This species can be distinguished from others in the key by the combination of the dorsal keel – undeveloped – and gonoporal process notch – notched.

Anacroneuria toriba **Froehlich, 2002** (Fig. 11B)

Examined material. Brazil, Rio de Janeiro: Teresópolis, Parque Nacional da Serra dos Órgãos, Trilha Pedra do Sino #2, 8.xii.2024, light trap, 1 male, RB Gastaldo leg. (UFVB – PL00596); **São Paulo:** Campos do Jordão, Parque Estadual Campos do Jordão, Córrego Galharada, 22º41'39"S 45º27'41"W, 1580m, 19.vii.2023, 1 male, by hand, PN Taniguti leg. (UFVB – PL00215).

Remarks: This species occurs in São Paulo, Espírito Santo and Rio de Janeiro. It may be identified especially by the combination of a developed dorsal keel and notched gonoporal process.

Anacroneuria tupi **Bispo & Froehlich, 2004** (Fig. 11C)

Examined material. Brazil, São Paulo: Iporanga, Parque Estadual de Intervales, Rio do Carmo, 09.ii.2017, light trap, 1 male, LH Almeida leg. (CIACGF – DP142); Iporanga, Parque Estadual de Intervales, Rio do Carmo, 09.ii.2017, light trap, 1 male, LH Almeida leg. (CIACGF – DP143).

Remarks: This species occurs in São Paulo. The photographed specimen is teneral, so comparison with the photographs should be done with care in relation to the maculation pattern. The character states of this species are included in the possible character states for *A. ruschii*, and when identifying *A. tupi* the key won't exclude *A. ruschii* as a possible species. However, upon observing the photographs of both species, they are easily distinguished.

Anacroneuria uyara **Froehlich, 2002** (Fig. 11D)

Examined material. Brazil, São Paulo: Salesópolis, Estação Biológica da Boracéia, Rio Claro, 17.i.2024, light trap, 1 male, PN Taniguti leg. (CIACGF – DP1183).

Remarks: See the remarks for *A. debilis*.

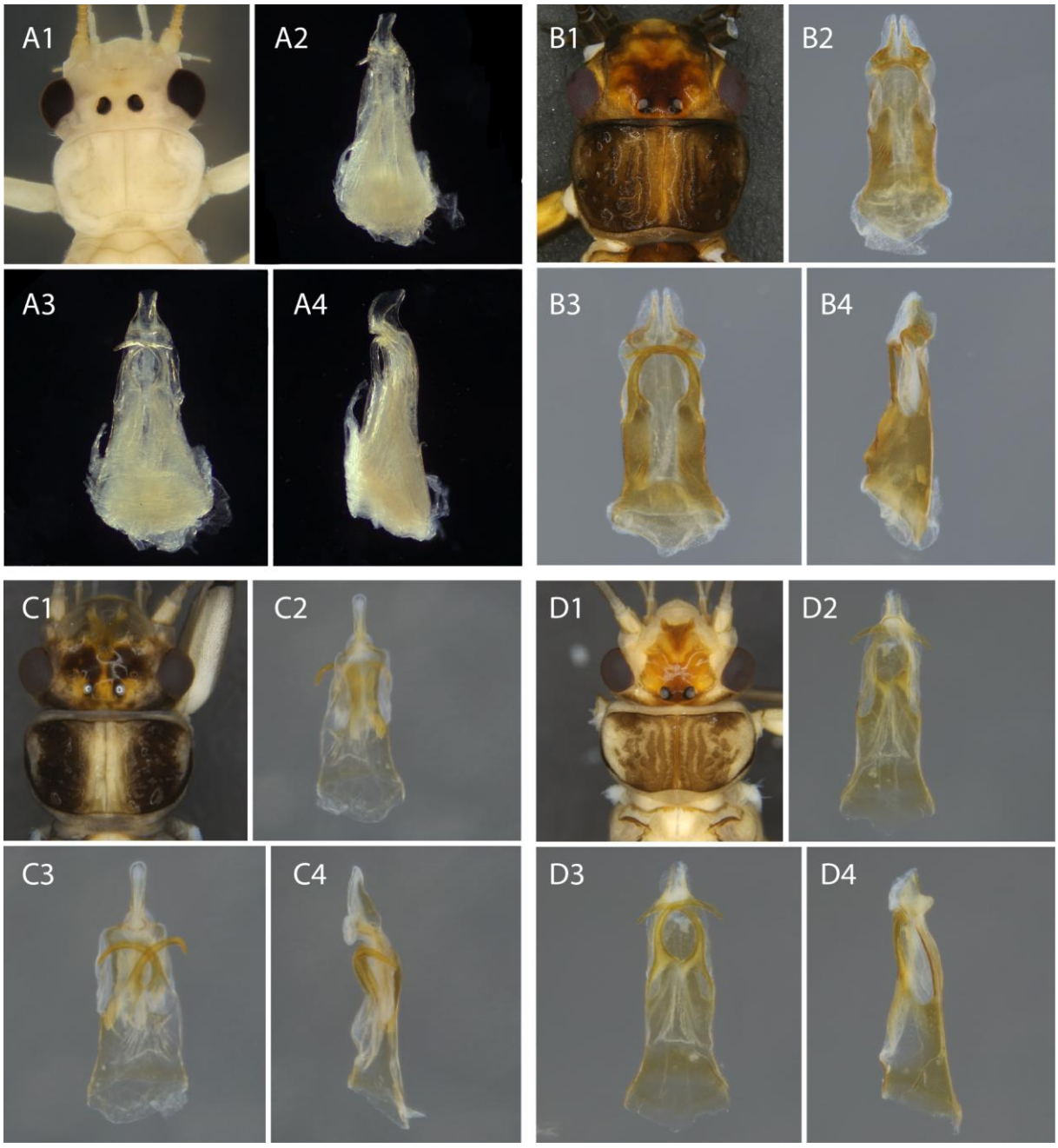

Figure 11 – Figures provided in the AnacroIndex key. Each figure shows (1) head and pronotum, penial armature in (2) dorsal, (3) ventral and (4) lateral views. (A) *Anacroneuria terere*, photographs by MLS Rippel; (B) *Anacroneuria toriba*; (C) *Anacroneuria tupi*; (D) *Anacroneuria uyara*.

***Anacroneuria vanini* Froehlich, 2004** (Fig. 12A)

Examined material. Brazil, Minas Gerais: Araponga, Parque Estadual da Serra do Brigadeiro, 20º43'52"S 42º27'50"W, 1110m, 3.x.2022 – 7.xii.2022, malaise, 1 male, MLS Rippel leg. (UFVB – PL245).

Remarks: See the remarks for *A. sallesi.*

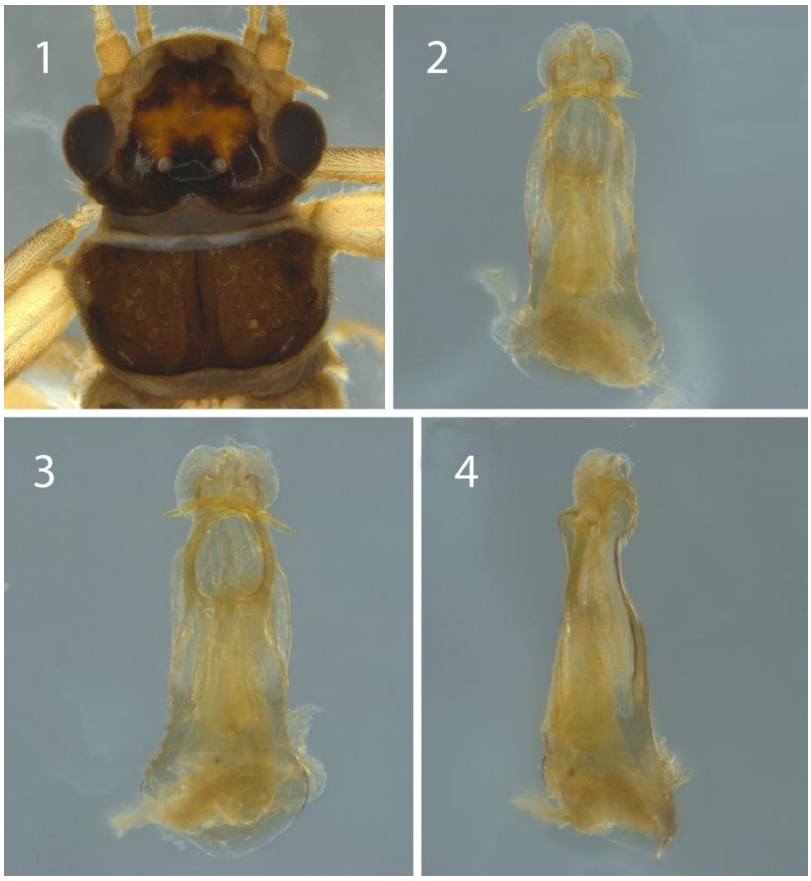

Figure 12 – Figure provided in the AnacroIndex key for *Anacroneuria vanini*. (1) head and pronotum, penial armature in (2) dorsal, (3) ventral and (4) lateral views.

3.1 Recommendations

When using the AnacroIndex key, there are a couple of suggestions we would like to point out. For those unfamiliar with *Anacroneuria* penial morphology and terminology, we recommend reading the corresponding section of Gastaldo et al. (2025). One practical suggestion in the use of the AnacroIndex key in Xper3 is the frequent submission of the key. After submission, the key will eliminate impossible characters and recommend characters that are critical for the next step of the identification process. As such, if the user fills in too many

characters without submitting, the key has less opportunities to suggest characters to the user, increasing the probability of running into dead ends.

One of the advantages of our key is that almost all species have their head, pronotum and penial armature photographed and available at all times to the user through clicking the species name on the list on the right of the screen (Fig. 1). In our experience, users find it considerably harder to fill the last couple of characters when only a few possible species are left. Considering this, if the user is having trouble or facing uncertainty in filling the last characters when only 5 or fewer species are possible, we encourage the user to briefly check on the provided photographs for the remaining potential taxa. This might be quicker and easier to identify a specimen than to confidently fill the last, harder characters in the key. When comparing external appearance to the provided photographs, always keep in mind the tenerality of your specimen, the possible tenerality of the photographed specimen, as commented when pertinent, and how well-preserved they are.

4 – Discussion

The key developed in this work provides a couple of advantages over dichotomous keys for *Anacroneuria*. The flexibility and editing potential of an interactive key is unmatched by dichotomous keys, as adding new characters or species is as simple as adding a new row or column in the description matrix (Dallwitz et al., 2002). When paired with the cooperative nature of the Xper3 platform, in which other researchers may add data, this makes that the key may be continuously worked upon and improved as new species are recognized and taxonomy is updated. Thus, the key as is described in this work may be consistently worked upon, streamlined and made to encompass more species.

Another advantage of this interactive key over other, previously published dichotomous keys is its character efficiency. In the keys of Stark & Kondratieff (2004) or Duarte & Lecci (2016) for example, the proportion of the number of used characters by the number of included species ranges around 0.85–1.4. The present key has a number of used characters by number of included species proportion of 0.38, meaning that fewer characters are needed to identify a proportionally larger number of species.

Including 39 species so far, this is already one of the largest keys for *Anacroneuria*. Considering the growth potential of this key and flexibility in species and character inclusion, we expect it to soon become a fundamental tool in identifying the *Anacroneuria* species across a wide geographical range. The next steps in development of the key include the coverage of the species found in Northeastern Brazil, which Duarte and Lecci (2016) have already made a

precise dichotomous key for, and Southern Brazil, as many of the species found in this region are also found in Southeastern Brazil and are already included in the key. Thus, we expect that the next version of the key to be released will include more than half of the species found in Brazil, leaving only the few species of the Midwest and the Amazonian species remaining for a full coverage of Brazil.

5 – References

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GENERAL CONCLUSION

This work has brought about the first dedicated illustrated atlas to Anacroneuriini stoneflies and one of the most comprehensive identification keys for the genus *Anacroneuria* to date. While it is easy to see taxonomy as only the description of new taxa, the frequent revision of what is already known and the updating of older information is equally as important to the field. Standardizing current knowledge is a must for good taxonomic practice.

In this way, this dissertation fits into the revision and standardization side of taxonomy. The first section of this work – the morphological atlas of Anacroneuriini – summarizes the information found in dozens of species descriptions and morphological textbooks into an illustrated format and may provide a strong basis for new descriptions to include detailed information and accurate terms for every body part of these insects. Further research could, then, make use of the provided atlas for their descriptions and add illustrations and terminology for whatever new characters may be found to be useful for describing species in Anacroneuriini. In this way, a citable standardized jargon was established, so that researchers feel confident in sharing and comparing information.

In the second section of this dissertation, the AnacroIndex key already has one of the largest species coverages for the genus while maintaining high levels of character efficiency in its first iteration. The highly pictorial aspect of the key, with pictures of all characters and most species being included, also provides some of the first photographs for many species and updates the knowledge on these species' identity. In the future, we expect to continue adding species to the AnacroIndex project, eventually covering more species and geographical regions. Thus, this dissertation provided tools for identifying the known species richness in Southeastern Brazil, as well as for any potential species descriptions or redescriptions. We hope to be able to help make working with *Anacroneuria* an easier task, facilitating future taxonomic, biomonitoring, ecological and any other work that may need identification to take place.

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