

Article

New Species and Old Semaphoronts: Updating Taxonomic Knowledge of *Kempnyia* Klapálek, 1914 (Plecoptera: Perlidae) with an Integrative Approach

Lucas Henrique de Almeida ^{1,*} , Rodrigo Braga Gastaldo ^{2,3} , Frederico Falcão Salles ³ 
and Pitágoras da Conceição Bispo ¹

¹ Laboratório de Biologia Aquática, Faculdade de Ciências e Letras de Assis, Universidade Estadual Paulista, Assis 19806-900, SP, Brazil; pitagoras.bispo@unesp.br

² Programa de Pós-Graduação em Entomologia, Universidade Federal de Viçosa (UFV), Viçosa 36570-900, MG, Brazil; rodrigo.gastaldo@ufv.br

³ Museu de Entomologia, Departamento de Entomologia, Universidade Federal de Viçosa (UFV), Viçosa 36570-900, MG, Brazil; frederico.salles@ufv.br

* Correspondence: lucasalmeida768@yahoo.com.br

Abstract: *Kempnyia* Klapálek, 1914 (Plecoptera: Perlidae) is the only genus within its family endemic to Brazil, being present in the Atlantic Forest and sections of the Cerrado. Taxonomic knowledge of the genus is incomplete, especially for nymphs. An integrative approach to the description of species and semaphoronts is essential for further studies. We describe a new species of *Kempnyia* based on morphological and molecular data, associate and describe the nymphs of both the new species and *Kempnyia umbrina* Froehlich, 1988, as well as expand its distribution and correct past mistakes in identification of *K. umbrina*. Specimens were identified morphologically and sequenced for the barcode region of the cytochrome oxidase subunit I (COI) mitochondrial gene, resulting in eight new sequences for three species of the genus. Both morphological and molecular analyses revealed a new species, *Kempnyia krenaki* sp. nov. Its nymph and that of *K. umbrina* are also described. With our additions, the number of known *Kempnyia* species rises to 41, of which 26 have COI sequences available in GenBank. Although only 11 nymphs have been associated and described for the genus so far, as the *Kempnyia* sequence database continues to grow, studies associating and describing nymphs will become more common, gradually reducing the Haeckelian deficit.

Keywords: barcoding; biodiversity; neotropical region; stoneflies; taxonomy



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1. Introduction

Perlidae (Plecoptera) is the family with the greatest richness of species within its order, representing over 30% of global diversity and at least 60% of Brazilian species [1,2]. While most of its genera are well distributed across the Amazon and Atlantic Forests, with a range which spans several countries, a single genus in this family is endemic to Brazil—*Kempnyia* Klapálek, 1914 [2–4].

Like all other Brazilian Perlidae, *Kempnyia* is a member of Anacroneuriini [5]. It has a dorsoventrally flattened body, relatively poor flight capability, a well-developed hammer, and a distinct pair of chitinated hooks in the penial armature [5,6]. *Kempnyia* species range in body size from medium to large (8 mm to over 24 mm length of forewing), have two or three ocelli, and are typically yellow or light brown to black in coloration [6]. The 40 known species are distinguished based on their size, wings, maculation patterns and,

especially, the morphology of their genitalia [4]. Although the penial armature is crucial for distinguishing species, in some cases they may appear similar, and often only small morphological differences allow the separation of two species, which can be clarified with the aid of molecular data [4].

Kempnyia presents several taxonomic challenges, primarily involving species with old or insufficient descriptions [4]. However, one of the major current gaps in knowledge about the genus concerns the nominal identification of immatures. Only nine of the forty species have nymphs which have been associated and described [4]: *Kempnyia colossica* (Navás, 1934) [7]; *K. couriae* Avelino-Capistrano, Barbosa and Takiya, 2016 [8]; *K. goiana* Bispo and Froehlich, 2004 [9]; *K. gracilentia* (Enderlein, 1909) [10]; *K. neotropica* (Jacobson and Bianchi, 1905) [11]; *K. obtusa* Klapálek, 1916 [12]; *K. reticulata* (Klapálek, 1916) [12]; *K. tijucana* Dorvillé and Froehlich, 1997 [13]; and *K. varipes* Klapálek, 1916 [12]. Among the described nymphs, six were associated based on rearing of immatures in situ [14–17] or deductive association [18], and three were associated with the aid of molecular data [8,19].

Kempnyia was the first Brazilian genus of Plecoptera to be studied using molecular data, primarily employing the barcode region of the mitochondrial gene cytochrome c oxidase subunit I (COI) [19]. Since then, three additional studies have been conducted using the same approach to describe new species, discuss morphological variations, propose synonymy of species, and associate immatures [4,8,20]. As a result, a robust molecular database has been created for the genus, with DNA sequences for 23 species available in GenBank [4,8]. In this study, based on the available molecular data and automatic molecular species delimitation methods, we describe a new species and its respective immature stage, *K. krenaki* sp. nov., as well as the immature stage of *K. umbrina* Froehlich, 1988. A general overview on the current knowledge of *Kempnyia* taxonomy is also provided.

2. Materials and Methods

Specimens were collected following standard Brazilian legal procedures, with licenses from the Authorization and Information System in Biodiversity (SISBIO–55428-16, 65213-11, 92744-1 and 70090-5) and the Environmental Research Institute of São Paulo State (IPA/SIMA–SMA 15054/2022 and 5579/2023). Collecting methods followed the normative instructions of the Chico Mendes Institute for Biodiversity Conservation (ICMBio, Brasília, Brazil). Sampling of adults was carried out using traditional Malaise and Pennsylvania traps, while nymphs were collected with D-nets. Specimens were kept in absolute ethanol at a standard temperature of $-25\text{ }^{\circ}\text{C}$ for DNA preservation. Studied specimens were deposited in the Entomology Museum of the Federal University of Viçosa (UFVB, Viçosa, Brazil) and the Aquatic Insect Collection “Prof. Dr. Cláudio Gilberto Froehlich” (CIACGF at Aquatic Biology Laboratory, São Paulo State University–UNESP, Assis, SP, Brazil). The type series of the new species was deposited in the UFVB collection.

Males had their abdomens severed between segments seven and eight, and were then clarified overnight in a 10% potassium hydroxide (KOH) solution for identification. For neutralization of the reaction, abdomens were placed in acetic acid (10%) and washed in 80% ethanol. The penial armatures were extracted to identify the species by morphological comparison with penial armatures described in the literature [21]. To characterize the gills of the nymphs, the nomenclature proposed by Shepard and Stewart [22] was used. Species descriptions followed the terminology proposed by Gastaldo et al. [23]. Structures were photographed with a digital camera (MC170 HD) mounted on a Leica M205A stereomicroscope. Pictures were treated in Adobe Photoshop 2024®.

Molecular analyses utilized the data provided in Almeida et al. [4], with the addition of new sequences (Table 1). Total DNA was extracted from specimens using the DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany) with standard man-

ufacturer's protocol. Extraction utilized two legs from each specimen. A voucher number (Table 1) was given for each specimen and its respective sequence. For amplifying the barcode region of the mitochondrial gene cytochrome c oxidase subunit I (COI) [24,25] through polymerase chain reaction (PCR) the following primers were used: LCO-1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO-2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') [26]. PCR was performed with a first denaturation step at 94 °C for 5 min and 5 subsequent cycles of denaturation at 94 °C for 1 min, annealing at 45 °C for 1 min, and extension at 72 °C for 1 min 30 s, followed by 35 cycles of annealing at 50 °C for 1 min, and a final extension step at 72 °C for 5 min. Amplicons were purified with the ExoSAP-ITTM factory protocol. Bidirectional sequencing was performed by the Center for Biological Resources and Genomic Biology (CREBIO/UNESP, Jaboticabal, Brazil). COI sequences used and provided by this study are available on GenBank (Table 1).

Table 1. Specimens with DNA barcoding obtained in this study, with added voucher codes, collection, and GenBank codes of the COI barcode region sequences. M—male; N—nymph.

Species (Life Stage)	Voucher Code	Collection	COI GenBank Accession
<i>Kempnyia krenaki</i> sp. nov. (M)	PL00246	UFVB	PV553778
<i>Kempnyia krenaki</i> sp. nov. (M)	PL00247	UFVB	PV553777
<i>Kempnyia krenaki</i> sp. nov. (N)	DP873	CIACGF	PV553779
<i>Kempnyia umbrina</i> (M)	PL00592	UFVB	PV553774
<i>Kempnyia umbrina</i> (N)	DP869	CIACGF	PV553776
<i>Kempnyia umbrina</i> (N)	DP870	CIACGF	PV553775
<i>Kempnyia vanini</i> (M)	DP918	CIACGF	PV553772
<i>Kempnyia vanini</i> (M)	DP919	CIACGF	PV553773

MEGA 11 software (version 11.0.13) [27] was used for editing chromatograms and attaining consensus sequences. ClustalW [28] in MEGA 11 was used to align the consensus sequences. The distance matrix inference followed the Kimura-2-parameter model using the following parameters in MEGA 11: *d*: Transitions + Transversions, 1000 bootstrap replicates, uniform rates, and pairwise deletion. A total of 56 sequences from the COI barcode region had a 503-base pair (bp) alignment for 23 *Kempnyia* species. Phylogenetic analyses used maximum-likelihood methods performed using RAXML software (version 8) [29]. Searches (1000) for the best tree were made using the GTR+G evolution model and a bootstrap of 5000 replications. Six Perlidae species were used as outgroups, two per genus (*Anacroneuria*, *Enderleina*, and *Macrogynoplax*), along with one species from Gripopterygidae. All species were from South America.

The following methods for species delimitation based on molecular data were used: Automatic Barcode Gap Discovery (ABGD) [30]; Assemble Species by Automatic Partitioning (ASAP) [31]; and Poisson Tree Processes (PTP) [32]. These analyses were performed using online servers (i.e., ABGD, <https://bioinfo.mnhn.fr/abi/public/abgd> ASAP, <https://bioinfo.mnhn.fr/abi/public/asap/>; and PTP/bPTP, <http://species.h-its.org/ptp/>; All accessed on 12 September 2024). The ABGD and ASAP analyses utilized the default parameters of Kimura (K80) TS/TV and a relative gap width of 1.0 (for ABGD). The PTP models utilized the default settings with the resulting maximum-likelihood trees.

3. Results

3.1. Molecular

Based on the COI fragment, the *Kempnyia* species were strongly supported in the maximum-likelihood (ML) analysis (Figure 1). The results of the species delimitation methods (ABGD, ASAP, and PTP) largely aligned with the prior morphological identification,

validating the description of a new species, *K. krenaki* sp. nov. Among the delimitation methods, only PTP showed inconsistency compared with the others. PTP suggested that the adult specimens of *K. krenaki* sp. nov. (PL00246 and PL00247) are not cohesive with the nymph *Kempnyia* sp. (DP873) and should be classified as two distinct species. However, both ABGD and ASAP, along with the consensus of all methods, supported associating the nymph with the adults of *K. krenaki* sp. nov. The results also confirmed the association of the *K. umbrina* nymphs (DP869 and DP870) with the adult (PL00592), allowing description of the immatures.

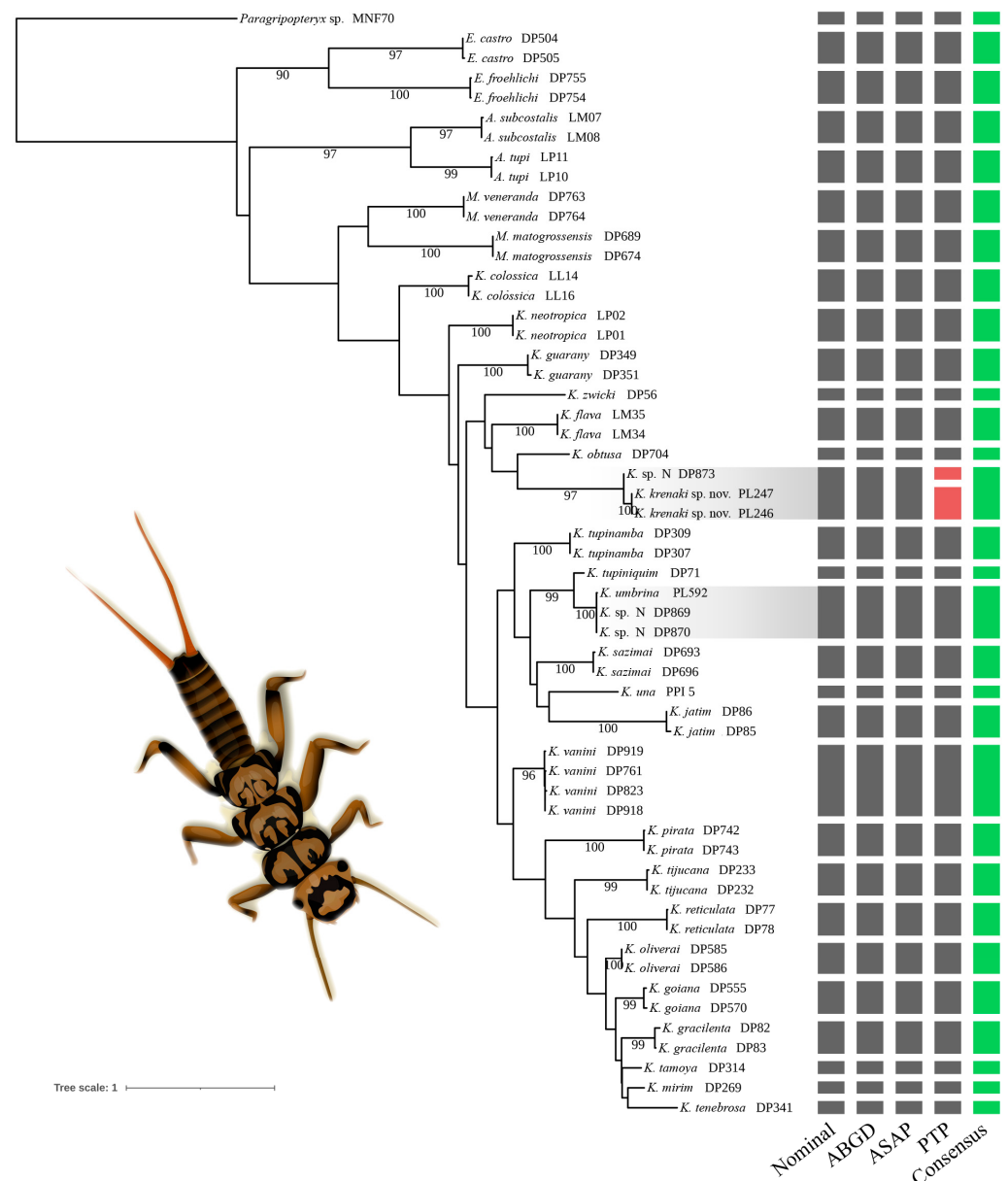


Figure 1. Molecular analysis. Maximum-likelihood tree of *Kempnyia* obtained using mitochondrial cytochrome c oxidase subunit I (COI) sequences (503 bp). Values represent ML bootstrap replications.

The consensus from our molecular analyses provided strong clade support for the morphological identification of the 23 *Kempnyia* species in our sample, and revealed a clear barcoding gap. The minimum intraspecific and interspecific divergences remained consistent with those reported by Almeida et al. [4], at 0% and 8.1%, respectively (Table S1). Meanwhile, the maximum intraspecific and interspecific divergences increased to 1.8%

between *K. krenaki* sp. nov. (PL00246 and PL00247) and (DP873), and 27.7% between *K. umbrina* (PL00592) and *K. mirim* (DP269) (Table S1).

3.2. Morphological Taxonomy

Kempnyia vanini Froehlich, 1988 [21]

Kempnyia vanini Froehlich 1988 [21]: 164, description; Bispo and Froehlich 2004 [9]: 2, record; Stark 2001 [33]: 415, checklist; Stark et al., 2009 [6]: 124, checklist; Froehlich 2010 [34]: 182, catalog; Froehlich 2011a [35]: 3, checklist; Taniguti et al., 2023 [36]: 10, checklist; Almeida et al., 2024 [4]: 19, COI sequence, misidentified as *K. umbrina*.

Material examined. Holotype/MZUSP. Brasil, São Paulo state: Salesópolis, Reserva Casa Grande (Estação Biológica de Boracéia), Ribeirão Coruja, 26.i.1974, at light, 1 male; Estação Biológica de Boracéia, Córrego Venerando, 09.xii.2022, 1 female, Almeida and Taniguti leg (CIACGF–DP761); Córrego Coruja, 22.i.2023, 1 female, Almeida and Taniguti leg. (CIACGF–DP823); 16.i.2024, 2 males, Taniguti and Sabino leg. (CIACGF–DP918, DP919).

Remarks. Froehlich (1988) [21] described *Kempnyia alterosarum*, *K. vanini*, *K. umbrina*, and *K. sazimai*, noting that they are very similar species and likely form a natural group. Since then, identifying these species has been a challenging task, mainly due to the preservation of the holotypes, which has resulted in the loss of the species' maculation patterns. Almeida et al. [4] included in their study two females identified as *K. umbrina* (DP761, DP823). However, after incorporating males into our molecular analysis, identified through comparisons with the holotypes, we determined that the females used by Almeida et al. [4] actually belong to *K. vanini*. Therefore, we provide this minor correction to the work of Almeida et al. [4], and present the real COI barcode region sequence for *K. umbrina*.

Kempnyia umbrina Froehlich, 1988 [21] (Figures 2 and 3)

Kempnyia umbrina Froehlich 1988 [21]: 164, description; Stark 2001 [33]: 415, checklist; Stark et al., 2009 [6]: 124, checklist; Froehlich 2010 [34]: 182, catalog; Froehlich 2011 [35]: 3, checklist; Taniguti et al., 2023 [36]: 10, checklist; Almeida et al., 2024 [4]: 18, misidentification.

Material examined. Holotype/MZUSP. Brasil, Minas Gerais state: Serra do Cipó, Km 126 on road to Conceição do Mato Dentro, 17.xii.1974, at light, 1 male; Brumadinho, Córrego Laranjeira (0 Km), 20°07'39" S 44°08'48" W, 15.xii.2022, 1 male, Rocha leg (UFVB–PL00592); Santana do Riacho, PARNA Serra do Cipó, Córrego Indaiá, 19°15'19" S 43°31'15" W, 27.ix.2023, 2 nymphs, Almeida, Taniguti and Sarmiento leg (CIACGF–DP869, DP870).

Morphometric data. Male (n = 1): forewing length, 9.8 mm.

Nymph description. General color: dark brown. **Head:** Figure 2A,D–H. A yellow stripe between compound eyes and light yellow narrow stripe on anterior margin (Figure 2A). Labrum with a light medial notch (Figure 2A). M-Line inconspicuous, present as a shallow embossing (Figure 2A). Median ocellus absent (Figure 2A). Antennae light yellow. Mandibles pale yellow, outer margin dark brown; three dense brushes of bristles in molar area; dorsal and ventral brushes with bristles longer toward apex, medial brush with similarly sized bristles; five teeth, from the outer to the inner margin second bigger than first and third (Figure 2D,E). Maxillae yellow dorsally, light yellow ventrally (Figure 2G,H). Maxillary palp 3-segmented (Figure 2G,H). Labium brown to very light yellow; submentum and mentum brown, prementum very light yellow; paraglossa with rounded distal margin (Figure 2F). **Thorax:** Figure 2A,B,I,J. Pronotum with posteromedial region yellow (Figure 2A). Meso- and metanotum with light yellow pattern; wing pads darker, apex without bristles (Figure 2B). Legs light yellow (Figure 2I). Femora covered with sparse

bristles; outer margin with a fringe of long hairs (Figure 2I). Tibiae with a row of hairs on the outer margin (Figure 2I). **Abdomen:** Figure 2C,J. Abdomen brown dorsally; ochraceous ventrally (Figure 2C). Cerci yellow with a ring of bristles on each segment; segments differ in shape and size from base to apex (Figure 2J). **Gills:** ASC [1], PSC [1–3], AT [2,3], PT [3], A [1], and SL.

Morphometric data. Nymph (n = 2): head width, 3.10–3.15 mm; body length, 9.8–10.0 mm; length of antenna, 7.8 mm; length of the cerci, 9.0 mm.

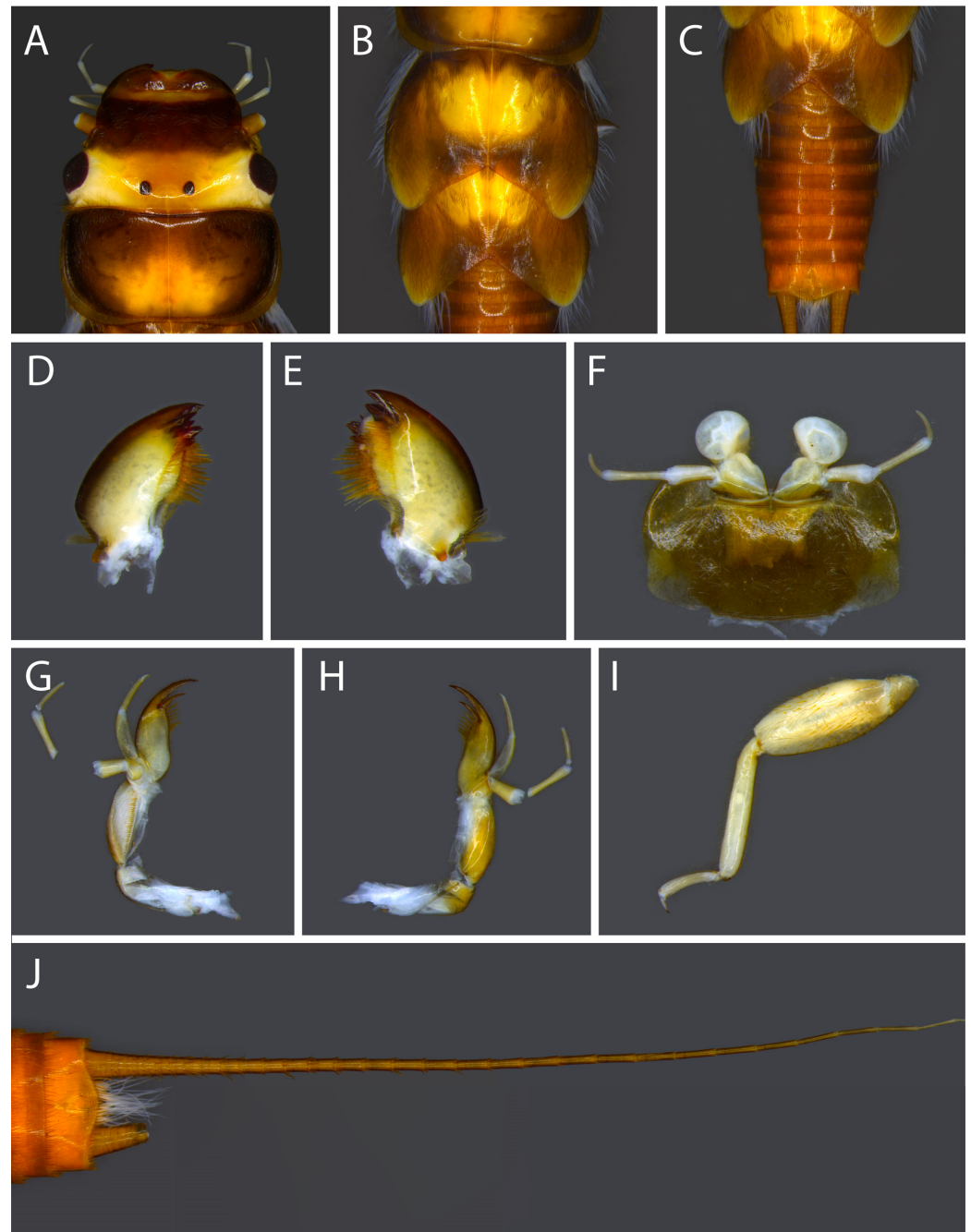


Figure 2. *Kempnyia umbrina* nymph: (A) head and pronotum; (B) thorax; (C) abdomen; (D,E) mandible in ventral and dorsal views; (F) labium; (G,H) maxilla in dorsal and ventral views; (I) foreleg; (J) cercus.

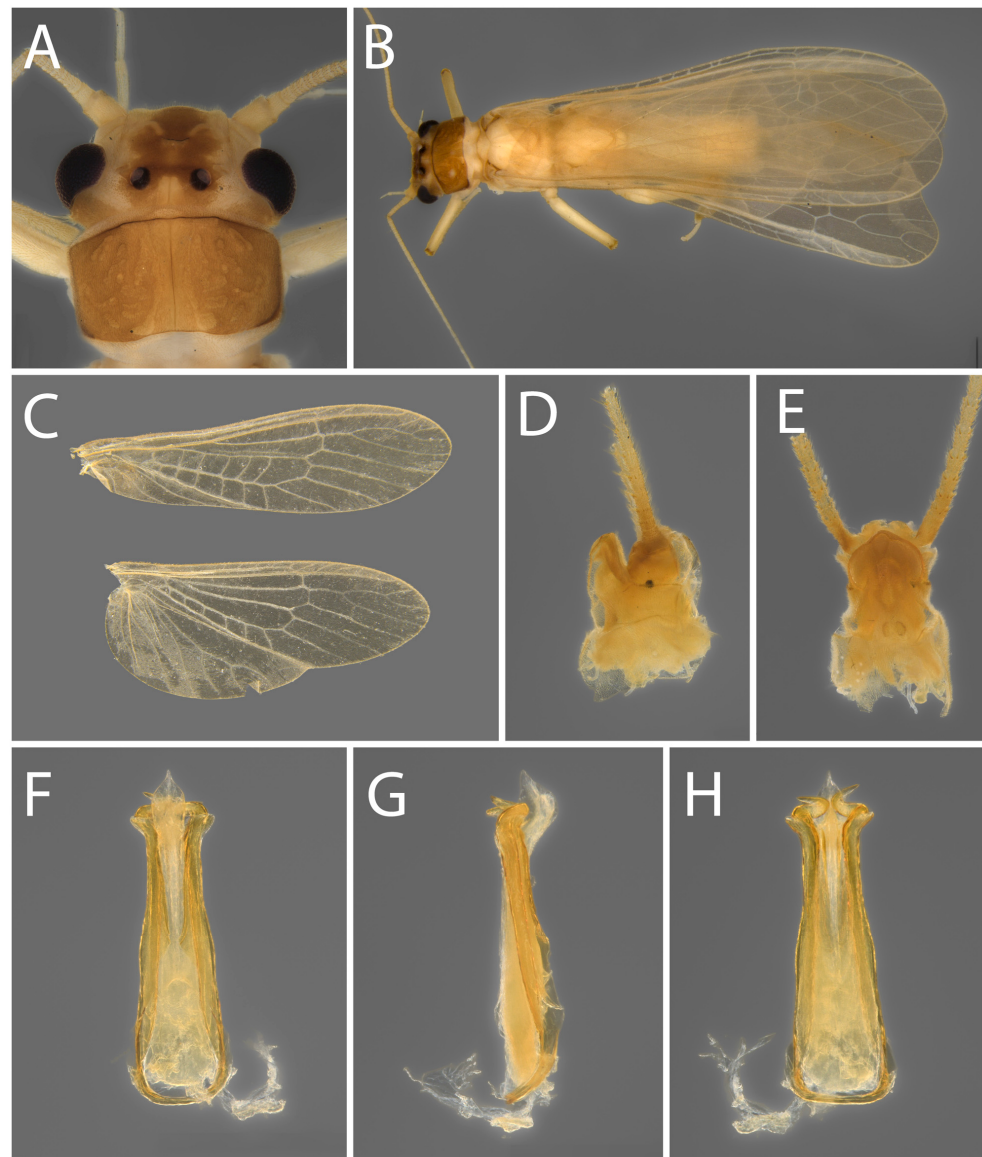


Figure 3. *Kempnyia umbrina* male adult: (A) head and pronotum; (B) habitus; (C) wings; (D,E) hammer in lateral and ventral views; (F–H) penial armature in ventral, lateral, and dorsal views.

Diagnosis. Nymph: Body dark brown with light yellow maculation (Figure 2A–C). Labrum dark brown with a distinctly light medial notch (Figure 2A). Head brown with light yellow stripe between compound eyes and on anterior margin; frontoclypeus with sparse light setae; third ocellus absent (Figure 2A). Labium brown to light yellow; submentum and mentum brown, prementum light yellow (Figure 2F). Abdominal terga with a lateral row of bristles in the posterior margin (Figure 2C).

Remarks. *Kempnyia umbrina* is a species that is challenging to identify. The male adult exhibits morphological similarities with other species, as mentioned in the previous “Remarks” section and by Frøehlich [21]. In this context, after confirming the identification of the collected specimen by comparing it with the holotype, we expanded the known distribution of the species in the state of Minas Gerais and obtained photographs of the species maculation pattern and penial armature to assist with its identification (Figure 3). It is important to make it clear that the specimen identified here is teneral, and may present some variation in the color pattern, according to Almeida and Bispo [20]. Additionally, using the COI marker, we established the association of immature specimens collected from the species’ type locality (Figure 2). The nymph of *K. umbrina* nymph has a head maculation

pattern similar to that of *K. neotropica*, primarily due to a light yellow stripe extending between the compound eyes. However, the median ocellus is absent in *K. umbrina* (it is present in *K. neotropica*), and the body color pattern, except for the head, appears to be unique among the species of *Kempnyia*.

***Kempnyia krenaki* sp. nov.** Gastaldo, Almeida, Salles and Bispo (Figures 4 and 5)
 urn:lsid:zoobank.org:act:A31DD275-0E70-4777-A065-E70D981C71D5

Material examined. Holotype/UFVB (PL00246). BR, MG: Mariana, Rio Gualaxo do Norte, 20°16'28" S, 43°25'29" W, 29.iii.2022, Piva et al. col., 1 male; Same data as holotype, 1 male (Paratype/UFVB PL00247); Santana do Riacho, PARNA Serra do Cipó, Córrego Indaiá, 27.ix.2023, 1 nymph, Almeida, Taniguti and Sarmiento leg (CIACGF-DP873).

Male description. General color: brown. **Head:** Figure 4A,B. Frontoclypeus darker than the rest of the head; lappets darker posteriorly and lighter anteriorly, slightly translucent; M-Line incomplete, severed medially, light brown; median ocellus present, lighter anteriorly and reaching the base of M-Line; tentorial scars light brown (Figure 4A). Vertex with ecdysial suture reaching anterior margin of paired ocelli; area surrounding medial arm of ecdysial suture slightly lighter in color; remainder of vertex darker lateroposteriorly (Figure 4A). Genae anterior margin distinctly dark brown (Figure 4A). Scape and pedicel darker distally; flagellomeres lighter distally (Figure 4A). Maxillary palp with 3 similarly sized palpomeres and a fourth palpomere roughly $\frac{1}{2}$ the length of the proximal palpomeres. Labial palp with 3 palpomeres subequal, distal palpomere slightly shorter. **Thorax:** Figure 4A–C. Pronotum surface uniform in color with distinct rugosities, sutures dark brown (Figure 4A). Longitudinal suture of mesoscutum dark brown. Furcasternum darker than basisternum; Y-ridge distinctly vertical in mesosternum. Femora light proximally, darkening distally with a thin apical dark band; outer margin of fore femora with distinct darker ring at base; outer margin of median and posterior femora with a darker band (Figure 4B). Tibiae uniform in color, darker than femora. Wings with brown venation; light brown membranes; RA and CuA veins slightly darker than remaining veins in both wings; unpigmented streaks in the wing membrane between RP and M veins and under the CuP vein in the forewings, and between M and CuA veins and under the CuP vein in the hind wings (Figure 4C). **Abdomen:** Figure 4D–H. Cercomeres banded in brown and light brown (Figure 4D). Tergum 10 uniform in color and longitudinally divided. Projection of subgenital plate about twice as broad as long; hammer piriform with transversal rugosities; posterior margin of subgenital plate corrugated (Figure 4D,E). Paraproct rounded with a mesoapical tooth. Endophallic spiked pads absent. Penial armature straight and long; basal ring complete and rounded; basal region and apical region of armature similar in size; median ventral process absent; straight row of spiniform projections present laterally in the base of the hooks; dorsal fold short but visible in lateral view in between the hooks; hooks short and regularly curved in a corkscrew manner, with acute tips pointed dorsally; gonopore projected ventrally and curved apically (Figure 4F–H).

Female. Unknown.

Nymph description. General color: light brown. **Head:** Figure 5A,D–H. Light brown, except for a pair of spots over the apex of M-Line, M-line, bilobed spot lateral to posterior ocelli, and vertex pale yellow; covered with sparse darker setae, with pale glabrous markings. Labrum uniformly light brown (Figure 5A). M-Line incomplete and severed medially (Figure 5A). Median ocellus present (Figure 5A). Vertex covered in sparse setae; anterior margin light brown, with pale glabrous area around the medial arm of the ecdysial suture (Figure 5A). Antennae pale yellow with very short setae. Mandibles pale yellow, outer margin brown; three dense brushes of bristles in the molar area; dorsal and ventral brushes with bristles longer toward apex, medial brush with bristles longer toward base; five teeth,

from the outer to the inner margin the second being bigger than the first and the third (Figure 5D,E). Maxillae and labium pale yellow (Figure 5F,G). Maxillary palp 4-segmented (Figure 5A). Paraglossa with rounded distal margin (Figure 5H). **Thorax:** Figure 5B,I,J. Pronotum covered with sparse dark brown setae, with pale yellow glabrous markings (Figure 5B). Meso- and metanotum with pale yellow markings, mostly covered in dense dark brown setae (Figure 5B). Legs pale yellow (Figure 5I,J). Femora covered with sparse bristles; longitudinal line without setae in the anterodorsal surface; high density of thick bristles near inner and outer margins; outer margin with a fringe of long hairs (Figure 5I,J). Tibiae with a row of hairs in the outer margin (Figure 5I,J). **Abdomen:** Figure 5C. Abdomen brown dorsally; light brown ventrally; last abdominal tergum with a light brown medial glabrous spot. Cerci yellow with a ring of bristles on each segment; segments differ in shape and size from base to apex. **Gills:** ASC [1], PSC [1–3], AT [2,3], PT [3], A [1], and SL.

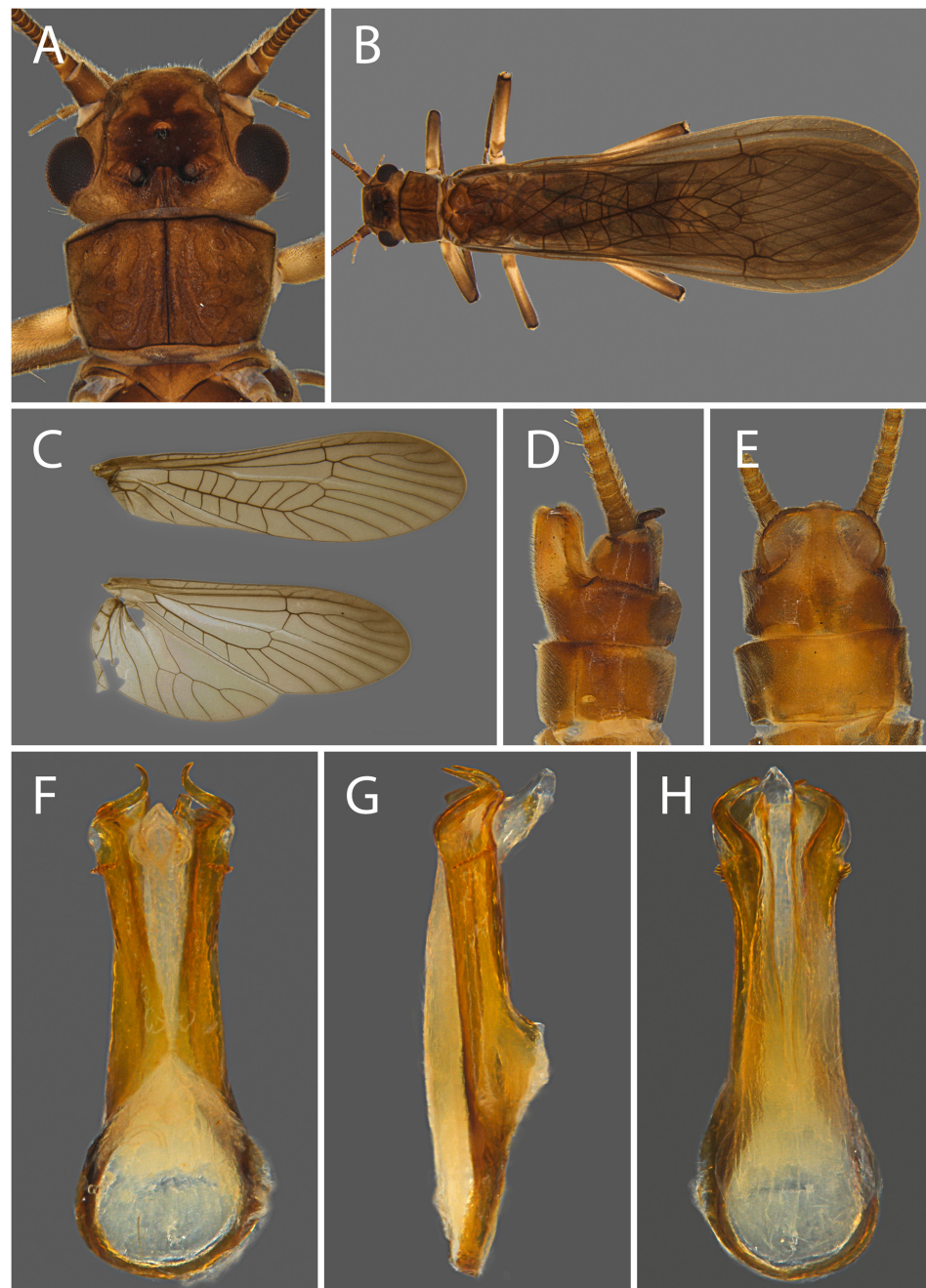


Figure 4. *Kempnyia krenaki* sp. nov. male adult: (A) head and pronotum; (B) habitus; (C) wings; (D,E) hammer in lateral and ventral views; (F–H) penial armature in ventral, lateral, and dorsal views.

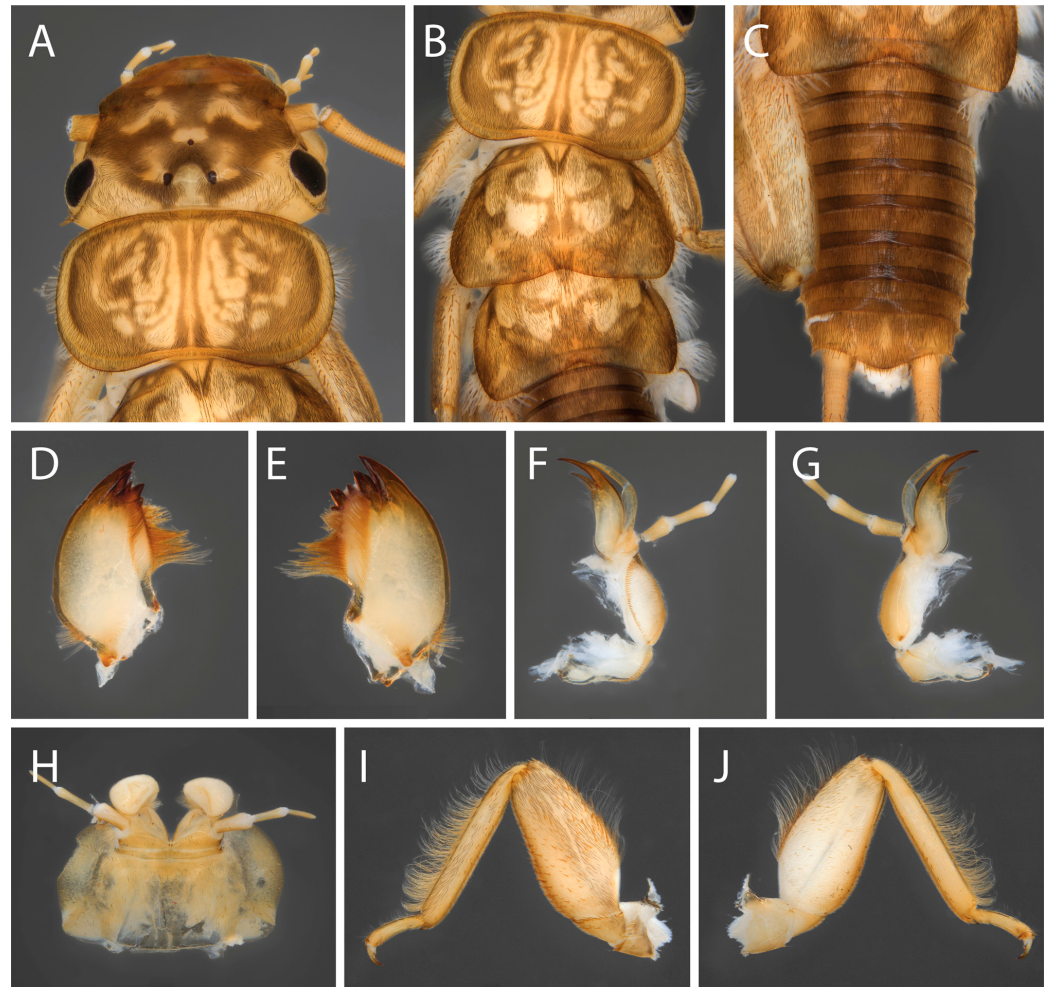


Figure 5. *Kempnyia krenaki* sp. nov. nymph: (A) head and pronotum; (B) thorax; (C) abdomen; (D,E) mandible in ventral and dorsal views; (F,G) maxilla in dorsal and ventral views; (H) labium; (I,J) foreleg in dorsal and ventral views.

Morphometric data. Male ($n = 2$): forewing length, 10.8–11.9 mm. Nymph ($n = 1$): head width, 3.5 mm; body length, 12.1 mm.

Diagnoses. Male: Brown in overall color, third ocellus present and with a distinctly light anterior margin (Figure 4A). Conspicuous darker ring in the base of the external margin of anterior femur (Figure 4B). Penial armature with a straight row of spiniform projections laterally in the base of the hooks, dorsal fold short but visible in lateral view (Figure 4G). **Nymph:** Light brown body with sparse darker setae, with distinct pale glabrous markings. A pair of light spots anterior to M-Line; M-Line pale and incomplete, severed medially; third ocellus present, with darker intraocellar region in the frontoclypeus; pale glabrous spot lateral to posterior ocellus, with bilobed appearance (Figure 5A). Vertex pale yellow and covered with sparse setae, but with anterior margin light brown; clear glabrous area around the medial arm of the ecdysial suture (Figure 5A).

Remarks. *Kempnyia krenaki* sp. nov. differs morphologically from other species primarily in the overall shape of the penial armature. The combination of a pair of straight rows of spiniform projections laterally at the base of the hooks and a short, rounded dorsal fold in the penial armature is unique among *Kempnyia* (Figure 4A,B). Only *K. flava* Klapálek, 1916 [12] shares the same row of spiniform projections; however, it has a pointed dorsal fold, whereas in *K. krenaki* sp. nov., the dorsal fold is rounded. Additionally, *K. flava* is a bright yellow species, while *K. krenaki* is brown. The external margin of the anterior femur,

with a conspicuous darker ring at the base, distinguishes it from all other described species of the genus (Figure 4B). Additionally, the nymph's maculation pattern also appears to be unique among the known nymphs of the genus (Figure 5).

Etymology. Eponym. The name honors the Krenak people from Brazil, who still live in indigenous areas in Minas Gerais state.

4. Discussion

Utilizing molecular data as an additional taxonomic tool has proven to be a reliable and important methodology in an integrative approach, especially after the publication of the DNA barcode technique [24,25]. For insects, morphological taxonomy alone often does not solve particular problems such as potential synonymy of similar species or semaphoront association. Particularly for immature description in Plecoptera, a group in which nymphs often do not resemble their adult counterparts and lack the structures most utilized in species descriptions, association without molecular data frequently is only possible through rearing, a cumbersome method for sensitive insects such as stoneflies in a warm country like Brazil.

Molecular tools have made nymphal associations relatively straightforward for Plecoptera in Brazil [4,8,19,20,37,38], providing great insight into species biology and a clear way to lessen the Haeckelian shortfall [39]. Over the past decade, *Kempnyia* has shown itself as a model genus for integrating molecular data into the taxonomy and systematics of Plecoptera in Brazil, with more than half of its species having DNA sequences registered in GenBank [4,8,20].

As a pioneer in DNA studies in Brazil [19], *Kempnyia* has progressively had its systematic issues revealed through this approach, serving as a robust example of integrative taxonomy for other Plecoptera genera in Brazil and, potentially, other aquatic insect groups. In the study of *Kempnyia*, for instance, the barcode region of the COI mitochondrial gene has proven particularly useful for species delimitation methods [4], supporting the description of new species, including on the basis of females (e.g., *Kempnyia una* Almeida and Bispo, 2024 [4]), which would not have been described based solely on morphology. Moreover, the integration of morphological and molecular data has facilitated differentiation between teneral and fully mature adults, reducing taxonomic inconsistencies and reshaping the understanding of the Perlidae morphology [20]. The application of the same approach initially used for *Kempnyia* appears to be effective for other perlid genera as well [20,37,38,40,41].

With this study, the number of known and described *Kempnyia* species has risen to 41, 26 of which have DNA sequences registered in GenBank (Table 2). Among the sixteen remaining known species, at least eight are considered problematic due to their outdated descriptions and the fact that they have never been recollected (Table 2). In this context, until the problematic species are reassessed, new COI sequences are expected to emerge for the other eight remaining species and for newly described species. Among the thirty-three species considered non-problematic, only ten have all semaphoronts described, twelve have both males and females described, one was described based on a male and a nymph, and eleven were described solely based on males. Additionally, only 11 species have associated and described nymphs (Table 2). Over time, as the *Kempnyia* sequence database continues to grow, studies like this will become increasingly common, and we hope that the Haeckelian shortfall will gradually be reduced.

Although we are working towards a fairly robust knowledge of *Kempnyia* species and their semaphoronts, a few challenging species groups remain. Some groups of species have very similar species and descriptions, such as the *K. colossica* group (*K. brasiliensis*, *K. colossica*, and *K. guassu*, to name a few) [20,42] and the *K. vanini* group (*K. alterosarum*, *K. tupinamba*, and *K. sazimai*, to name a few) [21,43]. As such, there is still much to study

about *Kempnyia*, but we are making progress toward a deeper understanding of species identity and delimitation in certain species groups, and integration of molecular data in the study of these groups is sure to help elucidate the differences and similarities between their species.

Table 2. Current taxonomic knowledge of *Kempnyia* species, including COI sequences available in GenBank. M—male; F—female; N—nymph. Distribution follows Brazilian state codes: Bahia (BA), Espírito Santo (ES), Goiás (GO), Minas Gerais (MG), Paraná (PR), Rio de Janeiro (RJ), Rio Grande do Sul (RS), Santa Catarina (SC), São Paulo (SP), and Tocantins (TO).

Species	COI	Life Stages			Distribution
		M	F	N	
<i>K. alterosarum</i> Froehlich, 1988 [21]	•	•	•	•	BA; MG; RJ.
<i>K. auberti</i> Froehlich, 1996 [42]		•			PR; SP.
* <i>K. brasílica</i> (Navás, 1932) [44]			•		RJ.
<i>K. brasiliensis</i> (Pictet, Kollar. Mss., 1841) [45]		•			Brazil.
<i>K. colossica</i> (Navás, 1934) [7]	•	•	•	•	MG; PR; RJ; SC; SP.
<i>K. couriae</i> Avelino-Capistrano, Barbosa and Takiya, 2016 [8]	•	•	•	•	RJ.
<i>K. flava</i> Klapálek, 1916 [12]	•	•	•		ES; MG; RJ; SP.
<i>K. goiana</i> Bispo and Froehlich, 2004 [9]	•	•			GO; TO.
<i>K. gracilentia</i> (Enderlein, 1909) [10]	•	•	•	•	BA; ES; MG; RJ; SP.
<i>K. guarany</i> Almeida, Gonçalves and Bispo, 2024 [4]	•	•			SC.
<i>K. guassu</i> Froehlich, 1988 [21]	•	•	•		RJ.
<i>K. jatim</i> Froehlich, 1988 [21]	•	•	•		BA; ES; MG; RJ; SP.
* <i>K. kaingang</i> Froehlich, 2011 [35]		•	•		SC.
* <i>K. klugii</i> (Pictet, 1841) [45]		•			Brazil.
<i>K. krenaki</i> sp. nov. Gastaldo, Almeida, Salles and Bispo	•	•		•	MG.
<i>K. mirim</i> Froehlich, 1984 [46]	•	•	•		MG; SC; SP.
<i>K. neotropica</i> (Jacobson and Bianchi, 1905) [11]	•	•	•	•	BA; ES; GO; MG; PR; RJ; RS; SC; SP.
<i>K. obtusa</i> Klapálek, 1916 [12]	•	•	•	•	ES; MG; RJ; SP.
* <i>K. ocellata</i> Froehlich, 2011 [35]		•			RJ.
<i>K. oliveirai</i> Bispo and Froehlich, 2004 [9]	•	•	•		GO.
* <i>K. petropolitana</i> (Navás, 1929) [47]			•		RJ.
<i>K. pinhoi</i> Froehlich, 2011b [43]		•	•		SC.
<i>K. pirata</i> Froehlich, 2011 [35]	•	•	•		ES; RJ; SP.
<i>K. puri</i> Avelino-Capistrano, Souza and Nessimian, 2013 [48]		•			RJ.
<i>K. reichardti</i> Froehlich, 1984 [46]		•	•		MG; SP.
* <i>K. remota</i> (Banks, 1920) [49]			•		RJ.
<i>K. reticulata</i> (Klapálek, 1916) [12]	•	•	•	•	ES.
<i>K. sazimai</i> Froehlich, 1988 [21]	•	•			MG.
<i>K. serrana</i> Navás, 1936 [50]		•			ES; RJ; SP.
* <i>K. sordida</i> Klapálek, 1916 [12]			•		Brazil.
<i>K. tamoya</i> Froehlich, 1984 [46]	•	•	•		SP.
* <i>K. taunayi</i> Navás, 1936 [50]			•		RJ.
<i>K. tenebrosa</i> Klapálek, 1916 [12]	•	•	•		RJ; SC.
<i>K. tijucana</i> Dorvillé and Froehlich, 1997 [13]	•	•	•	•	RJ.
<i>K. tupinamba</i> Froehlich, 2011 [35]	•	•	•		SP.
<i>K. tupiniquim</i> Almeida, Gonçalves and Bispo, 2024 [4]	•	•			ES.

Table 2. Cont.

Species	COI	Life Stages			Distribution
		M	F	N	
<i>K. umbrina</i> Froehlich, 1988 [21]	•	•	•	•	MG; RJ; SP.
<i>K. una</i> Almeida and Bispo, 2024 [4]	•		•		SP.
<i>K. vanini</i> Froehlich, 1988 [21]	•	•	•		GO; MG; SP.
<i>K. varipes</i> Klapálek, 1916 [12]		•	•	•	RJ.
<i>K. zwicki</i> Almeida, Gonçalves and Bispo, 2024 [4]	•	•			ES.

* Problematic species.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d17060416/s1>, Table S1: Estimates of Evolutionary Divergence between Sequences. References [27,51] are cited in the supplementary materials.

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Data Availability Statement: The DNA sequences generated in the present study were deposited in GenBank and can be accessed using the codes shown in Table 1.

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