First record of the genus *Claudiotendipes* Andersen, Mendes & Pinho (Diptera: Chironomidae) from Colombia, with notes on its altitudinal range and COI barcode

Narcís Prat[1] and Diana C. Hoyos-Jaramillo[1,2]

[1]*FEHM-Lab (Freshwater Ecology, Hydrology and Management), Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona (UB), Barcelona, Spain.*
E-mail: nprat@ub.edu, dicahoyosja@ub.edu
[2]*Fundación FUNMAJO, EBA, RAIEC, Biodiversity Branch, Boyacá, Colombia.*

Abstract

The genus *Claudiotendipes* Andersen, Mendes & Pinho (2017) includes species from Brazil, Costa Rica, and Bolivia typically occurring at altitudes between 220 and 1,525 m a.s.l. To date, no records of the genus exist for Colombia, and molecular data for the group remain absent from public databases. During a large-scale survey of 52 high Andean streams sites spanning Peru, Ecuador, and Colombia (altitudinal range: 2,200–4,500 m a.s.l.) we sorted hundreds of chironomid specimens and collected a single larva exhibiting this unique morphotype. This specimen came from the Cauca River basin (Caldas, Colombia) at 2,515 m a.s.l. We examined the specimen morphologically and sequenced the barcode region of the mitochondrial cytochrome c oxidase subunit I (COI) gene. Morphological characters—including the six-segmented antenna with alternate Lauterborn organs and the mentum with four pale median teeth—place the specimen within *Claudiotendipes*. This record extends the known altitudinal range of the genus above 2,000 m a.s.l. and represents the first record for Colombia. The partial COI sequence (GenBank: PX924818) provides the first DNA barcode for the genus. Maximum Likelihood analysis confirms its position as a distinct lineage within the Chironomini, clustered in a clade with *Oukuriella* and an unidentified Chironomidae sequence, though the deeper nodes supporting these relationships remain weak. This study contributes to the knowledge of Neotropical chironomid biodiversity and provides a molecular baseline for future taxonomic and ecological studies of *Claudiotendipes* in the Andes.

Introduction

The genus *Claudotendipes* (Diptera: Chironomidae) was erected by Andersen et al. (2017) based on material collected from Atlantic rainforest streams in Brazil, at altitudes ranging from 220 to 1,450 m a.s.l. Larvae were obtained from leaf litter in first-order forested streams. The type species, *Claudiotendipes froehlichi*, is the only species for which all life stages (adult male, pupa and larva) are known. In the same study, a second species, *C. epleri*, was described based solely on the adult male and is currently known from Costa Rica (Guanacaste Conservation Area, 1,000 m a.s.l.). Subsequently, Andersen (2018) described *C. bolivianus*, also known only from the adult male, collected with a drift net in a stream in the La Paz district, Bolivia, at 1,525 m a.s.l. More recently, Pinho and Fusari (2023) described *C. gilbertoi* from Brazil, based on material collected at the Serra Geral mountain range in the state of Rio Grande do Sul.

Larvae of *Claudiotendipes* possess a long, six-segmented antenna with alternate Lauterborn organs, a plumose S1 seta, and a mentum with four pale median teeth (the central pair being significantly reduced). The presence of three inner mandibular teeth, the specific shape of the ventromental plates, and a simple pecten epipharyngis with approximately 12 teeth distinguish this genus from the morphologically similar *Omisus* and *Paratendipes* (Andersen et al. 2017).

During the BIQURA project (https://www.fehm.cat/rios-altoandinos/), in 2011, hundreds of Chironomidae larvae were collected from 52 high Andean streams sites across Peru, Ecuador and Colombia. Among this material, we recovered a single *Claudiotendipes* larva from a stream in Colombia (Magallo River, Campoalegre sub-basin) at 2,515 m a.s.l. At the time of collection, this larva was tentatively identified as *Microtendipes*, as the genus *Claudiotendipes* had not yet been erected. A recent re-examination of the larval head capsules from the material allowed us to confidently assign this specimen to *Claudiotendipes*. This finding represents the first record of the genus from Colombia and extends its known altitudinal range which was previously restricted to elevations below 1,525 m a.s.l.

Andersen et al. (2017) investigated the relationships of *Claudiotendipes* with morphologically similar genera using a comprehensive morphological phylogenetic analysis. In their study, *Claudiotendipes* was recovered as closely related to *Oukuriella*, *Beardius*, *Endotribelos* and *Sigmoitendipes*, whereas *Paratendipes* and *Microtendipes*, despite larval similarities, were placed more distantly. The aim of the present study is to describe the larval morphology of the Colombian specimen and to contribute to the systematics of *Claudiotendipes* and related genera by providing the first cytochrome c oxidase subunit I (COI-5P, mtDNA) sequence for the genus and comparing its molecular placement with the previously proposed morphology-based phylogeny of Andersen et al. (2017). This integrative approach provides new molecular data for a poorly known Neotropical genus and contributes to ongoing efforts to link larval morphology and DNA barcodes in Chironomini.

Material and methods.

Sampling site and sampling methods.

We conducted sampling in the Magallo River (Caldas, Colombia; Campoalegre sub-basin, Cauca River basin: 4.853477°N, 75.531267°W) at an altitude of 2,515 m a.s.l. on 29 August, 2011. Using a Surber net, we collected benthic macroinvertebrates following the protocol of Villamarín et al. (2013). We obtained a composite sample from various microhabitats, including rocks, mosses, leaf litter, and fine sediments. In the field, we sorted larval Chironomidae and preserved them in 96% ethanol.

In the laboratory, we examined the larvae under a stereomicroscope and assigned them to morphotypes using the identification keys of Prat et al. (2012). We slide-mounted representative individuals of each morphotype in Euparal for morphological examination and utilized the remaining body parts for DNA extraction. Mounted material is deposited in the collection of the senior author and the Centre de Recerca de Biodiversitat Animal (CRBA), University of Barcelona.

DNA extraction, PCR amplification and sequencing

We followed the DNA extraction, PCR amplification, and sequencing protocols described by Prat et al. (2013). Among the sorted material we identified a single larva (n = 1) representing a unique morphotype belonging to the genus *Claudiotendipes*.

We obtained, edited, and assembled a 497 bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI-5P, mtDNA) gene from this specimen using the primers C1-J-1718 and C1-N-2191 (mt6/Nancy) (Simon et al. 1994) in Geneious Prime 2024.0.5 (Dotmatics; http://www.geneious.com). To confirm its identification, we conducted similarity searches against the NCBI and BOLD databases, incorporating the morphological phylogeny proposed by Andersen et al. (2017) into our taxonomic assessment.

We retrieved available COI sequences for closely related genera from GenBank and BOLD Systems. Due to limited public data, the analysis only included sequences from *Oukuriella*, *Endotribelos*, *Paratendipes*, *Microtendipes*, and *Sergentia*; *Polypedilum* was selected as the outgroup. Although Andersen et al. (2017) suggested *Sigmoitendipes* and *Beardius* as closely related to *Claudiotendipes*, no COI barcode sequences were available for these genera in public databases, and they were consequently excluded from the molecular analysis.

We aligned the sequences using the command-line version of MAFFT (Katoh and Standley, 2013) and subsequently checked for stop codons and trimmed the alignments in Mesquite v.3.70 (Maddison and Maddison, 2021). Although we initially sequenced a 670 bp fragment, we trimmed the final alignment to 426 bp to match shorter sequences from related genera in GenBank and BOLD, ensuring a high-quality matrix for comparison. Finally, to infer phylogenetic relationships between *Claudiotendipes* and the reference sequences retrieved from GenBank, we performed a Maximum Likelihood (ML) analysis in IQ-TREE. The best-fit substitution model and optimal partitioning scheme were automatically selected using the ModelFinder (MFP) feature (Kalyaanamoorthy et al., 2017). To ensure a robust tree search, heuristic settings were optimized by increasing search intensity parameters (-ninit 500 -nbest 25 -nstop 500 -allnni). Nodal support was evaluated using 1000 ultrafast bootstrap (UFB) replicates (Hoang et al., 2018). To evaluate genetic divergence, pairwise genetic distances among the included taxa were calculated in MEGA 12 (Kumar et al., 2024) utilizing the Kimura 2-parameter (K2P) substitution model. Standard errors were computed through 1,000 bootstrap replicates, and missing data or alignment gaps were handled using the pairwise deletion option to maximize the retention of informative sites.

Results

Characteristics of the larva

The single larval head capsule examined (n = 1), exhibits a uniform yellowish coloration, including the post-occipital margin, and measures approxi-

mately 300 µm in length. The postmentum reaches 105 µm, while the antenna—with a ratio (AR) of 0.68—possesses segments of 88.6, 50.2, 57.9, 5.5, 10.2, and 6.2 µm (A1–A6). The antennal blade measures 86 µm, and the ring organ sits 57.6 µm from the base of the first segment. Seta S1 appears plumose and reaches 21.5 µm in length.

Regarding the mouthparts, the bifid premandible (46.7 µm long) bears an internal brush. The mandible measures 83 µm and features three inner teeth (12.1, 8.1, and 7.3 µm, respectively) and a single 6.8 µm dorsal tooth; the subdental seta reaches 22 µm, and the seta interna displays two branches. The 81 µm-wide mentum possesses eight pairs of teeth: the four median teeth appear pale (with the central pair significantly reduced), whereas the lateral teeth show a brownish tint. Tooth lengths range from 4.3 to 13.8 µm (specifically: 4.3, 8.4, 6.8, 13.8, 9.9, 8.5, 6.9, 6.9 µm). The curved ventromental plates measure 80 µm in width and maintain a 34.2 µm separation (Fig. 1)

Due to the specimen's condition, we could not confidently assess the pecten epipharynx; similarly, we utilized the abdomen for DNA extraction, rendering it unavailable for morphological study. Overall, this specimen closely resembles *Claudiotendipes froehlichi* (Andersen et al. 2017) (Fig. 1). However, a strict morphometric comparison (Table 1) shows remarkable diagnostic discrepancies: our specimen exhibits a shorter first antennal segment (A1= 88.6µm vs.113µm in *C. froehlichi*) but substantially longer second and third segments (A2 = 50.2 µm and A3 = 57.9 µm vs. 35 µm and 37 µm respectively). This structural elongation of the intermediate segments results in a drastically lower antennal ratio (AR = 0.68) compared to the Brazilian type species (AR = 1.06). Additionally, the ventromental plates in the Colombian larva are notably wider (80 µm vs. 66 µm).

Distribution and Ecology

We collected the single Colombian *Claudiotendipes* larva from the Magallo River (Campoalegre sub-basin) a small, unpolluted stream at 2,515 m a.s.l. in a substrate sample dominated by mosses. At the time of sampling, the water reached a temperature of 12.8ºC, with a conductivity of 72 µS and a flow of 40.24 L/s. Dissolved oxygen showed high oversaturation (18.5 mg/L; 183%), and the water remained slightly alkaline (pH 7.58) with low concentrations of calcium (6.38 mg/L) and sodium (5.36 mg/L). The riparian habitat showed pristine conditions, with a well-developed forest (QBR = 100), and the instream habitat maintained excellent quality (IHF = 82) (Acosta et al. 2009).

Barcode analysis

The Maximum Likelihood (ML) analysis—based on the COI barcode trimmed to 426 bp—included the Colombian *Claudiotendipes* larva and 28 sequences from related genera (Fig. 2). The resulting ML tree recovered well-supported clusters (UFB ≥ 95%) for most genera, including *Paratendipes*, *Oukuriella*, *Sergentia*, and *Microtendipes*. In contrast, the Colombian *Claudiotendipes* specimen emerged as a distinct lineage characterized by a relatively long branch.

The *Claudiotendipes* sequence did not cluster within any other genus included in the analysis. Instead, in the ML tree, it was recovered within a

Table 1. Morphological comparison between the *Claudiotendipes* larva from Colombia and *C. froehlichi* (Andersen et al. 2017). All measurements are in micrometers (µm) unless otherwise stated.

Character	***Claudiotendipes* sp. (Colombia)**	***Claudiotendipes froehlichi* (Andersen et al. 2017)**
Head capsule length (µm)	~300	340
Postmentum length (µm)	105	115
Antennal segment A1 (µm)	88.6	113
Antennal segment A2 (µm)	50.2	35
Antennal segment A3 (µm)	57.9	37
Antennal segments A4–A6 (µm)	5.5, 10.2, 6.2	8, 17, 10
Antennal ratio (AR)	0.68	1.06
Mandible length (µm)	83	94
Mental teeth (pairs)	8	8
Mentum width (µm)	81	83
Ventromental plate width (µm)	80	66
Distance between plates (µm)	34.2	35

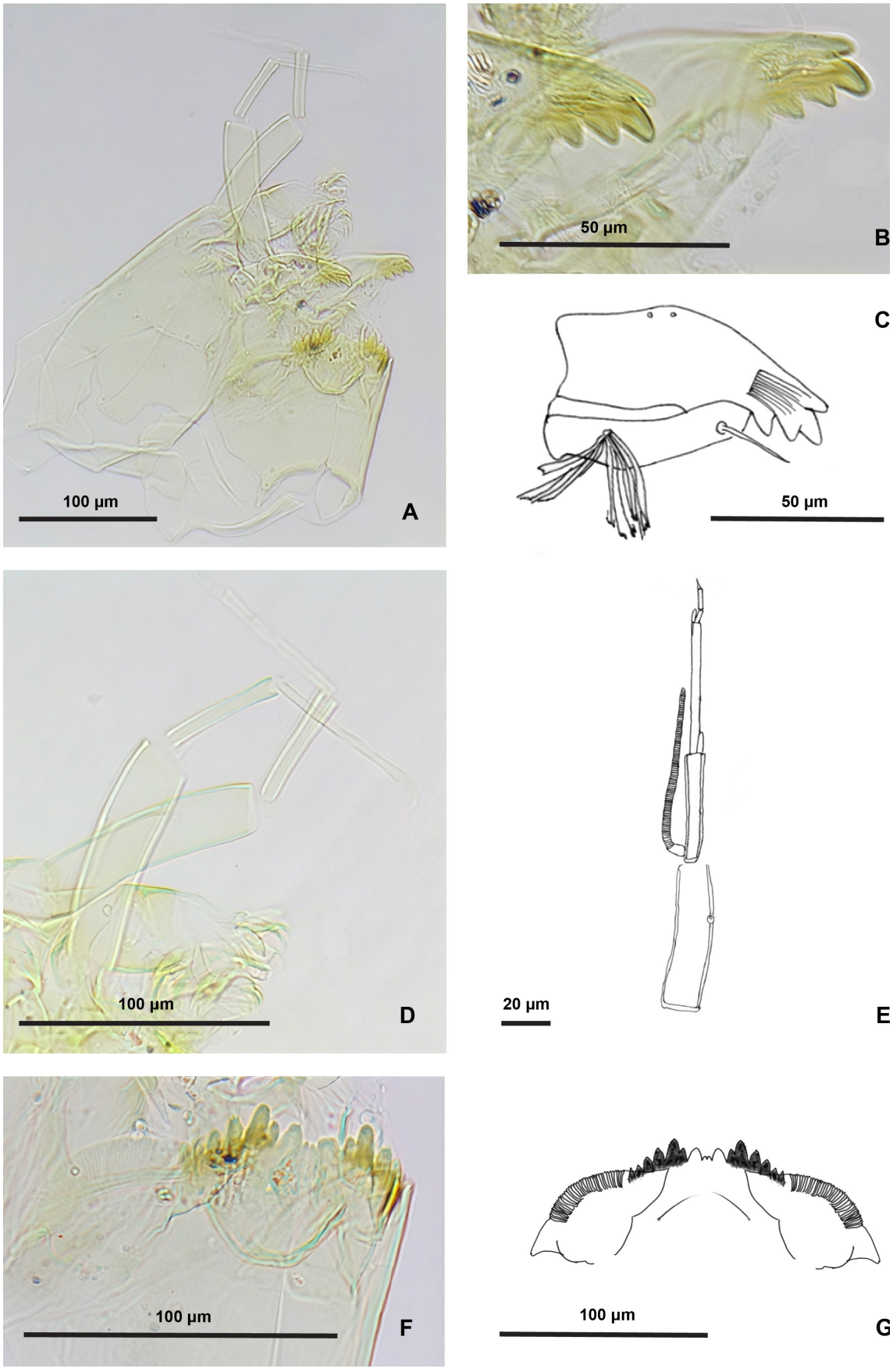

Figure 1. Larval structures of *Claudiotendipes* sp. from Colombia. A) head capsule, ventral view; B-C) antenna; D-E) mandible; F-G) mentum and ventromental plates.

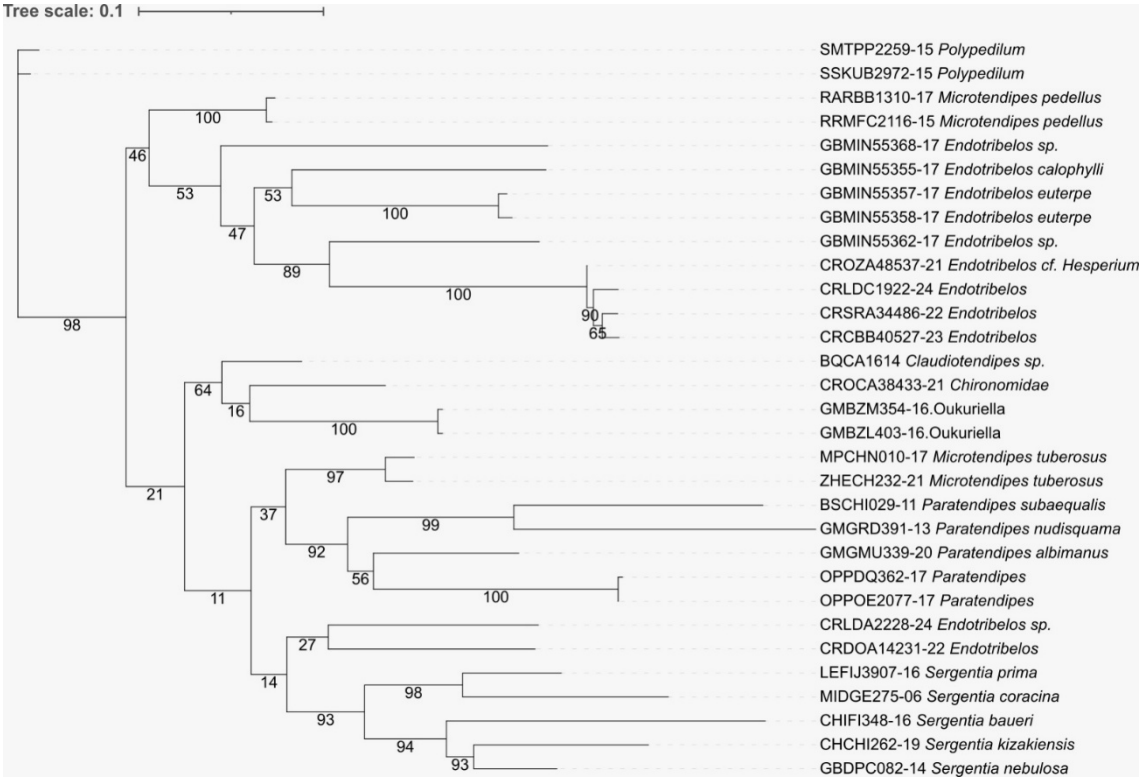

Figure 2. Maximum Likelihood (ML) tree of *Claudiotendipes* and related Chironomini genera based on COI barcode sequences (426 bp). Node values represent ultrafast bootstrap (UFB) support based on 1,000 replicates. The tree was rooted using *Polypedilum* as the outgroup following the morphology-based phylogeny of Andersen et al. (2017).

single clade comprising *Oukuriella* and an unidentified Chironomidae sequence, although the deeper nodes lacked robust support. Within this group, the *Oukuriella* sequences formed a highly supported monophyletic cluster (100% UFB), which then grouped with the unidentified Chironomidae lineage; *Claudiotendipes* emerged as the sister taxon to this combined assemblage with low to moderate support (64% UFB). Pairwise K2P genetic distances between the Colombian larva and the database sequences ranged from 9.8% to 20.9% (see Supplementary Table S1 for the full distance matrix). The unidentified Chironomidae specimen showed the lowest divergence (9.8%), followed by a *Microtendipes* lineage and the outgroup *Polypedilum* (~11.5%). Conversely, *Sergentia* (20.9%), *Endotribelos* (20.2%), and *Paratendipes* (19.8%) showed the highest genetic distances. Despite their proximity in the ML tree, *Claudiotendipes* and *Oukuriella* maintained a mean genetic distance of 11.8%.

Discussion

The morphology and molecular placement of the Colombian larva align with the phylogenetic position and descriptive framework established by Andersen et al. (2017). Although our analysis and biological notes rely on this single specimen (n = 1), its recovery is of significant interest because it was the only individual of this morphotype found across an extensive regional survey of 52 high Andean streams. The characteristic mentum guided our initial identification, as it features four pale median teeth—with a distinctly reduced central pair—and curved, striated ventromental plates (Fig. 1). Antennal structure further confirms this identity, specifically the third segment being longer than the second (A3 > A2) and the notably reduced fourth and fifth segments. By combining these characters, we can confidently distinguish the Colombian specimen from morphologically similar taxa such as *Paratendipes*, *Microtendipes*, and *Omisus* (Andersen et al. 2017). However, as our study relies on a single larval specimen, we cannot yet determine whether the observed differences in antennal measurements (A1 and AR) represent natural intraspecific variation or indicate a distinct, undescribed species. Confirming the taxonomic status of the Colombian population will require additional sampling of both larvae and adult life stages to provide a more comprehensive morphological and molecular assessment.

To date, the genus *Claudiotendipes* has been recorded in only four countries—Brazil, Costa Rica, Bolivia, and now, Colombia—with global litera-

ture records accounting for fewer than 25 specimens (23 males, one pupal exuviae, and one larval exuviae) (Andersen et al. 2017; Andersen 2018; Pinho and Fusari 2023). Consistently, we detected this genus in only one out of 52 sampled sites during our extensive BIQURA survey, yielding a single larval specimen. These combined metrics clearly demonstrate that *Claudiotendipes* populations are highly localized and exceptionally low in abundance across the Neotropics, with *C. froehlichi* from Brazil comprising most of the documented individuals. Regarding collection methods, light or Malaise traps yielded most of these known adults; however, a notable exception includes the *C. bolivianus* holotype, which Fittkau intercepted using a drift net in a Bolivian stream at 1,525 m a.s.l. (Andersen 2018).

Although physico-chemical profiles for previous collection sites remain undocumented, macrohabitat descriptions reveal clear ecological parallels with our Colombian locality (Andersen et al. 2017; Andersen 2018). *Claudiotendipes* exhibits a strong affinity for pristine, shaded, low-order lotic systems with high microhabitat complexity. In Brazil, larvae inhabit leaf litter debris in first-order forested streams within the Atlantic Forest, spanning from lowlands (220 m) to montane areas (over 1,450 m a.s.l.) (Andersen et al. 2017), whereas in Costa Rica and Bolivia, the genus occurs in small, shallow, high-quality streams. Our Colombian specimen from the Magallo River fits this profile perfectly, inhabiting an unpolluted, highly oxygenated stream (183% saturation, 12.8 °C) characterized by pristine riparian conditions (QBR = 100), excellent instream quality (IHF = 82), and dense instream mosses. This combination of wide Neotropical distribution and persistent low-density mirrors other specialized Chironomini like *Oukuriella* and *Stenochironomus*, whose larvae are exceptionally rare in standard benthic samples due to strict microhabitat restrictions (Borkent 1984; Fusari et al. 2014; Bellodi et al. 2016). Thus, *Claudiotendipes* likely acts as an environmental specialist tracking physically intact, oxygenated headwaters rather than being constrained by strict altitudinal boundaries (220–2,515 m a.s.l.). We suspect the genus is widely distributed across other South American basins at lower altitudes but remained undetected in the rest of the BIQURA project due to the high-altitude threshold targeted (>2,000 m a.s.l.). Furthermore, the widespread tendency in South American water quality assessments to identify Chironomidae larvae only to the family or subfamily level has historically masked the true geographic range of such low-abundance lineages (Zúñiga and Cardona 2009; Hamerlík et al. 2022).

Molecular phylogenetic studies of Neotropical Chironomini remain limited (Silva and Farrell, 2017; Hoyos-Jaramillo et al. 2025) and currently include only a subset of the genera morphologically close to *Claudiotendipes* (Cranston et al. 2012). Moreover, the lack of publicly available COI barcode sequences for several relevant genera, notably *Beardius* and *Sigmoitendipes*, restricts comprehensive molecular comparisons. Consequently, COI reference data for *Claudiotendipes* remained entirely absent from public databases until this study.

Our COI barcode analysis provides independent molecular support for the evolutionary distinctiveness of our Columbian specimen while morphological characteristics place it in *Claudiotendipes*. Although the Maximum Likelihood analysis recovered *Claudiotendipes* in association with *Oukuriella* and an unidentified Chironomidae lineage, support for the deeper relationships within this group was low. Therefore, the present data does not allow a robust inference of the phylogenetic position of *Claudiotendipes*, which in any case would be unexpected using COI (Ekrem et al. 2010). However, the recovered placement is broadly consistent with the morphology-based phylogenetic hypothesis proposed by Andersen et al. (2017). Pairwise K2P genetic distances between *Claudiotendipes* and the available reference sequences ranged from 9.8% to 20.9%, with a mean divergence of 11.8% from *Oukuriella*. These values are consistent with intergeneric divergences previously reported for Chironomidae (Hebert et al., 2003a,b; Ekrem et al., 2007) and support the recognition of *Claudiotendipes* as a distinct evolutionary lineage.

We observed a conflict between the morphological phylogeny and the raw genetic distances, specifically regarding the placement of the outgroup *Polypedilum* and certain *Microtendipes* lineages. These taxa showed lower K2P values (~11.5%) than expected for distantly related groups. We attribute this pattern to an artifact of mutational saturation at the COI locus. In many insect groups, including Chironomidae, mitochondrial DNA reaches a substitution plateau at deeper taxonomic levels, where multiple hits at the same nucleotide positions mask the true evolutionary distance (Ekrem et al. 2007; Cranston et al. 2012; Silva and Farrell, 2017). This saturation explains the lack of resolution at the base of the ML tree, despite the inclusion of *Polypedilum* as an explicit out-

group to polarize the analysis. Nevertheless, and despite the inherent limitations of using a single mitochondrial marker for deep phylogenetic inference, the COI barcode successfully discriminated the Colombian specimen and supported its generic distinctiveness.

Furthermore, this record at 2,515 m a.s.l. significantly extends the known altitudinal range and geographical distribution of *Claudiotendipes* into the northern Andes. These findings suggest that the genus is not restricted to lowland or sub-Andean Atlantic rainforests but also colonizes high-altitude Andean streams.

Declaration on the use of AI in scientific writing

During the preparation of this manuscript, the authors used AI to improve the English language, grammar, and stylistic flow of the text. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the accuracy and integrity of the final version of the manuscript.

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