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A new hematophagous Sycoracinae and an estimation of the evolutionary history of blood-feeding in Psychodidae (Diptera)

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Abstract

Background

Hematophagy is known in two Psychodidae subfamilies: Phlebotominae and Sycoracinae. While Phlebotominae exploits a wide range of vertebrate hosts, Sycoracinae feeds primarily on amphibians. However, the evolution of blood-feeding within Psychodidae is poorly understood. Here, we describe *Sycorax hylodiphila* **sp. nov.** and estimate the evolutionary history of blood-feeding across Psychodidae.

Methods

Males and females of *Sycorax hylodiphila* **sp nov.** were collected in association with calling *Hylodes phyllodes* during daytime, some females with visible blood meals. Males and unengorged females were slide-mounted, legs were dissected for amplification of a *cytochrome c oxidase subunit I* (*COI*) fragment. Blood-fed females used for blood meal identification. Phylogenetic analyses used a concatenated dataset comprising *COI*, *Peregrin* and *18S* sequences and 25 terminals. Bayesian inference was conducted in BEAST2, the resulting topology was used for estimation of ancestral state using *ape* and *phytools* packages in R environment.

Results

Sycorax hylodiphila **sp nov.** is morphologically similar to *S. striga* Rossito, Pinho & Andrade, and females show distinct adaptations consistent with hematophagy. Blood meal sequence matched human DNA; however, this result should be interpreted cautiously. Sycoracinae recovered as paraphyletic in relation to Horaiellinae, though with low support. Ancestral state estimation suggests a scenario with multiple origins of blood-feeding within Psychodidae.

Conclusion

Estimating hematophagy onto a phylogeny framework offers useful context for exploring the evolutionary history and diversification of blood-feeding. Hematophagy in Psychodidae likely evolved independently in Sycoracinae and Phlebotominae, however, we highlight the need for additional studies aimed at improving Psychodidae phylogeny as well as improving our morphological and behavioral knowledge about its species.

Background

Hematophagy has likely evolved multiple times throughout the evolutionary history of flies, especially among lower Dipteran lineages [1]. Within Psychodidae, blood-feeding is known in two of the seven subfamilies: Sycoracinae and Phlebotominae [2]. Phlebotominae comprises 1,063 species included in 40

genera and is well known for its hematophagous females and medical and veterinary importance as vectors of a range of pathogens, including arboviruses, *Bartonella* Strong, Tyzzer & Sellards and, mainly, *Leishmania* Ross, the etiological agents of leishmaniasis [3]. Female phlebotomines feed on a variety of cold- and warm-blooded vertebrates, although host preferences differ among species [4, 5]. In contrast, Sycoracinae comprises 71 species [6] classified in four genera, three of which include species known to exhibit hematophagous behavior [7]. Although records are scarce and geographically widespread, available evidence indicates that Sycoracinae females feed mainly on amphibians [8–11].

Amphibians have a long history of interactions with blood-feeding flies and may have constituted crucial vertebrate hosts during the evolution of hematophagy in dipteran lineages, such as the mosquitoes [12]. Interactions between flies and amphibians evolved with varying degrees of specialization, however, their ecological and historical contexts are still unknown in many instances [13, 14]. Within Phlebotominae, species of *Sciopemyia* Barretto have been reported feeding on *Bokermannohyla* Faivovich, Haddad, Garcia, Frost, Campbell & Wheeler and *Scinax* Wagler [15]. In addition, females of *Evandromyia* Mangabeira, *Lutzomyia* França, and *Psathyromyia* Barretto have been found infected with amphibian-specific trypanosomatids, suggesting that these phlebotomines may also feed on frogs [16].

The first record of hematophagy in Sycoracinae dates from 1942 in Touraine, France [8]. The author observed females of *Sycorax silacea* Haliday feeding on the ranid *Pelophylax esculentus* (Linnaeus) and proposed that these flies could act as vectors of the filarial worm *Icosiella neglecta* (Diesing) among *P. esculentus*. This remains the only report of *Sycorax* Haliday associated with the transmission of a parasite. Subsequent observations have provided additional evidence of blood-feeding among Sycoracinae. In Brunei, Ježek *et al*. [11] reported two *Sycorax* species feeding on specimens of *Ansonia* Stoliczka. Similarly, in Ecuador, Bravo and Salazar-Valenzuela [10] observed *Falsosycorax wampukrum* (Bravo & Salazar-Valenzuela) feeding on frogs of the genus *Atelopus* (Agassiz). In Chile, Curler *et al*. [10] successfully sequenced blood-meal DNA from the abdomen of females of *Aposycorax* Tonnoir and identified two species of *Batrachyla* Bell frogs as their vertebrate hosts. More recently, in Brazil, a male *S. manauara* Cordeiro & Bravo was found on the dorsal surface of an *Anomaloglossus* Grant, Frost, Caldwell, Gagliardo, Haddad, Kok, Means, Noonan, Schargel, and Wheeler frog; however, no blood-feeding behavior was observed [17].

Here, we describe a new species of *Sycorax* based on females and males collected at *Parque Estadual Serra do Mar – Núcleo Bertioga*, Brazil. All specimens were collected while interacting with males of *Hylodes phyllodes* Heyer & Cocroft. This provides the first documented record of hematophagy by *Sycorax* Haliday in Brazil. We further incorporate the new species into a phylogenetic hypothesis of Psychodidae, building upon the framework of Curler and Moulton [18], and used ancestral-state estimation to examine the distribution and evolution of hematophagy across the family.

Material and Methods

Data sampling and host species

Female and male specimens of *S. hylodiphila* **sp. nov.** were collected in an Atlantic Rainforest reserve in the municipality of Bertioga, estate of São Paulo, southeastern Brazil, at *Parque Estadual Serra do Mar – Núcleo Bertioga*. All specimens were collected using an entomological aspirator while interacting with calling males of *H. phyllodes* Heyer & Cocroft (Fig. 1A). Sampling was conducted during the daytime, at 10:00 a.m. and 4:00 p.m. *Hylodes* are small-bodied anurans belonging to the Atlantic Forest endemic family Hylodidae [19, 20]. As in other congeneric species, *H. phyllodes* exhibits predominantly diurnal activity and reproductive activity associated with rivers and streams, where males vocalize along stream margins to attract females [21]. No frog specimens were collected during sampling.

Morphological study

Specimens previously stored in ethanol were treated with 10% KOH and mounted on slides with Canada balsam [22]. Illustrations were prepared using a camera lucida coupled to a Leitz Dialux 22 optical microscope. Measurements are given in micrometers (μm). Scale bars represent 0.1 mm in all figures, except for the wings, for which the scale bars represent 0.5 mm. The description is based primarily on the male holotype, and measurements were obtained from one specimen of each sex. General morphological terminology follows the Manual of Afrotropical Diptera [2, 23]. Terminology for the anterior protuberance of the mid coxae bearing pores used as in *Psychomasina* Ježek (Psychodinae) [24], whereas terminology for post spiracular setae follows Young [25]. Terminology for the male and female terminalia [10, 26], and terminology for the female cibarium [11, 27] follows recent Sycoracinae literature.

Type specimens were deposited at *Coleção Entomológica Pe. Jesus Santiago Moure* (DZUP) at *Universidade Federal do Paraná* (UFPR), and at *Coleção Entomológica Mítia Heusi Silveira* (CE-MHS) at the *Universidade Federal de Santa Catarina* (UFSC).

DNA amplification and sequencing

Prior slide mounting, the legs of **s**pecimens were dissected and placed individually in 0.2 mL microtubes for direct DNA amplification. Polymerase chain reactions (PCR) was used to amplify a 658 bp fragment of the mitochondrial cytochrome c oxidase I (*COI*) gene. Each 10 μL PCR reaction contained 5 μL of GoTaq™ Green Master Mix (PROMEGA), 4.6 μL of ultrapure water, and 0.2 μL of each primer at 10 mM: LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') [28]. PCR cycling conditions consisted of an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 minutes. Negative and positive controls consisted of 1 μL of ultrapure water and 1 μL of previously amplified DNA from *Sc. sordellii* (Shannon & Del Ponte), respectively.

PCR products were visualized by electrophoresis on a 1% agarose gel stained with GelRed (Biotium Inc.). Positive PCR products were purified using the ExoSAP-IT™ PCR Product Cleanup kit (ThermoFisher, Santa Clara, California, USA). Purified products were submitted to the DNA Sequencing *Plataforma da Fundação Oswaldo Cruz Minas* (RTP018) for bidirectional Sanger sequencing using an ABI 3730xL Analyzer.

Blood meal source identification

Genomic DNA was extracted from a single engorged female using the Norgen Biotek Corp Kit (cat #24770) according to the manufacturer's instructions. Before DNA extraction, the female was mechanically homogenized. The first incubation step was performed at 37°C for 2 h under agitation at 520 rpm using a ThermoMixer (Kasvi, Brazil), followed by a second incubation at 55°C for 1 h under the same agitation conditions. DNA was eluted from the silica column in 30 µL of the elution buffer provided with the kit and stored at − 20°C until further analysis. DNA concentration (ng μL^{-1}) and purity, assessed using the A260/A280 and A260/A230 ratios, were determined with a NanoDrop® spectrophotometer.

Host DNA was amplified using a nested PCR protocol targeting an approximately 500-bp fragment of the mitochondrial cytochrome *b (cyt b*) gene. The first-round PCR employed the primers M13BC-FW and BCV-RV1 described by [29], whereas the second-round PCR used the primers VF and VR described by [30]. All primers were synthesized by Integrated DNA Technologies (IDT, USA). The first-round PCR was performed in a final volume of 15 µL containing approximately 8 ng of genomic DNA, 1× reaction buffer, 1.6 mM $MgCl_2$, 0.5 mM of each dNTP, 0.6 µM of each primer, 0.7 U of Taq DNA polymerase (Sigma-Aldrich), and nuclease-free water to volume. Thermal cycling conditions consisted of an initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 40 s, annealing at 45°C for 40 s, and extension at 72°C for 60 s, followed by a final extension at 72°C for 7 min. A no-template negative control was included in the PCR run. The second-round PCR was carried out in a final volume of 25 µL containing 1 µL of the first-round PCR product, 1× reaction buffer, 1.6 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of each primer, 0.6 U of Platinum Taq DNA polymerase (Invitrogen), and nuclease-free water to volume. Cycling conditions consisted of an initial denaturation at 95°C for 3 min, followed by 20 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 30 s. Followed by a final extension at 72°C for 5 min. PCR products were visualized by electrophoresis on 1.2% agarose gels stained with Safer Dye (Kasvi, Brazil).

The amplicon was purified and subjected to Sanger sequencing by GoGenetic (Brazil) (https://www.gogenetic.com.br/gogenetic-science). Forward and reverse chromatograms were assembled, edited, and inspected manually using the software Geneious (11.1.5) [31]. The consensus sequence was compared against reference sequences available in the NCBI GenBank database using the BLASTn algorithm. Host identification was assigned based on the top BLASTn match, considering sequence identity values greater than 99.5%.

Phylogenetic inference

Additional *COI*, large ribosomal subunit (*18S*) and bromodomain and PHD finger-containing protein (*Peregrin*) sequences were obtained from GenBank (www.ncbi.nlm.nih.gov/genbank) (Additional File 1). Both *18S* and *Peregrin* were used in previous investigations of [18] relationships within Psychodidae. The *18S* sequence of *Epiphragma fasciapenne* (Say) (Diptera: Limoniidae) was obtained from GenBank and combined with *COI* and *Peregrin* sequences of *Metalimnobia* sp. (Diptera: Limoniidae). This composed terminal was designated 'Limnophilinae' was included to ensure our root possessed sequences for all three genes. A single *COI* sequence identified as *Gondwanotrichomyia* sp. was obtained from BOLD Systems (versions 5) and combined with *Gondwanotrichomyia* kenricki (Duckhouse) *18S* sequence in a composed terminal.

Alignments were carried out in MUSCLE algorithm [32]. The final alignments consisted of 2.240 bp for *18S*; 2.113 bp for *Peregrin* and 717 bp for *COI*. Aligned sequences were concatenated using program AMAS [33]. The final matrix consisted of 25 taxa with five partitions, one for the *18s* gene, and two for each protein coding gene, treating the third codon position separately. Final alignment consisted of 5.070 aligned nucleotide positions. Substitutions models were inferred during the MCMC analysis in with the bModelTest package [34]. Phylogenetic reconstruction was conducted in BEAST2 software [35]. clock and tree models of all partitions were linked. We used the Optimized relaxed clock model with fixed mean clock rate, for tree prior we used Yule model with uniform distribution. For clock and bModelTest parameters we used default priors. Two MRCA (Most Recent Common Ancestor) priors were used, one rooting the three in Limnophilinae and the second constraining Psychodidae as a monophyletic group. MCMC chain consisted of 50 million generations sampled every 5000 with no pre-burnin. DensiTree [36] was used to visualize a set comprising 20% of sample trees as an alternative to access uncertainty in the topology. Maximum clade credibility tree visualized in Interactive Tree of Life (iTOL) online tool [37].

Coding for hematophagy

In Psychodidae, hematophagy has been recorded only in species of Phlebotominae and Sycoracinae [2]. Despite adaptations associated with hematophagy reported in some sycoracine species [*e.g*. 6, 10], these adaptations are not shared by all members of the subfamily [26]. Some female Horaiellinae possess serrate mandibles and hypopharynx, although hematophagy has not been documented in the subfamily [38]. Accordingly, all taxa outside Phlebotominae, Sycoracinae and Horaiellinae were coded as non-hematophagous, whereas some *Sycorax* and the *Horaiella* terminals were coded as uncertain (see below)

Ancestral State Estimation

Hematophagy was treated as a binary character, with species being either hematophagous or non-hematophagous. To account for uncertainty in the available biological information, uncertain terminals

were presented using a probability matrix, with equal probabilities (0.5) assigned to both character states [39]. This coding was applied to *S. paranaensis* Rossito, Andrade & Pinho and *H. iota* Curler.

Four alternative models of character evolution were evaluated: Equal Rates ("ER"), All Rates Different ("ARD") and two irreversible models. The first irreversible model allowed the loss of hematophagy but prohibited its reacquisition, treating the non-hematophagous state as irreversible. The second was the opposite case, where gains of hematophagy were allowed but its subsequent loss was prohibited. Models were compared using Akaike's Information Criterion (AIC). In addition, model averaging was performed using the relative frequencies provided by the Akaike model weights. Models were fitted with function *fitMk* and marginal ancestral states estimations were inferred with *ancr* function, both from *phytools* package [39]. All analyses were conducted in R version 4.3.3 [40].

Results

Taxonomy

Genus Sycorax Haliday in Curtis, 1839

Sycorax hylodiphila sp. nov. Rossito, Pinho & Andrade

Figures 1B; 2A–H; 3 A–G.

Diagnosis. *Sycorax hylodiphila* **sp. nov.** can be differentiated from all other *Sycorax* species by the following character combination. *Both male and female*: First flagellomere more than two times the length of the second and more than 1.6 times the length of the palpus (PI–PIV). *Male*: Gonocoxite about 3 times longer than wide; paramere trilobed, with an external, medial and internal lobe; dorso-medial process narrow; aedeagus presenting sclerotized filaments, curved at base. *Female*: Cibarium presenting sclerotized folds; dorsal portion of the cibarium 0.8 times the length of the labial fork stem; genital furca stem sclerotized, extended anteriorly in a straight line; posterior region of the genital furca conical, with sinuous posterior margin.

Description. Male. Head (Fig. 2A–D). Wider than long (270–226); coronal suture present, sclerotized, reaching the vertex. Clypeus wider than long (46–41), anterior margin inconspicuous. Scape cylindrical, shorter than spherical pedicel. Antenna complete: flagellomeres 1–13 presents and a terminal apiculus; flagellomere 1 (154) 2.2 times the length of the second and 1.6 times the total length of palps; apiculus 0.4 times the length of the last flagellomere and bearing two apical, with blunt apex, sensilla. Ascoids present in flagellomeres 1–4, with asymmetrical implantation. Hyaline spiniform sensilla present at fourth and subsequent flagellomeres. Palps with four segments (29, 29, 26.1, 20.3), second papal segment with hyaline sensilla on the internal surface, sensilla unordered. Cibarium with sclerotized folds, similar but weaker than females.

Thorax (Fig. 2E). Upper anepisternum bare, setae present in anepimerum and metepisternum. Post-notum sub-rectangular, posterior margin straight. Four long post-spiracular setae present, directed anteriorly almost reaching the posterior spiracle margin. Coxa II with apical porous protuberance. Coxa I: 209, Coxa II: 189, Coxa III: 160. Wing (Fig. 2F). Length: 1203 – width: 574. Sub costa complete, reaching the wing's anterior margin; transversal veins r-m, m-m and m-cu present, but m-m less evident. Radial fork basal in relation to the apex of R_1. Medial fork basal in relation to the apex of M_4. Anal vein with tubular structure developed, sclerotized and bearing setae scars; anal vein ending free.

Terminalia (Fig. 2G–I). Epandrium wider than long (61–139). Cerci shorter than epandrium, pilose, with longer setae on the posterior margin. Hypoproct not reaching the apex of cerci, micropilose and rounded apex. Gonocoxite straight; about three times longer than wide (171–55), with longer setae in the apical half of the internal margin. Gonostylus 0.7 times the length of gonocoxite; sub-terminal setae present, submedian; apical spiniform setae present, about the same length as the gonostylus. Parameres with three lobes; medial lobe bare, digitiform and longer than external and internal ones; external lobe dorsally covered in setae scars and a macro-seta on the ventral surface, sub-apically; internal lobe small and with two sub-apical setae; dorsal apodeme extended anteriorly, arched and heavily sclerotized, fused to dorso-medial processes; dorso-medial process narrow and straight, sclerotized through all its extension, ending near the apex of the medial lobe of the paramere; medial bridge present as a sclerotized arch, adjacent to the internal dorsal lobe of the paramere. Aedeagus bifid, in the shape of sinuous sclerotized filaments; filaments curved basally and ending before reaching the apex of dorso-medial process. Ejaculatory apodeme (122) straight, shorter than the length of paramere.

Female. Head (Fig. 3A–D). Wider than long (288–307); coronal suture present, sclerotized, reaching the vertex. Clypeus wider than long (64–70), anterior margin inconspicuous. Scape and pedicel as in male. Antenna complete: flagellomeres 1–13 and a terminal apiculus present; flagellomere 1 (218) 2.5 times the length of the second and 1.6 times the total length of palps. Ascoids present in flagellomeres 1–11, after flagellomere 4 only one ascoid present; remaining characters as in male. Palps as in male except the second segment with swollen aspect. Labrum-epipharynx with four sub-apical sensilla; sensilla with rounded apex, wider than basal portion. Maxilla with wide base, narrow towards the apex; apex spoon-like with at least one row of teeth present internally. Mandibles present, serrated and with round apex, with uniform width along its length. Hypopharynx with well delimited teeth. Cibarium developed, dorsal portion longer than ventral; dorsal portion with seven sclerotized and arched folds parallel to the lateral margin of the cibarium, anterior teeth scattered in the lateral portion of the dorsal portion of the cibarium; dorsal portion 0.8 times the length of the labial fork and 1.9 times longer than wide. Pharynx heavily sclerotized.

Thorax. as in male. Coxa I: 247, Coxa II: 200, Coxa III: 177. Wing (Fig. 3E). Length: 1.665 – width: 758. Radial fork basal in relation to the apex of R_1. Medial fork at the same level as the apex of M_4. Anal vein as in male.

Abdomen (Fig. 3F, G). Sternite VIII bipartite in two lobes; lobes setulose, with longer setae towards the apex. Hypoproct with rounded apex and micropilose. Cercus rounded, longer than wide. Genital fork steam, sclerotized and developed anteriorly, straight; posterior region cone-shaped, with posterior margin sinuous. Spermatheca was not discernible in any specimen.

Type material. Holotype. m# BRAZIL. SP. Bertioga. Parque Estadual Serra do Mar - Núcleo Bertioga. 23°42'29" S 40°02'13" W. Collected on *Hylodes phyllodes*. 25.x.2022. 10h. G. Augusto-Alves (DZUP).

Paratypes. 2m# as holotype (CE-MHS: D3744). 3m# 2#f as holotype (DZUP). 1f# as holotype except for 16h (CE-MHS: D3745, CE-MHS: D3746). 2m# as previous (DZUP).

Etymology. The scientific epithet *hylodiphila* is derived from the generic name *Hylodes* and the Greek suffix *-phila*, meaning "having affinity for". We intend to highlight the association of the new species with the frog *H. phyllodes*.

Distribution. Known only from type-locality: State of São Paulo, "*Parque Estadual Serra do Mar – Núcleo Bertioga*", at the Atlantic Forest in Southeastern Brazil.

Comments. All specimens of *S. hylodiphila* **sp. nov.** were collected while in contact with individuals of *H. phyllodes* during the day, both in the morning and afternoon periods. Males and females were collected in both periods of the day. Some of the females (n = 2) were visually engorged with blood (Fig. 1B).

Blood meal source identification

Host DNA was successfully amplified from one engorged female. BLASTn analysis of the *cytochrome b* sequence revealed 99% similarity with *Homo sapiens* L. mitochondrial DNA. Alignment against the complete human mitochondrial genome (GenBank accession no. MF523030.1) showed seven nucleotide mismatches and no gaps across a 496 bp fragment, supporting a human origin of the blood meal.

Molecular phylogeny

Bayesian inference produced a moderate-supported phylogeny of Psychodidae, including Tanyderinae (Fig. 4). Subfamilies Phlebotominae, Trichomyiinae and Psychodinae were well supported with high (≥ 0.9) posterior probability. Phlebotominae was recovered as a sister group to Bruchomyiinae with moderate supported ((≥ 0.7). The clade (Phlebotominae+Bruchomyiinae) recovered as sister to Psychodinae also with moderate support, and this clade sister to (Trichomyiinae+Tanyderinae), thought poorly supported (> 0.7). Subfamily Sycoracinae was recovered as paraphilic in relation to Horaiellinae but poorly supported.

Ancestral State Estimation

Four alternative models of character evolution were compared using Akaike's Information Criterion (AIC). The two models best explained our data: the irreversible-hematophagy model (Irr2; AIC = 20.29436) and

equal rates model (ER; AIC = 21.47038). The Akaike' weights were respectively 0.4289137 and 0.2382323. Remaining models played a less significant role during stochastic sampling with Akaike's weights 0.1577885 for all-rates-different model (ARD) and 0.1750654 for irreversible non-hematophagy (Irr1).

Under our best fit mode (Irr2), a hematophagous ancestral shared by Phlebotominae and Sycoracinae is not possible because losses of the trait are prohibited (Fig. 5). This scenario was very similar to the one suggested by all rates different model (ARD) (Additional file 2, Figure S1). Under the equal-rates model (Fig. 5), the probability of a hematophagous ancestor of Sycoracinae + Horaiellinae was 63%, whereas the marginal probability of hematophagy at the most recent common ancestor of Phlebotominae and Sycoracinae was 41%. When all models were averaged, we achieved a similar result, although with 27% marginal probability of a hematophagous ancestor to both subfamilies (Additional file 2, Figure S2). The irreversible losses model suggests at least two losses of blood feeding within Psychodidae and an obligate hematophagous ancestor for all psychodids (Additional file 2, Figure S3).

Discussion

Ancestral state estimation depends heavily on the underlying phylogenetic hypothesis. Our topology differs from previous analyses based on a similar dataset [18, 41], highlighting the instability of Psychodidae phylogeny. The position of subfamilies represented by a single taxon was unstable (Additional file 3). This behavior is probable responsible for the position of Horaiellinae nested whiting Sycoracinae. In previous inferences [41], the clade (Bruchomyiinae+Horaiellinae) might also be an artifact of the same nature. Nevertheless, Sycoracinae was not recovered as sister group of Phlebotominae in any of the previous hypotheses (18, 41) neither was a clade sample frequently in ours. This phylogenetic placement is noteworthy, as these lineages include the only currently recognized hematogenous representatives of Psychodidae.

Under an equal-rates model (Fig. 5), results indicate moderate (41%) of a blood-feeding ancestor between the two subfamilies. Although symmetric rates are a simplification and true rates are probably asymmetric, as expected for non-neutral traits [42]. The scenario of independent origin of hematophagy is even more evident when assuming irreversible losses of hematology (Fig. 5) our best fit model. In contrast, a scenario allowing losses but not reacquisition (Dollo's law), is the only one where a blood-feeding ancestral to Psychodidae is highly supported (Additional file 2: Figure S3). The application of Dollo's law is often expected for specialized traits [42], however, hematophagy independently appeared in multiple "nematocerans" and "lower Brachycera" lineages [1], taking multiple forms.

Within Sycoracinae were recovered with low or moderate support, but this might be the result of *Horaiella* position inside the subfamily. Under ER model there is 61% marginal probability of a hematophagous ancestral to all Sycoracinae and Horaiellinae, while this falls to 345 in irreversible loses model. Further study of the blood-feeding habit is limited by the absence of phylogenetic hypotheses and the few widespread records of hematophagy [7]. Morphological evidence that could be used to infer

hematophagy is likewise limited, both to *Sycorax* [6] and *Horaiella* [38]. Both species from the Brazilian Atlantic Forest, *S. hylodiphila* and *S. paranaensis* were recovered as sister groups with low support (Fig. 4). Both present pronounced morphological divergences. *Sycorax paranaensis* is characterized by plate-like parameres, short aedeagus, truncated dorso-medial projection and faintly sclerotized folds in the cibarium. In contrast, the new species closely resemble *S. striga* Rossito, Pinho & Andrade sharing, among other features, trilobed parameres with the external lobe bearing a macrosetae, an aedeagus with long sclerotized filaments, a developed dorso-medial projection, conspicuous sclerotized arched folds and anterior teeth in the cibarium [6].

The presence of sclerotized arched folds in the cibarium is not restricted to *S. striga* and the new species; these structures also occur in *S. konopiki* Ježek, *S. salamannai* Morelli, and possibly in *S. similis* Müller and *Aposycorax chilensis* (Tonnoir) [10, 43. 44]. In the new species, these folds are similar to those in *S. striga*: longitudinal eccentrically curved sclerotized lines, occupying most of the dorsal portion of the cibarium. A similar pattern is present in *S. konopiki*, though only the male is figured. In contrast, folds in *S. salamannai* occupy the lower part of the dorsal portion of the cibarium, are more lateralized and inclined towards the cibarium midline. These structures are conspicuous in females and very likely an indicative of hematophagous behavior [6]. The serrated mandibles, toothed maxillae, and labrum-epipharynx bearing well developed subapical sensilla are present in the new species, supporting the relationship between these structures and hematophagy.

Although evidence for hematophagy in females is clear for some Sycoracinae, available observations consistently report males outnumbering females at host associated aggregations [9, 11]. This pattern may indicate lekking behavior, with females potentially visiting aggregation of displaying males and opportunistically feeding on the frog's blood [45]. Lekking systems are characterized by female choice among several displaying males defending small territories. These are known within Psychodidae, particularly in the hematophagous Phlebotominae, when males of *Lutzomyia longipalpis* (Lutz & Neiva) form leks near or on the surface of the vertebrate host [46]. Current evidence suggests that at least some species of Sycoracinae may exhibit similar aggregations.

These aggregations have been observed at different times of the day. In both Brazil and Ecuador, *Sycorax* and their anuran hosts were active during the day; the male of the hylid *Hyloscirtus lindae* (Duellman and Altig) was active calling while flies were present in its surface [45]. A similar pattern was observed during our sampling, as all female and male flies were collected in association with vocally active males of *H. phyllodes* during the day. In contrast, *Sycorax* spp. in Brunei were active at night [11], when species of *Ansonia* are active, with males calling from low vegetation near rocky streams [47]. However, Bravo and Salazar-Valenzuela [9] reported *F. wampkrum* active during the night, despite the bufonid host genera *Atelopus* Duméril and Bibron comprising mostly diurnal species [48]. The temporal overlap between fly and host activity is relevant because the mechanism by which Sycoracinae locate or are attracted to frogs remains poorly understood. One possible mechanism is eavesdropping on frog calls, supported by the results of [49] from traps baited with sound, that captured an impressive number of *Sycorax* sp. females, including blood-engorged individuals.

Blood-fed females have also been reported in Sycoracinae–amphibian interactions [9, 11] but attempts to identify the blood source are few. The first to identify blood meals in Sycoracinae were [10], who investigated the blood meals of *A. chilensis* using *16S* mtDNA, tracing the blood to two species of *Batrachyla*. Subsequently, [49] targeted engorged females of *Sycorax* sp. but were unable to identify the blood-meal. Our results provide the first molecular evidence of a human blood meal in *Sycorax*. This contrasts with females and males found in direct contact with *H. phyllodes* and the previous natural history of Sycoracinae, always associated with amphibians. Residual DNA from a previous blood meal obtained approximately 24h before collection could have been amplified [50]. Alternatively, opportunistic feeding on exposed blood or wounds might bring *Sycorax* females in contact with human blood, as often is reported for male of sand flies found with traces of a blood meal [51]. Nevertheless, the present result should be taken with caution, and additional evidence is needed to associate humans as a regular host of *Sycorax* females.

Conclusion

Hematophagy in Psychodidae likely evolved independently in more than one lineage. In this context, Phlebotominae and Sycoracinae should exhibit distinct morphological and ecological adaptations associated with blood-feeding, including host location. This hypothesis aligns with the prolific events of hematophagy evolution in lower dipterans, however, requires further support. Psychodidae is in dire need of a well-supported backbone phylogeny, which can only be obtained by sampling it's smaller subfamilies: Bruchomyiinae, Horaiellinae, Sycoracinae and Trichomyiinae. Detailed comparative studies of mouthpart morphology, particularly homology assessment of cibarial structures should be investigated. Beyond describing *S. hylodiphila* and expanding the limited evidence of Sycoracinae-amphibian interactions, estimating the hematophagous state onto a phylogeny framework offers useful context for exploring the evolutionary history and blood-feeding adaptations in the family.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Author Contribution

Conceptualization: LR, LCP and AJA. Data curation LR, APC and SCJ. Funding acquisition: GAA and AJA. Investigation: LR, GAA, APC and SCJ. Methodology: LR, APC and SCJ. Project administration: LR. Resources: GAA, LFC and MNS. Supervision: LCP and AJA. Visualization: LR, GAA and LCP. Writing—original draft preparation: LR, GAA, APC and SCJ. Writing—review and editing: LR, GAA, APC, LFC, LCP and AJA. All authors read and approved of the final manuscript.

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Data Availability

The newly generated sequences were deposited in the GenBank database under the following accession nos. PZ859128, PZ859129. Raw files including alignment, tree file and states assigned for each terminal are available in Zenodo repository: https://doi.org/10.5281/zenodo.23050713

References

1. Wiegmann BM, et al. Episodic radiations in the fly tree of life. Proc Natl Acad Sci U S A. 2011. doi: 10.1073/pnas.1012675108.
2. Kvifte GM & Wagner R. Psychodidae. In: Kirk-Spriggs, A.H. & Sinclair, BJ, editors. Manual of Afrotropical Diptera. Volume 2. Nematocerous Diptera and lower Brachycera. Suricata 5. South African National Biodiversity Institute, Pretoria. 2017; pp. 607–632.

3. Galati EAB et al. Phlebotomine sand flies (Diptera, Psychodidae) of the world. Parasites Vectors. 2026; 18, 220. https://doi.org/10.1186/s13071-025-06748-55
4. Roque AL, Jansen AM. Wild and synanthropic reservoirs of *Leishmania* species in the Americas. Int J Parasitol Parasites Wildl. 2026; 3(3):251–62. doi: 10.1016/j.ijppaw.2014.08.004.
5. Dutra-Rêgo F, Silva MSD, Isnard AP, Medeiros JF, Andrade Filho JD, Freire ML. You are what you eat: a systematic review exploring the interaction between Brazilian sand flies and their vertebrate food sources. Mem Inst Oswaldo Cruz. 2024; doi: 10.1590/0074-02760240055
6. Rossito L, Pinho LC & de Andrade A.J. Increasing the diversity of *Sycorax* Haliday, 1839 (Diptera: Psychodidae) in the Neotropical region: New species from the Brazilian Atlantic Forest. Austral Entomology. 2026; 65(1), e70051. https://doi.org/10.1111/aen.70051
7. Bravo F, Araújo MX, Vilarinho N. A new genus and new species of Sycoracinae (Diptera: Psychodidae) from the Neotropical region with keys to the extant genera of the subfamily and males of Neotropical species of *Sycorax*. Neotrop Entomol. 2023; https://doi.org/10.1007/s13744-023-01040-6
8. Desportes C. *Forcipomyia velox* Winn. et *Sycorax silacea* Curtis, vecteurs d'*Icosiella neglecta* (Diesing) filaire commune de la grenouille verte. Annales de Parasitologie humaine et comparée, 1942; 19(1-2-3); 53–68.
9. Bravo F, Salazar-Valenzuela D. A new species of *Sycorax* Curtis (Diptera, Psychodidae, Sycoracinae) collected on harlequin frogs (Anura: Bufonidae, *Atelopus*) in the Ecuadorian Andes. Zootaxa. 2009; 2093, 37–42.
10. Curler GR, Moulton JK, Madriz RI. Redescription of *Aposycorax chilensis* (Tonnoir) (Diptera, Psychodidae, Sycoracinae) with the first identification of a blood meal host for the species. Zootaxa. 2015; 4048, 114. https://doi.org/10.11646/zootaxa.4048.1.7
11. Ježek J, Wahab RA, Ševčík J, Wahab RA, Ševčík J. Two new species of *Sycorax* (Diptera: Psychodidae: Sycoracinae) from the Oriental Region. Zootaxa. 2015; 4057, 539. https://doi.org/10.11646/zootaxa.4057.4.4
12. Soghigian J et al. Phylogenomics reveals the history of host use in mosquitoes. Nat Commun. 2023. doi: 10.1038/s41467-023-41764-y.
13. Campos LLF, Pinho LC, Neckel-Oliveira S, Bernal XE. The Diverse and Intricate Interactions Between Flies and Amphibians: A Systematic Review of Their Host-Use Patterns and Strategies. Annual Review of Entomology. 2026; 71, 577–595. https://doi.org/10.1146/annurev-ento-121423-013418
14. Campos LLF, Pinho LC, Neckel-Oliveira S, Bernal XE. Amphibian Strategies Against Attacks by Flies: Host-Specificity and Threats. Ecology and Evolution. 2026; 16: e72737. https://doi.org/10.1002/ece3.72737
15. Costa JCR, Marchi GH, Santos CS, Andrade MCM, Chaves Junior SP, Silva MAN, Melo MN, Andrade AJ. First molecular evidence of frogs as a food source for sand flies (Diptera: Phlebotominae) in Brazilian caves. Parasitol Res. 2021; 120, 1571–1582. https://doi.org/10.1007/s00436-021-07154-3

16. Ferreira RC, de Souza AA, Freitas RA, Campaner M, Takata CSA, Barrett TV, Shaw JJ, Teixeira MMG. A Phylogenetic Lineage of Closely Related Trypanosomes (Trypanosomatidae, Kinetoplastida) of Anurans and Sand Flies (Psychodidae, Diptera) Sharing the Same Ecotopes in Brazilian Amazonia 1. J Eukaryotic Microbiology. 2008; 55, 427–435. https://doi.org/10.1111/j.1550-7408.2008.00342.x
17. Cordeiro DP, Bravo F, Ramires AC. New species of Sycorax (Diptera: Psychodidae) from the Brazilian Amazon. Pap. Avulsos Zool. 2023; 63, e202363037. https://doi.org/10.11606/1807-0205/2023.63.037
18. Curler G, Moulton J. Phylogeny of psychodid subfamilies (Diptera: Psychodidae) inferred from nuclear DNA sequences with a review of morphological evidence for relationships. Syst Entomol. 2012; 37:603–16. https://doi.org/10.1111/j.1365-3113.2012.00634.x.
19. Augusto-Alves G, Dena S, Toledo LF. Acoustic allometry, background stream noise and its relationship with large-bodied and voiceless rheophilic frogs. Zool. Anz. 2021; 295: 156–162.
20. Vittorazzi SE et al. Paraphyly in the giant torrent-frogs (Anura: Hylodidae: *Megaelosia*) and the description of a new genus. Salamandra, 2021; 57: 274–284.
21. Augusto-Alves G, Toledo LF. Communication across multiple sensory modes: quantifying the rich behavioural repertoire of a Neotropical torrent frog. Behaviour. 2022; 159, 351–375.
22. Randrianambinintsoa, FJ et al. Processing and mounting phlebotomine sand flies: a consensus guideline. Parasite. 2026; 33, 18. https://doi.org/10.1051/parasite/2026009
23. Cummings JM, Wood DM. Adult Morphology and Terminology. In: Kirk-Spriggs AH, Sinclair BJ, editors. Manual of Afrotropical Diptera. Vol. 1. Introductory chapters and keys to Diptera families. Suricata 5, South African National Biodiversity Institute, Pretoria, 2017; pp. 89–133.
24. Kvifte GM. Review of the genus *Psychomasina* (Diptera: Psychodidae: Psychodinae), with descriptions of two new species. Acta Entomol Musei Natl Pragae. 2015; 55:495–503.
25. Young DG. A review of the bloodsucking Psychodid flies of Colombia (Diptera: Phlebotominae and Sycoracinae). Tech. Bull. 806, Agric. Exp. Station IFAS, Univ. Florida, Gainesville, 1979; p 226.
26. Curler GR, Jacobson AJ. New species of Psychodidae (Diptera) from Australasia, with a checklist of the world species of Bruchomyiinae and Sycoracinae. Zootaxa. 2012; 3552, 43. https://doi.org/10.11646/zootaxa.3552.1.3
27. Galati EAB. Phlebotominae (Diptera, Psychodidae): Classification, Morphology and Terminology of Adults and Identification of American Taxa. In: Rangel EF, Shaw JJ, editors. Brazilian Sand Flies. Springer International Publishing, Cham, Switzerland, 2018.
28. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology. 1994; 3, 294–299.
29. Alcaide M, Rico C, Ruiz S, Soriguer R, Muñoz J, Figuerola J. Disentangling vector-borne transmission networks: A universal DNA barcoding method to identify vertebrate hosts from arthropod bloodmeals. PLoS One. 2009; 4: e7092. https://doi.org/10.1371/journal.pone.0007092

30. Townzen JS, Brower AVZ, Judd DD. Identification of mosquito bloodmeals using mitochondrial cytochrome oxidase subunit I and cytochrome b gene sequences. Medical and Veterinary Entomology. 2008; 22: 386–393. https://doi.org/10.1111/j.1365-2915.2008.00760.x
31. Kearse M, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics (Oxford, England); 2012. https://doi.org/10.1093/bioinformatics/bts199
32. Edgar RC. Muscle5: High-accuracy alignment ensembles enable unbiased assessments of sequence homology and phylogeny. Nat Commun. 2022; 13, 6968. https://doi.org/10.1038/s41467-022-34630-w
33. Borowiec ML. AMAS: a fast tool for alignment manipulation and computing of summary statistics. PeerJ 4:e1660. 2016
34. Bouckaert, R. R., & Drummond, A. J. bModelTest: Bayesian phylogenetic site model averaging and model comparison. BMC Evolutionary Biology. 2017; 17(1), 42.
35. Bouckaert R., Vaughan T.G., Barido-Sottani J., Duchêne S., Fourment M., Gavryushkina A., et al. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLoS computational biology. 2019; 15(4), e1006650.
36. Remco R. Bouckaert & Joseph Heled. DensiTree 2: Seeing Trees Through the Forest bioRxiv. 2014. http://dx.doi.org/10.1101/012401
37. Letunic I, Bork P. Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. Nucleic Acids Res. 2024. doi: 10.1093/nar/gkae268.
38. Tonnoir AL. Descriptions of remarkable Indian Psychodidae and their early stages, with a theory of the evolution of the ventral suckers of dipterous larvae. Records of the Zoological Survey of India; 1933; 53–75.
39. Revell LJ. phytools 2.0: an updated R ecosystem for phylogenetic comparative methods (and other things) PeerJ. 2024; doi.org/10.7717/peerj.16505
40. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2024; URL https://www.R-project.org/.
41. Azar D, Maksoud S, Huang D-Y, Maalouf M, Cai C-Y. A new fossil psychodomorphan fly from Lower Barremian Lebanese amber elucidates the relationship of the Tanyderinae stat. nov. within the Psychodidae. Carnets Géol. 2024; 24 (6), 113–126.
42. Cunningham CW. "Some limitations of ancestral character-state reconstruction when testing evolutionary hypotheses." Systematic biology. 1999; 48.3, 665–674.
43. Morelli A. Two new species of *Sycorax* Haliday in Curtis, 1839 from peninsular Italy (Diptera, Psychodidae, Sycoracinae). Annali del Museo Civico di Storia Naturale 'Giacomo Doria'. 2021; 114, 349–365.
44. Morelli A, Biscaccianti AB. Contribution to the Knowledge of; 'Non-Phlebotomine' Psychodidae (Diptera Nematocera) from Central; Italy. Redia. 2022; 105, 123–129.

45. González K, Culebras J. Tree frogs serve as a hotel for moth flies. Frontiers in Ecol & Environ. 2023; 21, 498–498. https://doi.org/10.1002/fee.2692
46. Jones TM, Quinnell RJ, Balmford A. Fisherian flies: benefits of female choice in a lekking sandfly. Proc Biol Sci. 1998; https://doi.org/10.1098/rspb.1998.0484
47. Noda A, Fukuyama I, Kimura K, Fukuyama R, Kojima Y, Hossman M Y, Nishikawa K. Daily movement and microhabitat use of the spiny slender toad, *Ansonia spinulifer* (Mocquard, 1890) in Sarawak, Borneo. Herpetology Notes. 2021; 14, 833–837.
48. Rueda Solano L, & Warkentin K. Foraging behavior with possible use of substrate-borne vibrational cues for prey localization in *Atelopus laetissimus* (Ruiz-Carranza, Ardila-Robayo and Hernández-Camacho, 1994). Herpetology Notes. 2016; 9. 191–195.
49. Cutajar TP, Rowley JJL. Surveying frogs from the bellies of their parasites: Invertebrate-derived DNA as a novel survey method for frogs. Global Ecology and Conservation. 2020; 22, e00978. https://doi.org/10.1016/j.gecco.2020.e00978
50. Kent RJ, Norris DE. Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome B. Am J Trop Med Hyg. 2005; Aug;73(2):336–42.
51. Silva OS & Grunewald J. First detection of blood in the gut of wild caught male sandflies in southern Brazil, Studies on Neotropical Fauna and Environment. 2000; 35:3, 201–202.

Figures

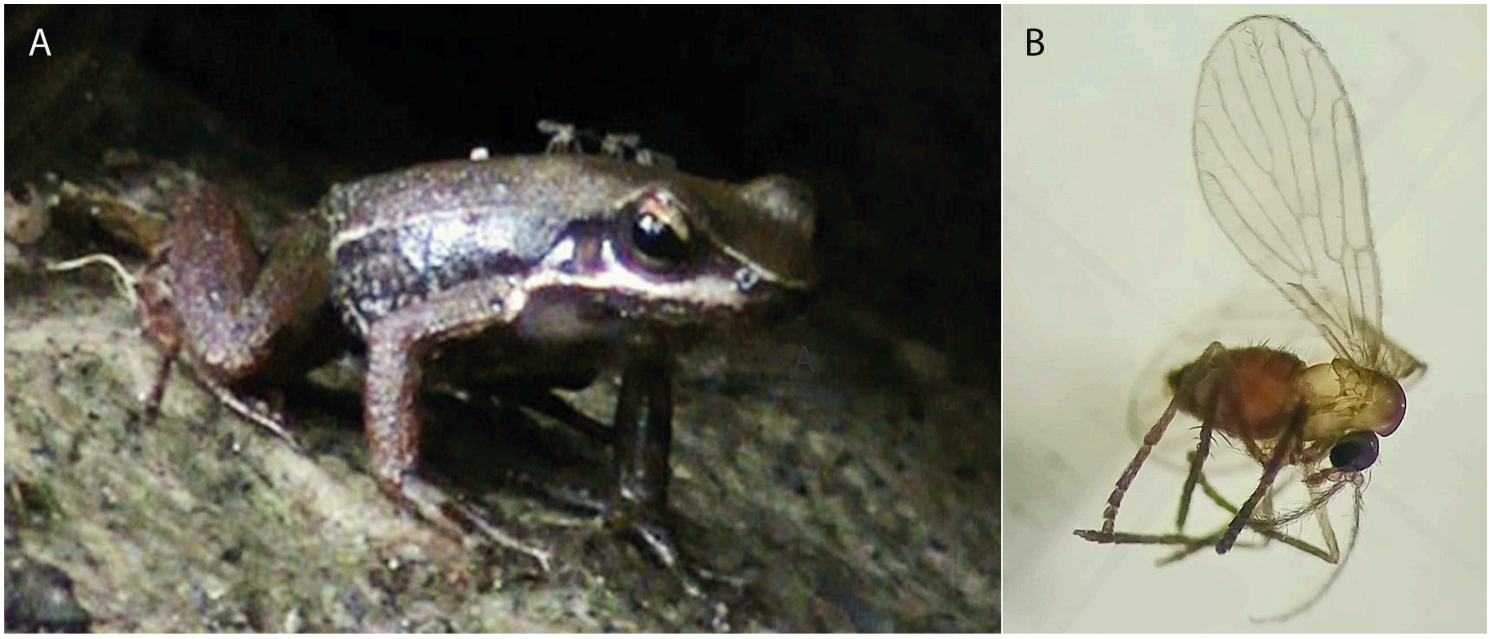


Figure 1

(A) *Hylodes phyllodes* male with Sycoracinae individuals on the dorsal region of the body. The photograph was taken at the same locality where the specimens described herein were collected, and the individuals shown likely belong to the *Sycorax* species described in this study. However, because

these individuals were not collected, their identity could not be confirmed. (B) Female *Sycorax hylodiphila* **sp. nov.**with traces of a blood meal visible in the abdomen.

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