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Phylogenomics of the family Lachesillidae (Insecta: Psocodea: Psocomorpha)

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Abstract

Lachesillidae is one of the largest families of bark lice and includes more than 420 described species, in 26 genera and three subfamilies. This family belongs in the suborder Psocomorpha, infraorder Homilopsocidea. The classification of Lachesillidae is based on male and female genital morphologies, but questions remain regarding the monophyly of the family and some of its genera. Here, we used whole genome and transcriptome data to generate a 2060 orthologous gene data matrix of 2,438,763 aligned bp and used these data to reconstruct the phylogenetic relationships of species of Lachesillidae and relatives. Taxon sampling included 24 species from Lachesillidae and 23 additional species belonging to related families from the infraorders Homilopsocidea and Caeciliusetae. Phylogenetic relationships reconstructed with maximum likelihood and coalescent-based analyses indicated paraphyly of Lachesillidae, and monophyly of the tribe Graphocaeciliini and the genus *Lachesilla* were also never recovered. Instability was observed in the position of *Eolachesilla chilensis*, which was recovered either as sister to Elipsocidae or to Mesopsocidae species, so we cannot conclusively determine the position of this genus within the Homilopsocidea. Given our results, a reclassification is necessary, but more taxon sampling of other species in Mesopsocidae and Peripsocidae would be useful to add to a tree in future before proposing a new classification.

KEYWORDS
Eo. chilensis, genomics, Graphocaeciliini, *Lachesilla*, phylogenetic relationships

INTRODUCTION

The parasitic lice and the free-living bark lice belong to the insect order Psocodea. This order is divided into three suborders: Trogiomorpha, Troctomorpha and Psocomorpha (Lienhard & Smithers, 2002; Yoshizawa & Johnson, 2014). Within the largest suborder, Psocomorpha, six infraorders have been recognized: Archipsocetae, Caeciliusetae, Epipsocetae, Philotarsetae, Psocetae and Homilopsocidea (Yoshizawa & Johnson, 2014). Homilopsocidea includes one of the largest families of bark lice, Lachesillidae, which was previously named Pterodelidae by Pearman (1936) and included the genus *Lachesilla* Westwood. This group was subsequently amended and named Lachesillidae by Badonnel (1951).

Currently, Lachesillidae includes more than 420 described species, in 26 genera and three subfamilies; Eolachesillinae Mockford & Sullivan, Lachesillinae Mockford & Sullivan and Cyclolachesillinae Li (Li, 2002; Mockford & Sullivan, 1986). Primary classification of the subfamily Eolachesillinae was based on morphological similarities and included nine genera and 29 species, with the number of described genera and species eventually reaching 17 and 44, respectively (García Aldrete et al., 2012; García Aldrete et al., 2014; González Obando et al., 2020). The subfamily Lachesillinae includes eight genera, mainly distributed in the Americas and Asia, with *Lachesilla* Westwood being one of the most species rich genus among all bark lice, with 349 species and at least 100 undescribed species (García Aldrete & da Silva-Neto, 2020). This genus has a Pantropical distribution and its representatives exhibit a remarkable morphological diversity (García Aldrete, 1999, 2017; Li, 2002; Lienhard & Smithers, 2002; Mockford & Sullivan, 1986; Yoshizawa, 2002). Recently, Li (2002) erected Cyclolachesillinae to include a monospecific *Cyclolachesillus ningxiaensis* Li from China.

Morphology has played an important role in the systematics of Lachesillidae, and the monophyly of the family is in part supported by morphological data. Yoshizawa (2002) established the monophyly of the family based on morphological systematics, but once the genus *Eolachesilla* Badonnel (Eolachesillinae) was included, the monophyly of the family was called into question. In a similar way, (Schmidt & New, 2004) established the phylogenetic relationships of the family Elipsocidae, with *Eolachesilla chilensis* New & Thornton being the sister group of the elipsocids. This analysis gave support to the previous hypothesis, based on the male genital characters, that this genus is more closely related to Elipsocidae than to Lachesillidae (New & Thornton, 1981).

Within Lachesillinae, the highly diverse genus *Lachesilla* has been divided into 20 species groups based on morphological similarity (Garcia Aldrete, 1974; García Aldrete, 2014; García Aldrete & Mockford, 2011). However, a morphological phylogenetic analysis revealed paraphyly of the genus *Lachesilla*, with the *pedicularia* species group + genus *Nadleria* Badonnel standing apart from the remaining *Lachesilla* species, which are closely related to the genus *Hemicaecilius* Enderlein (Saenz Manchola et al., 2019). Similarly, several Asian genera of the subfamily Lachesillinae have been discussed, and their diagnosis may need a major taxonomic revision. For example, the genus *Dicrolachesillus* was placed in synonymy with *Lachesilla* by Lienhard (2003), whereas the monotypic genus *Cyclolachesillus* (Cyclolachesillinae) could possibly be an elipsocid based on the illustrations of *C. ningxiaensis* Li (García Aldrete, 2006).

As with morphological data, prior molecular phylogenetic analyses based on Sanger sequencing have not recovered the monophyly of Lachesillidae, with the genus *Lachesilla* being the main source of instability within the infraorder Homilopsocidea (Yoshizawa & Johnson, 2014). This Sanger sequence-based phylogeny recovered *Lachesilla* as sister to Peripsocidae, whereas *Eolachesilla* + *Anomopsocus* were recovered as sister to Elipsocidae. Recently, a phylogenomic study of higher level relationships within Psocodea revealed generally stable relationships within Psocomorpha, although the monophyly of the infraorder Homilopsocidea was not supported nor was the monophyly of Lachesillidae and Elipsocidae (de Moya et al., 2021). It should be noted that these Sanger and phylogenomic analyses were not focused on resolving relationships within Lachesillidae. However, recent mitochondrial genomics (Saenz Manchola et al., 2021) and UCE (Saenz Manchola et al., 2022) data sets with extensive taxon sampling of Lachesillidae also did not recover monophyly of the family. These data sets also had aspects of instability for some higher level relationships within Psocomorpha, including the relationships among the major clades of Lachesillidae, Elipsocidae and Mesopsocidae.

Here, we used whole genome and transcriptome data to generate a 2060 orthologous gene data matrix to reconstruct the phylogenetic relationships of bark louse species of Lachesillidae. Taxon sampling included 24 species from the family Lachesillidae, plus 23 additional species belonging to related families in the infraorders Homilopsocidea and Caeciliusetae. We performed concatenated (including examination of different codon positions, Binary RY based coding and reduced gene data matrix) and coalescent base methods to explore phylogenetic relationships of this group.

MATERIAL AND METHODS

Taxon sampling

Genomic and transcriptomic data belonging to 47 species from the suborder Psocomorpha were available for this study. Sampling of Lachesillidae included 24 species belonging to 13 genera from the subfamilies Eolachesillinae and Lachesillinae. Also, we included 23 species from the infraorder Caeciliusetae and from the families Elipsocidae, Mesopsocidae, Ectopsocidae and Peripsocidae, plus distant outgroup species belonging to the infraorders Psocetae, Epipsocetae and Philotarsetae. To circumvent alignment difficulties and potential Long Branch attraction (LBA) artefacts, we avoid outgroups that are highly divergent from the ingroup.

Genomic sequencing

For 35 species, whole genome sequence (WGS) data were generated by extracting total genomic DNA using a Qiagen DNeasy Microkit. Library preparation and Illumina sequencing were conducted at the Roy J. Carver Biotechnology Centre at the University of Illinois. A Covaris M220 machine was used to sonicate DNA fragments to approximately 300–500 bp. Libraries were prepared using a Hyper Library construction kit from Kapa Biosystems. Libraries were quantified by qPCR and pooled for sequencing using Illumina HiSeq2500 or NovaSeq6000 S4 lanes for 151 cycles. Pooling was done to achieve between around 30–60X coverage based on an estimated (but unknown) genome sizes of 200–400Mbp, using genome sizes of other members of Psocodea for which genome size is known. The bcl2fastq v2.20 Conversion Software was used to demultiplex and generate FASTQ files. Raw reads were deposited in the NCBI Sequence Read Archive (Table 1).

Gene assembly

Transcriptome data used in this study were previously published by Johnson et al. (2018). Gene assembly was performed with aTRAM2 v2.2.0 (Allen et al., 2018), using a gene set of 2395 protein-coding orthologs as reference. This gene set was identified in the annotated genome of the human body louse, *Pediculus humanus* Linnaeus and previously used for phylogenomic at deep levels for the hemipteroid

TABLE 1 Taxa included in this study with their number of genes assembled and GenBank accession numbers

Infraorder	Family	Subfamily/tribe	Species	N. Raw reads	Genes assembled	Accession
Homilopsocidea	Lachesillidae	Eolachesillinae/Eolachesillinii	*Eolachesilla chilensis* Badonnel, 1951	226,604,268	2194	PRJNA555288
		Eolachesillinae/Graphocaeciliini	*Acantholachesilla* sp.	206,068,144	2164	PRJNA555306
			Anomolachesilla palaciosi García Aldrete et al., 2012	237,957,484	2170	PRJNA555303
			Anomopsocus amabilis Walsh, 1862	114,281,976	2307	SRR5308259
			Anomopsocus sp.	200,613,008	2173	PRJNA555296
			Dagualachesilla anchicayaensis García Aldrete et al., 2012	237,259,818	2171	PRJNA555301
			Dagualachesilloides caliensis García Aldrete et al., 2012	213,331,430	2163	PRJNA555308
			Graphocaeciliini gen. nov2.	232,838,878	2167	PRJNA555309
			Graphocaeciliini gen. nov1.	230,317,566	2183	PRJNA555300
			Graphocaeciliini gen. nov1.	225,307,562	2174	PRJNA555305
			Graphocaecilius interpretatus Roesler, 1940	242,249,482	2142	PRJNA555307
			Prolachesilla sp.	208,624,224	2172	PRJNA555299
		Eolachesillinae/Waoraniellini	*Waoraniella jarlinsoni* Saenz Mancholaet al., 2018	206,587,416	2158	PRJNA555304
		Lachesillinae	*Hemicaecilius mockfordi* García Aldrete et al., 2012	200,271,622	2203	PRJNA555302
			Hemicaecilius smithersi García Aldrete et al., 2012	207,407,542	2198	PRJNA555310
		Lachesillinae/pedicularia	*Lachesilla pedicularia* Linnaeus, 1758	207,382,824	2199	PRJNA555268
		Lachesillinae/corona	*Lachesilla picticeps* Mockford & Sullivan, 1986	228,676,462	2188	PRJNA555295
		Lachesillinae/andra	*Lachesilla punctata* Banks, 1905	259,656,526	2177	PRJNA555297
		Lachesillinae/rufa	*Lachesilla rufa* Walsh, 1863	223,235,000	2187	PRJNA555292
		Lachesillinae/rufa	*Lachesilla abiesicola* García Aldrete, 1990	16,521,720	2106	SRR2051497[a]
		Lachesillinae/forcepeta	*Lachesilla* sp. Ghana	221,948,032	2197	PRJNA555272
		Lachesillinae/forcepeta	*Lachesilla contraforcepeta* Chapman, 1930	28,197,282	2279	SRR1821927[a]
		Lachesillinae/pedicularia	*Lachesilla* sp. Malasya	261,424,186	2191	PRJNA555289
		Lachesillinae/Q	*Lachesilla* sp. 7	198,924,722	2175	PRJNA555293
		Lachesillinae/texcocana	*Lachesila texcocana* García Aldrete, 1972	230,954,950	2173	PRJNA555294
	Ectopsocidae	–	*Ectopsocopsis cryptomeriae* Enderlein, 1907	214,952,998	2167	PRJNA555282
		–	*Ectopsocus briggsi* McLachlan, 1899	16,725,136	2125	SRR645929[a]

(Continues)

TABLE 1 (Continued)

Infraorder	Family	Subfamily/tribe	Species	N. Raw reads	Genes assembled	Accession
	Peripsocidae	–	*Kaestneriella* sp.	203,192,336	2143	
		–	*Peripsocus phaeopterus* Stephens, 1836	15,667,64	2038	SRR2051507[a]
	Elipsocidae	Elipsocinae	*Kilauella* sp.	122,187,158	2327	SRR5308272
			Elipsocus kuriliensis Vishnyakova, 1986	16,530,462	2158	SRR2051485[a]
		Nepiomorphinae	*Nepiomorpha* sp.	118,461,218	2322	SRR5308276
		Propsocinae	*Propsocus pulchripennis* Perkins, 1899	102,301,880	2323	SRR5308281
	Mesopsocidae	–	*Idatenopsocus orientalis* Vishnyakova, 1986	111,986,150	2316	SRR5308271
		–	*Mesopsocus unipunctatus* Müller, 1764	11,695,630	1987	SRR2051502[a]
Caeciliusetae	Asiopsocidae	–	*Asiopsocus sonorensis* Mockford & García Aldrete, 1976	123,267,446	2315	SRR5308261
	Caeciliusidae	Caeciliusinae	*Dypsocus coleoptratus* Hagen, 1858	214,452,066	2165	PRJNA555285
		Fuelleborniellinae	*Fuelleborniella* sp.	229,903,730	2145	PRJNA555311
	Paracaeciliidae	–	*Paracaecilius japonicus* Enderlein, 1906	243,594,290	2145	PRJNA555290
		–	*Xanthocaecilius sommermanae* Mockford, 1955	95,115,648	2298	SRR5308288
	Amphipsocidae	Amphipsocinae	*Polypsocus corruptus* Hagen, 1861	223,302,824	2128	PRJNA555266
			Amphipsocus japonicus Enderlein, 1906	19,173,370	2177	SRR2051466[a]
	Dasydemellidae	–	*Teliapsocus coterminus* Walsh, 1863	218,677,290	2138	PRJNA555312
		–	*Matsumuraiella radipicta* Enderlein,1906	18,092,976	2212	SRR2051500[a]
	Stenopsocidae	–	*Graphopsocus cruciatus* Linnaeus, 1768	19,778,312	2095	SRR2051490[a]
Epipsocetae	Epipsocidae	–	*Bertkauia* sp.	13,140,324	1939	SRR2051473[a]
Philotarsetae	Pseudocaeciliidae	Pseudocaeciliinae	*Heterocaecilius solocipennis* Enderlein, 1907	13,992,850	2126	SRR2051493[a]
Psocetae	Psocidae	Psocinae/Ptyctini	*Ptycta johnsoni* Bess & Yoshizawa, 2007	52,403,206	2006	SRR1821962[a]

[a] Refer to transcriptome data.

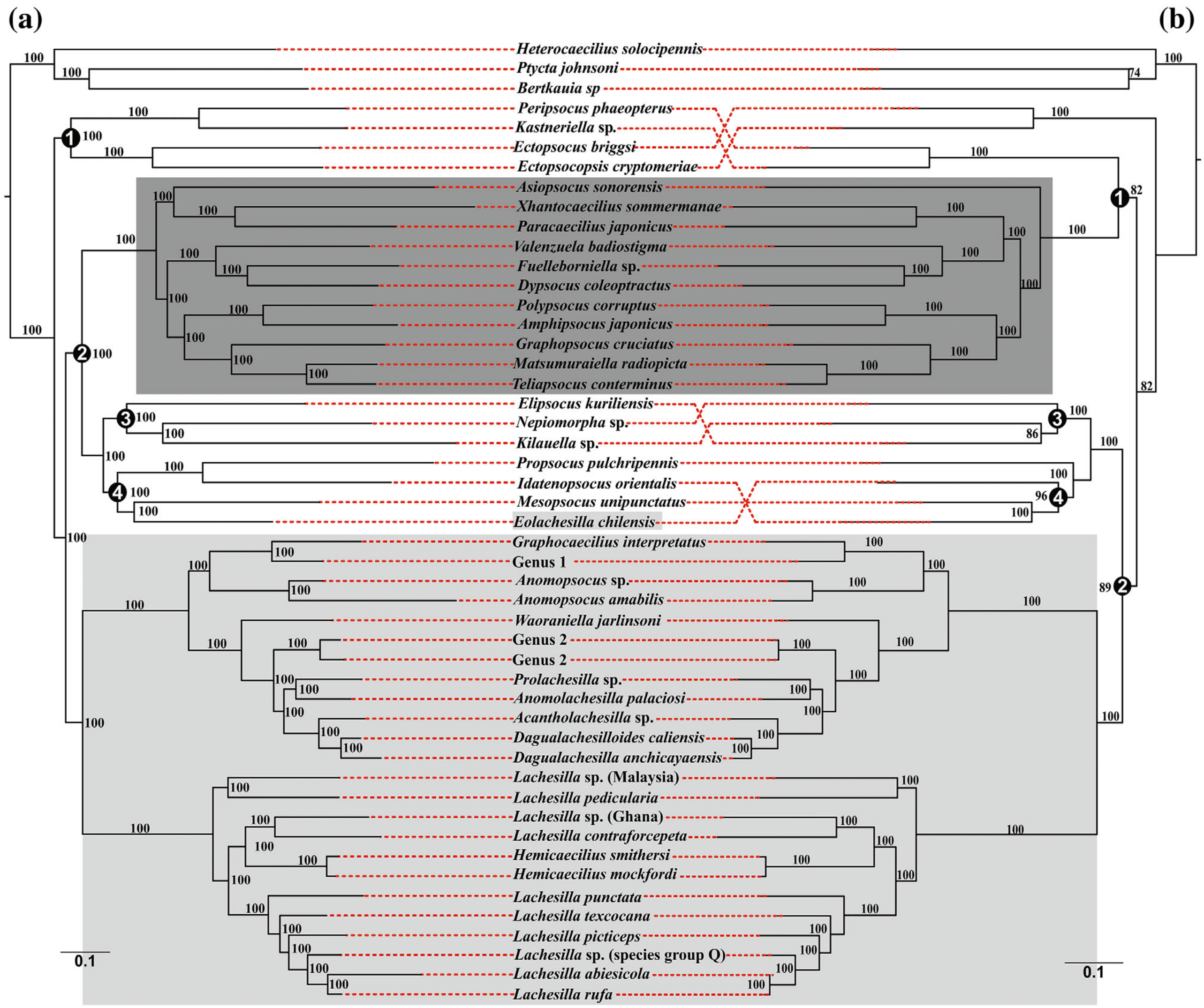

FIGURE 1 ML topologies inferred with (a) full 2060 concatenated super matrix, and (b) reduced 690 concatenated matrix. Numbered nodes indicate 1. Position of Ectopsocidae + Peripsocidae species, 2. Sister relationships associated to *E. chilensis* + Elipsocidae + Mesopsocidae species, 3. Sister relationships associated to *E. kuriliensis* + *Nepiomorpha* sp. + *Kilauella* sp, 4. Sister relationships associated to *E. chilensis* + *P. pulchripennis* + Mesopsocidae species. Numbers associated with branches indicate UFB support. Light grey and dark grey squares indicate Lachesillidae and Caeciliusetae species, respectively

insects (Johnson et al., 2018); Hemiptera: Auchenorrhyncha, (Skinner et al., 2020); bark lice and parasitic lice order Psocodea (de Moya et al., 2021), and shallow levels for the bird louse, genus *Penenirmus* (Johnson et al., 2021). aTRAM2 was set to 1 iteration, using the amino acid sequences of the reference genes to assembly target genes with ABySS assembler (Simpson et al., 2009). The resulting exon sequences for each gene were stitched together with Exonerate (Slater & Birney, 2005) implemented in aTRAM2.

Individual gene sequences were translated to amino acid sequences using EMBOSS Transeq v6.6.0 (Rice et al., 2000). Transcripts and translated genes were concatenated and aligned based on the amino acid sequences using PASTAL v1.8.6 with default parameters (Mirarab et al., 2015). Resulting amino acid alignments were back translated to nucleotide sequences with PAL2NALv14 (Suyama et al., 2006). Nucleotide and amino acids sequences were trimmed with trimAl v.1.4 (Capella-Gutiérrez et al., 2009) with a gap threshold of 0.4. In order to reduce missing data, we used a customized Python script to filter the data, including at least one outgroup taxa and at least 50% of the ingroup Lachesillidae species. A final data set of 2060 genes was used to generate a nucleotide and amino acids concatenated super matrices with PHYUTILITY (Smith & Dunn, 2008) using default parameters and memory usage increased to 4050 MB. Concatenated data sets were manually checked for possible errors in codon frames, whereas stop codons were removed with MACSE v2.06 (Ranwez et al., 2018).

Phylogenomics

Analyses were conducted with the full concatenated matrix (Supplementary data 1) using IQTREE2 v2.1.3 (Minh et al., 2020), under a

Maximum likelihood (ML) approach. The best fit model was estimated with ModelFinder (Kalyaanamoorthy et al., 2017), immediately followed by tree reconstruction (Nguyen et al., 2015) using the estimated best partitioning scheme (−m TESTNEWMERGE). We used the fast relaxed clustering algorithm (− rclusterf 10) (Lanfear et al., 2017) parameter to maximizing computational efficiency, whereas tree support was estimated using ultrafast bootstrapping with UFBoot2 (−bb 1000) (Hoang et al., 2018; Minh et al., 2013).

Additionally, to explore possible impact of molecular biases (GC bias), LBA and/or Incomplete Lineage Sorting (ILS) on phylogeny, several methods were implemented. We generated a binary RY coding-based data set from the full concatenated matrix with a customized python script (Braun & Kimball, 2021), which was analysed under an ML approach, using the same parameters as those for the full concatenated data matrix on IQTREE2. Similarly, two additional matrices based on the codon positions of the full concatenated matrix were generated with PAUP v4.0a (Swofford, 2003); first and second codon positions combined (Supplementary data 2) and second codon positions only (Supplementary data 3). We performed a coalescent species tree analysis with ASTRAL–III v5.7.7 (Zhang et al., 2018) using as input the individual gene trees generated with IQTREE2 (−m MFP) and computing local posterior probabilities (LPP) for branch support (Sayyari & Mirarab, 2016). Finally, TreeShrink v1.3.9 (Mai & Mirarab, 2018) with default parameters was used to prune potential outlier species with abnormally long branches. Based on the TreeShrink analysis, we generated a reduced data set which was analysed under ML with IQTREE2, whereas individual gene trees pruned were used as input for an additional coalescent species tree analysis with ASTRAL–III.

RESULTS

From the 2395 single copy ortholog genes used as reference, aTRAM 2 assembled, on average, 2199 genes for the species generated in this study (Table 1). The final concatenated data matrix included 2060 single copy ortholog genes and 2,438,763 aligned bp (general statistics per gene associated to each data matrix can be found in Table S1). Similarly, derived from the TreeShrink prune analysis, a reduced concatenated data matrix with 690 outlier-free genes containing 858,528 aligned bp was generated. Based on these concatenated data matrices, with the exception of the AA and the second codon only data sets, the majority of the ML analyses did not recover *Eolachesilla chilensis* Badonnel in a monophyletic clade with the remaining species of the family Lachesillidae. Also, monophyly of the infraorder Homilopsocidea was generally unsupported and relationships were generally unstable, especially at deep phylogenetic levels. In contrast, the infraorder Caeciliusetae always was recovered as monophyletic, regardless the data set and analysis used.

Excluding *E. chilensis*, the remaining species of Lachesillidae were grouped as a monophyletic clade with both concatenated and the majority of the ML analyses (Figures 1a,b, S1 and S2). The second codon position (Figure S3) and AA (Figure S4) analyses, clustered *E. chilensis* in a monophyletic subfamily Eolachesillinae, sister to the Elipsocidae + Mesopsocidae clade, rather than the subfamily Lachesillinae, whereas RY coding data set (Figure S5), clustered *E. chilensis* sister to the Elipsocidae + Mesopsocidae clade, rendering Lachesillidae polyphyletic. Within the subfamily Eolachesillinae (which currently also includes *E. chilensis*), the tribe Graphocaeciliini was always recovered as paraphyletic, with *Graphocaecilius interpretatus* Roesler grouped with an undescribed genus from Colombia (Genus 1), plus the species of the genus *Anomopsocus* Roesler. The remaining species of Graphocaeciliini were closely related to *Waoraniella jarlinsoni* Saenz Manchola, González Obando & García Aldrete, a species belonging to tribe Waoraniellini. Here, the recently described genera from the southwest low lands of Colombia (Valle del Cauca department), *Acantholachesilla* García–Aldrete, Saenz Manchola & González Obando, *Dagualachesilla* García Aldrete, González Obando & Carrejo and *Dagualachesilloides* García Aldrete, González Obando & Carrejo were clustered together, whereas another Colombian genus, *Anomolachesilla* García Aldrete, González Obando & Carrejo was recovered as sister to *Prolachesilla* Mockford & Sullivan (Figure 1).

TABLE 2 Current classification and number of Lachesillidae species at subfamily and genus level

Subfamily	Tribe	Genus	No. species
Eolachesillinae	Eolachesillini	*Eolachesilla*	1
	Waoraniellini	*Waoraniella*	4
	Graphocaeciliini	*Amazolachesilla*	1
		Antilachesilla	1
		Acantholachesilla	1
		Anomolachesilla	2
		Anomopsocus	2
		Cuzcolaquesilla	1
		Dagualachesilla	2
		Dagualachesilloides	1
		Garcialdretiella	1
		Graphocaecilius	11
		Nanolachesilla	7
		Notolachesilla	2
		Mesolachesilla	1
		Prolachesilla	13
		Tricholachesilla	2
Lachesillinae		*Ceratolachesillus*	1
		Ectolachesilla	1
		Hemicaecilius	7
		Homoeolachesilla	2
		Lachesilla	349
		Nadleria	4
		Zangilachesilla	1
		Zonolachesillus	14
Cyclolachesillinae		*Cyclolachesillus*	1

The subfamily Lachesillinae was always recovered as monophyletic (Figures 1a,b, S1–S5). For Lachesillinae, this study included two species of the genus *Hemicaecilius* Enderlein and ten species of the highly diverse genus *Lachesilla* Westwood, representing seven species groups, but monophyly of *Lachesilla* never was recovered. All data sets and analysis recovered the species group forcepeta (two species included here) sister to *Hemicaecilius*, whereas the species group pedicularia (which include the family, genus and group type species *Lachesilla pedicularia* Linnaeus) was recovered as sister to the forcepeta + *Hemicaecilius* clade, plus the remaining species of *Lachesilla*. Similarly, the majority of the topologies clustered the species group andra (represented by *Lachesilla punctata* Banks) sister to the species groups texcocana + picticeps + Q + rufa, the latter including two species as a monophyletic clade (species group rufa).

At the level of infraorder, monophyly of Homilopsocidea was not recovered in the majority of the analysis and data sets. With the full 2060 gene concatenated data set, the families Ectopsocidae + Peripsocidae were recovered as sister to a clade which includes the species belonging to the infraorder Caeciliusetae sister to the Elipsocidae + Mesopsocidae + *E. chilensis* clade (Figure 1a, node 1, UFB = 100%), whereas the 690 reduced gene data set, recovered Ectopsocidae sister to the Homilopsocidea + Caeciliusetae species and Peripsocidae sister to Caeciliusetae (Figure 1b, node 1, UFB = 82%). In contrast, with the third codon position excluded data set, monophyly of Homilopsocidea was recovered with low UFB (67%, Figure S2), being the Ectopsocidae + Peripsocidae clade, sister to the remaining Homilopsocidea species. With the second codon position only data set, deep relationships within Homilopsocidea obtained poor UFB, with the family Peripsocidae recovered sister to the Caeciliusetae, with low UFB branch support (75%), whereas Eolachesillinae (including *E. chilensis*) was recovered as sister to a clade that clustered Elipsocidae + Mesopsocidae species (both families paraphyletic).

With the AA data set, deep relationships received high UFB (100–97%). Here, Ectopsocidae species were recovered sister to the remaining Homilopsocidea + Caeciliusetae, with Peripsocidae begin sister to the later infraorder (Figure S4). Some differences were observed between full 2060 ASTRAL tree and the reduced 690 ASTRAL tree regarding the position of Ectopsocidae and Peripsocidae; the former data set recovered Peripsocidae as sister to Caeciliusetae + remaining species of Homilopsocidae (LPP = 0.45, Figure S1, node 1), whereas Ectopsocidae was recovered as sister to a clade which includes Caeciliusetae plus Elipsocidae + Mesopsocidae + *E. chilensis*, but with low branch support (LPP = 0.43, Figure S1, node 2–3). The latter data set recovered Peripsocidae sister to Caeciliusetae (LPP = 0.81, Figure S1, node 1) and Ectopsocidae sister to the remaining Homilopsocidea species (LPP = 0.58, Figure S1, node 2–3).

DISCUSSION

Relationships within family Lachesillidae have been discussed in the past based on morphology, but a phylogenetic framework has not been proposed until recently. Mockford and Sullivan (1986) recognized the subfamilies Eolachesillinae and Lachesillinae, the former including *Eolachesilla* and "graphocaeciliines" (now including the species of the tribe Graphocaeciliini plus the genus *Waoraniella*) and the latter primarily including the genera *Lachesilla* and *Nadleria* Badonnel & García–Aldrete. Eolachesillinae currently includes 17 genera and 44 species, whereas Lachesillinae includes eight genera, four endemic to the Oriental region, three from the Neotropical region. The genus *Lachesilla* (Table 2) is the most specious genus, with more than 340 described and at least 100 undescribed species (García Aldrete & da Silva-Neto, 2020) and a nearly cosmopolitan distribution.

The phylogenomic tree of the family Lachesillidae presented here provides a new framework to better understand the phylogenetic relationships of some genera of the family. For example, the genus *Eolachesilla* has been an issue since Badonnel (1951) included this genus within Lachesillidae. This genus was transferred by New and Thornton (1981) to Elipsocidae and subsequently placed back into Lachesillidae by Mockford and Sullivan (1986) based on morphological characters. It was not until Yoshizawa (2002) that a morphological systematic approach explored the phylogenetic relationships of suborder Psocomorpha and found that the monophyly of the family Lachesillidae including *Eolachesilla* is uncertain and noticed that *Eolachesilla* may represent its own family, close to Lachesillidae.

Our results support the hypothesis that *Eolachesilla* is closely related to the Elipsocidae + Mesopsocidae rather than to Lachesillidae, as was suggested by Schmidt and New (2004). However, the systematic position of *Eolachesilla* is unstable across the data sets and analysis; sometimes, recovered as sister to *Mesopsocus unipunctatus* Müller (rendering the family Mesopsocidae paraphyletic) with the full 2060 and reduced 690 data sets (Figure 1a,b, node 4), whereas the ASTRAL trees recovered it sister to a monophyletic Mesopsocidae (Figure S1), suggesting that ILS (deep coalescence) occurred. This is not surprising because internal branches are short in this part of the ML topology. Codon and amino acid analyses also recovered an ambiguous position of *Eolachesilla*, being sister to the Elipsocidae + Mesopsocidae clade (third codon exclude data set, Figure S2) or in a monophyletic clade with the remaining species of the subfamily Eolachesillinae (second codon position only and amino acid data sets, Figures S3 and S4, respectively). Similar instability was observed in recent mitophylogenomics and UCE phylogenomics analyses (Saenz Manchola et al., 2021, 2022), for which taxon sampling was also heavily focused in Lachesillidae species (Figure 2b,c).

Such discordance between topologies (considering data sets and gene tree-species trees) may be the result of several issues that can affect phylogenetic estimation. These include faster substitution rate in nonadjacent phylogenetic lineages, poor taxon sampling due to extinction or unavailability of some taxa, and unsuitable models of sequence evolution that do not account for base compositional heterogeneity can be associated with long-branch attraction biases (LBA, Lartillot et al., 2007; Qu et al., 2017). Here, the topological conflict observed between nucleotide vs amino acid analysis could be caused by a model misspecification, which has been proposed as source of inaccurate phylogeny estimation and potentially resulting in conflicting topologies hypothesis under nucleotide vs amino acid (Gillung et al., 2018).

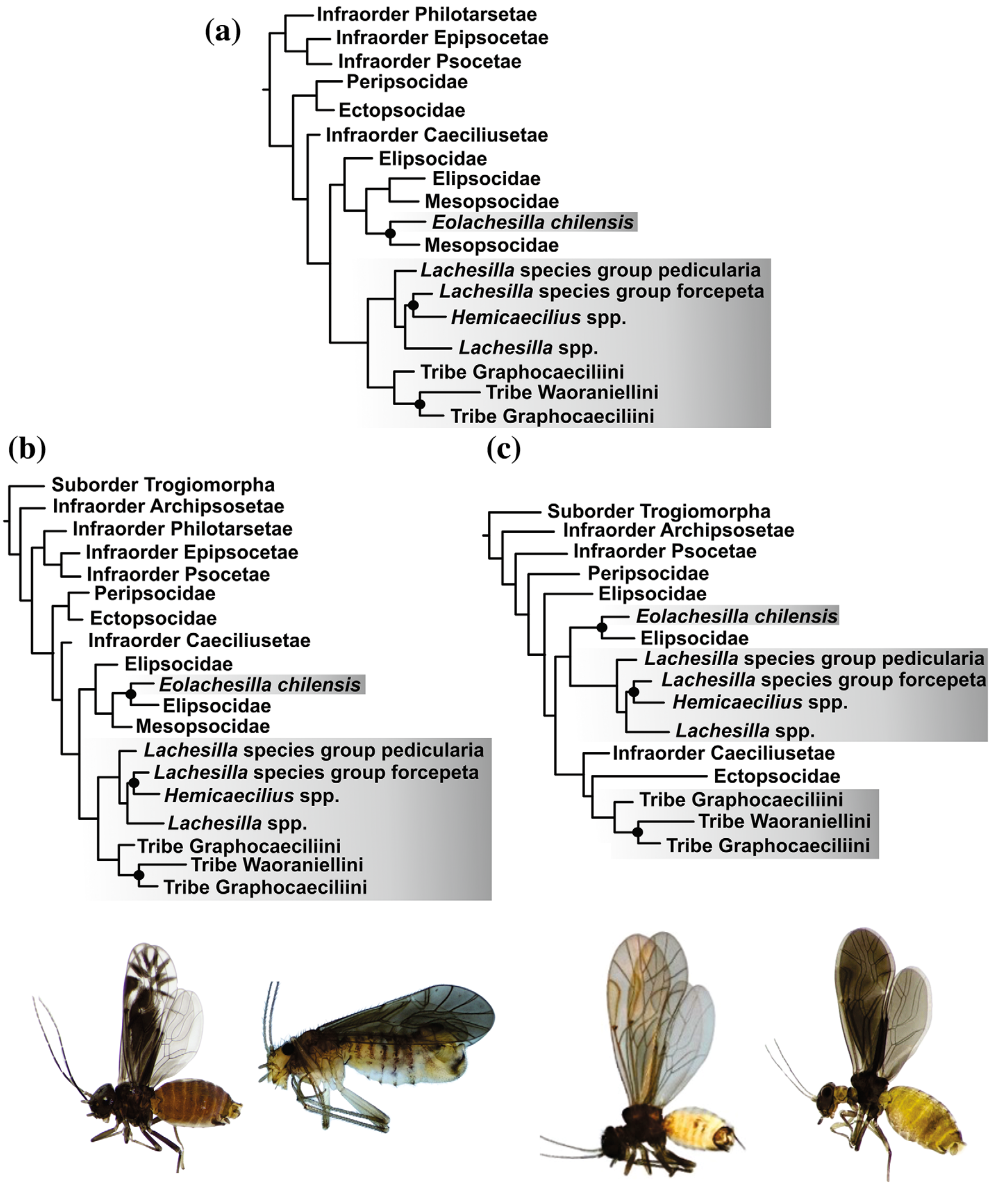

FIGURE 2 Tree topologies for Lachesillidae species obtained with ML analysis for: (a) 2060 nuclear ortholog gene data set, (b) 2081 UCE loci data set and (c) 37 mitochondrial genes data set. Grey squares indicate Lachesillidae species. Black circles indicate monophyly not supported for Lachesillidae groups. Species pictures from left to right, tribe Graphocaeciliini: *Graphocaecilius* and *Dagualachesilla*. Subfamily Lachesillinae: *Lachesilla* (*pedicularia* species group) and *Hemicaecilius*. Credits to Dr. Ranulfo González

However, conflict among nucleotides or amino acids topologies is relatively common in phylogenomics, affecting Lepidoptera, Hymenoptera, Coleoptera, Hemiptera, amount others groups (see Rota et al., 2022, for a summary of insect phylogenomics with topologies conflict).

One of the proposed strategies to reduce the effects of LBA is to exclude long-branch taxa and/or fast-evolving genes from the analysis (Lartillot et al., 2007; T. Li et al., 2014) which was implemented here with the TreeShrink analysis. These resulted in both ML and coalescent analyses highly congruent, especially at shallow levels, whereas ASTRAL 2060 vs 690 data sets showed some incongruence, especially for Ectopsocidae + Peripocidae species and their position at deep levels (Figure S1, nodes 1–2). Unlike for the 2060 vs 690 concatenated data sets, relationships between *E. chilensis* and Mesopsocidae + Elipsocidae differs (Figure 1a,b, nodes 3–4), which may be associated with the topology impact of the outlier taxa and genes in the phylogeny.

On the other hand, base composition bias (GC bias) may cause erroneous phylogenetic estimation, which has been identified in the past as a source of conflict in phylogenetic studies of psocids (de Moya et al., 2021; Saenz Manchola et al., 2022; Yoshizawa & Johnson, 2014). In our current data set, *E. chilensis* and *M. unipunctatus* contain the largest average and codon-specific GC content among all the species analysed (Table S2). Both species were recovered as sister taxa in the full data set topology (which contains 54.44 and 50.82 GC %, respectively), whereas they were recovered as more distantly when the third codon was excluded and with the second codon position only data sets (containing 39.27 and 38.52 GC %, respectively, Table S2). An additional strategy we implemented to avoid GC bias was the RY coding-based analysis (Figure S5), and this result was similar to that obtained with the second codon position only and the amino acid data sets (i.e. subfamilies Eolachesillinae and Lachesillinae not clustered together in a monophyletic clade). However, the RY data set resulted in *E. chilensis* sister to the Mesopsocidae + Elipsocidae species rather that Eolachesillinae, which could be an indicator of the impact of GC bias has on the conflicting positions of this species in the phylogeny.

Given this instability, it is difficult to clearly establish a systematic position for *Eolachesilla* within Homilopsocidea, but we consider that a reclassification is likely necessary, thus excluding *Eolachesilla chilensis* from Lachesillidae and declaring this genus *incertae sedis* within Elipsocidae + Mesopsocidae. Additionally, we strongly suggest that more taxon sampling of other species in Elipsocidae and Mesopsocidae would be useful to add to a tree in future before finalizing a new classification for this problematic genus.

Apart from the unstable position of *Eolachesilla*, highly supported clades were found within Lachesillidae. For example, monophyly of tribe Graphocaeciliini was not recovered with any of the data sets or analysis (Figures 1a,b and S1–S5), and these results also were supported by previous UCE and Mitophylogenomics phylogenies (Figure 2b,c). The grouping *G. interpretatus* + *Anomopsocus* spp. + Genus 1 was recovered in a clade apart from the remaining genera and species of graphocaeciliines with high branch support (UFB =100, LPP = 1, Figure 1a,b). A close relationship between the genera *Graphocaecilius* and *Anomopsocus* was recognized by Mockford and Sullivan (1986), based on the phallosome and epiproct morphology. The arrangement of the mitochondrial genome indicates that this clade also shares a unique mitochondrial gene rearrangement, which supports the close relationships between these species (Saenz Manchola et al., 2021). The remaining species of graphocaeciliines were clustered in a clade with *W. jarlinsoni* (belonging to tribe Waoraniellini) as sister. Within this group, the genus *Acantholachesilla* was recovered sister to *Dagualachesilla* + *Dagualachesilloides*, which support previous morphological hypotheses about this clade (García Aldrete et al., 2014). Similarly, the clustering of *Prolachesilla* + *Anomolachesilla* supports the close relationship of both genera based on the genital morphology of both sexes found by García Aldrete et al. (2012), for which *Anomolachesilla* could be assignable to *Prolachesilla* were it not for the forewing venation.

In the subfamily Lachesillinae, monophyly of *Lachesilla* was never recovered. The species group forcepeta was recovered as sister to *Hemicaecilius*, with high UFB and LPP branch support (100/1, Figure 1a,b, node 3). *Hemicaecilius* is a genus from the Neotropical Andean region, whereas the species group forcepeta is the largest within *Lachesilla*, with 105 species, mainly distributed in the Neotropic and Neartic regions, but with few species in the Ethiopian region (García Aldrete & da Silva-Neto, 2020; Lienhard, 2020). Considering the close relationship with *Hemicaecilius*, also supported by mitochondrial, UCE topologies (Figure 2b,c) and the male claspers–phallosome structure, we suggest that the species group forcepeta could be considered as a separate entity from the remaining species of *Lachesilla*. Similarly, with the results presented here, the remaining species groups of *Lachesilla* should be re-evaluated, considering the species in the pedicularia species group, which includes the type species of the genus (*L. pedicularia*), as *Lachesilla* sensu strictu.

CONCLUSIONS

Since Mockford and Sullivan (1986), this current phylogenomic study is one of the first attempts to resolve phylogenetic relationships within Lachesillidae. Our findings give support to the hypothesis that the genus *Eolachesilla* is closer to the clade Elipsocidae–Mesopsocidae rather than Lachesillidae. However, considering the relatively sparse taxon sampling for Elipsocidae and Mesopsocidae, we cannot conclude decisively the position of *Eolachesilla* within the Homilopsocidea and we consider that expanding the taxon sampling within these families will help to resolve this problem. Similarly, monophyly of Lachesillidae excluding *Eolachesilla* is strongly supported, thus we suggest excluding *Eolachesilla chilensis* from the Lachesillidae and declare the genus *incertae sedis*. Finally, the paraphyly of *Lachesilla*, supported by our analysis, agrees with previous phylogenomic studies and we suggest considering the species group forcepeta as a different entity from *Lachesilla*.

Similarly, these results, plus previous nuclear ortholog genes and UCE phylogenomics (de Moya et al., 2021; Saenz Manchola et al., 2022), give strong support to the paraphyly of infraorder Homilopsocidea. A 2370 nuclear ortholog gene analysis resulted in a topology with Peripsocidae or Ectopsocidae grouped with the Caeciliusetae, depending on the method of analysis (de Moya et al., 2021), whereas UCE phylogenomics recovered both families sister to the remaining Caeciliusetae + Homilopsocidea species (Saenz Manchola et al., 2022). Here, the systematic position of the Peripsocidae and Ectopsocidae is unstable, depending on the data sets and analysis. These families are sometimes grouped as sister to the remaining species Caeciliusetae + Homilipsocidea (full 2060 data set, Figure 1a), with each one grouped either to Caeciliusetae, with the remaining Homilopsocidea species (second codon only data set, Figure S3), or as sister to the remaining species in a monophyletic infraorder Homilopsocidea (third codon excluded data set, Figure S2). Giving our taxon sampling is not possible to establish whether either Ectopsocidae or Peripsocidae (or both) are sister to the Caeciliusetae and the remaining Homilopsocidea species, thus we consider important to focus on this infraorder in future phylogenetic studies.

AUTHOR CONTRIBUTION

Oscar Fernando Saenz Manchola: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing–original draft, Visualization. Stephany Virrueta-Herrera: Data Curation, Formal analysis, Writing–original draft. Lorenzo Mario D'Alessio: Data Curation, Formal analysis. Alfonso Neri García Aldrete: Conceptualization, Resources, Writing–original draft, Supervision. Kevin P. Johnson: Conceptualization, Methodology, Resources, Validation, Writing–original draft, Writing– Reviewing and Editing, Funding acquisition.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

DATA AVAILABILITY STATEMENT

All supplementary data are available from the figshare repository https://doi.org/10.6084/m9.figshare.20346285.v1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Coalescent-based trees inferred using ASTRAL-III for (A) full 2060 concatenated super matrix, (B) reduced 690 concatenated matrix. Numbered nodes indicate 1. Position of Peripsocidae species, 2. Position of Ectopsocidae species Paraphyly of tribe Graphocaeciliini, 3. Sister relationships associated to *E. chilensis* + Elipsocidae + Mesopsocidae species. Light grey and dark grey squares indicate

Lachesillidae and Caeciliusetae species, respectively. Numbers associated with branches indicate UFBoot (A) and local posterior probability

Figure S2. ML phylogenetic tree inferred with the third codon position excluded data set. Numbers associated with branches indicate UFB support. Light grey and dark grey squares indicate Lachesillidae and Caeciliusetae species, respectively. Light grey and dark grey squares indicate Lachesillidae and Caeciliusetae species, respectively

Figure S3. ML phylogenetic tree inferred with the second codon positions only data set. Numbers associated with branches indicate UFB support. Light grey and dark grey squares indicate Lachesillidae and Caeciliusetae species, respectively

Figure S4. ML phylogenetic tree inferred with the amino acid data set. Numbers associated with branches indicate UFB support. Light grey and dark grey squares indicate Lachesillidae and Caeciliusetae species, respectively

Figure S5. ML phylogenetic tree inferred with the RY-based coding data set. Numbers associated with branches indicate UFB support. Light grey and dark grey squares indicate Lachesillidae and Caeciliusetae species, respectively

Table S1. General statistics per gene associated to each data matrix used in this study

Table S2. Nucleotide composition and AT–GC % for species analysed in this study. Bold species refer to the highest GC %

Supplementary Data 1. 2060 loci full concatenated and aligned data matrix used in this study

Supplementary Data 2. First and second codon position combined data matrix used in this study

Supplementary Data 3. Second codon positions only data matrix used in this study

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