Antonio Marcelino do
Carmo Neto

Cladistic Analysis of Lestremiinae (Diptera: Cecidomyiidae)

Análise Cladística de Lestremiinae (Diptera: Cecidomyiidae)

Single Volume

SÃO PAULO

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Original Version

Thesis submitted to the Graduate Program of the Museu de Zoologia da Universidade de São Paulo in partial fulfillment of the requirements for the degree of Doctor of Science (Systematics, Animal Taxonomy and Biodiversity).

Advisor: Prof. Dr. Carlos José Einicker Lamas

Co-Advisor: Prof. Dr. Maria Virginia Urso Guimarães

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RESUMO

Pequenos mosquitos micófagos da família Cecidomyiidae são facilmente ignorados em pesquisas pois são inconspícuos, ao contrário das espécies galhadoras que deixam vestígios vistosos de sua presença na forma de galhas entomogênicas e foram estudados de forma mais consistente. Apesar disso, as espécies micófagas representam 25% da riqueza de espécies dessa família muito diversificada (6.590 espécies em 812 gêneros conhecidos), e são onipresentes no ambiente natural como parte da reciclagem de nutrientes por sua associação com matéria vegetal em decomposição e fungos. A subfamília Lestremiinae *sensu* Jaschhof & Jaschhof é uma das cinco dentre os Cecidomyiidae que são inteiramente compostas por espécies micófagas. Esta subfamília é um dos clados mais antigos de Cecidomyiidae, incluindo 13 gêneros existentes e 103 espécies existentes e três fósseis. A delimição dos gêneros é frequentemente dificultada porque muitos caracteres morfológicos usados para definir os táxons de Lestremiinae carecem de estudos abrangentes e comparativos sob abordagens modernas. As sensilas antenais são de grande importância taxonômica, mas não há estudo de sua ultraestrutura para nenhum Cecidomyiidae micófago. Da mesma forma, não há análise quantitativa dos muitos caracteres de uso tradicional que variam de forma contínua. Entre os vários táxons novos encontrados e aguardando descrição, há alguns que não corresponderam a nenhum dos gêneros conhecidos como são atualmente definidos, assim levantamos a hipótese de potenciais novos gêneros. Como a validade, delimitação e inter-relações dos gêneros de Lestremiine não são estabelecidos, este estudo teve como objetivo revisar a diversidade taxonômica e morfológica de Lestremiinae, testar a monofilia da subfamília, apresentar uma hipótese de relações filogenéticas entre os gêneros, posicionar os novos táxons neotropicais e descrever eventuais novos táxons supraespecíficos. Os espécimes estudados provêm de amostras de todo Brasil, além de espécimes de coleções da América do Norte e Europa. Espécimes de seis gêneros foram estudados com Microscopia Eletrônica de Varredura para análise da ultra-estrutura das sensilas da antena e do palpo. A matriz de dados foi composta por 54 espécies do grupo interno, de 12 gêneros, mais 21 espécies do grupo externo representando todas as outras subfamílias de Cecidomyiidae e as famílias Bibionidae, Mycetophilidae e Sciaridae. As análises de parcimônia foram realizadas no TNT 1.5, por meio de buscas com “Novas Tecnologias”, sob pesagem implícita estendida. Os resultados incluem a revisão dos gêneros *Insulestremia* e *Anaretella* na região Neotropical, incluindo a descrição

de novas espécies; 15 subtipos de sensilas encontrados na cabeça das espécies de lestremiinae, sendo dois deles exclusivos; uma matriz com 282 caracteres morfológicos, sendo 162 quantitativos, que permitiu a reconstrução de uma única Árvore Mais Parcimoniosa estável a partir de K=10, que é a primeira filogenia de Lestremiinae. Onze dos gêneros reconhecidos de lestremiine foram recuperados como monofiléticos (*Neolestremia* não foi incluído na análise). Espécies anteriormente colocadas em *Allarete* foram divididas em quatro gêneros, incluindo três novos. *Conarete* é considerado sinônimo júnior de *Anarete*. Um novo táxon neotropical foi corroborado como representante de um novo gênero. Duas espécies fósseis de *Lestremia* foram encaixadas na estrutura da filogenia, sendo elevadas como compondo um novo gênero fóssil.

Palavras-chave: Bibionomorpha. Biodiversidade. Evolução. Região Neotropical. Taxonomia integrativa.

ABSTRACT

Tiny mycophagous midges of the family Cecidomyiidae are easily overlooked by researchers as they are inconspicuous, unlike the galling species that leave showy traces of their presence in the form of entomogen galls and have been studied more consistently. Nevertheless, mycophagous species represent 25% of the species richness of this very diverse family (6,590 species in 812 known genera), and they are ubiquitous in the natural environment as part of the recycling of nutrients by their association with decaying vegetal matter and fungi. The subfamily Lestremiinae *sensu* Jaschhof & Jaschhof is one of the five within Cecidomyiidae that are entirely composed by mycophagous species. This subfamily is one of the most ancient clades of Cecidomyiidae, including 13 extant genera and 103 extant and three fossil species. Generic boundaries are often hampered because many morphological characters used to define Lestremiinae taxa lack comprehensive and comparative studies under modern approaches. Antennal sensilla are of great taxonomic importance, but there is no study of its ultrastructure for any mycophagous Cecidomyiidae. Likewise, there is no quantitative analysis of the many characters of traditional usage in the taxonomy of Lestremiinae that varies continuously. Among the several new taxa found and waiting to be named, there are some that did not correspond to any of the described genera as they are currently defined, thus we hypothesized the discovery of new genera. As the validity, boundaries, and interrelationships of lestremiine genera were largely unclear, this study aimed to revise Lestremiinae taxonomic and morphological diversity, test the monophyly of the subfamily, presenting a hypothesis of phylogenetic relationships among genera, placing the new neotropical taxa and erecting and describing new supraspecific taxa accordingly. Specimens studied were sampled from all over Brazil, besides specimens from collections in North America and Europe. Specimens of six genera were studied with Scanning Electron Microscopy in order to assess the ultrastucture of the sensilla of antenna and palpus. The data matrix consisted of 54 ingroup species of 12 genera, plus 21 outgroup species representing all the other subfamilies of Cecidomyiidae and the families Bibionidae, Mycetophilidae and Sciaridae. Parsimony analysis were performed in TNT 1.5, through searches with “New Technologies”, under extended implied weighting. The results include the revision of the genera *Insulestremia* and *Anaretella* in the Neotropics, including description of new species; fifteen subtypes of sensilla found in the head of lestremiinae species, two of them being exclusive; a matrix with 282 morphological

characters, 162 quantitative, that allowed the reconstruction of a single, stable Most Parsimonious Tree under K=10, which is the first phylogeny of Lestremiinae. Eleven of the recognized lestremiine genera were recovered as monophyletic (*Neolestremia* was not included in the analysis). Species previously placed in *Allarete* were split into four genera, including three new. *Conarete* is considered a junior synonym of *Anarete*. A new neotropical taxon was corroborated as the representant of a new genus. Two fossil *Lestremia* species were fitted within the framework of the phylogeny, being raised as composing a new, fossil genus.

Keywords: Bibionomorpha. Biodiversity. Evolution. Integrative taxonomy. Neotropical region.

GENERAL INTRODUCTION

Cecidomyiidae is the most diverse family within the Bibionomorpha and one of the most diverse within the Diptera, with 6,590 species in 812 known genera (Gagné & Jaschhof, 2021). Studies indicate that the family is potentially even the most diverse among all Diptera (Borkent et al. 2018; Brown et al., 2018; Hebert et al., 2016). The family has been recovered as monophyletic in phylogenies based on both morphological and molecular data (Amorim & Rindal, 2007; Ševčík et al., 2016; Sikora et al., 2019). It is positioned in one of the ancient branches of the order Diptera, in the infraorder Bibionomorpha, superfamily Sciaroidea, although its relationship with the other families has been controversial (Matile, 1997; Chandler, 2002; Hippa & Vilkamaa, 2005; Hippa & Vilkamaa, 2006; Wood & Borkent, 1989, Amorim & Rindal, 2007; Ševčík et al. 2016; Sikora et al., 2019).

The synapomorphies of Cecidomyiidae are the loss of tibial spurs, the reduction of the larval thoracic capsule with modifications to the mouthparts, including elongated and styliform mandibles specialized in sucking food, and the presence of a dermal structure in the larva called prothoracic spatula. Their larvae have three instars, and their habits range from mycophagous, as the remainder Sciaroidea, to phytophagous and predators of small arthropods. The galling habit is predominant in the family. Galling cecidomyids belong to the Lasiopteridi and Cecidomyiidi supertribes, within the subfamily Cecidomyiinae, including about 75% of the known diversity (Gagné & Jaschhof, 2021).

Tiny mycophagous midges of this family are easily overlooked by researchers as they are inconspicuous, unlike the galling species that leave showy traces of their presence in the form of entomogen galls and have been studied more consistently all around the globe (Gagné & Jaschhof 2021). Nevertheless, the mycophagous species represent 25% of the species richness, and they are ubiquitous in the natural environment as part of the recycling of nutrients by their association with decaying vegetal matter and fungi (Mamaev & Krivosheina 1965; Jaschhof & Jaschhof, 2009). They are grouped in the subfamilies Catotrichinae, Lestremiinae, Micromyiinae,

Winnertziinae, Porricondylinae, and two supertribes of Cecidomyiinae, Brachineuridi and Stomatosematidi.

The subfamily Lestremiinae *sensu* Jaschhof & Jaschhof (2009) is one of the five within Cecidomyiidae that are entirely composed by mycophagous species. This subfamily is one of the most ancient clades of Cecidomyiidae (Sikora et. al., 2019), including 13 extant genera, and 103 extant and three fossil species (Gagné & Jaschhof 2021). Their members are recognized by the following combination of characters: first tarsomere longer than the second, the relatively short vein R5 that joins the Costa well before the wing apex (except in the genera *Buschingomyia* Jaschhof, *Eomastix* Jaschhof, and *Gongromastix* Enderlein), and the forked vein M1+2, the later with the fork being longer than the stem (Fig. 1B).

The midges grouped in the tribe Lestremiini (Edwards, 1938) were split from the Micromyiidi (currently Micromyiinae) and raised as subfamily by Jaschhof & Jaschhof (2009). This group has been well circumscribed and always hypothesized as a monophyletic clade. This hypothesis still lacks the support of a cladistic analysis, as it either was not addressed by cladistic methods or was a secondary question of the study, as in the recent phylogenies regarding early evolution of the Cecidomyiidae with minimal sampling of lestremiini taxa (Ševčík et al., 2016; Sikora et al., 2019).

Eomastix (1 sp.), *Buschingomyia* (1 sp.), *Gongromastix* (5 spp.), and *Mangogrostix* Mamaev (2 spp.), the genera with massive gonocoxites, are hypothesized as composing an early branch in the evolution of Lestremiinae (Jaschhof & Jaschhof, 2009; 2011). *Allaretella* Meyer & Spungis (1 sp.), *Anaretella* Enderlein (7 spp.), and *Insulestremia* Jaschhof (3 spp.), the genera with branched sensilla, are generally considered to be closely related (Jaschhof & Jaschhof, 2009). Those groupings are only vaguely defined, and there is no hypothesis regarding the interrelationships of the remainder genera.

Regarding generic circumscriptions, the genus *Allarete* Pritchard (12 spp.) is a grouping of species with very diverse genitalic structures, often because they just do not fit into other genera, and thus it is probably polyphyletic (Jaschhof & Jaschhof, 2009). The genera *Anarete* Haliday (38 spp.) and *Conarete* Pritchard (13 spp.) are quite

distinctive among the Lestremiinae, but rather vaguely from each other, as all characters used to separate them are often overlapping (Jaschhof & Jaschhof, 2011). *Neolestremia* Mani (3 spp.) lacks detailed information in its original description and a revision of its type specimens, thus its validity is questioned (Jaschhof & Jaschhof, 2009). Generic boundaries are often hampered because many morphological characters used to define Lestremiinae taxa lack comprehensive and comparative studies under modern approaches. Antennal sensilla are of great taxonomic importance, but there is no study of its ultrastructure for any mycophagous Cecidomyiidae; likewise, there is no quantitative analysis of the many characters of traditional usage in the taxonomy of Lestremiinae that varies continuously.

Although not the most diverse within Cecidomyiidae, the Lestremiinae are widespread (Jaschhof & Jaschhof 2011; Gagné & Jaschhof, 2021). The species *Anaretella defecta* Winnertz, *Lestremia cinerea* Macquart, and *Lestremia leucophaea* Meigen are considered to be virtually cosmopolitan (Gagné & Jaschhof 2021), and Jaschhof & Jaschhof (2009) argue that many other species of those genera are merely regional variants of them. The distinction among several species of *Allarete*, *Anarete* and *Conarete* are also questioned (Jaschhof & Jaschhof, 2009; 2011). A revision of the Lestremiinae outside the holarctics is still pending, mainly due to the unavailability of the material from the Oriental region (Jaschhof & Jaschhof, 2009; 2011).

Subfamily studies started with the description of *Lestremia cinerea* by Macquart, 1826. Only putative contributions would be added during the next 100 years, mainly by Felt and Kieffer (Gagné & Jaschhof, 2021). Edwards (1938) revised the Lestremiinae from the United Kingdom, setting the standard for descriptions and identification practice, prioritizing characters of the male terminalia and assessing intraspecific variation. An exponential growth in the volume of studies regarding Lestremiinae taxa would spawn during the second half of the 20th century, with the works of Mamaev and Berest in the Palearctic region, and of Pritchard and Kim in the Nearctic region. Pritchard was the first to appreciate the wide distribution of lestremiine taxa, but although he would then use this knowledge together with the known morphological variability of the Lestremiinae to synonymize species accordingly, he also described species and erected genera based on arbitrary measurement thresholds. Many studies would also be published regarding

the lestremiine taxa from the Oriental region, mainly by Grover and Rao (Gagné & Jaschhof, 2021). The fauna of the Holarctic region was later revised by Jaschhof & Jachhof (2009), recognizing 13 genera.

The study of the Lestremiinae in the Neotropical region began with the description of *Lestremia nigra* Blanchard, in 1852, from Chile. More than 60 years later, Felt described *Anarete buscki*, in 1915, from Cuba. In 1994, Gagné added the record of *Conarete eluta* Pritchard for Dominica. The last contribution was made by Jaschhof in 2004, recording the occurrence of *A. buscki* and erecting the monotipic genus *Insulestremia* to include the species *I. sinclairi* from the Galapagos Islands, Ecuador (Gagné & Jaschhof, 2021).

In Brazil, mycophagous taxa have been largely neglected, with some groups being completely unknown in the country until some years ago (Carmo-Neto et al., 2019). There were no previous records for the subfamily Lestremiinae (Maia, 2022). The study of these cecidomyids is being developed since the launch of the SISBIOTA-Diptera program in 2012, which was coordinated by CJEL and aimed to inventory the dipterofauna of the states of Mato Grosso, Mato Grosso do Sul and Rondônia. The presence of unrecorded and also undescribed taxa was noted, as expected, and it propelled an expanded search through samples from other localities.

We took advantage of several other taxonomic initiatives from Brazil. Additional material from the Afrotropical and Oriental regions was examined, part of the Diptera collection of NHRS, separated during visit of AMCN and loaned for study back in Brazil. Naturally, in Brazil researchers can draw upon an extremely diverse living environment. The acquired material includes samples from five major biomes in the country: the Amazon and Atlantic forests, the Cerrado (Brazilian savannah), the Pantanal (Brazilian wetlands), and the Caatinga (dry tropical forest). Those biomes are known to be both rich in endemics and highly threatened by landscape changes (Brazil Flora Group 2021; MMA 2018); for instance, the recent and tragical episodes of fires in the Amazon rainforest (in the states of Amazônia, Pará and Rondônia, in 2019 and 2020) and in the Pantanal (in the states of Mato Grosso and Mato Grosso do Sul, in 2020). The scenario

encompassing all the tropical regions of the globe is very similar, hence the increasing necessity of assessing this threatened biodiversity.

After the study of 1,303 adult male specimens of Lestremiinae (2,575 lestremiine specimens in total, from at least 117,539 cecidomyiid specimens) sorted from samples in ethanol, plenty of new records and species could be found. Among the several new taxa waiting to be named, there are some that did not correspond to any of the described genera as they are currently defined, thus we hypothesized them corresponding to four new genera. The lack of a system of classification and delimitation of genera based on cladistics makes it difficult to understand the diversity Lestremiinae, even more so if including taxa from the unexplored tropical regions. Being the validity, boundaries, and interrelationships of lestremiine genera still largely unclear, this study aimed to: 1) revise Lestremiinae taxonomic and morphological diversity; 2) test the monophyly of the subfamily Lestremiinae *sensu* Jaschhof & Jaschhof (2009); 3) present a classification system based on phylogenetic systematics (cladistic) of the lestremiine genera; 4) placing the new neotropical taxa within the phylogenetic hypothesis, erecting and describing new supraspecific taxa accordingly.

This document is divided into chapters that correspond to published papers or manuscripts. They resulted from the processes of revising described taxa of Lestremiinae and assessing morphological characters to compose a comprehensive data matrix for the cladistic analysis. Besides the main chapter with the phylogeny of Lestremiinae, we present chapters revising the neotropical genus *Insulestremia* and the cosmopolitan *Anaretella*, and a chapter with the first attempt of characterization of the ultrastructure of antennal and palpal sensilla and standardization of sensillar nomenclature for the Cecidomyiidae, specially the Lestremiinae. They are written in the format of research articles, each with their own, more specific sections of Introduction, Material and Methods, Results, and Discussion.

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#. CONCLUSIONS

This study presents the first records of the subfamily Lestremiinae and the genus *Insulestremia* Jaschhof in Brazil. *Insulestremia*, a previously monotypic genus described from the Galapagos Islands, has three species in Brazil: *I. sinclairi* Jaschhof, *I. amorimi* **sp. nov.** and *I. ament*i **sp. nov.** The new species are described, the generic concept is reviewed, and a key to the species of *Insulestremia* is provided.

The genus *Anaretella* in Brazil is represented by only one, new species. The new species is described, and the generic diagnosis of the genus is revised. Comparative studies indicate that *Anaretella* is closer to the neotropical genus *Insulestremia*, because of shared features of antennal sensilla.

It was possible to recognize thirteen subtypes within six types of antennal sensilla and two subtypes of one type of palpal sensilla in Lestremiinae. The data were compared with those in literature regarding other families of Diptera and the subfamily Cecidomyiinae. Two types of antennal sensilla are exclusive and characterized for the first time. The ultrastructure of palpal sensilla is presented for the first time for the family. A correlation between the names used across traditional morphological studies and the nomenclature of sensilla in studies of ultrastructure is provided. This study presents morphological characters that are key to the understanding of the relationships among Lestremiinae taxa and consequently of the early evolution of the Cecidomyiidae.

The first phylogeny of the subfamily Lestremiinae is presented, including comprehensive sampling of the included taxa and a substantial number of morphological characters, both quantitative and qualitative. Quantitative characters were analyzed as continuous and proven to be important to understanding the evolution of the group. Eight nodes that represent major steps in the evolution of Lestremiinae are discussed in depth. 11 of the recognized lestremiine genera were recovered as monophyletic (*Neolestremia* was not included in the analysis). Species previously place in *Allarete* were split into four genera, including three new. *Conarete* is considered a junior synonym of *Anarete*. A new neotropical taxon was corroborated as the representant of a new genus. Two fossil *Lestremia* species were fitted within the

framework of the phylogeny, being raised as composing a new, fossil genus.