SYSTEMATICS, TAXONOMY, AND DIVERSITY OF NEOTROPICAL LEAF-MINER FLIES (DIPTERA: AGROMYZIDAE)

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A THESIS SUBMITTED TO THE FACULTY OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES, MCGILL UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE DEGREE OF DOCTOR OF PHILOSOPHY

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ABSTRACT

The Neotropical region is home to most of the world's biodiversity but is experiencing a high rates of species extinction mainly due to habitat destruction and climate change. Unfortunately, our knowledge of tropical diversity is still very limited, and small often inconspicuous organisms, make up the bulk of this unknown diversity. The family Agromyzidae (Order Diptera), commonly known as leaf-miner flies, includes nearly 3200 species, distributed worldwide but with most species described from the Nearctic and Palaearctic regions. These flies can be economically important yet are small and difficult to identify. The Neotropical region, with approximately 500 described species, is poorly known, with some regions and habitats never surveyed for this taxon; this is a significant gap in biodiversity and biogeography knowledge and has consequences for long-term monitoring and conservation planning. This thesis explores the biodiversity, systematics, and taxonomy of the leaf-miner flies, collected in underexplored regions and habitats of the neotropics as part of two large and intensive biodiversity projects: the "Our Planet Reviewed" expedition in Mitaraka, (French Guiana) and the Zurquí All-Diptera Biodiversity Inventory project (ZADBI) (Costa Rica). The habitats surveyed consisted of lowland rainforest with rocky outcrops and mid-elevation (1600 m) cloud forests, respectively. This study has resulted in the identification of over 200 species and/or morphospecies of Agromyzidae, the first report of multiple genera for French Guiana (for which only four species in two genera were previously known), first report of the genus *Haplopeodes* Steyskal for Costa Rica and the first report of the genus *Metopomyza* Enderlein for the Neotropical region. Eight new species are formally described for French Guiana and many species are recognized as new to science in Costa Rica, including some apparently endemic to

cloud forests. Although multiple collecting techniques were used in both projects, the Malaise trap was by far the most successful in collecting Agromyzidae. Species delineation and identification were based mainly on morphological characters, but the utility of DNA barcoding to assist identification of Neotropical Agromyzidae was explored. This research represents a significant increase in knowledge about an important family of flies located in some of the most ecologically important, but also most threatened habitats in the world. This is foundational work that fills a critical gap as we endeavour to explore, define, and name the world's species.

RÉSUMÉ

La région néotropicale abrite la majeure partie de la biodiversité mondiale, mais connaît un taux élevé d'extinction d'espèces, principalement en raison de la destruction de l'habitat et du changement climatique. Malheureusement, notre connaissance de la diversité tropicale est encore très limitée et les petits organismes constituent l'essentiel de cette diversité inconnue. La famille des Agromyzidae (ordre des diptères), communément appelée mouche mineuse, comprend près de 3200 espèces, réparties dans le monde entier mais dont la plupart sont décrites dans les régions néarctique et paléarctique. Ces mouches peuvent être économiquement importantes, mais elles sont petites et difficiles à identifier. La région néotropicale, avec environ 500 espèces décrites, est mal connue; certaines régions et habitats n'ayant jamais été étudiés pour ce taxon. Il s'agit d'une lacune importante dans les connaissances sur la biodiversité et la biogéographie, qui pourrait entraîner des conséquences sur la surveillance à long terme et la planification de la conservation. Cette thèse explore la biodiversité, la systématique et la taxonomie des mouches mineuses, collectées dans des régions et des habitats sous-explorés des néotropiques dans le cadre de deux grands projets intensifs de biodiversité : l'expédition "La planète revisitée" dans le massif du Mitaraka, (Guyane française) et le projet Zurquí All-Diptera Biodiversity Inventory (ZADBI) (Costa Rica). Les habitats étudiés consistaient respectivement en une forêt tropicale humide de basse altitude avec des affleurements rocheux et des forêts de nuages (forêts humides en montagne) à moyenne altitude (1600 m). Cette étude a abouti à l'identification de plus de 200 espèces et/ou morphoespèces d'Agromyzidae, le premier signalement de plusieurs genres pour la Guyane française (dont seulement 4 espèces dans deux genres étaient auparavant connues), le premier signalement du genre *Haplopeodes* Steyskal pour Costa Rica et le premier signalement du genre *Metopomyza* Enderlein pour la région néotropicale. Huit nouvelles espèces sont

officiellement décrites pour la Guyane française et de nombreuses espèces sont reconnues comme nouvelles pour la science au Costa Rica, y compris certaines apparemment endémiques des forêts de nuages. Bien que plusieurs techniques de collecte aient été utilisées dans les deux projets, le piège Malaise a été de loin le plus efficace pour la collecte d'Agromyzidae. La délimitation et l'identification des espèces reposaient principalement sur des caractères morphologiques, mais l'utilité du code-barres ADN pour faciliter l'identification des Agromyzidae néotropicaux a été explorée. Cette recherche représente une augmentation significative des connaissances sur une importante famille de mouches située dans certains des habitats les plus écologiquement importants et menacés au monde. Il s'agit d'un travail fondamental qui comble une lacune critique alors que nous nous efforçons d'explorer, de définir et de nommer les espèces du monde.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my initial supervisor, the late Terry Wheeler, who, although no longer with us, has inspired me to become a scientist. Terry's love of flies and nature was contagious, and he had a great influence on my past and present work on leaf-miner flies and taxonomy. I reassured him that I would (one day!) finish the PhD …it took a certain time, and it hasn't been always easy, but giving up was not an option. Thank you for believing in me and for your constant support.

And of course, this thesis would not have been possible without my current supervisor Christopher Buddle. Thank you so much, Chris, for taking me on, part way into the project, and for your guidance and your constructive criticism that greatly help improving the thesis. Thank you for taking the time (even when you had no time!) especially in the last little miles, allowing me to complete this work.

Thank you also to my committee member Jeffrey Cumming (Canadian National Collection of Insets) for his valuable input, editing and commenting on the various chapters. Thank you, Jeff for the fast responses and easy communication. I really appreciate your help.

Thank you to the small agromyzid community for discussion, revision, and comments on my manuscripts, principally Michael von Tschirnhaus, Owen Lonsdale, Sonja Scheffer and Viviane de Sousa.

Thank you also to Kenji Nishida for collaboration with the leaf-miners of Costa Rica and for allowing me to use some of his pictures in this thesis.

I would also like to thank the Lyman Entomological Museum's past and present staff, students, and associates (the Lymanites!), especially those with whom I still have regular contact today: Amélie Grégoire-Taillefer, Marjolaine Giroux, Jade Savage, Cyrena Riley, Julia Mlynarek, Anna Solecki, Valérie Lévesque-Beaudin, George Hsiung, Jessica Gillung, Morgan Jackson, Catherine Scott, Alice Assmar, Ruishen Zhang, Linley Sherin, Vinko Culjak, Javahery (Jay), thank you for past and present discussions, and for your encouragement.

Thank you to my parents for their love, constant support and for telling me to always go after my dreams. My brother Stephane, his wife Cammie, my in-laws, Aimé and Denise, my friends Nathalie, Éric, Carole: thank you all for your support and encouragement and thank you Carole for your friendship and for sending me essential oils during the tough times!

Finally, thank you to my family: My husband Vincent and my kids, Maélie and Nicolas. Deciding to do a PhD in my middle adulthood with a part-time job and a family had many challenges. But I had a constant support from my husband Vincent, even though he had to do a few extra rides for the kids and extra household duties! Thank you for being in my life.

My research was supported by a NSERC CGS-D (Natural Science and Engineering Research Council of Canada, Alexander Graham Bell Canada Graduate Scholarship). Thank you also to QCBS for the Excellence award and McGill University for the GREAT award (Graduate research enhancement and travel awards) that allowed me to attend the International Congress of Dipterology, in Namibia during my PhD.

PREFACE

This thesis is Manuscript-based. Chapter 1 and Chapter 2 have been published in the journal Zoosystema and ZooKeys, respectively. Chapter 3 is formatted for submission to Zoosystema and Chapter 4 is formatted for submission to Zootaxa.

Note. In accordance with the International Code of Zoological Nomenclature, new species names described in this thesis are not valid until they are published in the primary literature.

Contribution to knowledge

This thesis provides important knowledge of the Neotropical fauna of Agromyzidae with the following specific contributions:

1. This was the first study looking at the agromyzid fauna of French Guiana and the first study identifying Agromyzidae from an inselberg (rocky outcrop).

2. A total of 50 species/morphospecies has been identify from French Guiana, which is an important contribution to our previous knowledge that included only four species. Eight genera are for the first time recorded for French Guiana (*Melanagromyza*, *Ophiomyia*, *Phytobia*, *Japanagromyza*, *Calycomyza*, *Agromyza*, *Cerodontha*, *Phytoliriomyza*).

3. Eight species of Agromyzidae were newly described for French Guiana, including DNA Barcode for some of them. New distribution records are given for *Phytoliriomyza jurgensi* and *Calycomyza grenadensis*, two species previously known from the Caribbean and for the first time reported in French Guiana.

4. This is the first study investigating and discussing the diversity and uniqueness of the agromyzid fauna from Costa Rican cloud forests, collected over a full year. The identification resulted in one genus (*Metopomyza*) being newly reported for the Neotropical region and one genus (*Haplopeodes*) newly reported for Costa Rica. In addition, many species were recorded in Costa Rica for the first time and many new species were recognized.

5. DNA barcoding was for the first time tested as an identification tool in a context of biodiversity survey in the Neotropical region.

6. This study contributed 24 new CO1 sequences on GenBank and to the Barcode of Life data system for Neotropical Agromyzidae.

Contribution of authors

Chapter 1. The leaf-miner flies (Diptera: Agromyzidae) of Mitaraka, French Guiana.

The authors of this study are Stéphanie Boucher and Marc Pollet.

SB did the specimens preparation (drying, mounting, labelling), genitalic dissections, and all the identifications, faunistic analysis and writing of the first draft of the manuscript.

MP participated to the expedition in Mitaraka as member of the first team (22 February-11 March 2015), and as Diptera coordinator. He sorted all Diptera material and distributed the specimens to respective experts (to SB for the Agromyzidae). Marc also contributed to comments and editing of the manuscript.

Chapter 2. DNA barcoding of the leaf-miner flies (Diptera: Agromyzidae) of Mitaraka, French Guiana.

The authors of this study are Stéphanie Boucher and Jade Savage.

> **SB** Tissue sampling of the specimens sent to Lifescanner. Analysis of dataset on Bold. Responsible for the Neighbor-joining tree, tables, data analysis, photographs and writing of the first draft. **JS** Tissue sampling for specimens sent to Centre for biodiversity genomics (CBG), provided financial support for the barcoding at CBG and provided advice, comments and editing of the manuscript.

Chapter 3. New species and new records of leaf-miner flies (Diptera: Agromyzidae) from rainforest and inselberg at Mitaraka (French Guiana).

The author of this study is Stéphanie Boucher.

I was responsible for all the identifications, taxonomic decisions, species descriptions, writing of the chapter and photographic additions. Comments and editing of the manuscript were provided by my supervisor Christopher Buddle and my committee member Jeffrey Cumming.

Chapter 4. The leaf-miner flies (Diptera: Agromyzidae) of Costa Rican cloud forests.

The author of this study is Stéphanie Boucher.

I was responsible for all the morphospecies and species identifications, taxonomic decisions, and discussion, writing of the chapter and elaboration of tables, and all photographic additions. Comments and editing of the manuscript were provided by my supervisor Christopher Buddle and my committee member Jeffrey Cumming.

INTRODUCTION

We are in a world where biodiversity loss is significant and has been cited as a crisis (Sepkoski 2020; Wilson 1985). Our poor knowledge of the world's biodiversity is a major obstacle in understanding the biodiversity crisis and how species are affected by various threats such as climate change and habitat loss (Costello 2015; Dubois 2003; Sunderland 2012; Wagner et al. 2021; Wilson 2017). Notable is the slow rate at which species are discovered and named (their taxonomy), and the relatedness among species (systematics), which is also critical when it comes to biodiversity conservation (Giangrande 2003). Although the total number of world species is still a matter of debate, a general agreement is that only 15-20% of the living eukaryotic species have been described (Mora et al. 2011; Wilson 2017). The insects make up the bulk of the species awaiting discovery (Mora et al. 2011; Myers et al. 2000; Stork 2018; Wagner et al. 2021) and when we consider where most of the well documented animals are found, it is in the tropics that most species are expected (Stork 2018).

Insects have high diversity generally, but within the insects, some orders are 'megadiverse', and these include the Coleoptera, Hymenoptera, Lepidoptera and Diptera. The Flies (Order Diptera) have nearly 160,000 described species worldwide (Courtney et al. 2017). Although they have significant impacts on humans (positively and negatively), they remain one of the least studied of the megadiverse insect orders (Brown 2005; Hebert et al. 2016). The Neotropical Diptera fauna is particularly poorly known (even "grossly deficient" (Brown 2005)), especially when compared to the Nearctic and Palaearctic faunas (Amorim 2009).

Flies are found in a variety of aquatic and terrestrial ecosystems. They are one of the most ecologically diverse groups of insects with the larval stage developing sometimes as scavengers, predators, parasitoids, parasites, or herbivores (Ferrar 1987; Courtney et al. 2017).

The herbivorous (or phytophagous) comprise approximately 30% of the Diptera fauna (Price et al. 2011). Among the 24 most diverse families of Diptera (representing about 80% of the flies) listed in Brown (2009), the family Agromyzidae is the only family that is exclusively phytophagous.

It is well known that plant diversity is highest in the tropics (Brummitt et al. 2020), as well as most insect taxon that are associated with plants (Lewinsohn and Roslin 2008; Novotny et al. 2006; Zhang et al 2016). But this pattern of diversity is different in the family Agromyzidae which reaches their highest diversity in temperate areas of the Palaearctic and Nearctic regions (Černý and Tschirnhaus 2014). This is mainly due to the high diversity of Agromyzidae found in the Palaearctic (about 1200 species, including 950 species in Europe), which is reflecting over 100 years of intensive work from experts in this region. In comparison, the Neotropical agromyzid fauna is considered poorly known with less than 500 species.

This poor representation of Agromyzidae in the Neotropical region may well represent an artifact of research effort but is creating an important gap in biodiversity and biogeography knowledge which is especially critical for a region that includes some of the most threatened habitats of the world. This lack of knowledge also restricts their use for long-term monitoring program and conservation policies.

Spencer (1972) mentioned that the family Agromyzidae was widely distributed throughout the world but with significantly less species in the southern hemisphere. But a few studies (Étienne and Martinez 2003; Spencer et al. 1992) have revealed that the family may be more species-rich in the neotropics than previously known. For example, a single species of Agromyzidae was known from Guadeloupe in 1985 but collecting effort in the region has increased the known fauna to 85 species, ranking the Agromyzidae in first place out of 23 Diptera families reported in Guadeloupe (Meurgey and Ramage 2020).

The lack of taxonomic and sampling efforts in other regions is apparent. For example, only four species have been reported in French Guiana (EPPO 2021), although the region is known for a rich insect fauna (Brûlé and Touroult 2014). Costa Rica, which likely has the highest species density in the world (Kohlmann 2011, Obando 2007), has only 65 agromyzid species reported, which is less than what is reported for Guadeloupe (85 species), although Costa Rica has a much larger land area: 51 042 km2 (Kohlmann 2011) compared to only 1434 km^2 for Guadeloupe (Meurgey and Ramage 2020) and a diversified array of terrestrial ecosystems (Kappelle 2016b).

It is without any doubt that additional sampling and taxonomic effort in different regions of the neotropics is required to obtain a better representation of species richness across the continent. But this has some challenges, due to the difficulty in identification of these small flies, the lack of identification keys for the Neotropical region, and the large number of undescribed species.

THESIS OBJECTIVES

My proposed study of the Systematics, taxonomy, and diversity of Neotropical leaf-miner flies (Diptera: Agromyzidae) has four chapters with four main objectives:

1) Document generic composition and species richness of Agromyzidae present at the Mitaraka Massif, French Guiana, by performing morphospecies identification for all specimens collected during the expedition “Our planet Reviewed- French Guiana”.

Morphospecies identification will be based on the detailed study of morphological characters and the structure of the male genitalia when possible. (Chapter 1)

2) Investigating whether DNA barcoding could assist with species identification, species delineation, male/ female association, and diversity assessment of Agromyzidae specimens collected in Mitaraka, French Guiana, a tropical region where the Agromyzidae fauna is completely unknown. (Chapter 2)

3) Reporting the presence of new species to science from Mitaraka, French Guiana and describing them based on habitus and male genitalia and reporting new distribution data for previously known species. (Chapter 3)

4) Providing a faunistic analysis and discussion of the species richness of Agromyzidae collected from three different cloud forest sites in Costa Rica as part of the ZADBI project. (Chapter 4)

LITERATURE REVIEW

The family Agromyzidae

Biology and host plant

Larvae of all species of Agromyzidae are internal plant feeders. Various plant parts may be chosen as feeding site, including roots, stems, seeds, flower head, trunk and twigs, but the majority of species with known biology are leaf-miners and occur in between the epidermis of leaves forming a feeding channel, the leaf mine. The mines produced by Agromyzidae can be of different forms: linear, serpentine, blotch-like or irregular. These feeding patterns are species-specific and often helpful in the identification of Agromyzidae (Eiseman et al. 2021; Spencer 1981). A few species of Agromyzidae are known as gall inducers, causing an abnormal plant outgrowth in which they develop. Most galls are associated with stems, petioles, or leaf veins of vascular plants (Dempewolf 2005).

Host plants are known for approximately 42% of the described species of Agromyzidae with a total of 146 families of plants recorded as hosts (Benavent-Corai et al. 2005). Agromyzidae have succeeded in colonizing most types of plants except mosses (Spencer 1990). Host plants are better known for species living in temperate area of the world. For example, nearly 50% of the Agromyzidae species recorded in the United States have known host plants (Eiseman and Lonsdale 2018). In the Neotropical region the host plants are known for approximately 35% of the species (Benavent-Corai et al. 2005).

The great majority of the Agromyzidae shows a high specificity in their choice of host plant, with some species considered “extreme specialists” (Benavent-Corai et al. 2005). Only 16 species (less than 2%) of Agromyzidae, are polyphagous and feed regularly on plants of unrelated families (Spencer 1990). Although most Agromyzidae are probably true specialists, further work is required to understand the real pattern in host use, as many species have incomplete host data, including species described from a single rearing event (Scheffer et al. 2021). Due to their high host-specificity, Agromyzidae are good study organisms for evolutionary and ecological questions in insect-plant relationships (Scheffer et al. 2021; Spencer 1990; Hébert et al. 2016), but their largely unknown taxonomy and biology, especially in the neotropics, limit their use.

Economic importance

Agromyzidae are best known for the damage they cause to agricultural, horticultural and ornamental plants. There are over 150 agromyzid species of particular economic importance (Spencer 1973a). The polyphagous species include the most important agromyzid pests worldwide such as *Phytomyza horticola* (Goureau) and various species of *Liriomyza* (Spencer 1973a). In the Neotropical region, the most important pest species of vegetable and ornamental crops are also in the genus *Liriomyza*. These include *L. brassicae* (Riley), *L. huidobrensis* (Blanchard), *L. sativae* Blanchard and *L. trifolii* (Burgess) which have now spread in many regions of the world (EPPO 2021).

When present in large numbers, leaf-miners may affect the general health of the plant by reducing its photosynthetic capacity (Spencer 1973a). They also cause aesthetic damage due to their highly visible feeding traces (Figs. 1-3), reducing the commercial value of ornamental plants (Parrella 1987). Stem-borers cause also considerable damage by affecting the vascular system of the plant, disturbing water and nutrient supplies. This is especially injurious when the larval feeding occurs in young plants or seedlings (Dempewolf 2004; Spencer 1973a).

Although most of the damage to plants is caused by the agromyzid larvae, the adult females may also injure plants by inserting their well-sclerotized ovipositor into the plant tissues (Fig. 4). This is primarily for egg-laying purposes, but the females use these scars also as feeding sites, sucking plant sap through the punctures (Spencer 1973).

Under normal condition, most agromyzids are naturally controlled by parasitoid wasps. The uses of insecticides often disrupt this natural balance and results in major agromyzid outbreaks (Spencer 1973a; Oatman and Kennedy 1976; Reitz et al. 2013). This is due to the agromyzids having a higher resistance to chemicals than the parasitoids (Dempewolf 2004;

Spencer 1973). In greenhouse, a combination of natural control methods including the release of parasitoid wasps with the release of sterile agromyzid males, has shown to be effective (Kaspi and Parrella 2006).

Some agromyzid species have a beneficial impact as well, as some species are known to feed on noxious and invasive plants and have been used as natural control agents for those plants (April et al. 2021; Klein and Kroschel 2002; Madire et al. 2011; Nzama et al. 2014).

Morphological identification (Imago)

Agromyzidae (Brachycera: Cyclorrhapha; Schizophora), also known as leaf-miner flies are considered a taxonomically challenging family of flies, mainly due to their small size and their morphological similarity (Lonsdale 2021; Nartshuk and von Tschirnhaus 2015; Nowakowski, 1962). Members of the family are minute to medium-sized flies, generally measuring between 2-4 mm in wing length, but extremes range from 0.5 mm to 6.5 mm. They are often yellow and/or black, brown or grey; a few have some metallic greenish, bluish or coppery colouration. Most have clear wings, but they may be patterned or infuscated in a few tropical species. Other family diagnosis characters include: Vibrissae present; one to seven frontal bristles; costal break present at the apex of Sc; cell *cup* small; vein A_1 not reaching wing margin; anterior part of abdominal segment 7 in female forming an oviscape (Spencer 1987). Although identification at the family level is normally relatively simple, especially for the females that are easily recognizable by their characteristic oviscape, the identification at the genus and species level is more challenging. In some genera, there are no external apomorphies known to date. In these cases, identification and monophyly can only be confirmed by specific internal characters, mainly from the male genitalia, a complex structure described in detail by Frick (1952) and later found to be extremely useful in taxa recognition and establishment of phylogenetic relationships (Nowakowski 1962). For example, the symmetrical phallus of *Melanagromyza*, the L-shaped subepandrial sclerites of *Cerodontha* species and the patch of spines at the hind corner of the epandrium in *Calycomyza* are all of great importance in the identification of these genera. Identification at the species level also relies mainly on complex male genitalic characters, often with minor interspecific differences, which sets a high degree of difficulty for species recognition, especially for non experts (Kamiji and Iwaizumi, 2013; Scheffer et al. 2006; Shahreki et al. 2012). The lack of reliable diagnosis characters for female Agromyzidae has the consequence of leaving females unidentified or undescribed in various studies (e.g. Černý and Bächli 2018; Eiseman and Lonsdale 2018; Sousa and Couri 2016a). In some cases, species-level identification is not possible, even for males, due to a high species richness, lack of identification keys or due to a largely undescribed fauna, which is the reality of tropical countries. In these cases, morphospecies identification (the recognition of a distinct taxonomic unit, but unnamed), could be used as an alternative in biodiversity surveys, as seen for other insect taxa (Derraik et al. 2002; Kremen et al. 1993).

Morphological identification (Larvae)

Agromyzid species descriptions often include the larval and/or pupal stage when these are known, although no formal species descriptions are usually based on immature stages only. Allen (1957) provided a good review of larval morphology and its use in identification at the generic and species level. Dempewolf (2001) also found valuable morphological characters in the larval stages of multiple species that were used in interpreting species relationships. Important morphological characters of the larva are mostly found in the larval cephalopharyngeal skeleton, the mandible and the spiracles. Larval characters are often useful in separating species that are morphologically similar in the adult stage. For example, two *Liriomyza* species feeding on the leaf of the same host plant, showed noticeable differences in the size of their cephalopharyngeal skeleton and shape of the larval posterior spiracles but very subtle differences externally in the adult stage (Boucher and Nishida 2014). Nowadays, it is difficult or even impossible to confirm species identification from larval or pupal stage using morphological characters, as so many species have unknown host plant and larval stages.

DNA barcoding

To overcome some of the difficulties of morphological identification addressed above, DNA barcoding, the sequencing of a short fragment of DNA sequence of the mitochondrial cytochrome c oxidase 1 (CO1) gene (Hebert et al. 2003), has been increasingly used to identify Agromyzidae, especially economically important species (Bhuiya et al. 2011; Blacket et al. 2015; Czepak et al. 2018; Firake et al. 2018; Scheffer et al. 2006; Xu et al. 2021). DNA barcoding has also been used to identify Agromyzidae from juvenile stages (Amin et al. 2014; Scheffer et al. 2006), or even from recently vacated leaf mine (Mlynarek et al. 2016). In addition to these, DNA barcoding has been useful to uncover and identify cryptic species (e.g. Mlynarek and Heard 2018; Scheffer and Lewis 2006; Scheffer et al. 2014; Weintraub et al. 2017), and to discover new species (e.g. Scheffer and Wiegmann 2000). DNA barcoding has also been used to estimate Agromyzidae species richness in Canada (Savage et al. 2019) and provide species identification of Agromyzidae collected during large biotic surveys in Ontario (deWaard et al. 2018; Telfer et al. 2015). DNA barcoding has never been used as a tool to identify or to assess biodiversity of the Neotropical Agromyzidae fauna.

Diversity and distribution

The family Agromyzidae, contains nearly 3200 species (von Tschirnhaus 2021) occurring from the tropics to the high arctic although most of the diversity is found in the Palaearctic region with over 1200 species (Černý et al. 2020), followed by the Nearctic region with nearly 880 species (north of Mexico) (Eiseman et al. 2021; Lonsdale 2021), Neotropical region with nearly 500 species (details below), Oriental region with 390 species (Černý and von Tschirnhaus 2014), Afrotropical region with 325 species (Lonsdale and von Tschirnhaus 2021) and Australia/Oceania region with 270 species (Černý and von Tschirnhaus, 2014).

Synopsis of the Neotropical fauna of Agromyzidae

Following Morrone's (2014) biogeographical regionalisation of the Neotropical region, the Neotropics include most of Central and South America, southern and central Mexico, and the Antilles. Southern Florida, which has been considered part of the Neotropical region by some authors is excluded. The Mexican Transition Zone (MTZ) includes the mountainous areas of central and southern Mexico and is an area where the Neotropical and Nearctic regions overlap (Morrone 2014). A few Agromyzidae are known to occur in the MTZ, more specifically from the Sierra Madre Occidental, and the Transmexican Volcanic Belt province, these include the six *Cerodontha* s. str., one *Cerodontha (Icteromyza)* and one *Liriomyza* described from high elevation (between 7000 ft and 9000 ft) in Durango and Toluca, Mexico (Spencer 1977; Boucher 2002). These eight species are so far endemic to these Mexican mountains. The six *Cerodontha* s. str. were considered as part of the Nearctic region in Boucher 2002, and this is how they are considered here as well. Another species, *Amauromyza abnormalis* (Malloch) widespread in the Nearctic region (Canada and U.S.A.) (Boucher 2012) is also known from the Transmexican Volcanic Belt province (part of the MTZ) in Mexico (Montecillo and San Miguel del Milagro, Tlaxcala) (Bautista-Martinez et al. 1997), and is here considered a Nearctic species. Finally, species recorded in Florida that were listed in Martinez and Étienne (2002) but are not yet recorded from the Caribbean or other Neotropical regions are also considered Nearctic.

Considering the above specifications, the current literature reports 497 species of Agromyzidae from the Neotropical region, distributed into 14 genera (species counts are normally using Martinez and Étienne's list of Neotropical species (2002a) as a starting reference):

Agromyza Fallén. This genus of 203 species in the world (Lonsdale and von Tschirnhaus 2021) is poorly represented in the Neotropical region with only 14 species (Martinez and Étienne 2002a (11 species); Sasakawa 2005 (1 species); Sousa and Couri 2016a (2 species)).

Amauromyza Hendel. There are 60 world species of *Amauromyza*, including 20 species in the Nearctic region (Boucher 2012) but only one species in the Neotropical region: *Amauromyza boliviensis* Sasakawa (1992a). A further undescribed species has been reported form Cuba (Spencer and Stegmaier 1973).

Calycomyza Hendel. This genus of 98 species worldwide, is essentially restricted to the New World. Only 12 species have been recorded in the Palaearctic region (Lonsdale and von Tschirnhaus 2021). The genus is very well represented in the Neotropical region with 71 species (Martinez and Étienne 2002a (63 species); Étienne and Martinez 2003 (1 species); Korytkowski 2014 (3 species); Monteiro and Esposito 2017 (2 species); Spencer 1963 (1 species); Sasakawa 2005 (1 species)).

Cerodontha Rondani. There are presently 30 species of Neotropical *Cerodontha* (Martinez and Étienne 2002a (26 species); Boucher 2003 (1 species); Boucher 2005 (2 species); Boucher and Wheeler 2014 (1 species)) from most subgenera (*Cerodontha* (10 species); *Dizygomyza* (8 species); *Butomomyza* (7 species); *Icteromyza* (2 species); *Poemyza* (2 species); *Xenophytomyza* (1 species)), except *Phytagromyza* which is exclusively Holarctic. The subgenus *Xenophytomyza* was also exclusively Holarctic until *C. (Xenophytomyza) biseta* (Hendel) was reported from Jamaica (Boucher 2003). There are currently 295 species in the world (Lonsdale and von Tschirnhaus 2021).

Haplopeodes Steyskal. This genus of only 18 species is restricted to the New World, except for one South African species with uncertain generic status (Lonsdale and von Tschirnhaus 2021; Steyskal 1980). The genus is most diverse in the Neotropical region with 14 species (Martinez and Étienne 2002a (13 species); Korytkowski 2014 (1 species)), with most of them recorded from Argentina and Brazil. The genus is not officially reported in Central America yet, although female specimens possibly belonging to this genus have been reported from Costa Rica (Boucher 2010). This genus includes some of the smallest Agromyzidae known, with many species averaging 1.0 mm in wing length.

Japanagromyza Sasakawa. This genus is well represented in the Neotropical region with 35 species (Martinez and Étienne 2002a (26 species); Boucher and Hanson 2006 (1 species); Étienne and Martinez 2003 (1 species); Korytkowski 2014 (2 species); Monteiro et al. 2015 (1 species); Sasakawa 2005 (2 species); Sousa and Couri 2017a (2 species)) out of 83 species in the world (Lonsdale and von Tschirnhaus 2021). Only 8 species are known in the Nearctic region.

Liriomyza Mik. This is the second largest genus of Agromyzidae with 453 species (Lonsdale and Tschirnhaus 2021). Although *Liriomyza* is usually considered a north temperate genus (Lonsdale and Tschirnhaus 2021; Spencer 1972; Spencer and Steyskal 1986), it is the most diverse agromyzid genus in the Neotropical region with 104 species described (Martinez and Étienne 2002a (87 species, including 3 species previously in *Galiomyza*); Boucher and Wheeler 2014 (1 species); Boucher and Nishida 2014 (2 species); Carvalho-Filho et al. 2016 (1 species); Korytkowski 2014 (9 species); Martinez and Étienne 2002b (1 species); Sasakawa 2005 (1 species); Sousa and Couri 2018 (1 species): Zlobin 2001 (1 species)), compared to 157 species recorded in the Nearctic (north of Mexico) (Lonsdale 2021).

Melanagromyza Hendel. This agromyzid genus is in 3rd place for its diversity with 380 world species (Lonsdale and von Tschirnhaus 2021) and is generally well represented in the tropics. In the Neotropical regions, this is the second largest genus (after *Liriomyza*) of Agromyzidae with 93 species known (Martinez and Étienne 2002a (82 species); Boucher and Wheeler 2014 (1 species); Braun et al. 2009 (5 species); Korytkowski 2014 (3 species); Martinez-Alava et al. 2016 (1 species); Sasakawa 2005 (1 species)), compared to 80 species recorded in the Nearctic (north of Mexico) (Lonsdale 2021).

Nemorimyza Frey. This small genus of 5 species is mainly restricted to the New World, except for one Holarctic species (*N. posticata* (Meigen)). All five species are present in the Neotropical region (Martinez and Étienne 2002a). A further species from China (Chen and Wang 2008) appears to have been described in the wrong genus.

Ophiomyia Braschnikov. There are 324 species of *Ophiomyia* in the world (Lonsdale and von Tschirnhaus 2021) including 51 species in the Neotropical region (Martinez and Étienne 2002a (48 species); Étienne and Martinez 2003 (1 species); Monteiro et al. 2019 (1 species); Sasakawa 2005 (1 species)).

Phytobia Lioy. Thirty-two species of *Phytobia* are currently known from the Neotropical region (Martinez and Étienne 2002a (16 species); Sasakawa 2005 (1 species); Sousa and Couri 2017b (14 species); Zlobin 2008 (1 species), out of 117 world species (Lonsdale and von Tschirnhaus 2021). This genus includes some of the largest Neotropical agromyzids, for example, *P. lanei*

Spencer (1966) from Brazil has a wing length of 5.1 mm. Others are much smaller, such as *P. multisetosa* Sousa and Couri (2017b) with a wing length of 1.3 mm.

Phytoliriomyza Fallén. There are 114 species in this genus (Lonsdale and von Tschirnhaus 2021), including 27 species in the Neotropical Region, (Martinez and Étienne 2002a (26 species); Martinez and Étienne 2002b (1 species)). The same number (27) is reported from the Nearctic region (north of Mexico) (Lonsdale 2021).

Phytomyza Fallén. This is the largest agromyzid genus with 774 species (Lonsdale and von Tschirnhaus 2021) including 275 species in the Nearctic region (north of Mexico). *Phytomyza* is comparatively weakly represented in the Neotropical region with 19 species (Martinez and Étienne 2002a (15 species, including 3 *Chromatomyia* species); Korytkowski 2014 (2 species); Monteiro et al. 2019 (1 species); Norambuena et al. 1999 (1 species)).

Pseudonapomyza Hendel. This genus includes 102 species worldwide (Lonsdale and von Tschirnhaus 2021), but only four species are known from the New World (Boucher, 2004), including a single species (*P. asiatica* Spencer) in the Neotropical region. Additional Neotropical species are apparently present, including an undescribed species from Argentina (Madire et al. 2011) and other undescribed species from temperate areas of the Neotropics (Lonsdale and von Tschirnhaus 2021). This genus was reported for the first time in the Neotropical region in 1992, from unidentified female specimens from Guadeloupe (Spencer et al. 1992). Its presence in Guadeloupe was confirmed in 2003 with the identification of a male specimen representing *P. asiatica* Spencer (Étienne and Martinez, 2003) and the same species was later reported from Costa Rica, Venezuela (Boucher, 2004), and Brazil (Monteiro et al. 2019).

Nearctic genera currently unreported in the Neotropical regions

Three further genera, present in the Nearctic region, are still unreported in the Neotropical region. These include:

Aulagromyza Enderlein. This genus includes 52 species (Zlobin 2007a), with most of the species (42) reported from the Palaearctic region (Gil-Ortiz et al. 2010). Nearctic species are mostly found in Canada and Northern United-States, with a few species reaching as far south as Colorado, Kansa, California (Spencer and Steyskal, 1986).

Euhexomyza Lonsdale. The recent synonymy of the genus *Hexomyza* Enderlein with *Ophiomyia* has resulted in the description of the new genus *Euhexomyza* (Lonsdale 2014) to accommodate a few species previously assigned to *Hexomyza* but not recombined as *Ophiomyia*.

This genus includes only eight species which are all restricted to the Holarctic region, except one species known from New Zealand (Lonsdale 2014, 2021).

Metopomyza Enderlein. This genus of 21 species (Zlobin 2007b (20 species); Çikman and Sasakawa 2011 (1 species)) is mostly restricted to the Nearctic and Palaearctic regions except for one species recorded from Nepal (Oriental region) (Zlobin 2007b). Seven species are known from the Nearctic region, of which two are Holarctic.

Recent progress in knowledge of the Neotropical fauna

Although the family Agromyzidae is considered poorly studied in the Neotropical region (Monteiro et al. 2019), there has been increased interest and progress in our knowledge of this fauna in recent years. Spencer (1963), published an important monograph on Neotropical Agromyzidae, redescribing 70 previously known Neotropical species in addition to the description of 54 new species. Since then, the known diversity has increased by more than three times due to a series of papers describing the fauna from Argentina (Sasakawa 1992b; Valladares 1981, 1992, 1998), Bolivia (Sasakawa 1992a), Brazil (Braun et al. 2009; Carvalho-Filho et al. 2016; Esposito 1994; Esposito and Prado 1993a, b; Monteiro and Esposito 2017; Monteiro et al. 2015; Monteiro et al. 2019; Sasakawa, 1992a; Sousa and Couri 2016a, 2017a,b; Spencer 1966), Chile (Sasakawa 1994; Spencer 1982), Colombia (Sanabria de Arévalo 1993a,b, 1994; Sasakawa 1992c; Steyskal 1972; Spencer 1984), Costa Rica (Boucher 2005; Boucher and Hanson 2006; Boucher and Nishida 2014; Spencer, 1983; Spencer and Stegmaier 1973; Woodley and Janzen, 1995), Ecuador (Boucher and Wheeler 2014; Sasakawa 1992d; Steyskal 1972), El Salvador and Guatemala (Sasakawa, 2005), Peru (Korytkowski 2014; Sasakawa, 1992a), Venezuela (Sasakawa 1992a; Spencer 1973b; Spencer and Havranek 1989), Guadeloupe (Étienne and Martinez, 2003; Martinez 1994; Spencer et al., 1992), and other Caribbean islands (Étienne and Martinez, 1996; Spencer and Stegmaier 1973; Zlobin, 1996).

With approximately 115 species, Brazil has the highest known diversity of Neotropical Agromyzidae, followed by Chile (approximately 100 species); Guadeloupe (85 species) (Étienne and Martinez 2003); Venezuela (approximately 85 species); Argentina (approximately 83 species); Peru (77 species) (Korytkowski 2014) and Colombia (74 species) (Sousa and Couri, 2016b). This higher species richness is primarily the result of major collecting effort in some of these regions, and is particularly impressive in Guadeloupe, considering the small size (1628 km^2) of this island compared to larger territory (e.g., Argentina 2.78 million km^2) with lower agromyzid species richness probably because they were less intensively studied.

Long-term or short-term intensive biodiversity surveys can provide a rapid way of increasing our knowledge of the species present in a region. Very few biodiversity surveys have documented the Agromyzidae fauna of the Neotropical region. But one of them, known as the

SISBIOTA Diptera project (Lamas et al. 2014) has resulted in the description of 17 new species of Agromyzidae for Brazil (Sousa and Couri 2016, 2017a,b) which was an important contribution to the knowledge of the Brazilian fauna of this family.

Biodiversity Projects in the Neotropics

My thesis will be investigating the Agromyzidae fauna of French Guiana and Costa Rica as part of two large and recent biotic surveys: 1) Our planet Reviewed- French Guiana expedition in Mitaraka and 2) Zurqui All-Diptera Biodiversity Inventory (ZADBI) project, in Costa Rica. The primary objective of the "Our planet reviewed – French Guiana" project was to accelerate the discovery and description of new species (with a focus on the largely neglected invertebrate fauna) by sampling a largely unexplored region in French Guiana, known as the Mitaraka Massif, where the environment was dominated by lowland tropical forests with rocky outcrops (Touroult et al. 2018). The primary objective of the ZADBI project was to determine the number of all dipteran species present at the primary site (Zurquí) a mid-elevation, small patch of cloud forest in Costa Rica, sampled intensively for a full year. The Diptera diversity from two additional nearby mid-elevation cloud forest sites was also investigated, although not as comprehensively (Borkent and Brown 2015).

Current knowledge of the Agromyzidae of French Guiana

Although, French Guiana is very well studied compared to other neotropical territories (Touroult et al. 2018), the agromyzid fauna of French Guiana remains almost completely unexplored. There are no specific studies dealing with the Agromyzidae of French Guiana and only four economically important species have been previously recorded: *Liriomyza huidobrensis* (Blanchard); *Liriomyza trifolii* (Burgess); *Liromyza sativae* Blanchard and

Nemorimyza maculosa (Malloch) (EPPO 2021; Martinez and Étienne 2002). The presence of these species in French Guiana has been reported by various agricultural or plant protection organisations (e.g., EPPO), rather than from biodiversity surveys. Although our current knowledge of the agromyzid fauna of French Guiana is very limited, many other Diptera families have been studied from the region, with approximately 577 species of flies described in thirty-two families (Brûlé and Touroult 2014). There are no studies that have specifically looked at the agromyzid fauna of French Guiana and the specimens collected as part of "Our planet reviewed "project in Mitaraka will be an excellent opportunity to study them.

Current knowledge of the Agromyzidae of Costa Rica

Very few studies have dealt specifically with the Costa Rican fauna of Agromyzidae. In 1973, K.A. Spencer examined a large and important collection of Costa Rican Agromyzidae consisting of approximately 1000 specimens collected by H. Schmidt in the 1930's from La Caja, near San José and housed in the Deutsches Entomologisches Institut in Eberswalde, Germany. A total of 21 species was identified, including two newly described (Spencer 1973c). Additional new species from the same collection were described in Spencer and Stegmaier (1973). In 1983, nine new species were described from Costa Rica in various genera (*Melanagromyza*, *Ophiomyia*, *Japanagromyza*, *Liriomyza* and *Phytoliriomyza*) and 18 species were newly recorded for the region, giving a total of 53 Costa Rican Agromyzidae (Spencer, 1983). These species were the result of a three week visit to Costa Rica by the late K.A. Spencer to assess and report the Agromyzidae fauna present in ornamentals and vegetable farms in addition to sporadic collecting in the area, mainly along roadsides (Spencer 1983). Further Costa Rican records of Agromyzidae can be found in Woodley and Janzen, 1995; Boucher, 2004;

Boucher, 2005; Boucher and Hanson, 2006; Boucher and Nishida, 2014 for a total of 65 Costa Rican Agromyzidae species currently recorded. No previous studies have specifically looked at the agromyzid species found in mid to high elevation cloud forests of Costa Rica. The specimens collected as part of the ZADBI project in Costa Rica will be an excellent opportunity to study them.

Figures

Figures 1-4. Leaf mines (1-3) on various plants, found in Monteverde, Costa Rica. (4) female Agromyzidae laying an egg in the leaf. (Photos K. Nishida)

CONNECTING STATEMENT

The introduction and literature review provided the background information on many aspects of the biology, morphology and identification of the family Agromyzidae in addition to the state of knowledge of the Neotropical fauna. Chapter 1 will be discussing the preliminary results of the expedition to the Mitaraka Massif, in a completely remote and uninhabited region of French Guiana. Only four economically important species are currently reported from French Guiana, creating a large biodiversity and biogeographic gap for this section of the neotropics.

The following chapter has been published in *Zoosystema*.

Boucher S, & Pollet M (2021). The leaf-miner flies (Diptera: Agromyzidae) of Mitaraka, French Guiana. Zoosystema 43(6): 113-125. https://doi.org/10.5252/zoosystema2021v43a6

CHAPTER 1. THE LEAF-MINER FLIES (DIPTERA: AGROMYZIDAE) OF MITARAKA, FRENCH GUIANA

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1.1 Abstract

Preliminary data on the agromyzid fauna (Diptera: Agromyzidae) collected during the "Our Planet Reviewed" expedition in Mitaraka (French Guiana) is provided. A total of 138 specimens representing 10 genera and 50 morphospecies are recorded. *Melanagromyza* Hendel, 1920 and *Ophiomyia* Braschnikov, 1897 were the most diverse genera with 15 and 14 species respectively, followed by *Phytobia* Lioy, 1864 (5 species), *Japanagromyza* Sasakawa, 1958 and *Calycomyza* Hendel, 1931 (4 species each), *Liriomyza* Mik, 1894 (3 species), *Nemorimyza* Frey, 1946 (2 species), *Agromyza* Fallén, 1810, *Cerodontha* Rondani, 1861, *Phytoliriomyza* Hendel, 1931 (1 species each). Except for *Liriomyza* and *Nemorimyza*, all of these genera are recorded from French Guiana for the first time. Nearly 90% of the specimens were collected with a 6 m long Malaise trap installed for 8 days on a rocky outcrop (site "savane roche 2") during the third phase of the expedition in August 2015. Prior to this expedition, only four species of Agromyzidae were reported from French Guiana.

Key words: Diptera, Agromyzidae, Neotropical, French Guiana, Mitaraka, savane-roche, inselberg, rain forest, Amazon

1.2 Résumé

Des données préliminaires sur la faune des agromyzides (Diptera : Agromyzidae) collectées dans le cadre du programme « La planète revisitée », expédition Mitaraka (Guyane) sont fournies. Un total de 138 spécimens représentant 10 genres et 50 morpho-espèces sont répertoriés. *Melanagromyza* Hendel, 1920 et *Ophiomyia* Braschnikov, 1897 étaient les genres les mieux représentés avec respectivement 15 et 14 espèces, suivis par *Phytobia* Lioy, 1864 (5 espèces), *Japanagromyza* Sasakawa, 1958 et *Calycomyza* Hendel, 1931 (4 espèces chacun), *Liriomyza* Mik, 1894 (3 espèces), *Nemorimyza* Frey, 1946 (2 espèces), *Agromyza* Fallén, 1810, *Cerodontha* Rondani, 1861, *Phytoliriomyza* Hendel, 1931 (1 espèce chacun). Tous ces genres, à l'exception de *Liriomyza* et *Nemorimyza,* sont recensés en Guyane pour la première fois. Près de 90% des spécimens ont été récoltés d'un piège Malaise de 6 m de long, installé durant 8 jours sur un affleurement rocheux (site « savane roche 2 ») lors de la troisième phase de l'expédition, en août 2015. Seules quatre espèces d'Agromyzidae avaient auparavant été recensées de la Guyane.

Mots clés: Agromyzidae, Diptères, Néotropical, Guyane, Mitaraka, savane-roche, inselberg, forêt tropicale, Amazonie

1.3 Introduction

Approximately 18,000 insect species have been described from French Guiana, although about 100,000 species are estimated to occur there. Approximately 75% of these described species belong to the order Lepidoptera (moths and butterflies) and Coleoptera (beetles) while Diptera with only 577 species and 2 subspecies is listed among the most poorly known orders of French Guiana insects (Brûlé & Touroult 2014). The fly family Agromyzidae, commonly known as leaf-miner flies, has nearly 3,200 species worldwide (von Tschirnhaus 2021) and has been relatively well studied in some regions of the Neotropics with nearly 500 species recorded; The fauna of French Guiana, on the contrary, remains almost completely unknown with only four species previously recorded: *Liriomyza huidobrensis* (Blanchard); *L. trifolii* (Burgess); *L. sativae* Blanchard and *Nemorimyza maculosa* (Malloch) (EPPO 2020; Martinez & Étienne 2002). These species are well known agromyzid pests and their presence in French Guiana has been reported by various agricultural or plant protection organisations (e.g. EPPO), rather than from biodiversity surveys.

During 2015, an expedition to the Mitaraka massif, a mostly unexplored region of French Guiana, was organized to assemble information on the biodiversity of the area, with a focus on freshwater and terrestrial invertebrate biodiversity (Pascal et al. 2015; Biotope 2014). The expedition was part of a large-scale biodiversity program known as “Our planet Reviewed” (La Planète Revisitée) led by the National Museum of Natural History (MNHN, France) and Pro-Natura international (Pollet et al. 2014; Muséum national d’Histoire naturelle 2016). The main objective of this program is to accelerate the discovery and description of new species by concentrating efforts in regions considered a priority for nature conservation (Muséum national d’Histoire naturelle 2016).

The Mitaraka massif is situated in the southwesternmost part of French Guiana directly bordering Surinam and Brazil (Fig. 1). It is part of the Guiana Amazonian Park, which forms one of the largest protected rain forest areas in the world, together with the adjacent Tumucumaque National Park in Brazil (Shelley 2013). The Mitaraka massif is a completely remote, uninhabited region consisting mainly of lowland tropical rain forest with scattered isolated rocky outcrops of Precambrian granite known as inselbergs (Fig. 2) (Touroult et al. 2018). Although these inselbergs are sometimes described as being part of a larger mountain range, known as Tumuc-Humac, the truth is that this mountain range does not exist, at least not as it was visualized by French explorers. Indeed, these "mountains" are no more than isolated inselbergs varying in size from 100 to 800 meters (Hurault 1973, 2000; Chavance 2015).

Inselbergs ("Island mountains" in German) have recently received increased interest for biodiversity research as these "islands" are known to serve as refuges for unique flora and fauna (Vitt 1993; Porembski & Barthlott 2000; Vlasáková et al. 2008; Jocque & Giupponi 2012). The unique diversity and structure of their plant communities have been studied in both tropical and temperate regions (Porembski & Barthlott 2000; Sarthou et al. 2003; Porembski 2007). Contrary to the rich flora of the surrounding moist forest, inselbergs are considered "micro-environmental deserts" (Phillips 1982) where vegetation grows on exposed granite rock with very little or no soil cover that is often exposed to extreme weather conditions. In French Guiana, the characteristic vegetation of inselbergs is called "savane-roche" (Gasc et al. 1998; Sarthou et al. 2003). More than 600 plant species have been reported from these savanes-roche vegetations. Most of these species belong to the following plant families: Orchidaceae Juss., Rubiaceae Juss., Melastomataceae Juss., Poaceae Barnhart, Cyperaceae Juss., Bromeliaceae Juss., Araceae Juss., Myrtaceae Juss., and Euphorbiaceae Juss. (Gasc et al. 1998), with some variation from one inselberg to the other (Sarthou et al. 2003). In French Guiana, inselbergs are located in undisturbed rain forest and their flora includes some species that are rare and restricted to these rocky outcrops in this part of the Neotropics (Sarthou et al. 2003). Until recently, little was known on the insect fauna associated with inselbergs (Mares & Seine 2000). The recent expedition to the Mitaraka massif has produced some important new data on the insect fauna of the French Guiana inselbergs and surrounding rain forest habitats (eg: Dalens & Touroult 2015; Touroult & Dalens 2015; Fleck 2017; Vicente & Robillard 2017; Mantilleri 2018; Campos & Desutter-Grandcolas 2020; Desutter-Grandcolas & Faberon 2020; Pocco & Cigliano 2020), including important progress on our knowledge of the Diptera fauna (Krolow et al. 2017; Brooks et al. 2018; Gomes & Carvalho 2018; Mortelmans & Pollet 2018; Pollet et al. 2018; Runyon & Pollet 2018; Ale-Rocha & Pollet 2019; Marques et al. 2019; Pirani & Grimaldi 2019; Vieira et al. 2019; Curler 2020; Mederos & Pollet 2019; Silva & Pollet 2020).

The objective of this paper is to document on the diversity of agromyzid species collected during the Mitaraka expedition in a southern lowland rain forest with special attention to the fauna of a minor inselberg and to provide a first insight into the composition of French Guiana Agromyzidae.

1.4 Materials and Methods

1.4.1 Research team and timeframe

Due to the dense vegetation and remote aspect of the Mitaraka massif, a landing area (the drop zone) was cleared by the army prior to the arrival of the research crew by helicopter. A base camp was also established for the duration of the expedition. Three different teams of researchers were involved during three different sampling periods in 2015: February 23 to March 11, March 11-27 and August 12-20 (Touroult et al. 2018). The main mission was conducted during the rainy season (February – March 2015) by 32 researchers, each studying a specific taxonomical group e.g., higher plants, earthworms, birds, fish, or particular insect taxa. A third shorter visit by a smaller team of 10 researchers to the site during the beginning of the dry season focused on insects only, and was organized by the Entomological Society of Antilles-Guyane (SEAG). MP was the only Diptera worker involved in fieldwork and participated in the first sampling period.

1.4.2 Collecting sites and collecting methods

The area under investigation consisted of the base camp, the drop zone and four main trails of approximately 3.5 km each, leaving in different directions from the base camp (Fig. 3). A total of 37 sampling sites were investigated (Pollet et al. 2018) but only a few of them produced agromyzid specimens, including a river bank forest (MIT-A-RBF1) (samples 186, 189), the drop zone (MIT-DZ) (sample 218), a minor inselberg with savane-roche vegetation (MIT-E- savane roche 2) (Fig. 4) (sample 230), the base camp, and trails and other sites in tropical moist forest. Some samples were pooled from different sites and traps but were collected with the same method (samples 227, 229) (Table 1).

A wide array of methods was used to collect invertebrates (Pascal et al. 2015; Touroult et al. 2018) and multiple traps of particular types were employed e.g., 280 pan traps and 33 SLAM traps, but not all trapping methods were used during all three sampling periods of the expedition (e.g., pan traps were only used during the first sampling period). Among all techniques used, only a few were successful in collecting Agromyzidae: a 6 m long Malaise trap (MT(6M)) (Fig. 5c), the Sea and Land Air Malaise traps (SLAM) (Fig. 5b), and blue Polytrap automatic light traps (PVB) (flight intercept trap using blue LED light, and suspended in canopy) (Fig. 5a) (Table 1). Further details on sampling methods can be found in Touroult et al. (2018).

1.4.3 Specimen preparation and identification

A total of 223 invertebrate samples were examined, with non-pan trap samples being sorted to insect orders and families at the SEAG office, and pan trap samples sorted by MP. This process produced nearly 2,200 subsamples, containing 28 Diptera families and superfamilies (Pollet et al. 2018) that were disseminated among experts worldwide, including the 6 samples of Agromyzidae (Table 1) sent to SB. The Agromyzidae samples were initially stored in 70% ethanol and subsequently dried using hexamethyldisilazane (HMDS), mounted and labelled (see Table 1). Morphospecies identifications were based on external morphological characters and male genitalic characters. Male genitalia were examined by detaching the abdomen from the remainder of the specimen, clearing it in 85% lactic acid heated in a microwave oven for 2-3 intervals of 30 seconds each, separated by a cooling period, or cleared outside the microwave (later in the project) following procedures outlined in Boucher (2019). The abdomen was subsequently transferred to a glycerin solution for further dissection and examination and was ultimately stored in a microvial pinned below the corresponding specimen. The format of the morphospecies names used here are: genus name + species number, preceded by the prefix MIT (for Mitaraka).

1.5 Results

A total of 138 specimens of Agromyzidae from 6 samples (different collection events) were examined. Sample 230 was by far the largest sample with 123 specimens (Table 1) and was collected at the Mit-E-savane roche 2 site during the third period of the expedition (August 13-20, 2015) by researchers of SEAG. Sample 229 with 6 specimens was also collected during this period, resulting in a total of 129 specimens out of 138 specimens (93.48%) from this period (Fig. 6). Seven specimens (samples 186, 218, 227) were collected during the first period of the expedition (Feb 23-March 11), and only 2 specimens (sample 189) during the second period (March 11-27) of the expedition (Fig. 6). Fifty morphospecies in 10 genera were recognized in these 138 specimens, with 43 males and 95 females (Table 2).

The following eight agromyzid genera are recorded for the first time from French Guiana: *Agromyza* Fallén, 1810; *Calycomyza* Hendel, 1931; *Japanagromyza* Sasakawa, 1958; *Melanagromyza* Hendel, 1920; *Ophiomyia* Braschnikov, 1897; *Phytobia* Lioy, 1864; *Cerodontha* Rondani, 1861; *Phytoliriomyza* Hendel 1931.

The genus *Melanagromyza* with 67 specimens was by far the most abundant, followed by *Ophiomyia* (24 specimens), *Liriomyza* Mik, 1894 (15 specimens), *Japanagromyza* (11 specimens), *Calycomyza* (7 specimens), *Nemorimyza* Frey, 1946 and *Phytobia* (5 specimens each), *Phytoliriomyza* (2 specimens), *Cerodontha* and *Agromyza* (1 specimen each) (Fig. 7). The most abundant species was *Melanagromyza* Mit-2 (26 specimens), followed by *Melanagromyza* Mit-3 (13 specimens), *Liriomyza* Mit-1 (12 specimens), *Melanagromyza* Mit-6 (8 specimens), *Japanagromyza* Mit-1 (7 specimens); *Melanagromyza* Mit-4 (5 specimens) (Table 2).

The genus *Melanagromyza* and *Ophiomyia* were the most diverse with 15 and 14 species respectively, followed by the genus *Phytobia* (5 species), *Japanagromyza* and *Calycomyza* (4 species each), *Liriomyza* (3 species), *Nemorimyza* (2 species) and *Phytoliriomyza, Cerodontha, Agromyza* (1 species each) (Fig. 7).

Six species were collected during the first sampling period, 2 species during the second period, and most species (43 species out of 50) during the last period of the expedition (Fig. 6), with very little or no species overlap between the different periods (Fig. 9).

Only two species were represented in more than one sample: *Japanagromyza* Mit-1 was retrieved from samples 229 and 230, and *Melanagromyza* Mit-3 from samples 218, 229, 230

(Table 2). Among the 50 species identified, 27 species were represented by females only and most species (38/50) were represented by only 1 or 2 specimens (Table 2).

Three collecting methods successfully caught Agromyzidae, but the 6 m long Malaise trap (MT(6M)) was by far the most successful with a total of 48 species (Fig. 8). Three agromyzid samples (186, 189, 230) were collected with this 6 m Malaise trap (Table 1), which was installed in tropical lowland rain forest over the Alama River (samples 186, 189) in February – March and on a minor inselberg in August (sample 230). The first two samples (1-25 March 2015) contained 5 different species (*Japanagromyza* Mit-4; *Melanagromyza* Mit-12; *Ophiomyia* Mit-8, *Phytobia* Mit-1, *Phytobia* Mit-2) while the latter 8 day's sample encompassed an astonishing 43 agromyzid species (Table 2). The other 2 trapping methods, the SLAM (samples 218, 229) and blue polytrap automatic light traps (PVB) (sample 227) caught 3 (*Liriomyza* Mit-3; *Japanagromyza* Mit-1; *Melanagromyza* Mit-3) and 1 species (*Ophiomyia* Mit-14) respectively (Table 2; Fig. 8). The Malaise trap produced 46 unique species, while the SLAM and PVB traps collected one unique species each (*Liriomyza* Mit-3 and *Ophiomyia* Mit-14 respectively) (Fig. 8).

1.6 Discussion

Given this relatively short survey (40 days total) and the low number of specimens examined (138 specimens), alpha diversity of Agromyzidae in Mitaraka appears quite high with a total of 50 species in 10 genera. Surprisingly, most of these agromyzids were collected from a trap set up for only 8 days. A recent survey in a very small patch of cloud forest in Zurquí de Moravia, Costa Rica, yielded 812 specimens of 117 species of Agromyzidae in 12 genera, but this was the result of a whole year of sampling (Brown et al. 2018).

With 67 specimens and 15 species, *Melanagromyza* was the most abundant and most diverse genus in Mitaraka. It is also the second largest genus in the Neotropical region with 93 described species. Its diversity is substantially lower in temperate regions of the world. As an example, only 37 species are reported in the USA (Shi & Gaimari 2015). *Liriomyza*, the most speciose Neotropical agromyzid genus with 105 described species, was not well represented in Mitaraka with only 3 species. The other most diverse genera in the Neotropical region are *Calycomyza* (73 described species) and *Ophiomyia* (50 species). The latter genus was represented in the Mitaraka samples with 14 species, the former with only 4 species.

The highest agromyzid specimen and species numbers were retrieved from a single sample (code 230), which was collected with a 6 metre long Malaise trap on one of the minor inselbergs (site MIT-E- savane roche 2). This site was described as a rocky outcrop (471 m a.s.l.) covered with herbaceous vegetation (including *Pitcairnia* L'Hér.), scattered shrubs and exposed rocks (Touroult et al. 2018; Pollet et al. 2018) (Fig. 4). *Pitcairnia* is a speciose genus of Bromeliaceae present mainly in Mexico, Central and South America and the Caribbean (Saraiva et al. 2015), with some species known to be restricted to inselbergs (Sarthou et al. 2001). This plant genus has never been recorded as host for any Agromyzidae, and only one species of Agromyzidae is known to feed on Bromeliaceae, *Melanagromyza rosales* Woodley, a leaf-miner in *Bromelia pinguin* L. in Costa Rica (Woodley & Janzen 1995). However, none of the Mitaraka *Melanagromyza* specimens correspond to this species.

Only two species (*Melanagromyza* Mit-3 and *Japanagromyza* Mit-1) were present in more than one sample, with *Melanagromyza* Mit-3 collected in two distinct habitats (savane-roche and drop zone (cleared moist forest)). Surprisingly, other sites similar to the "Mit-E-savane roche 2" investigated at Mitaraka (including other minor rocky outcrops (390-470 m a.s.l.) and major rocky outcrops- 540-570 m a.s.l.)), did not yield any Agromyzidae. This is most probably due to the fact that no 6 m Malaise trap was operational on these inselbergs, and that no agromyzids were actively searched for.

The success of the 6 m Malaise trap reflects previous knowledge that Malaise traps are an effective method for collecting flying insects, including flies (Lamarre et al. 2012; Borkent et al. 2018). But this method is also known to be female-biased for Agromyzidae (Scheirs et al. 1997), which concurs with the high proportion of agromyzid females collected during the expedition. As compared to the very productive trap on the minor inselberg in August 2015 (43 species, 123 specimens), the other habitat examined with this trap in March (samples 186, 189) proved much less species rich, with only 5 species and 6 specimens. Reasons for this difference might include: season, habitat type and host vegetation, position of the trap, or, a combination of these factors. Among the other collecting methods used, the SLAM trap is not commonly applied for Agromyzidae, but when installed on land (as it was the case here) it works in a similar way as a Malaise trap, although being smaller (Touroult et al. 2018). This method collected few specimens and added only one unique species (*Liriomyza* Mit-3) (Fig. 8) in phase 1 of the project. The SLAM traps that were installed for 9 days during the last sampling period (August) in a range of sites, including Mit-E-savane roche 2 (sample 229), were much less successful than the 6 m Malaise trap (sample 230) installed during the same period in the savane roche 2 site. The reasons for this difference remain unclear as this trap type was successful for collecting other Diptera families (Krolow et al. 2017; Pollet et al. 2018; Vieira et al. 2019). The PVB (automatic light trap with blue LED) also added one unique species (*Ophiomyia* Mit-14) (Fig. 8) represented by one female specimen. The automatic light traps were installed to collect nocturnal flying insects from various insect orders, but not targeting Diptera (Touroult et al. 2018). Agromyzidae are known to be diurnal and are not normally collected by light traps so this specimen is possibly an accidental visitor to the trap. Despite being established as a successful device to collect Diptera, including Agromyzidae (Scheirs et al. 1997), the 280 pan traps used did not yield a single agromyzid in Mitaraka. This absence of Agromyzidae could be explained by the heavy rainfall which also affected yields of Dolichopodidae (Pollet et al. 2018). Although savane roche 2 and at least 29 other sites were investigated by MP using a sweep net during the first phase of the expedition, no Agromyzidae were gathered because an on sight collecting approach was used to mainly target Dolichopodidae. Sweeping was also performed during the last phase of the expedition (Touroult et al. 2018), but again this approach was unsuccessful for Agromyzidae, most probably for the same reason.

All species are here identified as morphospecies as a first step in the species recognition process. Species identification of small flies like the Agromyzidae (averaging 2-3 mm in wing length but sometimes as small as 1 mm) can be challenging, even for trained taxonomists. This is particularly true for species living in inadequately studied regions like French Guiana for which no reference work nor identification keys are available and where many undescribed species are expected. Although morphospecies identification is often performed by non-specialists (or “parataxonomists”) for rapid biodiversity assessment (Derraik et al. 2002), it was here performed by a trained agromyzid taxonomist (SB) using elaborate external non-genitalic and genitalic characters when possible, which certainly adds to the accuracy of the morphospecies determination. Nevertheless, females often remain difficult to differentiate from each other or to assign to a male morphospecies.

Further taxonomic work is in progress and will allow the assignment of some of the morphospecies to described species, while others will be confirmed as new species and will be described. Some morphospecies will remain unidentified until further material is available, and this will include most of the 27 species represented by female specimens only.

Although additional work is required for species level identification, we can confirm that the agromyzid species collected during the Mitaraka expedition do not correspond to any of the 4 species (*Nemorimyza maculosa*, *Liriomyza huidobrensis*, *L. trifolii*, *L. sativae*) previously recorded from French Guiana. The Mitaraka survey thus increases the known agromyzid fauna of this French Département d'Outre Mer to 54 (morpho)species.

These 50 morphospecies of Agromyzidae recorded from Mitaraka are a significant addition to the other Diptera species so far recorded from the Mitaraka survey. This list includes 24 species of Tabanidae (Krolow et al. 2017), 2 species of Sciomyzidae (Mortelmans & Pollet 2018), 244 species of Dolichopodidae (Pollet et al. 2018; Runyon & Pollet 2018), 2 species of Ropalomeridae (Ale-Rocha & Pollet 2019), 15 species of Pipunculidae (Marques et al. 2019), 44 species of Asilidae (Vieira et al. 2019), and 11 species of Sepsidae (Silva & Pollet 2020), apart from the description of multiple new or extraordinary species (Gomes & de Carvalho 2018; Mederos & Pollet, 2019; Pirani & Grimaldi 2019; Curler 2020).

1.7 Acknowledgments

All the material for this study was collected during the "*Our Planet Reviewed*" Guyane-2015 expedition in the Mitaraka range, in the core area of the French Guiana Amazonian Park, organized by the MNHN and Pro-Natura international. The expedition was funded by the European Regional Development Fund (ERDF), the Conseil régional de Guyane, the Conseil général de Guyane, the Direction de l'Environnement, de l'Aménagement et du Logement and by the Ministère de l'Éducation nationale, de l'Enseignement supérieur et de la Recherche. It was realized in collaboration with the Parc amazonien de Guyane and the Société Entomologique

Antilles-Guyane (SEAG). MP participated to this expedition as member of the first team (22 February-11 March 2015), hereby supported financially by MNHN and Pro-Natura international. We would also like to thank in particular Pierre-Henri Dalens and his team for collecting the major part of the agromyzid material during the August mission. Also Olivier Pascal (MNHN), Julien Touroult (MNHN), Stéphane Brûlé (SEAG), Xavier Desmier, Maël Dewynter and the Service Géomatique du Parc Amazonien de Guyane for providing pictures.

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1.9 Figures and Tables

Figure 1. Map showing locality of Mitaraka study site, in southwest French Guiana.

Figure 2. The Sommet-en-Cloche, an example of a major inselberg amid tropical lowland rain forest in the Mitaraka area (Photo Xavier Desmier).

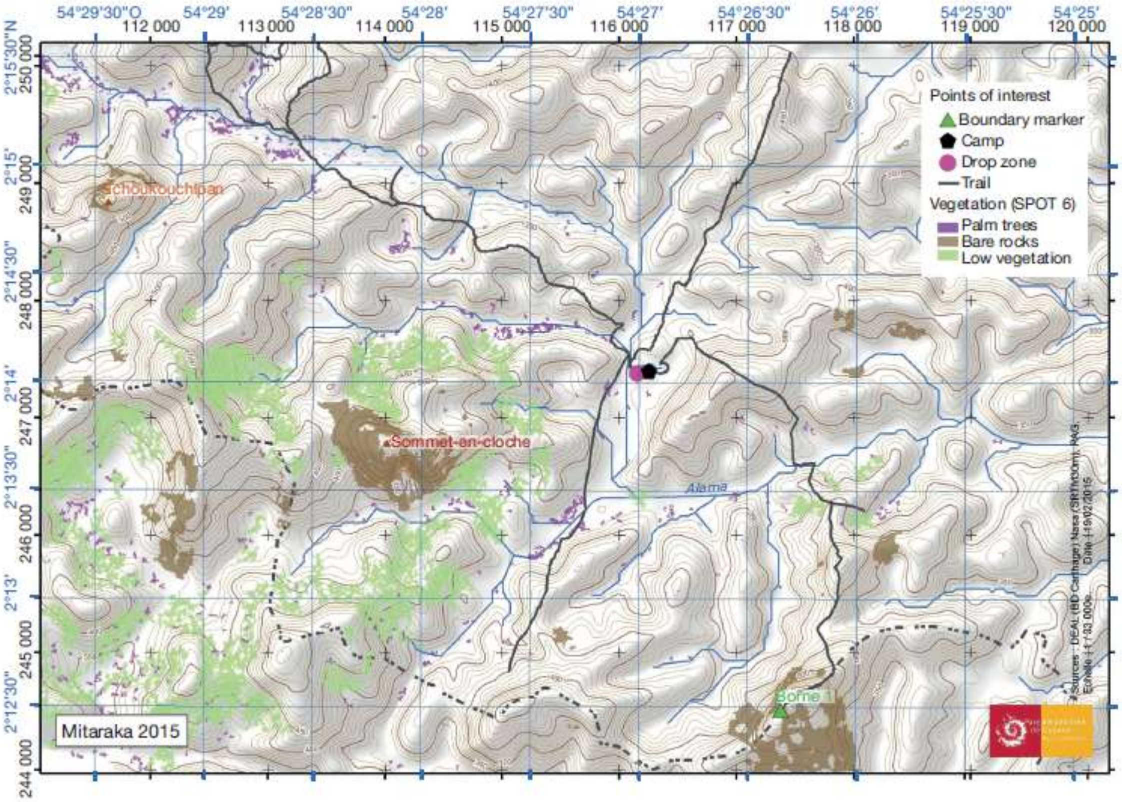

Figure 3. Mitaraka study site showing location of base camp, drop zone and main collecting trails (source: Maël Dewynter; Service Géomatique du Parc Amazonien de Guyane).

Figure 4. Mitaraka study site MIT-E-savane roche 2 (Photo Marc Pollet).

Figure 5. Collecting techniques that yielded Agromyzidae samples in Mitaraka. A. PVB light trap (Photo Julien Touroult); B. 6 m long Malaise trap; C. SLAM traps (Photos B, C Stéphane Brulé).

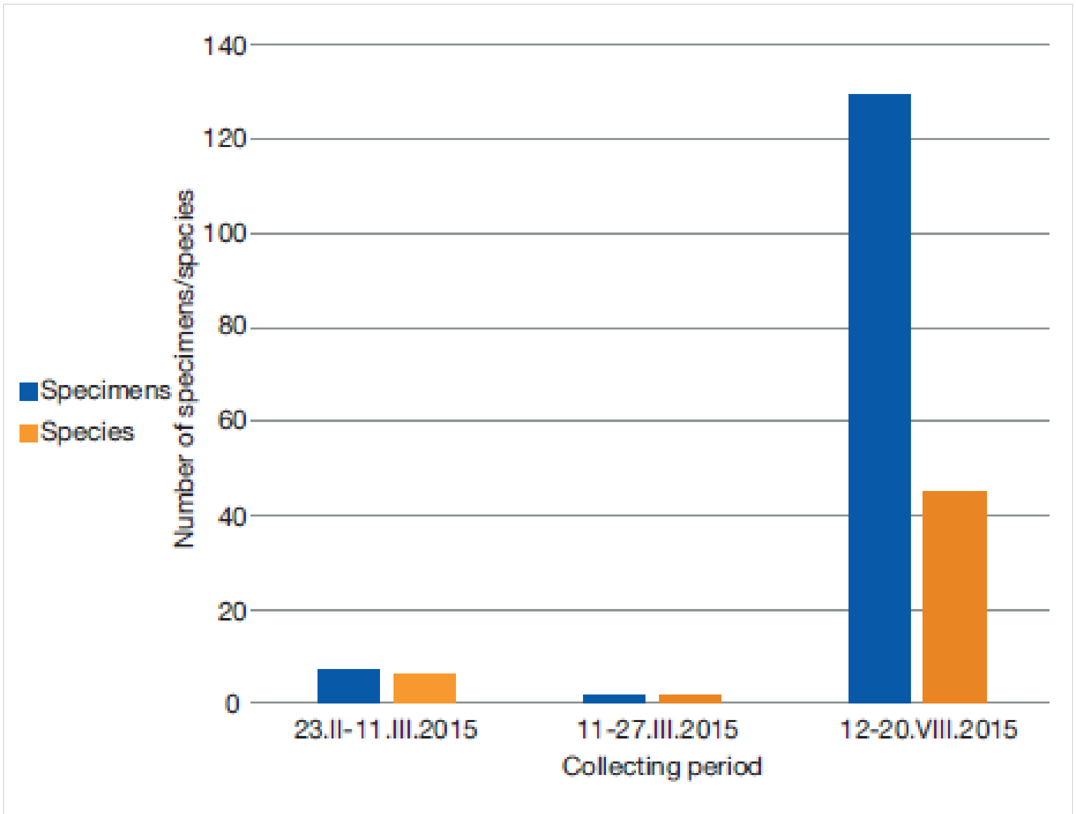

Figure 6. Number of Agromyzidae specimens and species collected during the different periods of the expedition.

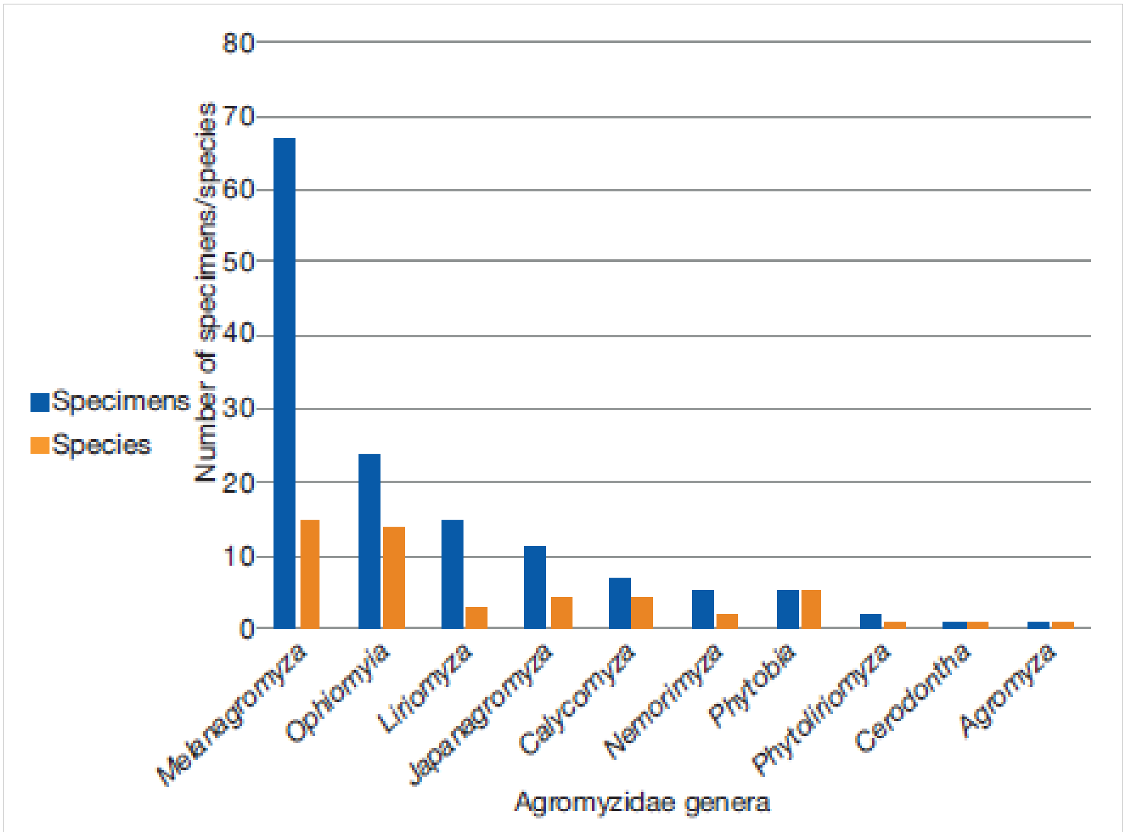

Figure 7. Abundance (number of specimens) vs diversity (number of species) for each Agromyzidae genus collected during the Mitaraka expedition.

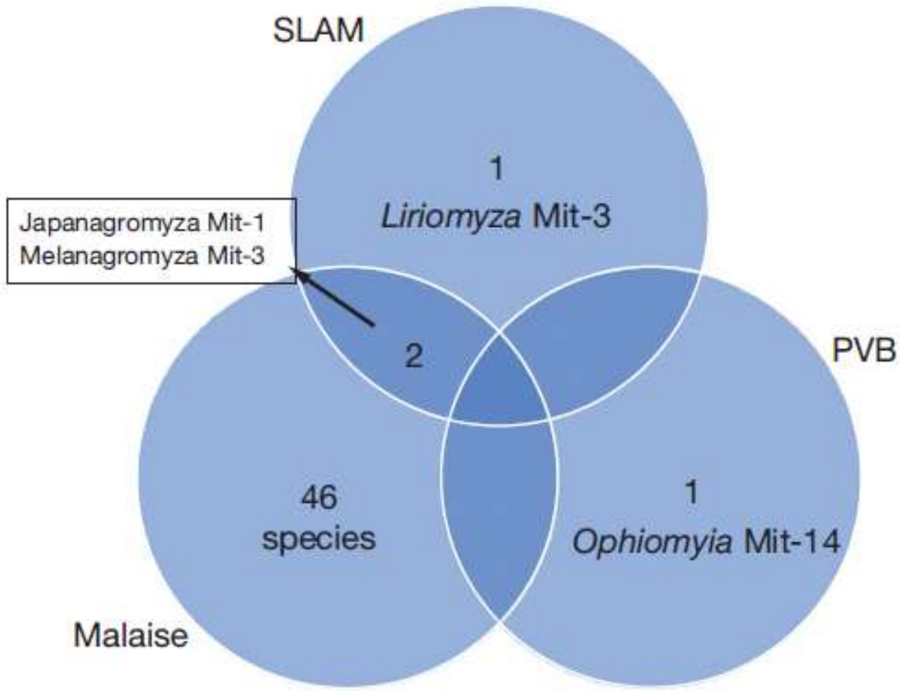

Figure 8. Venn diagram showing number of species collected from different trapping methods.

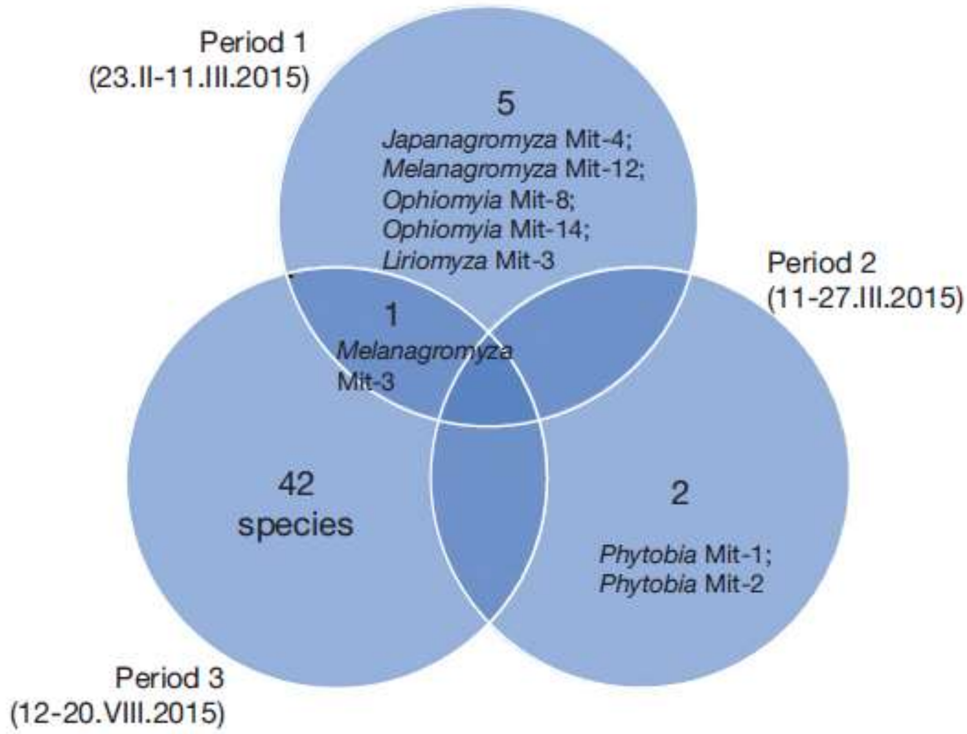

Figure 9. Venn diagram showing number of species collected during the different sampling periods.

Tables.

Table 1. Labels of the six Agromyzidae samples from Mitaraka with specimens and species number per sample. Collecting method for each sample is highlighted in bold.

Sample codes	Label data	Specimens	Species
186	(FR-GU) Guyane Française, Mitaraka, nr MIT-A-RBF1, river, 02°14'11.4"N, 54°27'07.0"W, 306 m, 1.iii.2015-7.iii.2015, **MT(6m)**, leg. Julien Touroult & Eddy Poirier (FR-GU/Mitaraka/2015) - sample code: MITARAKA/186 (sorted by Marc Pollet, 2015)	4	3
189	(FR-GU) Guyane Française, Mitaraka, nr MIT-A-RBF1, river, 02°14'11.4"N, 54°27'07.0"W, 306 m, 7-25.iii.2015, **MT(6m)**, leg. Julien Touroult & Eddy Poirier (FR-GU/Mitaraka/2015) - sample code: MITARAKA/189 (sorted by Marc Pollet, 2015)	2	2
218	(FR-GU) Guyane Française, Mitaraka, MIT-DZ, 02°14'01.8"N, 54°27'01.0"W, 306 m, tropical moist forest (plateau-slope - cleared), 1.iii.2015, **SLAM**, leg. Julien Touroult & Eddy Poirier (FR-GU/Mitaraka/2015) - sample code: MITARAKA/218 (sorted by Marc Pollet,2015)	2	2
227	(FR-GU) Guyane Française, Mitaraka, different sites nr base camp and along trails, tropical moist forest (different sites), 3.iii.2015, **PVB**, leg. Julien	1	1

	Touroult & Eddy Poirier (FR-GU/Mitaraka/2015) - sample code: MITARAKA/227 (sorted by Marc Pollet, 2015)		
229	(FR-GU) Guyane Française, Mitaraka, different sites nr base camp and along trails, open / partially opened areas around base camp and dropzone, and in savane roche 2, 12.viii.2015-20.viii.2015, **SLAM**, leg. Pierre-Henri Dalens (FR-GU/Mitaraka/2015) – sample code: MITARAKA/229 (sorted by M. Pollet, 2015)	6	2
230	(FR-GU) Guyane Française, Mitaraka, MIT-E-savane roche 2, 02°13'59.8"N, 54°27'46.5"W, 471 m, open / partially opened areas on savane roche 2, 13.viii.2015-20.viii.2015, **MT(6m)**, leg. Pierre-Henri Dalens (FR-GU/Mitaraka/2015) - sample code: MITARAKA/230 (sorted by Marc Pollet, 2015)	123	43

Table 2. Agromyzidae morphospecies list from Mitaraka with sample codes and number of specimens

Species #	Sample code	Morphospecies name	no. males	no. females	Total specimens
1	Mitaraka/229/230	Japanagromyza MIT-1		7	7
2	Mitaraka/230	Japanagromyza MIT-2		1	1
3	Mitaraka/230	Japanagromyza MIT-3		2	2
4	Mitaraka/186	Japanagromyza MIT-4		1	1
5	Mitaraka/230	Agromyza MIT-1	1		1
6	Mitaraka/230	Melanagromyza MIT-1	2		2
7	Mitaraka/230	Melanagromyza MIT-2	10	16	26
8	Mitaraka/218/229/230	Melanagromyza MIT-3	4	9	13
9	Mitaraka/230	Melanagromyza Mit-4	2	3	5
10	Mitaraka/230	Melanagromyza Mit-5	1		1
11	Mitaraka/230	Melanagromyza Mit-6		8	8
12	Mitaraka/230	Melanagromyza Mit-7		2	2
13	Mitaraka/230	Melanagromyza Mit-8		1	1

14	Mitaraka/230	Melanagromyza Mit-9		1	1
15	Mitaraka/230	Melanagromyza Mit-10		2	2
16	Mitaraka/230	Melanagromyza Mit-11		1	1
17	Mitaraka/186	Melanagromyza Mit-12	1	1	2
18	Mitaraka/230	Melanagromyza Mit-13		1	1
19	Mitaraka/230	Melanagromyza Mit-14		1	1
20	Mitaraka/230	Melanagromyza Mit-15		1	1
21	Mitaraka/230	Ophiomyia Mit-1	3		3
22	Mitraka/230	Ophiomyia Mit-2	1		1
23	Mitaraka/230	Ophiomyia Mit-3	1	1	2
24	Mitaraka/230	Ophiomyia Mit-4	2		2
25	Mitaraka/230	Ophiomyia Mit-5	2	2	4
26	Mitaraka/230	Ophiomyia Mit-6		1	1
27	Mitaraka/230	Ophiomyia Mit-7	1	1	2
28	Mitaraka/186	Ophiomyia Mit-8		1	1
29	Mitaraka/230	Ophiomyia Mit-9		1	1
30	Mitaraka/230	Ophiomyia Mit-10		1	1
31	Mitaraka/230	Ophiomyia Mit-11	1		1
32	Mitaraka/230	Ophiomyia Mit-12		2	2
33	Mitaraka/230	Ophiomyia Mit-13		2	2

34	Mitaraka/227	Ophiomyia Mit-14		1	1
35	Mitaraka/230	Calycomyza Mit-1	1	1	2
36	Mitaraka/230	Calycomyza Mit-2	1	1	2
37	Mitaraka/230	Calycomyza Mit-3		2	2
38	Mitaraka/230	Calycomyza Mit-4		1	1
39	Mitaraka/230	Cerodontha Mit-1	1		1
40	Mitaraka/230	Nemorimyza Mit-1	1	2	3
41	Mitaraka/230	Nemorimyza Mit-2		2	2
42	Mitraka/230	Phytoliriomyza Mit-1	1	1	2
43	Mitaraka/230	Liriomyza Mit-1	3	9	12
44	Mitaraka/230	Liriomyza Mit-2		2	2
45	Mitaraka/218	Liriomyza Mit-3		1	1
46	Mitaraka/189	Phytobia Mit-1	1		1
47	Mitaraka/189	Phytobia Mit-2		1	1
48	Mitaraka/230	Phytobia Mit-3		1	1
49	Mitaraka/230	Phytobia Mit-4	1		1
50	Mitaraka/230	Phytobia Mit-5	1		1
			Total: 43	Total: 95	Total: 138

CONNECTING STATEMENT

In Chapter 1, all specimens from Mitaraka were identified to morphospecies as a first step for species delineation. It was recognized that identifying Neotropical Agromyzidae in a region where no previous work had been done, can be extremely challenging due to the lack of keys and reference work. Also, the high number of female specimens present in the samples is increasing the difficulty in species delineation. In the next chapter, the use of DNA barcoding was explored in assisting with species identification, species delineation, and male/ female association.

The following chapter has been published in Zookeys.

Boucher S. & Savage J. 2022. DNA barcoding of the leaf-miner flies (Diptera: Agromyzidae) of Mitaraka, French Guiana. *ZooKeys* 1083: 147-168.

CHAPTER 2. DNA BARCODING OF THE LEAF-MINER FLIES (DIPTERA: AGROMYZIDAE) OF MITARAKA, FRENCH GUIANA

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2.1 Abstract.

Species level identification of Agromyzidae based on morphology is often challenging due to their small size and morphological homogeneity. DNA barcoding has been used regularly to assist with the identification of economically important species of Agromyzidae, but rarely as a tool for species delineation or identification in biodiversity surveys. The main objective of this study was to investigate whether DNA barcoding and the BIN (Barcoding Index) system could assist with species identification, species delineation, male/ female association, and diversity assessment of Agromyzidae material previously determined to morphospecies from Mitaraka, French Guiana. Amplification success was low, with sequences over 400 bp recovered for only 24 (48%) of the selected specimens. Sequences assigned to 17 morphospecies formed 16 distinct branches or clusters separated by very high (minimum of 10%) sequence divergence. Following the reassessment and subsequent reassignment of one specimen, congruence between morphology and DNA barcodes was high with a single instance of two morphospecies sharing identical sequences. While DNA barcoding did not assist with identification (none of our sequences matched those of named taxa in BOLD or GenBank), it did provide support for most of our morphospecies concepts, including male/female associations. The BIN system also provided access to information about the distribution and habitat preferences of several taxa. We conclude that DNA barcoding was a useful approach to study the species diversity of our samples but that much work remains to be done before it can be used as an identification tool for the Agromyzidae fauna of Mitaraka and the rest of the Neotropical region.

Keywords. Agromyzidae, DNA barcoding, Barcode Index Number (BIN), CO1, French Guiana, Neotropical

2.2 Introduction

The Agromyzidae is a family of small flies, measuring on average 2-4 mm in wing length, although they can be smaller than 1 mm or measure up to 6.5 mm. Their colouration is variable, from yellow and/or black, brown or grey, sometimes with metallic greenish, bluish or coppery colouration. Most have clear wings, but they may be patterned or infuscated in a few tropical species. The family contains approximately 3200 described species found worldwide (von Tschirnhaus 2021). The larvae of all species feed internally on living plant tissues, with most species with known biology developing inside leaves, hence their common name of leaf-miner flies. The family includes some important pest species of agricultural and ornamental plants, including three well known species occurring in many parts of the world, including South America: *Liriomyza huidobrensis* (Blanchard), *Liriomyza trifolii* (Burgess), *Liromyza sativae* Blanchard. Agromyzidae species identification based on morphology alone is a difficult task due to their small size and morphological homogeneity, but also due to their high diversity, presence of numerous undescribed species and lack of recent identification keys for many countries (Benavent-Corai et al. 2005; Boucher 2010; Boucher and Pollet 2021). Misidentification has happened repeatedly in the literature even when identification was performed by specialists (Scheffer and Winkler 2008). Examination of male genitalia through dissection is often required to confirm species identity, or to support morphospecies delineation in biodiversity surveys (Boucher and Pollet 2021), but this is not an easy process requiring laborious preparation and expertise. In addition to these challenges, species descriptions are often based on one sex only (more commonly males), making male/ female association difficult, especially when sexually dimorphic species are involved.

DNA barcoding, the sequencing of a short fragment of DNA sequence of the mitochondrial cytochrome c oxidase 1 (CO1) gene, is being increasingly used as an identification tool, especially for very diverse and/or morphologically difficult taxa. DNA barcoding was initially proposed as a tool for the identification of animal species (Hebert et al. 2003), but later found to be useful for many other applications in taxonomy and biodiversity studies including species delineation and biodiversity assessment (Hebert et al. 2016), the discovery of cryptic species, female identification, and male/female association (Janzen et al. 2009; Ekrem et al. 2010; Renaud et al. 2012; DeSalle and Goldstein 2019). The Barcode Index Number (BIN) system (Ratnasingham and Hebert 2013) implemented in the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007) is used to group similar COI sequences into genetic clusters (Molecular Operational Taxonomic Units: MOTUs) that can be used as proxy for species. These genetic clusters are assigned unique identifiers (BINs) and include any barcoded specimens on BOLD (even from unrelated projects) with similar sequences, sometimes providing useful metadata such as locality, elevation, habitat type, sex, picture of the specimen, collection date, sampling technique, and taxonomic assignment if named reference sequences are included in the BIN. This could provide important information for biodiversity inventories and revisionary taxonomic studies (Telfer et al. 2005; Ratnasingham and Hebert 2013).

In the family Agromyzidae, the use of the CO1 gene has been used mainly as a tool to differentiate and identify economically important and invasive species (e.g. Scheffer et al. 2006; Bhuiya et al. 2011; Blacket et al. 2015; Czepak et al. 2018; Firake et al. 2018; Xu et al. 2021), to uncover and identify cryptic species (e.g. Scheffer and Lewis 2006; Scheffer et al. 2014; Weintraub et al. 2017; Mlynarek and Heard 2018), to discover new species (e.g. Scheffer and Wiegmann 2000) and to elucidate Agromyzidae phylogenetic relationships (e.g. Scheffer and Wiegmann 2000; Scheffer et al. 2007; Winkler et al. 2009).

DNA barcoding has rarely been used as a tool for Agromyzidae species identification, morphospecies delineation or gender association in biodiversity surveys, although its use could provide faster and more accurate identification results. Two large biotic surveys occurring in Ontario have used barcoding to provide species identification of thousands of taxa including 21 species (Telfer et al. 2005) and 13 species (deWaard et al. 2018) of Agromyzidae without the expertise of an agromyzid specialist.

A recent and relatively short biotic survey conducted in 2015 at the Mitaraka massif, a mostly unexplored region of French Guiana (Touroult et al. 2018), resulted in 138 agromyzid specimens (43 males; 95 females), delineated into 50 morphospecies (Boucher and Pollet 2021). Based on a combination of external and genitalic characters, male specimens could be delineated into 23 morphospecies, but 69% of the specimens collected were females and morphospecies delineation and male/ female association were highly challenging due to the lack of external diagnostic characters. This problem was especially noticeable for the genera *Melanagromyza* and *Ophiomyia*, the two most abundant and diverse agromyzid genera at Mitaraka (Boucher and Pollet 2021).

Prior to the 2015 Mitaraka expedition, approximately 500 agromyzid species were recorded in the Neotropical region including only four species in French Guiana (*Liriomyza huidobrensis* (Blanchard), *Liriomyza trifolii* (Burgess), *Liromyza sativae* Blanchard, *Nemorimyza maculosa* (Malloch)) (EPPO 2021)). Morphological examination indicated that the Mitaraka agromyzids did not correspond to any of the named species previously recorded for French Guiana (Boucher and Pollet 2021), but some questions remained related to species delineation and identification for the Mitaraka specimens.

The main objective of this study was to investigate whether DNA barcoding could assist with species identification, species delineation, male/ female association, and diversity assessment of the Agromyzidae specimens collected from the Mitaraka Massif (French Guiana) and previously identified as morphospecies (Boucher and Pollet 2021). We also explored if the Barcode Index Number (BIN) system could provide information other than taxonomic assignment (e.g. distribution range, elevation, host plant, etc) in a region where most of the Agromyzidae fauna is unknown and expected to be undescribed.

2.3 Materials & Methods

Agromyzid specimens were collected in 2015 as part of the Mitaraka expedition, French Guiana (Touroult et al. 2018). The samples were stored in 70% ethanol and subsequently dried using hexamethyldisilazane (HMDS), mounted on cardboard points and identified to morphospecies. A total of 138 specimens representing 10 genera and 50 morphospecies were recorded (Boucher and Pollet 2021). Of these, 54 specimens from 5 genera (*Melanagromyza*, *Ophiomyia*, *Nemorimyza*, *Liriomyza*, *Cerodontha*) representing 33 morphospecies of Agromyzidae were selected for DNA barcoding (Tables 1, 2). The selection included 29 specimens of *Melanagromyza* representing all 15 morphospecies, 17 specimens of *Ophiomyia* representing all 14 morphospecies, two specimens of *Nemorimyza*, representing the two morphospecies, five specimens of *Liriomyza* representing one morphospecies, and one specimen of *Cerodontha*, representing the single *Cerodontha* specimen collected from Mitaraka (Boucher and Pollet 2021). In addition to these Mitaraka specimens, one paratype specimen of *Cerodontha (Diz) nigrihalterata* Boucher (2005) from Costa Rica and housed at the Lyman Entomological Museum was also selected for barcoding for possible comparison with the only *Cerodontha* collected in Mitaraka. The specimens were chosen based on ambiguities and uncertainties that arose during the morphospecies determination (further details below).

DNA amplification and Sanger sequencing were performed at the Centre for Biodiversity Genomics (CBG) (previously known as the Canadian Centre for DNA Barcoding (CCDB)) except for specimens #24, 25, 26, 32-34, 51-54 (Tables 1, 2) that were processed through the LifeScanner barcoding service. Tissue samples for DNA extraction, consisting of one or two leg(s) from each specimen, were sent to these institutions following their submission protocols (CBG: http://ccdb.ca/resources/); LifeScanner: http://lifescanner.net/). Primers C_LepFolF/C_LepFolR (Hernández-Triana et al. 2014) were used for DNA amplification of most specimens except the two specimens of *Liriomyza* (#25-26, Table 1) for which primer set MLepF1/C_LepFolR (Hajibabaei et al. 2006) was used. All COI sequences over 400bp were aligned using the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007) and subsequently uploaded in MEGA X (Kumar et al. 2018), where a neighbor-joining (NJ) tree (Saitou and Nei 1987) was built from a distance matrix computed using the Kimura 2-parameter method (Kimura 1980). The NJ tree provides a graphic representation of genetic distance between sequences from a selected dataset. All sequences retrieved from the Mitaraka specimens were compared to the reference sequence libraries of BOLD (using BOLD identification system) and GenBank (using the Basic Local Alignment Search Tool (BLAST)) for a possible match to a named species. All CO1 sequences were deposited in GenBank with accession number listed in Table 1. Collection data, sequences, and specimen photographs are available on the Barcode of Life Data System (BOLD) (dx.doi.org/10.5883/DS-AGROMIT). Specimens from Mitaraka are presently housed in the Lyman Entomological Museum, Ste-Anne-de-Bellevue, QC (LEMQ) but will eventually be deposited in the Muséum National d'Histoire Naturelle, Paris, France (MNHN).

2.4 Results

Amplification success was low (48%), with COI sequences recovered for only 26 of the 54 submitted specimens (Tables 1-2). Twenty sequences were recovered from *Melanagromyza* specimens, two from *Ophiomyia*, two from *Nemorimyza*, and two short ones of 356 bp from *Liriomyza* (Table 1). None of the COI sequences retrieved from the Mitaraka specimens matched a named species in BOLD or GenBank. In the NJ tree (Fig. 1), the 24 sequences of at least 400 bp representing 17 morphospecies formed 16 distinct clusters with pairwise K2P distances between clusters ranging from 10.7 % to 20.9 %.

Following the reexamination and subsequent reassignment of specimen #7 (Table 1) to *Melanagromyza* sp. Mit-4, the congruence between morphology and clustering patterns of DNA barcodes was very high, with a single instance of two morphospecies (*Melanagromyza* Mit-6 and *M*. Mit-7) being assigned to the same BIN (BOLD:ADW8881). A total of 15 BINs were assigned to the Mitaraka dataset (Fig. 1, Table 1), all of which were newly created except for BOLD:ACJ8134, BOLD:ADB0898 and BOLD:ADW8248 (Table 1). Even if none of these three BINs were associated to named species in BOLD the presence of sequences from specimens from other localities than Mitaraka provided information on the distribution range of *Melanagromyza* Mit-4, *M.* Mit-9, and *Nemorimyza* Mit-2 (Tables 3-5, Figs 19, 20).

Detailed results by genus are presented below.

2.4.1 *Melanagromyza* Hendel

Sequences over 500 bp were successfully recovered for 20 specimens (69%) belonging to 13 morphospecies and distributed into 12 BINs (Table 1); no sequences were recovered for specimens assigned to *Melanagromyza* Mit-5 and *Melanagromyza* Mit-8 (Table 2).

Sequences from one specimen each of *Melanagromyza* Mit-6 and *Melanagromyza* Mit-7 displayed identical barcodes and were therefore assigned to the same BIN (BOLD:ADW8881) (Table 1; Fig. 1). *Melanagromyza* Mit-7 (2 females) was separated morphologically from *M.* Mit-6 (8 females) by the weaker metallic reflection of the abdomen, ocellar triangle more extended and not as well defined, and body paler. While a BIN merge for *M.* Mit-6 and *M.* Mit-7 could indicate that *Melanagromyza* Mit-6 and Mit-7 are conspecific, it could also represent a case of misidentification for one specimen. Unfortunately, *M.* Mit-6 (specimen #10, Table 1) was lost in the process of tissue sampling, thereby precluding any further morphological comparison with specimen *M.* Mit-7 (specimen #11, Table 1), and no sequences were recovered from the other specimens of *M.* Mit-6 (3 females) and *M.* Mit-7 (1 female) submitted for barcoding (Table 2).

Of the six specimens of *Melanagromyza* Mit-2 submitted for barcoding, only one (#27, Table 2) failed to produce a sequence. Four sequences (2 males and 2 females, #2-5, Table 1) clustered together in BOLD:ADR6853 but one (female #7, Table 1) clustered with material of *Melanagromyza* Mit-4 in BOLD:ACJ8134 (Fig. 1). *Melanagromyza* Mit-2 and *M*. Mit-4 are very similar (Figs 2, 3, 6, 7) except for the shorter pubescence on the arista of *Melanagromyza* Mit-2 (Fig. 4). After re-examination, it was found that specimen #7 (Table 1), previously identified as *Melanagromyza* Mit-2, had long pubescence on the arista matching that of specimens assigned to *Melanagromyza* Mit-4 (Fig. 5). The identification of specimen #7 was therefore updated to *Melanagromyza* Mit-4 (Table 1). *Melanagromyza* Mit-2 was the most common of the Mitaraka

Agromyzidae (Boucher and Pollet, 2021), but morphological differences were observed between males and some females, including abdomen colouration (Figs 8-10) and number of mid-tibial bristles (Figs 11-12) which created some uncertainties in gender association. Having sequences from both male and female specimens clustering together in the same BIN (BOLD:ADR6853) with a low sequence divergence, ranging from 0.15 to 0.30% provided additional support for conspecificity.

Another case of uncertainty in morphospecies determination involved two female specimens (#13-14; Table 1) that were identified as *Melanagromyza* Mit-10 (Boucher and Pollet 2021), although they exhibited slight external differences (Figs 13-14) including a paler reddish-brown gena, paler lunule and paler anterior orbit for specimen #14. Identical sequences were retrieved for the two specimens and these were assigned to BOLD:ADW8248 (Fig. 1).

Although agromyzid male genitalia are usually species-specific, providing useful characters for species differentiation, it was not the case for males of *Melanagromyza* Mit-3 and *M.* Mit-4 who exhibited very similar genitalia. They were assigned to separate morphospecies based on a few subtle external characters, including a smaller size for *M.* Mit-4 and in spite of their morphological similarities, material from these morphospecies produced very distinct DNA barcodes with interspecific distances ranging from 11.99% to 12.60%.

When sequences were recovered for more than 1 specimen of a single morphospecies, as seen in *M.* Mit-2, *M.* Mit-4, *M.* Mit-10, and *M.* Mit-12, intraspecific divergences were low, with maximum intraspecific distance (0.37%) recorded in *Melanagromyza* Mit-4 (BIN (BOLD:ACJ8134) (Fig. 1). On the other hand, interspecific distances were high in this genus, ranging from 10.70% between *Melanagromyza* Mit-2 (specimen #4) and *M.* Mit-1 (specimen #1) and 20.90% between *Melanagromyza* Mit-15 (specimen #20) and *Melanagromyza* Mit-6 (specimen #10) (Fig. 1).

Of the 12 BINs assigned to the Mitaraka *Melanagromyza* specimens, most were new, except BOLD:ACJ8134 and BOLD:ADB0898 (Table 1) that were shared with specimens from other projects. BOLD:ACJ8134 included a total of 10 specimens: three specimens from Mitaraka, French Guiana (*Melanagromyza* Mit-4) and seven specimens (two public and five private records) collected in Guanacaste, Costa Rica and Formosa, Argentina (Table 3; Fig. 19). The other shared BIN: BOLD:ADB0898 included the single female specimen of *Melanagromyza* Mit-9 collected at Mitaraka and two specimens (one public record, one private) from Guanacaste, Costa Rica (Table 4; Fig. 20). Surprisingly, *Melanagromyza* Mit-2, the most commonly collected Agromyzidae at Mitaraka (Boucher and Pollet, 2021) was attributed a new BIN (BOLD:ADR6853) (Table 1).

2.4.2 *Ophiomyia* Braschnikov

Amplification success for *Ophiomyia* material was very low, with sequences retrieved from only two of the 17 selected specimens (Tables 1-2). These sequences (both from females), representing *Ophiomyia* Mit-10 and *Ophiomyia* Mit-12 (Table 1) were separated by an interspecific distance of 18.8% (Fig. 1). The short sequence for *Ophiomyia* Mit-12 (#22, Table 1) did not match an existing BIN and did not meet the 500 bp requirement for erecting a new BIN (Ratnasingham and Hebert 2013). *Ophiomyia* Mit-10 (BUIC-DIP1646) was assigned a new BIN (BOLD:ADW4594) (Table 1).

2.4.3 *Nemorimyza* Frey

The five *Nemorimyza* specimens (1 male, 4 females) collected in Mitaraka were originally treated as one morphospecies (*Nemorimyza* Mit-1), until subtle morphological differences were found in two females that were subsequently treated as a distinct morphospecies (*Nemorimyza* Mit-2) (Boucher and Pollet 2021). A sequence over 500 bp was successfully recovered for each of the *Nemorimyza* female specimens representing *Nemorimyza* Mit-1 and *N*. Mit-2 (Table 1). These were assigned to separate BINS, BOLD:ADW8176 and BOLD:ADB9391, and separated by a high interspecific distance of 13.9%. *Nemorimyza* Mit-1 (#23) was assigned a new BIN (BOLD:ADW8176), while *Nemorimyza* Mit-2 (#24) was assigned to BOLD:ADB9391 (Table 1) already containing 5 other BOLD records (1 public) from Guanacaste, Costa Rica (Table 5; Fig. 20).

2.4.4 *Liriomyza* Mik

One of the morphospecies (*Liriomyza* Mit-1) collected at Mitaraka was very similar to *Liriomyza sativae*, a species previously recorded in French Guiana, but was treated as distinct based on small male genitalic differences. Of the five male *L*. Mit-1 specimens selected for barcoding, only #25 and #26 produced short sequences of 356 bp (Table 1). These short identical sequences did not match any existing BINs or reference taxon in Genbank and did not meet the 500 bp requirement for erecting a new BIN (Ratnasingham and Hebert 2013). They also had over 11% genetic distance with reference sequences of *Liriomyza sativae* found in BOLD and GenBank, supporting the assignment of the material to a separate morphospecies.

2.4.5 *Cerodontha* Rondani

One morphospecies (*Cerodontha* Mit-1) (Fig. 17) was very similar to *Cerodontha (Dizygomyza) nigrihalterata* (Fig. 18) a species previously recorded from Costa Rica (Boucher 2005). While a few external characters differentiated *C.* Mit-1 from *C. nigrihalterata*, we could not investigate their genetic differences as no sequences were retrieved for either of the specimens representing these taxa (Table 2).

2.5 Discussion

There are several possible reasons explaining the low amplification success of the sampled specimens such as the fact that they were not freshly collected and had been kept in 70% ethanol before being dried and mounted, instead of 95% ethanol as recommended for DNA preservation (Nagy 2010). However, most of our specimens were very small (< 2.0 mm) and we suspect that the small amount of tissue submitted for DNA extraction (1-2 legs per specimen) may not have been enough.

While DNA barcoding is regularly used as a method of identification for economically important species of Agromyzidae (see introduction), it was not helpful in providing species identification for any of the Mitaraka specimens. This is in part due to the fact that some (if not most) of our material belongs to undescribed taxa. This has been confirmed at least for *Nemorimyza*, where *Nemorimyza* Mit-1 and *N.* Mit-2 do not match any of the five described species (including *N. maculosa*, a species previously reported from French Guiana (EPPO 2021) and with reference sequences available on BOLD from the Nearctic region). Another likely explanation for the absence of a match between our material and reference sequences is the under-representation of identified Neotropical Agromyzidae in BOLD (Fig. 21) and GenBank, making a match unlikely. For example, as of September 2021, there were 540 public records for *Melanagromyza* in BOLD, representing 18 species. More than half (326) of these records (including 319 records from Pakistan) represent *Melanagromyza obtusa* (Malloch), a well-known economically important species recently reported in the Americas, including Colombia (Martinez-Alava et al. 2016). Of the remaining 17 species, only one, *Melanagromyza minimoides* Spencer is from the Neotropical region and none of the barcoded Mitaraka specimens matched that species.

As for *Liriomyza*, most reference sequences in BOLD belong to economically important species and this barcode library is important to facilitate the identification of the most important agromyzid pests. As of September 2021, there were 3411 public records of *Liriomyza* in BOLD representing 49 species. More than half (1803) of these records belong to four agricultural pests: *L. sativae* (677 records); *L. trifolii* (668 records); *L. brassicae* (Riley) (339 records) and *L. huidobrensis* (119 records), all recorded from the Neotropical region. Other than these four species, no other named Neotropical species of *Liriomyza* have been barcoded, except for five specimens of *L. nigra* Spencer (with short sequences of 307 bp) belonging to a private project managed by the first author. The short sequence retrieved for *Liriomyza* Mit-1 did not match those of any *Liriomyza* species found in BOLD. Further investigation will be required to confirm the identity of *Liriomyza* Mit-1. The genus *Liriomyza* is the most diverse agromyzid genus in the Neotropical region with approximately 105 species known. Species level identification is difficult due to the lack of recent keys to the Neotropical species and the fact that some species that have been described based on female specimens only (e.g. *L. mikaniovora* Spencer from Venezuela; *L. pagana* (Malloch) from Argentina and *L. quinquevittata* Sasakawa from Chile).

Although DNA barcoding and the BIN system were not useful to assign names to any of our morphospecies, they did provide information relevant to the taxonomy and diversity of the Mitaraka agromyzid fauna. They allowed us to flag and reassess the identification of some specimens (see results under *Melanagromyza*) and assisted with male/female associations. Due to the importance of male genitalic character for species recognition in agromyzids, females are often left unidentified in taxonomic and faunistic studies (Černý and Bächli 2018; Eiseman and Lonsdale 2018), excluded from type series because of uncertainties in gender association (eg: *Calycomyza addita* Spencer (1983)) or left undescribed or unnamed in the absence of conspecific male (eg: *Liriomyza* sp. B (Boucher and Wheeler 2014); *Japanagromyza* “female 1” (Lonsdale 2013)). Females can be particularly abundant in biodiversity surveys, especially when Malaise traps are used (Scheirs et al. 1997). This was the case for the Mitaraka survey where 95 females and 43 males were collected (Boucher and Pollet 2021). In the present work, DNA barcoding supported the male/ female conspecificity of specimens assigned to three *Melanagromyza* morphospecies (*M*. Mit-2, *M*. Mit-4, *M*. Mit-12). Furthermore, the high sequence divergence measured between branches or clusters of barcoded morphospecies (Fig. 1) supported almost all the morphospecies assignments even when these were erected only based on female material. The sequencing of additional material will be needed to further investigate the grouping of *Melanagromyza* Mit-6 and *M.* Mit-7 in the same BIN (BOLD:ADW8881) due to the accidental destruction of the only specimen of Mit-6 with a DNA barcode.

Very little data was available on the agromyzid fauna of French Guiana before the 2015 Mitaraka survey. The high congruence between DNA barcodes/ BIN assignments and morphology presented here suggests that DNA barcoding is an effective approach to estimate the Agromyzidae species diversity of Mitaraka and beyond, especially when females are abundant in samples. Additional studies will be necessary to further evaluate the robustness of the approach since it is widely recognized that levels of congruence between species limits and DNA barcodes/ BINS vary according to the study group. While causes such as hybridization and incomplete lineage sorting (Funk and Omland 2003) are most commonly evoked, simple errors in morphology-based identification can also account for mismatches, especially in the case of morphologically challenging taxa such as agromyzid flies. An approach combining multiple data sources such as morphology, DNA sequences, and life history traits such host plants should therefore be favored whenever possible.

The genus *Melanagromyza* was the most diverse at Mitaraka with 15 morphospecies (Boucher and Pollet 2021). This diversity resulting from a short survey in a single locality of French Guiana was surprisingly high when compared to known diversity of *Melanagromyza* in different Neotropical countries such as Brazil (19 species), Venezuela (20 species) or Colombia (14 species). The diversity of *Melanagromyza* from the Mitaraka survey could even be greater considering that 70% of the identified specimens were not sequenced and could include cryptic species that failed to be differentiated morphologically. We therefore suspect that much is left to be discovered about the agromyzid fauna of French Guiana and the Neotropical region in general.

We also found that the Barcode Index Number (BIN) system, along with the metadata associated with each barcoded specimen in BOLD, provided important insight into the distribution pattern, habitats, and elevation preference of some species (Tables 3-5), in addition to allowing researchers to locate material easily for revisionary taxonomic studies.

Considering the difficulty associated with species-level identification of Neotropical Agromyzidae and the risks associated with the postal transport of type material, a reference library of DNA barcodes for named species of Neotropical Agromyzidae (including sequences from type material whenever possible) would not only help with identification but also reduce taxonomic errors that may lead to long lists of synonyms such as seen for several species of economic importance such as *L. sativae* and *L. brassicae*.

This study has contributed a total of 23 new barcode-compliant CO1 sequences (above 500 bp), of Neotropical Agromyzidae, distributed into 15 BINs (including 12 unique BINs). Although these sequences lack species-level determination, they set a stronger base for future taxonomic work and facilitate the discovery of conspecific supplementary material for morphological studies.

2.6 Acknowledgments

We would like to thank the lifeScanner team at the Centre for Biodiversity Genomics, in particular Megan Milton, Michelle Pyle and Sujeevan Ratnasingham who provided helpful information and support for the barcoding results obtained through their barcoding service. We would also like to thank Valerie Levesque-Beaudin at the Centre for Biodiversity Genomics for her great assistance with BOLD, and Andréanne Lessard for the lab assistance at Bishop's University. Financial support for DNA barcoding was provided by Bishop's University to J. Savage. All the material for this study was collected during the "*Our Planet Reviewed*" Guyane-2015 expedition in the Mitaraka range, in the core area of the French Guiana Amazonian Park, organized by the MNHN and Pro-Natura international. The expedition was funded by the European Regional Development Fund (ERDF), the Conseil régional de Guyane, the Conseil général de Guyane, the Direction de l'Environnement, de l'Aménagement et du Logement and by the Ministère de l'Éducation nationale, de l'Enseignement supérieur et de la Recherche. It was realized in collaboration with the Parc amazonien de Guyane and the Société Entomologique Antilles-Guyane (SEAG). We would also like to thank Marc Pollet for the invitation to SB for studying the Agromyzidae collecting during this project and for sending the specimens. Marc participated to this expedition as member of the first team (22 February-11 March 2015), hereby supported financially by MNHN and Pro-Natura international. We would also like to thank Pierre-Henri Dalens and his team for collecting the major part of the agromyzid material during the August mission.

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2.8 Figures and Tables

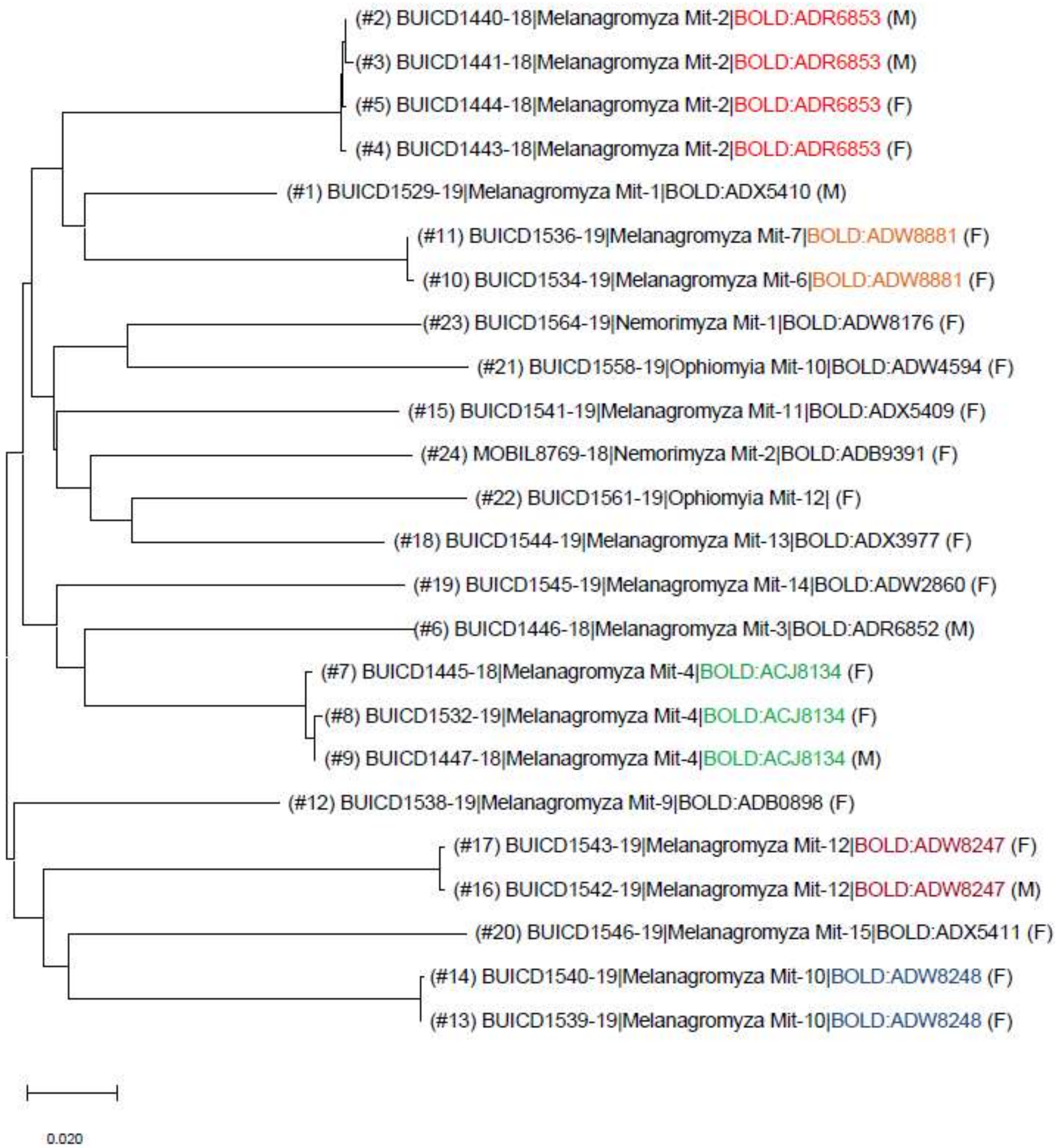
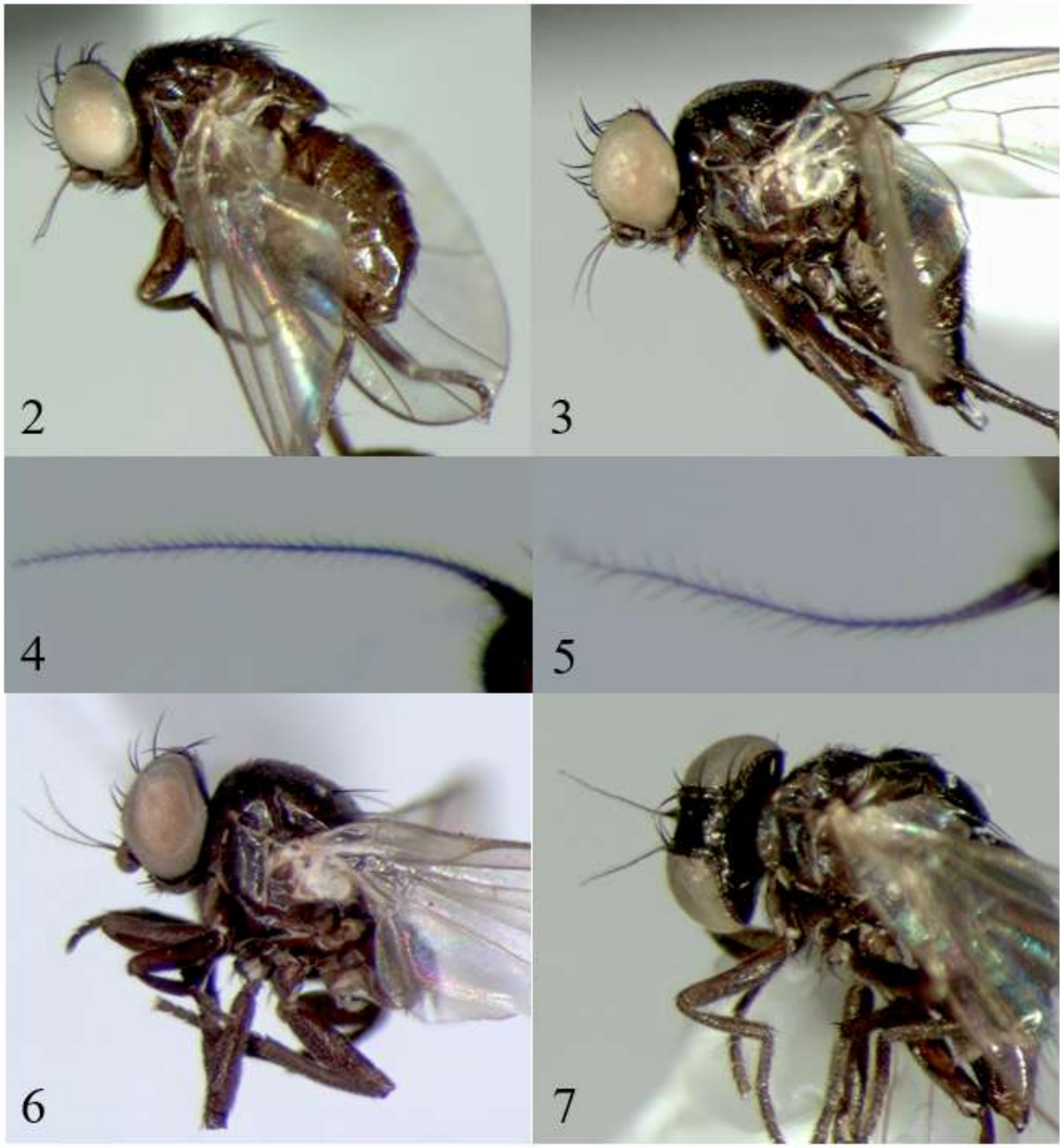
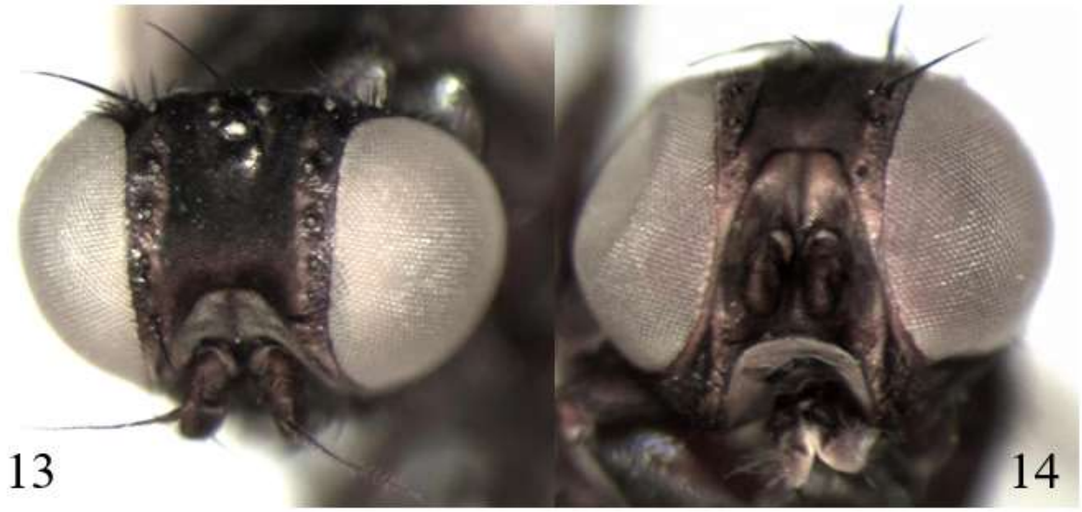


Figure 1. Neighbor-joining tree based on K2P-distance of the 24 specimens of Mitaraka Agromyzidae for which a sequence over 400 bp were retrieved. Information includes specimen number (from Table 1), BOLD process ID, morphospecies name, BIN number and sex. Colour text is used when more than one Mitaraka specimen were clustering together in the same BIN.



Figures 2-4. *Melanagromyza* Mit-2. (2). Specimen BUICD1441-18, lateral view; (3). Specimen BUICD1444-18, lateral view; (4). Arista showing short pubescence. **Figures 5-7**. *Melanagromyza* Mit-4. (5). Arista showing long pubescence; (6). Specimen BUIC1447-18, lateral view; (7) Specimen BUIC1445-18, lateral view.

Figures 8-10. Abdomen (colour variation) of *Melanagromyza* Mit-2. (8). Specimen BUICD1440-18; (9) Specimen BUICD1443-18; (10) Specimen BUICD1441-18. **Figures 11-12**. Midtibial bristles (number variation) of *Melanagromyza* Mit-2. (11) Specimen BUICD1441-18; (12) Specimen BUICD1444-18.



Figures 13-14. *Melanagromyza* Mit-10. (13) Specimen BUICD1539-19, head dorsal view; (14) Specimen BUICD1540-19, head antero-dorsal view.

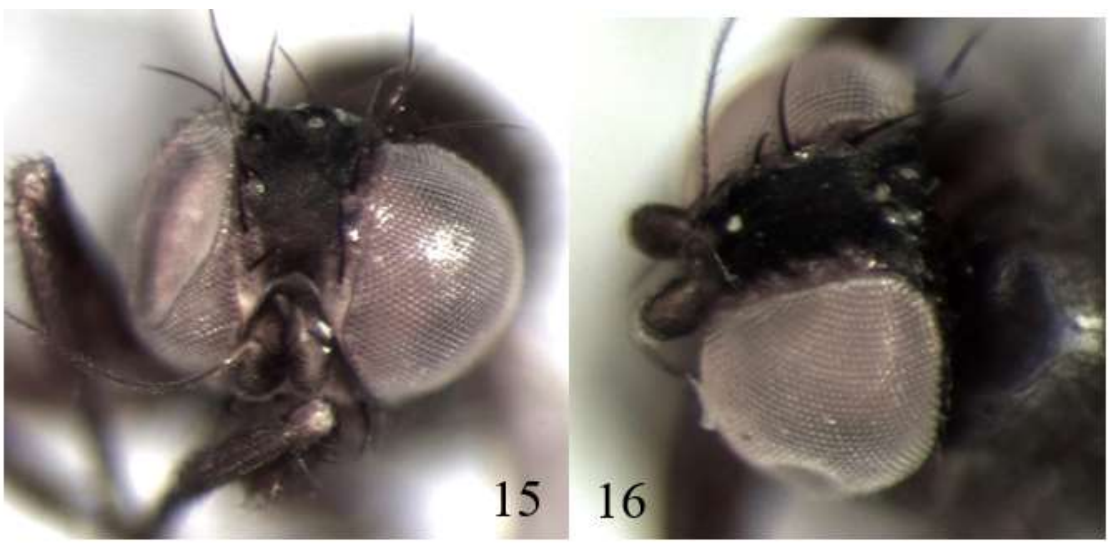


Figure 15. *Nemorimyza* Mit-1 BUICD1564-19, head dorsal view; **Figure 16**. *Nemorimyza* Mit-2 MOBIL8769-18, head latero-dorsal view.

Figure 17. *Cerodontha* Mit-1, lateral view; **Figure 18**. *Cerodontha nigrihalterara* Boucher , paratype, lateral view.

Figure 19. Distribution map for BOLD records for BIN:BOLD:ACJ8134 (*Melanagromyza* Mit-4). Distribution data points include: Guanacaste, Costa Rica; Formosa, Argentina and Mitaraka, French Guiana. (Created with SimpleMappr).

Figure 20. Distribution map for BOLD records for BIN:BOLD:ADB0898 (*Melanagromyza* Mit-9) and BIN (BOLD:ADB9391) (*Nemorimyza* Mit-2). Distribution data points include: Guanacaste, Costa Rica and Mitaraka, French Guiana. (Created with SimpleMappr).

Figure 21. Map of Agromyzidae species occurrence on BOLD. Map generated by BOLD (September 2021).

Figure 22. Map of *Melanagromyza* species occurrence on BOLD. Map generated by BOLD (September 2021)

Tables

Table 1. List of Mitaraka specimens sent for barcoding and for which a sequence was retrieved. Includes specimen number for in-text reference, morphospecies name (from Boucher and Pollet 2021), BOLD process ID, BIN assignment, sex, CO1 sequence length and GenBank accession number. Colour text is used when more than one Mitaraka specimen were clustering together in the same BIN (matching colour is used in Fig. 1 for easy reference).

Specimen #	Morphospecies	BOLD process ID	BIN assignment (*added for new BIN)	Sex	CO1 Sequence length	GenBank number
1	Melanagromyza Mit-1	BUICD1529-19	BOLD:ADX5410*	M	613	OK623732
2	Melanagromyza Mit-2	BUICD1440-18	BOLD:ADR6853*	M	658	OK623717
3	Melanagromyza Mit-2	BUICD1441-18	BOLD:ADR6853*	M	658	OK623728
4	Melanagromyza Mit-2	BUICD1443-18	BOLD:ADR6853*	F	631	OK623740

5	Melanagromyza Mit-2	BUICD1444-18	BOLD:ADR6853*	F	658	OK623741
6	Melanagromyza Mit-3	BUICD1446-18	BOLD:ADR6852*	M	658	OK623742
7	Melanagromyza Mit-4 (Previously identified as M. Mit-2)	BUICD1445-18	BOLD:ACJ8134	F	658	OK623727
8	Melanagromyza Mit-4	BUICD1532-19	BOLD:ACJ8134	F	549	OK623722
9	Melanagromyza Mit-4	BUICD1447-18	BOLD:ACJ8134	M	658	OK623729
10	Melanagromyza Mit-6	BUICD1534-19	BOLD:ADW8881*	F	602	OK623723
11	Melanagromyza Mit-7	BUICD1536-19	BOLD:ADW8881*	F	658	OK623726
12	Melanagromyza Mit-9	BUICD1538-19	BOLD:ADB0898	F	658	OK623739
13	Melanagromyza Mit-10	BUICD1539-19	BOLD:ADW8248*	F	571	OK623721
14	Melanagromyza Mit-10	BUICD1540-19	BOLD:ADW8248*	F	596	OK623733
15	Melanagromyza Mit-11	BUICD1541-19	BOLD:ADX5409*	F	555	OK623738
16	Melanagromyza Mit-12	BUICD1542-19	BOLD:ADW8247*	M	570	OK623737
17	Melanagromyza Mit-12	BUICD1543-19	BOLD:ADW8247*	F	570	OK623735
18	Melanagromyza Mit-13	BUICD1544-19	BOLD:ADX3977*	F	658	OK623724
19	Melanagromyza Mit-14	BUICD1545-19	BOLD:ADW2860*	F	658	OK623734
20	Melanagromyza Mit-15	BUICD1546-19	BOLD:ADX5411*	F	590	OK623736

21	Ophiomyia Mit-10	BUICD1558-19	BOLD:ADW4594*	F	564	OK623718
22	Ophiomyia Mit-12	BUICD1561-19	Not assigned	F	417	OK623725
23	Nemorimyza Mit-1	BUICD1564-19	BOLD:ADW8176*	F	590	OK623720
24	Nemorimyza Mit-2	MOBIL8769-18	BOLD:ADB9391	F	600	OK623730
25	Liriomyza Mit-1	MOBIL11198-20	Not assigned	F	356	OK623731
26	Liriomyza Mit-1	MOBIL11196-20	Not assigned	F	356	OK623719

Table 2. Specimens sent for barcoding for which no sequence was retrieved. Includes specimen number for in-text reference, morphospecies name (from Boucher and Pollet 2021), BOLD process ID and sex.

Specimen #	Morphospecies	BOLD Process ID	Sex
27	Melanagromyza Mit-2	BUICD1442-18	F
28	Melanagromyza Mit-4	BUICD1530-19	F
29	Melanagromyza Mit-4	BUICD1531-19	F
30	Melanagromyza Mit-5	BUICD1533-19	M
31	Melanagromyza Mit-6	BUICD1535-19	F
32	Melanagromyza Mit-6	Lifescanner Vial ID: BOLD AT1	F
33	Melanagromyza Mit-6	Lifescanner Vial ID: BOLD DM0	F
34	Melanagromyza Mit-7	Lifescanner Vial ID: BOLD 8E4	F
35	Melanagromyza Mit-8	BUICD1537-19	F
36	Ophiomyia Mit-1	BUICD1547-19	M
37	Ophiomyia Mit-1	BUICD1548-19	M
38	Ophiomyia Mit-2	BUICD1549-19	M

39	Ophiomyia Mit-3	BUICD1550-19	M
40	Ophiomyia Mit-3	BUICD1551-19	F
41	Ophiomyia Mit-4	BUICD1552-19	M
42	Ophiomyia Mit-5	BUICD1553-19	M
43	Ophiomyia Mit 6	BUICD1554-19	F
44	Ophiomyia Mit-7	BUICD1555-19	M
45	Ophiomyia Mit-8	BUICD1556-19	F
46	Ophiomyia Mit-9	BUICD1557-19	F
47	Ophiomyia Mit-11	BUICD1559-19	M
48	Ophiomyia Mit-12	BUICD1560-19	F
49	Ophiomyia Mit-13	BUICD1562-19	F
50	Ophiomyia Mit-14	BUICD1563-19	M
51	Liriomyza Mit-1	BUICD1449-18	M
52	Liriomyza Mit-1	BUICD1448-18	M
53	Liriomyza Mit-1	Lifescanner Vial ID: BOLD 5K8	M
54	Cerodontha Mit-1	Lifescanner Vial ID BOLD NO6	M
	Cerodontha nigrihalterata	Lifescanner Vial ID BOLD 1N9	F

Table 3. Specimen records (public) included in BIN(BOLD:ACJ8134) with associated specimen data.

BOLD identification	**BOLD Process ID**	**Sex**	**CO1 Sequence length**	**Locality/Coordinate/ Elevation**	**Habitat/collecting technique/sampling date**
Melanagromyza Mit-4	BUICD1445-18	F	658	Mitaraka, French Guiana, 2.233, -54.463, 471m	Minor inselberg with savane-roche vegetation /6 m Malaise trap/August 2015

Melanagromyza Mit-4	BUICD1532-19	F	549	Mitaraka, French Guiana 2.233, -54.463 471m	Minor inselberg with savane-roche vegetation /6 m Malaise trap/August 2015
Melanagromyza Mit-4	BUICD1447-18	M	658	Mitaraka, French Guiana 2.233, -54.463 471m	Minor inselberg with savane-roche vegetation /6 m Malaise trap/August 2015
Agromyzidae	GMAFN352-15	?	633	Reserva El Bagual. Formosa, Argentina -26.3028, -58.815 57m	Unknown/Malaise trap/November 2013
Agromyzidae	GMCRM972-13	F	658	Area de Conservacion Guanacaste. Guanacaste, Costa Rica 10.8438, -85.6138 300m	Forest/Malaise trap/May 2012

Table 4. Specimen records (public) included in BIN (BOLD:ADB0898) with associated specimen data.

BOLD identification	**BOLD Process ID**	**Sex**	**CO1 Sequence length**	**Locality- Coordinate-Elevation**	**Habitat/Collecting technique/Sampling date**
Melanagromyza Mit-9	BUICD1538-19	F	658	Mitaraka, French Guiana 2.233, -54.463 471m	Minor inselberg with savane-roche vegetation /6 m Malaise trap/August 2015
Agromyzidae	**JICAZ278-16**	F	543	Area de Conservacion Guanacaste. Guanacaste, Costa Rica 10.764, -85.335 828m	Subtropical/tropical moist lowland forest/Malaise trap/March 2014

Table 5. Specimen records (public) included in BIN (BOLD:ADB9391) with associated specimen data.

BOLD identification	BOLD Process ID	Sex	CO1 Sequence length	Locality/Coordinate/ Elevation	Habitat/Collecting technique/Sampling date
Nemorimyza Mit-2	MOBIL8769-18	F	600	Mitaraka, French Guiana/ 2.233, -54.463/ 471m	Minor inselberg with savane-roche vegetation /6 m Malaise trap/August 2015
Agromyzidae	JCCCY4402-16	F	576	Area de Conservacion Guanacaste. Guanacaste, Costa Rica 10.763, -85.334 820m	Subtropical/tropical moist lowland forest/ Malaise trap/ November 2014

CONNECTING STATEMENT

In Chapter 2, DNA barcoding offered good support for the morphology-based species delination, but unfortunately it was not helpful in providing species identification to any of the Mitaraka morphospecies. One of the reasons for this is the absence of a decent reference barcode library for Neotropical Agromyzidae and likely the presence of many undescribed species. A next critical step is to determine if some of the morphospecies belong to previously named species and to recognize which species are new to science and to describe them. This is fundamental work for all future biological and ecological studies. The next chapter provides the systematic and taxonomic treatment of the Mitaraka specimens of Agromyzidae in the genera *Agromyza*, *Calycomyza*, *Cerodontha*, *Liriomyza*, *Nemorimyza*, *Phytobia*.

Chapter 3 is formatted for submission to Zoosystema

CHAPTER 3. NEW SPECIES AND NEW RECORDS OF LEAF-MINER FLIES (DIPTERA: AGROMYZIDAE) FROM RAINFOREST AND INSELBERG AT MITARAKA (FRENCH GUIANA)

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3.1 Abstract

The Agromyzidae fauna of French Guiana has never been previously studied and only four well known and economically important species had been reported from the region. A recent expedition at the Mitaraka massif, a largely unexplored region of French Guiana, has resulted in an unexpected diversity of Agromyzidae. This study provides preliminary results of the taxonomic and systematic treatment of these agromyzids with the description of eight new species: *Agromyza mitarakensis* n.sp., *Calycomyza inselbergensis* n.sp., *Cerodontha pseudonigrihalterata* n.sp., *Liriomyza touroulti* n.sp., *Nemorimyza thanatos* n.sp., *Phytobia dalensi* n.sp., *Phytobia pluviasilvae* n.sp., and *Phytobia corona* n.sp. and the first report in French Guiana of two previously known Neotropical species: *Calycomyza grenadensis* Zlobin and *Phytoliriomyza jurgensi* Spencer. Diagnoses and systematic notes are provided for each species in addition to photographs of habitus and male genitalia. The agromyzid fauna of French Guiana now includes 14 described species.

Key words: Agromyzidae, Diptera, Neotropical, French Guiana, Guyane, new species, new records

3.2 Résumé

La faune Agromyzidae de Guyane française n'a jamais été étudiée auparavant et seules quatre espèces bien connues et économiquement importantes ont été signalées dans la région. Une expédition récente dans le massif du Mitaraka, une région largement inexplorée de la Guyane française, a abouti à une diversité inattendue d'Agromyzidae. Cette étude fournit des résultats préliminaires du traitement taxonomique et systématique de ces Agromyzidae avec la description de huit nouvelles espèces : *Agromyza mitarakensis* n.sp., *Calycomyza inselbergensis* n.sp., *Cerodontha pseudonigrihalterata* n.sp., *Liriomyza touroulti* n.sp., *Nemorimyza thanatos* n.sp., *Phytobia dalensi* n.sp., *Phytobia pluviasilvae* n.sp. et *Phytobia corona* n.sp. et le premier signalement en Guyane française de deux espèces néotropicales précédemment connues : *Calycomyza grenadensis* Zlobin et *Phytoliriomyza jurgensi* Spencer. Des diagnostics et des notes systématiques sont fournis pour chaque espèce ainsi que des photographies des habitus et des organes génitaux masculins. La faune des Agromyzidae de Guyane française compte aujourd'hui 14 espèces décrites.

Mots clés: Agromyzidae, Diptères, Néotropical, Guyane, nouvelle espèce, nouveau signalement

3.3 Introduction

Describing the world's biodiversity remains a critical priority, especially in the tropics where we know only a fraction of the diversity. Larger organisms like mammals and birds are relatively well known but smaller organisms like insects make up the bulk of the unknown diversity (Stork 2018). The expedition to the Mitaraka massif French Guiana (Touroult et al. 2018), was part of a large-scale biodiversity program known as "Our planet Reviewed" ("La planète revisitée") led by the National Museum of Natural History (France) and Pro-Natura International, which has a primary objective of accelerating the discovery and description of new species (Muséum national d'Histoire naturelle, 2016; Touroult et al. 2018). This expedition has already resulted in the description of 20 new Diptera species from various families (Touroult et al. 2021): Chloropidae (1 species) (Riccardi 2020); Dolichopodidae (7 species) (Runyon & Pollet 2018; Brooks et al. 2018); Keroplatidae (3 species) (Blagoderov & Pollet 2020); Muscidae (1 species) (Gomes & Varvalho 2018); Pipunculidae (1 species) (Marques et al. 2019); Psychodidae (2 species) (Curler 2020); Sciomyzidae (1 species) (Mortelmans & Pollet, 2018); Sepsidae (1 species) (Silva & Pollet 2020) and Tipulidae (3 species) (Mederos & Pollet 2019).

The Agromyzidae is a family of phytophagous flies found throughout the world with nearly 3200 species described (von Tschirnhaus 2021), including approximately 500 species in the Neotropical region. Prior to this expedition, very little was known about the agromyzid fauna of French Guiana with only four economically important species previously reported, namely *Liriomyza huidobrensis* (Blanchard), *Liriomyza trifolii* (Burgess), *Liromyza sativae* Blanchard and *Nemorimyza maculosa* (Malloch) (EPPO 2022; Martinez & Étienne 2002). The brief expedition of the Mitaraka massif resulted in a surprisingly diverse fauna consisting of 50 additional morphospecies belonging to 10 genera (Boucher & Pollet 2021).

The Mitaraka massif is a particularly interesting site for Agromyzidae. It is situated in an undisturbed rainforest environment in southwestern French Guiana where no agromyzids have ever been collected. It is part of the protected Guiana Amazonian Park, which is adjacent to the Amazon rainforest of the Tumucumaque National Park of Brazil, also with an unknown agromyzid fauna. This whole area, combined with Suriname to the west (also completely unexplored), represents a huge gap in our knowledge of Neotropical Agromyzidae. The environment of Mitaraka consists of lowland tropical rainforest with scattered rocky outcrops, known as inselbergs ("Island mountains" in German) (Touroult et al. 2018). Inselbergs have a characteristic patchy vegetation separated by bare rock, called "savane-roche" ("rock savanna") in French Guiana (Sarthou et al. 2003). The inselbergs offer an exposed xeric environment with extreme climatic variation to its inhabitants (Szarzynski 2000), which contrasts with the humid environment of the surrounding rainforests of Mitaraka. Due to their isolated nature, inselbergs are known for their unique flora and fauna (Mares & Seine 2000; Porembski 2007). The agromyzid fauna of inselbergs (of French Guiana or elsewhere in the world) is completely unknown. Interestingly, most of the Agromyzidae at the Mitaraka massif were collected from a minor inselberg (471 m) and a few species were collected in the lowland rainforest (Boucher & Pollet 2021).

The main objective of this study is to provide a systematic treatment of the Agromyzidae (all genera except *Melanagromyza* and *Ophiomyia* which will be treated separately) collected during the Mitaraka expedition, to describe the new species in the genera *Agromyza*, *Calycomyza*, *Cerodontha*, *Liriomyza*, *Nemorimyza* and *Phytobia*, and to record new distributional data for two previously known Neotropical species.

3.4 Materials & Methods

Specimens examined were collected in French Guiana as part of the Mitaraka expedition and were identified as morphospecies prior to this study (Boucher & Pollet 2021). Examination of male genitalia were made by removing the abdomen of specimens and clearing them in 85% lactic acid heated in a microwave oven for 2-3 intervals of 30 seconds each, separated by a cooling period, or cleared outside the microwave (later in the project) following the procedures outlined in Boucher (2019). Each abdomen was subsequently transferred to glycerin solution for dissection and identification and finally stored in a microvial pinned below the corresponding specimen. Morphological terminology follows Cumming & Wood (2009). Abbreviations are as follows: dc: dorsocentral setae (numbered starting posteriorly); ors: orbital setae; ori: frontal setae. Distribution of species is based on published data and on specimens examined. Asterisks in distribution lists indicate new locality records. Digital images of specimens were taken with a luminera infinity camera 1 mounted on a stereo microscope Leica MZ 12.5 or a compound microscope Leica DMLB. Image stacking were performed using Combine ZP software.

A few Mitaraka specimens used in the study were previously barcoded for a complimentary DNA barcoding project (Boucher & Savage 2022), with GenBank accession numbers provided under appropriate species.

The Mitaraka Agromyzidae, including the type specimens will be deposited at the Muséum National d’Histoire Naturelle, Paris, France (MNHN).

3.5 Systematic treatment (alphabetically).

3.5.1 Genus *Agromyza* Fallén

This genus is easily recognized by the presence of a stridulatory organ, in the form of a band of chitinized scales present on the side of the abdomen, along the margin of the fused tergites 1 and 2. The adults are usually completely black with white or yellow halter (except the Neotropical species *A. fusca* Spencer with black halter and *A. insolens* Spencer with brownish halter), they normally have three or more pairs of dorsocentral setae (dc) (a few species with only two well developed dc and a third small one. *A. megaepistoma* Sasakawa from Ecuador has only 2 dc with rarely a third small one) and well developed prescutellar acrostichal setae. A single specimen of *Agromyza* was collected from the Mitaraka massif (Boucher and Pollet 2021). This specimen is a male and represents a new species of *Agromyza* described below. This is the first species of the genus *Agromyza* recorded from French Guiana~~.~~

Agromyza mitarakensis n.sp.

(Figs. 1-5)

Type material. Holotype ♂. Guyane Française: Mitaraka, Sampling site: MIT-E-savane roche 2. 02°13'59.8"N, 54°27'46.5"W, 471 m. Open / partially opened areas. 13.viii.2015 - 20.viii.2015, MT(6m). leg. Pierre-Henri Dalens. MITARAKA/230 (sorted by M. Pollet) (MNHN).

Etymology. The specific name is referring to the type locality.

Diagnosis. This species can be distinguished from other Neotropical species of *Agromyza* by the strongly angulate gena, calypter greyish with brown fringe, halter dirty yellow, small epistoma, antennae yellowish, acrostichal setulae long, 2 well developed dc, and arista with normal pubescence.

Description. Frons not projecting in front of eye; 2 reclinate ors and 2 slightly weaker inclinate ori; orbital setulae sparse, short and reclinate, in one row; first flagellomere small with short white pubescence; arista long with distinct short pubescence; gena angulate, deeper at rear (Fig. 1), at midpoint about 0.11 times maximum eye height; clypeus with upper margin rounded (Fig. 2); small epistoma present (Fig. 2); only two well developed dc located posterior to supra-alar and a small anterior one; prescutellar acrostichal seta well developed; acrostichal setulae long and numerous, in about 8-9 rows; midtibia with 2 lateral setae; wing length in male: approximately 1.95 mm (wing bent); last section of CuA_1 0.6 times length of penultimate.

Colour. Frons and orbital plate pale brown, upper frons blackish at level of ocelli; scape and pedicel pale brown, first flagellomere yellowish; face and palpus brown; gena brown with yellowish spot at rear; clypeus brown, subshining; mesonotum and scutellum shiny brown; side of thorax (all pleura) brown; legs brown; calypter and fringe brown; halter yellow; abdomen yellowish-brown.

Male genitalia. Phallus (Figs. 4, 5) symmetrical, with distiphallus short and broad; hypandrium pointed at apex; ejaculatory apodeme with narrow blade (Fig. 3).

Distribution. French Guiana*.

Host. Unknown.

Comments. This new species has a characteristic ejaculatory apodeme with a narrow blade as seen for *A. simillima* Spencer, known from Brazil (Spencer 1963). The two species also have in common the presence of only two well developed dc. The phallus of *A. simillima* has never been illustrated. Pictures of the phallus (on permanent mount) and habitus of the holotype specimen of *A. simillima* (Figs. 6-8) were provided to the author by the Natural History Museum, London (NHM), and these confirm that the two species are distinct. The distiphallus of *A. simillima* is much narrower and elongated (Fig. 6). Furthermore, the arista of *A. simillima* is described as conspicuously plumose (Spencer 1963), which is not the case for *A. mitarakensis*. The head of the holotype of A. *simillima* is glued to a cardboard point separated from the rest of the specimen, but the aristae are missing (Fig. 8). The phallus of *A. mitarakensis* suggests close relationship to *A. animata* Spencer, known from Costa Rica (Spencer 1973a), but this species has 3 well developed dc and darker antennae. The ejaculatory apodeme of *A. animata* has not been described or illustrated.

3.5.2 Genus *Calycomyza* Hendel

Most species of *Calycomyza* are characterized by having a yellow frons; dark antennae, a yellow notopleuron, dark scutellum, pale yellowish halter and the presence of only 2 postsutural dc, although there are many exceptions. For example, *C. devia* Spencer from Venezuela and Costa Rica has a brownish frons and darker notopleural area; *Calycomyza parilis* Spencer and *C. palmaris* Spencer, both from Venezuela have brown halter and 3+1 dc. The male genitalia, with the epandrium and surstyli having a patch of short strong spines is characteristic. Seven specimens of *Calycomyza* were collected from the Mitaraka massif representing four species (Boucher & Pollet 2021). Two of them were represented by females only (Boucher & Pollet

2021) and their identity could not be confirmed. These two species are characterized by the presence of 4 dorsocentral setae (two weak anterior ones), a character rarely observed in *Calycomyza*, but present in a few Neotropical species (e.g. *C. compositana* Spencer; *C. illustris* Spencer; *C. steviae* Spencer). Of the other two species, one represents *C. grenadensis* Zlobin (morphospecies *Calycomyza* Mit-1 of Boucher & Pollet 2021), which was previously known from Grenada (Zlobin 1996) with further discussion below, and the other (morphospecies *Calycomyza* Mit 2 of Boucher & Pollet 2021) represents a new species, described below. These are the first *Calycomyza* species reported from French Guiana.

Calycomyza grenadensis Zlobin, 1996

(Figs. 9-12)

Calycomyza grenadensis Zlobin, 1996: 153; Martinez & Étienne 2002: 29.

Material examined: Guyane: Mitaraka, Sampling site: MIT-E-savane roche 2. 02°13'59.8''N, 54°27'46.5''W, 471 m. Open / partially opened areas. 13- 20.viii.2015, MT(6m). leg. Pierre-Henri Dalens. MITARAKA/230 (sorted by M. Pollet) (1 ♂, 1 ♀: MNHN).

Diagnosis: A distinctive characteristic of this species is the long strongly chitinized projection of the hind ventral lobe of the phallus (Zlobin 1996), prominent in the Mitaraka specimen (Fig. 11). The shape of the ejaculatory apodeme is also characteristic with the blade only 2.3 x larger (at its widest point) than the stalk at midpoint (Fig. 10).

Distribution: Grenada, French Guiana*.

Host: Unknown.

Comments: There are slight differences between the Mitaraka specimens and the original description of the species. They include the orbital plate darkened to upper ors only, continuing faintly on lateral margin to lower ors in male (Fig. 9) (darkened to lower ors in original description) and face darkened at base of antennae (face yellow in original description). Other characteristics worth mentioning, but not part of the original description, include the presence of 3 postsutural dorsocentral setae: 2 well developed dorsocentrals with a much shorter anterior one and mid tibia with three short lateral setae. This species was previously known only from the type material from Grenada. This species was identified as morphospecies *Calycomyza* Mit-1 in Boucher & Pollet (2021).

Calycomyza inselbergensis n.sp.

(Figs. 13-19)

Type material. Holotype ♂. Guyane: Mitaraka, Sampling site: MIT-E-savane roche 2. 02°13'59.8"N, 54°27'46.5"W, 471 m. Open / partially opened areas. 13- 20.viii.2015, MT(6m). leg. Pierre-Henri Dalens. MITARAKA/230 (sorted by M. Pollet) (MNHN).

Etymology: The specific name is referring to the habitat (inselberg) of the type specimen.

Diagnosis: This species can be differentiated from other Neotropical species of *Calycomyza* by the following features: halter and notopleuron pale brown; calypter and fringe brown; orbital plate brown to lower ors; postpronotum and anepisternum almost completely brown; male genitalia with a very large ring-like sclerite at the base of distiphallus.

Description. Frons approximately as wide as eye in dorsal view; frons not projecting in front of eye; 2 reclinate ors and 2 weaker inclinate ori; orbital setulae sparse, short and reclinate, in one row; first flagellomere small, rounded apically with short white pubescence; arista long with distinct pubescence (Fig. 13); gena at midpoint about 0.1 x eye height; clypeus narrow with anterior margin rounded (Fig. 14); small triangular epistoma present (Fig. 14); two well developed postsutural dc distant from each other, and one weaker third postsutural dc (on right side only); prescutellar seta absent; acrostichal setulae in about 7-8 rows; mid tibia with 2 approximate lateral setae; wing length in male: approximately 1.7 mm (wing bent) with last section of CuA_1 1.6 x penultimate section.

Colour. Frons yellow; upper orbital plate brown to lower ors (Fig. 15); antenna brown; face apparently partly pale brown (partly hidden by the antennae); palpus brown; clypeus dull brown, paler brown centrally; mesonotum and scutellum shiny brown; postpronotum brown except for narrowly yellow hind corner; notopleuron brownish (Fig. 13), slightly paler, yellowish ventrally; anepisternum, katepisternum and anepimeron brown; legs brown except for narrowly yellow fore knee; calypter and fringe brown; halter slightly darkened, pale brown (Fig. 13).

Male genitalia. Distiphallus in the shape of 2 long tubules with apical section bent to almost 90° and basal section surrounded by a large ring-like sclerite that extends much beyond the width of the tubules in lateral and ventral view (Figs. 16, 17); length of distal tubules anterior to the ring-like sclerite 0.1 mm (Fig. 16, a) equal to the length of mesophallus (Fig. 16, b); epandrium with numerous short internal spines (Fig. 18); surstyli with short spines and a few (4-5) long setae on upper margin (Fig. 18); hypandrium narrow and slightly constricted near midpoint; ejaculatory apodeme large with wide blade (Fig. 19).

Distribution. French Guiana*.

Host. Unknown. Possibly Verbenaceae (see comments).

Comments. The male genitalia of this new species is most similar to *C. verbenivora* Spencer, which feeds on various plants in the family Verbenaceae. The type specimen from Venezuela, illustrated in Spencer 1963, apparently has the lower tubule broken off (see comment in Spencer 1973b: 49), but the upper tubule is complete, and it can be observed that it is distinctly longer than the mesophallus, which is not the case in *C. inselbergensis*. Another difference is the ring-like sclerite at the base of the distiphallus which is distinctly wider in *C. inselbergensis*. Other illustrations of *C. verbenivora* from Argentina (Valladares 1981) and Peru (Korytkowski 2014) match more closely the phallus of the new species (except for the size of the ring-like sclerite), but *C. verbenivora* features significant external differences that are diagnostic: calypter yellow with fringe partially to completely yellow; arista virtually bare; orbital plate bright yellow; notopleuron, posterior half of postpronotum and upper posterior corner of anepisternum bright yellow. Externally *C. inselbergensis* is most similar to *C. verbenae* (Hering), a species known from southern United-States and Brazil (Spencer 1963, Esposito 1994), but the male genitalia of the two species are distinct. *Calycomyza verbenae* is separated externally from *C. verbenivora* by the darker, distinctly black calypteral fringe and black face (Spencer 1963). Like *C. verbenivora*, this species feeds on *Verbena spp*. (*Verbena* L.) and other plants in the family Verbenaceae (Benavent-Corai et al. 2005). A female specimen has tentatively been identified as *C. inselbergensis* (*C.* Mit-2) (Boucher & Pollet 2021) but is not included as a paratype specimen because of uncertain conspecificity. The female differs from the male in having a yellow halter, and a yellow face, the mid tibial setae are further apart, and the clypeus is shiny brown. This species was identified as morphospecies *Calycomyza* Mit-2 in Boucher & Pollet (2021).

3.5.3 Genus *Cerodontha* Rondani

The genus *Cerodontha* is classified into seven subgenera (*Butomomyza* Nowakowski, *Cerodontha*, *Dizygomyza* Hendel, *Icteromyza* Hendel, *Phytagromyza* Hendel, *Poemyza* Hendel, and *Xenophytomyza* Frey). All subgenera have been recorded in the Neotropical region, except *Phytagromyza*, which is exclusively Holarctic. The species are quite variable externally, but the male genitalia is characteristic with a well-developed mesophallus and long tubular distiphallus. In addition, all species in the genus *Cerodontha* have a unique L-shape sclerite inside the epandrium (the subepandrial sclerite). A single specimen of *Cerodontha* was collected from the Mitaraka massif and it belongs to the subgenus *Dizygomyza*, normally characterized by the large lunule and first flagellomere enlarged in males. It represents a new species closely related to *Cerodontha (Dizygomyza) nigrihalterata* Boucher. This is the first *Cerodontha* species reported from French Guiana.

Cerodontha (*Dizygomyza*) *pseudonigrihalterata* n.sp.

(Figs. 20-24)

Type material. Holotype ♂. Guyane Française: Mitaraka, Sampling site: MIT-E-savane roche 2. 02°13'59.8''N, 54°27'46.5''W, 471 m. Open / partially opened areas. 13.viii.2015 - 20.viii.2015, MT(6m). leg. Pierre-Henri Dalens. MITARAKA/230 (sorted by M. Pollet) (MNHN).

Etymology. “Pseudo” which means false in Greek refers to the similarity of this species to *Cerodontha* (*Dizygomyza*) *nigrihalterata* Boucher.

Diagnosis. This species can be differentiated from all other *Cerodontha* (*Dizygomyza*) species by the combination of the following characters: knob of halter completely brown, only one ors, wing infuscated in cell R_1 and R_{2+3}, presence of only 2 dc, male with first flagellomere small, patch of reclinate orbital setulae at level of ori; frons not projecting in front of eye in profile.

Description. Frons including orbital plate 0.30 mm; orbital plate broad, 0.37 width of frons at midpoint; orbital plate only slightly widening anteriorly; not projecting in front of eye in profile (Fig. 20); eye appears bare, at most with a few scattered hairs; lunule broad, partly covered by large orbital plate (Fig. 21); lunule and anterior orbital plate with a patch of silvery pubescence (Fig. 21) best seen in posterodorsal view; orbital plate and ocellar triangle shining; frons with black microtomentum; 1 reclinate ors and 3 ori (anterior 2 inclinate and upper ori lateroclinate on one side); orbital setulae reclinate, numerous, forming a patch at level of ori; first flagellomere small (Fig. 20), rounded apically with short white pubescence, longer posteriorly; arista long (0.5 mm) with long pubescence (Fig. 20); small raised keel present between antennae; gena at midpoint about 0.16 times maximum eye height; clypeus medium width with upper margin rounded, slightly projecting and distinctly visible in lateral view; palpus with 7-9 long hairs apically (Fig. 20); two postsutural dc; prescutellar acrostichal seta absent; acrostichals in about 7-8 rows; midtibia with 1 long apical seta; wing length in male: approximately 1.9 mm (wing bent); last section of CuA_1 approximately 0.7 times length of penultimate; wing conspicuously infuscated in cell R_1 and R_{2+3}.

Colour. Body completely brown, except for orbital plate yellowish anteriorly (Fig. 21); halter with knob completely brown, stalk paler; legs brown, tarsi at most a little paler; calypter yellow, fringe short and pale brown.

Male genitalia. Distiphallus in the form of two tubules, almost equal to the length of mesophallus and with a short, square, terminal process (Figs. 22, 23); hypandrium broad and rounded; epandrium with apical long hairs; surstylus with five spines; subepandrial sclerites narrow, elongated; anal projection of epandrium prominent; ejaculatory apodeme with short stalk (Fig. 24).

Distribution. French Guiana*

Host. Unknown.

Comments. This species is most similar to the Costa Rican species *C.* (*Diz.*) *nigrihalterata* Boucher (Figs. 25-29). An attempt was made to have both species barcoded, but no sequences were obtained (Boucher & Savage 2022). Externally the species have a few important similarities including the presence of only 1 ors and 3 ori, dark knob of halter, wing infuscated in cell R_1 and R_{2+3}, lunule and anterior orbital plate with silvery pubescence and mid tibia with a strong apical spine. The phallus of these species is also similar. The holotype of *C. nigrihalterata* illustrated in Boucher (2005) has the distal tubules of the phallus divergent, but it is parallel sided (as seen for *C.* (*Diz.*) *pseudonigrihalterara*) in a paratype shown in Fig. 28. *Cerodontha (Diz.) nigrihalterata* differ from the new species described here by having a projecting frons (Fig. 25), the palpus with normal setae, the first flagellomere slightly enlarged in male (Fig. 26), tarsal segments paler yellow, mesonotum with three dc, the ejaculatory apodeme with a long and narrow stalk (Fig. 29, paratype (slightly different than ejaculatory apodeme illustrated for the holotype in Boucher 2005)) and apex of distal tubule more contiguous with tubule, not appearing as a separate sclerite.

3.5.4 Genus *Japanagromyza* Sasakawa

(Figs. 30-33)

A total of 12 specimens was collected from the Mitaraka massif, representing four morphospecies (Boucher & Pollet 2021), all represented by female specimens (Figs. 30-33). All these species have a metallic sheen characteristic of the genus. The wing venation is also characteristic of the subfamily Agromyzinae with the subcostal vein joining with vein R_1 before reaching the costa and R_1 expanded near junction with costa (Fig. 33, inset). Most of these *Japanagromyza* species (*Japanagromyza* MIT-2; *J*. Mit-3 and *J*. Mit-4) lack the prescutellar bristles normally present in this genus. Out of the 35 species of *Japanagromyza* recorded in the Neotropical region, only 5 species (*J. aldrichi* (Frick); *J. lonchocarpi* Boucher; *J. polygoni* Spencer; *J. desmodivora* Spencer and *J. tingomariensis* Sasakawa) lack these bristles. Unfortunately, without male specimens the identification of the Mitaraka specimens could not be confirmed. This is the first record of the genus *Japanagromyza* from French Guiana.

3.5.5 Genus *Liriomyza* Mik

This is a very speciose genus, difficult to identified due to high morphological homogeneity across the species (both externally and in the male genitalia). Most species are very small, usually yellow (frons, part of pleura and scutellum, sometimes legs) and black (mesonotum, part of pleura) in colour. Although some exceptions exist. For example, *L. nigra* Spencer from Colombia has a dark frons and dark scutellum. The males of all *Liriomyza* have a characteristic feature: the stridulatory organ present on the lateral membranous portion of the abdomen. Although this structure is sometimes pale and difficult to see and may be secondarily lost in some species. A total of 15 specimens of *Liriomyza* was collected from the Mitaraka massif, representing 3 species. Two (*Liriomyza* Mit-2 and *L*. Mit-3) were represented by female specimens only, so identification could not be confirmed. The other species (*Liriomyza* Mit-1) represented by 9 females and 3 males represents a new species described below. Three *Liriomyza* species were previously known from French Guiana. These are widespread and well known agromyzid pests: *Liriomyza huidobrensis* (Blanchard), *Liriomyza trifolii* (Burgess) and *L. sativae* Blanchard.

Liriomyza touroulti n.sp.

(Figs. 34-38)

Type material. Holotype ♂. Guyane Française: Mitaraka, Sampling site: MIT-E-savane roche 2. 02°13'59.8''N, 54°27'46.5''W, 471 m. Open / partially opened areas. 13.viii.2015 - 20.viii.2015, MT(6m). leg. Pierre-Henri Dalens. MITARAKA/230 (sorted by M. Pollet) (MNHN). Paratypes: Same as holotype (2 ♂, 9 ♀; MNHN).

Etymology. The species name is a patronym in honour of Julien Touroult the PI for the entomological component of this Mitaraka survey.

Diagnosis: This species can be distinguished from other Neotropical species of *Liriomyza* by the orbital plate and frons all yellow; inner vertical bristle (vti) and outer vertical bristle (vte) on yellow background; thorax subshining black with yellow patch at hind corner; femora yellow, tibia and tarsi slightly darker (especially on mid and hind leg); clypeus pale brown; wing length in male of 1.5 mm and by the male genitalia with the distiphallus being only slightly longer than the mesophallus.

Description. Frons including orbital plate narrow, approximately 0.15 mm; orbital plate not projecting in front of eye in profile; eye bare; lunule small; 2 well developed reclinate ors and 2 well developed inclinate ori (anterior one at most only slightly shorter than posterior ori); orbital setulae sparse, short and reclinate, in one row; first flagellomere small, covered with short but dense white pubescence (Fig. 34); arista with distinct pubescence; gena slightly deeper at rear (Fig. 34), at midpoint about 0.16 times maximum eye height; clypeus with upper margin rounded; small epistoma present; 3+1 dc; prescutellar seta well developed; acrostichal setulae long in about 4-5 rows; midtibia with 2 lateral setae; wing length 1.5 mm in male and in female; last section of CuA_1 0.6 times length of penultimate.

Colour. Head yellow, except for slight greyish infuscation on the orbital plate; pleura yellow except for katepisternum mostly brown and anepisternum with a small brown patch; mesonotum shiny brown with small yellow patch at hind corner; legs yellow, tibiae and tarsi a little darker, pale brown; calypter yellow, fringe short and pale brown. Stridulatory organ present.

Male genitalia: Phallus small: length of distiphallus combined to mesophallus measuring approximately 0.8 mm; distiphallus (Fig. 35-a) approximately 1.5 times longer than mesophallus (Fig. 35-b) and approximately 1.6 times longer than wide (at largest point, Fig. 35-c); distal end of phallus somewhat claw-like in lateral view (Fig. 36); ejaculatory apodeme with long, narrow stalk: width of blade at largest point, about 5 times wider than stalk at midpoint (Fig. 37); epandrium and surstylus each with one spine (Fig. 38); spine of surstylus located near midpoint.

Distribution. French Guiana*.

Host. Unknown.

Comments: This new species is most similar externally to *L. sativae*, a species widely distributed in the Neotropical region (and elsewhere), and also present in French Guiana (EPPO 2022). These two species can be differentiated by their male genitalia: in *L. sativae*, the mesophallus is much shorter than the distiphallus and the ejaculatory apodeme has a shorter stalk and wider blade. Also, the spine on the surstylus is subapical (see Lonsdale 2021). The phallus of this new species is similar to the phallus of *L. geniculata* Sasakawa from Venezuela (Sasakawa 1992a), in lateral view, but it is quite different in ventral view, in addition to having 2 apical spines on the surstylus. Short DNA sequences (356 bp) were obtained for two female paratypes of *L. touroulti* n.sp., with the following GenBank accession numbers: OK623731 and OK623719 (Boucher & Savage 2022).

3.5.6 Genus *Nemorimyza* Frey

Nemorimyza is a small genus of 5 species (excluding *N. xizangensis* Chen & Jian from China with questionable status), all present in the Neotropical region. The genus is characterized by having a small lunule, often silvery or greyish dusted; body almost completely black with halter at least partially black (except male *N. posticata* (Meigen) with abdomen conspicuously yellow posteriorly, halter white and foreknee narrowly yellow). Most species have 3+1 dc, except *N. posticata* that has 3+0 dc (sometimes with an additional smaller pair close to suture). Some species have a well-developed lateral bristle on the foretibia. The male genitalia is characterized by having the subepandrial sclerite broadly fused with each other, with numerous microscopic spines centrally and a pair of strong spine-like processes, directed ventrally (Zlobin 1996).

Although the structure is inside the epandrium and sometimes difficult to see. One *Nemorimyza* species, *N. maculosa* (Malloch), has previously been reported from French Guiana (Martinez & Étienne 2002). A total of five specimens were collected from the Mitaraka massif, representing two species (Boucher & Pollet 2021; Boucher & Savage 2022). One of them (identified as morphospecies *N.* Mit-2 in Boucher & Pollet 2021) is known from female specimens only. *Nemorimyza* Mit-1 and *N.* Mit-2 can only be differentiated by subtle external differences, but DNA barcoding of these two species, supported the assignment to two different species (Boucher & Savage 2022). Although an additional specimen from Costa Rica (Guanacaste), collected by D. Janzen and W. Hallwachs was included in the same BIN (Barcode Index Number) as *N*. Mit-2 (BOLD:ADB9391) (Table 5, in Boucher & Savage 2021: the number of public domain specimens has now increased to four, as of March 2022, all from Guanacaste), this additional material consists of female specimens only, and the species description will be delayed until a male becomes available (most probably from the BioAlfa project at Guanacaste, Costa Rica (Janzen & Hallwachs 2019). *Nemorimyza* Mit-1 is described below based on 1 male and 2 female specimens.

Nemorimyza thanatos n.sp.

(Figs. 39-46)

Type material. Holotype ♂. Guyane Française: Mitaraka, Sampling site: MIT-E-savane roche 2. 02°13'59.8''N, 54°27'46.5''W, 471 m. Open / partially opened areas. 13.viii.2015 - 20.viii.2015, MT(6m). leg. Pierre-Henri Dalens. MITARAKA/230 (sorted by M. Pollet. Paratypes. Same as holotype (2 ♀: MNHN).

Etymology. The specific name comes from the Greek "Thanatos" which is the personification of Death (better known as the Grim Reaper), referring to the dark coloured body of the species, hooked apical structures of the distiphallus and the large scythe-like projections of the phallus in lateral view.

Diagnosis. This species can be differentiated from all other species of *Nemorimyza* by the combination of the following characters: gena narrow; knob of halter completely brown; 3+0 dc including only two well developed dc; wing length between 1.65-2.0 mm; absence of foretibial seta and anterior orbital plate yellow.

Description. Frons narrow, width including orbital plate 0.22 mm; orbital plate 0.20 width of frons at midpoint; orbital plate not projecting in front of eye in profile (Fig. 39); lunule small with silvery pubescence; frons and orbital plate mat; 2 reclinate ors and 2 ori (both reclinate on one side; anterior ori inclinate on other side); orbital setulae reclinate, in one distinct row; first flagellomere small, rounded apically with short white pubescence; arista long (0.48 mm) with short pubescence; gena at midpoint about 0.08 times maximum eye height; clypeus with narrow thickness and upper margin rounded; palpus with 3 long apical bristles. 3+0 dc with only 2 well developed dc, anterior dc about half the size of second bristle; prescutellar acrostichal seta present; acrostichals in about 7 rows; midtibia with 2 lateral setae and 1 long apical seta with 3-4 smaller apical setae; foretibial seta absent; wing length 1.65 mm in male to 2.0 mm in females; last section of CuA_1 approximately 0.8 times length of penultimate.

Colour. Frons mat black with reddish-brown undertone anteriorly; orbital plate brown posteriorly and yellow anteriorly (anterior to lower ori) (Fig. 40); antenna brown; base of arista yellow; thorax brown but notopleuron and part of postpronotum paler brown; halter with knob completely brown; legs brown; calypter yellow or pale greyish with black margin and fringe; abdomen brown, with tip of abdomen and epandrium paler brownish in male.

Male genitalia. Phallus ending in 2 short, hooked processes, visible in ventral view (Figs. 41, 43), in addition to two large scythe-like projections visible in lateral view (Figs. 42, 44); hypandrium with narrow arms; ejaculatory apodeme with narrow blade, only slightly expanding distally (Fig. 45); surstylus elongated and extended ventrally (Fig. 46); epandrium with a spine posteroventrally (Fig. 46).

Distribution. French Guiana.

Host. Unknown.

Comments. *Nemorimyza thanatos* is most similar to *N. ranchograndensis* (Spencer) but this latter species is larger (up to 3.0 mm in wing length), has 3+1 well developed dc, longer orbital setae and the presence of a foretibial seta (Sasakawa 1992b; Spencer 1973b). A barcoding sequence was obtained for a female paratype and was assigned the GenBank accession number: OK623720 (Boucher & Savage 2022).

3.5.7 Genus *Phytobia* Lioy

Phytobia species are generally dark coloured in the Nearctic and Palaearctic region, but a few species have contrasting yellow colouration in the Neotropical region. Most *Phytobia* have 3+1 well developed dc, wing with vein R_{4+5} ending nearer the wing tip than M_{1+2} or both veins equidistant from wing tip. The frons is normally dark, sometimes paler, reddish, the lunule is often silvery. The male genitalia usually have a broadly rounded hypandrium, the surstyli are usually broad, lobate. A few species differ from this description and were included in a separate group, the *Phytobia* unica group (Boucher 2010), discussed below. Five specimens of *Phytobia* were collected in Mitaraka, representing 5 species. Two of them (*P*. Mit-2 and *P*. Mit-3) are known from female specimens only (Boucher & Pollet 2021) and identity could not be confirmed. The other species, *Phytobia* Mit-1, *Phytobia* Mit-4 and *Phytobia* Mit-5 (Boucher & Pollet 2021) represent new species and are described below. Two of these new species (*P. dalensi* n.sp. and *P. corona* n.sp.) are part of the *Phytobia unica* group, along with four other previously known Neotropical species: *P. unica* Spencer, *P. mentula* Sasakawa, *P. pipinna* Sasakawa and *P. guatemalensis* Sasakawa. This species group is characterized by the presence of only two well developed dc, in addition to a very small third one in some species, a phallus ending in paired tubules, a well-defined cylindrical mesophallus, a narrow and elongated hypandrium, surstyli C-shape and covered with short spines, and narrowly connected to the posteroventral margin of the epandrium. Also, the wing venation of the *P. unica* group is very similar to that of the subfamily Agromyzinae due to the subcostal vein that joins R_1 before reaching the costa (also occurs in other *Phytobia*) and the distal margin of R_1 that is expanded near the junction.

Phytobia dalensi n.sp.

(Figs. 47-57)

Type material. Holotype ♂. Guyane Française: Mitaraka, Sampling site: MIT-E-savane roche 2. 02°13'59.8"N, 54°27'46.5"W, 471 m. Open / partially opened areas. 13.viii.2015 - 20.viii.2015, MT(6m). leg. Pierre-Henri Dalens. MITARAKA/230 (sorted by M. Pollet) (MNHN).

Etymology. The species name is a patronym in honour of Pierre-Henri Dalens, president of the Société Entomologique Antilles-Guyane (SEAG) who was present during phase 2 and 3 of the expedition and is the collector of most Agromyzidae of Mitaraka.

Diagnosis. This species can be differentiated from all other Neotropical species of *Phytobia* by the combination of the following characters: wing hyaline; 3+0 dc with anterior dc much reduced; frons yellowish-brown; anepisternum completely brown; mesonotum mostly black, with posterolateral corner narrowly yellow; femora yellow except narrowly brown apically on mid and hind legs; abdomen mostly yellow with black spots centrally and laterally on hind tergites, and by the male phallus.

Description. Frons width including orbital plate 0.35 mm at midpoint; orbital plate 0.2 width of frons at midpoint; orbital plate not projecting in front of eye in profile; lunule small, collapsed inside; ocellar triangle extended anteriorly to level of anterior ors; 2 reclinate ors and 2 ori : upper ori reclinate or lateroclinate and lower ori inclinate (Fig. 49); orbital setulae reclinate, in one row, except for 1-2 extra setulae; first flagellomere small, rounded apically with normal short pubescence; arista long with distinct pubescence; gena narrow, at midpoint about 0.05 times maximum eye height (Fig. 48); clypeus with medium thickness, with upper margin rounded; small epistoma present (Fig. 49); 3+0 dc with only 2 posterior ones well developed (Fig. 52), anterior dc about 0.33 length of second dc; prescutellar acrostichal setae present; acrostichal setulae numerous, in about 8-9 rows; midtibia with 2 lateral setae; wing length 2.28 mm in male; last section of CuA_1 approximately 0.6 times length of penultimate; vein R_{4+5} ending close to wing tip; distal margin of R_1 expanded near junction (Fig. 51).

Colour. Frons mat, yellowish-brown (Fig. 49); orbital plate and ocellar triangle subshining, darker brown except orbital plate yellowish at level of ori and below; antennae yellowish, slightly infuscated with brown (Figs. 48, 49); clypeus and palp yellowish-brown; face yellowish (Fig. 50); mesonotum shiny brown except posterolateral corner possibly narrowly yellow (thorax collapsed, difficult to see) (Fig. 52); scutellum brown; postpronotum yellow with small brown spot; notopleuron yellow; other pleura completely brown; fore leg yellow including coxa, tarsi a little darker yellowish-brown; mid and hind femora yellow except narrowly brown apically (Fig. 47); mid coxa brownish; mid and hind tibia and tarsi brown; halter yellow; calypter and margin yellow; fringe brown. Abdomen yellow with dark brown spots medially on tergites 3-6 in addition to lateral brown spots on tergites 5-6 as seen in Fig. 72 for *P. touroulti* n. sp. described below.

Male genitalia. Distiphallus (Fig. 54-a) separated into 2 tubules (Fig. 53) that are approximately 1.5 times longer than mesophallus (Fig. 54-b); hypandrium without apodeme, conspicuously constricted near midpoint (Fig. 55); surstyli with multiple spines (Fig. 56); ejaculatory apodeme with short stalk and medium size blade (Fig. 57).

Distribution. French Guiana*.

Host. Unknown.

Comments. This new species is part of the *Phytobia unica* group and is most similar externally to *P. mentula* Sasakawa described from Peru (Sasakawa 1992a), but the male phallus of these two species is distinct. *P. dalensi*, was identified as morphospecies *Phytobia* Mit-4 in Boucher & Pollet (2021).

Phytobia pluviasilvae n.sp.

(Figs. 58-66)

Type material. Holotype ♂. Guyane Française: Mitaraka, nr MIT-A-RBF1, river, 25.iii.2015, MT(6m), leg. Julien Touroult & Eddy Poirier (FR-GU/Mitaraka/2015) - sample code: MITARAKA/189 (sorted by Marc Pollet, 2015). (MNHN).

Etymology. This species (from sample 189) was collected from a 6 m Malaise trap installed in tropical lowland rainforest over the Alama River (Boucher & Pollet 2021). The specific name is referring to the habitat of the type specimen ("pluvia" for rain; "silva" for forest).

Diagnosis. This species can be differentiated from all other Neotropical species of *Phytobia* by the combination of the following characters: wing hyaline; presutural dc present; abdomen largely yellow; legs all brown; scutellum and prescutellar area yellow; anepisternum mostly brown, except narrowly yellow dorsally, and by the male phallus.

Description. Frons width including orbit 0.38 mm at midpoint; frons becoming narrower anteriorly; orbital plate 0.17 width of frons at midpoint; orbital plate not projecting in front of eye in profile; lunule small, wide and low; with silvery pubescence (Fig. 59); frons and orbital plate mat; ocellar triangle small, not extending beyond anterior ocellus; 2 (3 on one side) reclinate ors and 2 inclinate ori; orbital setulae reclinate, in one row; first flagellomere small, rounded apically with normal short white pubescence; arista long with short pubescence; gena narrow, at midpoint about 0.04 times maximum eye height; clypeus narrow, widely open with upper margin rounded; small epistoma present (Fig. 61); 4+1 dc with only 2 posterior ones well developed, anterior 3 dc less than half the size of second dc; prescutellar acrostichal seta absent; acrostichals numerous, in about 9-10 rows; fore femora with row of 3 long setae and a few shorter ones ventrolaterally; midtibia with 2 lateral setae; wing length 2.28 mm in male; last section of CuA_1 approximately 0.83 times length of penultimate; vein R_{4+5} ending very close to wing tip; subcostal vein extending to costa independently from R_1 and distal margin of R_1 straight, not expanded near junction (Fig. 60).

Colour. Frons mat black with small reddish spot near lunule; orbital plate mat brown except yellowish anterior to lower ori (Fig. 59); antennae brown; clypeus dark brown, subshining; palpus and face brown. Mesonotum brown except prescutellar area largely yellow and scutellum yellow (Fig. 62); brown area of mesonotum separated into 2 bands laterally; postpronotum yellow with large brown spot centrally (Fig. 58); notopleuron yellow; anepisternum mostly brown except narrowly yellow dorsally; all other pleura brown; legs brown (Fig. 58); halter yellow; calypter, margin and fringe brown. Abdomen yellow with brown spots medially and laterally on all tergites (Fig. 63).

Male genitalia. Phallus divided into 2 very long and coiled tubules (Figs. 64, 65); hypandrium narrow, U-shaped and without apodeme (Fig. 65); postgonites well developed; surstylus (Fig. 65, inset) with a few long hairs and separated from epandrium by suture; ejaculatory apodeme with small blade (Fig. 66).

Distribution. French Guiana*.

Host. Unknown.

Comments. Although the narrow, elongated hypandrium is unusual for this genus (broadly rounded in most *Phytobia*) and the phallus of this species is most similar to some Neotropical species of *Japanagromyza*, this new species belongs to the genus *Phytobia* on the basis of wing venation, shape of the surstyli, presence of 4+1 dc, fringe of calypter dark and postgonites present. This species was identified as morphospecies *Phytobia* Mit-1 in Boucher & Pollet (2021).

Phytobia corona n.sp.

(Figs. 67-77)

Type material. Holotype ♂. Guyane Française: Mitaraka, Sampling site: MIT-E-savane roche 2. 02°13'59.8"N, 54°27'46.5"W, 471 m. Open / partially opened areas. 13.viii.2015 - 20.viii.2015, MT(6m). leg. Pierre-Henri Dalens. MITARAKA/230 (sorted by M. Pollet) (MNHN).

Etymology: The specific name is derived from the Latin “corona” (crown), referring to the spinose ring-like sclerite on the distiphallus.

Diagnosis: This species is very similar to *P. dalensi* described above but can be differentiated by its bicolor frons (black posteriorly and orange anteriorly), and the male phallus.

Description. Frons width including orbital plate 0.32 mm at midpoint; orbital plate 0.14 width of frons at midpoint; orbital plate not projecting in front of eye in profile; lunule small; ocellar triangle extended anteriorly to level of anterior ors; 2 reclinate ors and 2 ori: upper ori reclinate and lower ori inclinate; orbital setulae reclinate, in one row; first flagellomere small, rounded apically with normal short pubescence; arista with long pubescence; gena extremely narrow, at midpoint about 0.03 times maximum eye height (Fig. 68); clypeus narrow, with upper margin rounded; no epistoma (Fig. 70); 3+0 dc with only 2 posterior ones well developed, anterior dc about 0.30 length of second dc; prescutellar acrostichal seta present; acrostichal setulae numerous, in about 8-9 rows; midtibia with 2 lateral setae; wing length 2.3 mm in male; last section of CuA_1 approximately 0.7 times length of penultimate; vein R_{4+5} ending close to wing tip; distal margin of R_1 expanded near junction (Fig. 67), similar to *P. dalensi* (Fig. 51).

Colour. Similar to *P. dalensi* with following differences: Frons mat, bicolor: black posteriorly at level of ocellar triangle and orange anteriorly (Fig. 69); orbital plate brownish-black to level of upper ori, yellowish below; clypeus black; face yellowish-brown; antennae yellow infuscated with brown (Fig. 68); palpus yellow; mesonotum shiny brown except posterolateral corner with distinct yellow patch (Fig. 71); calypter and margin yellow; fringe brown; abdomen yellow with dark brown spots medially on tergites 3-6 in addition to lateral brown spots on tergites 5-6 (Fig. 72).

Male genitalia. Distiphallus separated into 2 long tubules, longer than hypandrium and phallapodeme (Figs. 73-74) (distal end of phallus may be cut-off); tubules are straight, without distinct curvature; phallus with a ring-like sclerite armed with teeth (Figs. 73-75); ring-like sclerite located slightly below midpoint (Fig. 73); distal end of phallus (distal to ring-like sclerite) measuring 0.4 mm (possibly longer, as apex appears cut-off); distal end of phallus (distal to sclerite) about 3 times longer than basal part (arrows, Fig. 73); mesophallus short, oval, somewhat bean-shaped (Fig. 73); hypandrium narrow, constricted near midpoint, with distal end bent (Fig. 74, arrow); ejaculatory apodeme with very short stalk and medium size blade (Fig. 77); surstylus with multiple spines (Fig. 76).

Distribution. French Guiana*.

Host. Unknown.

Comments: This species is part of the *Phytobia unica*-group (Boucher, 2010) and is most similar to *P. guatemalensis* Sasakawa due to its bicolor frons and the characteristic spinose sclerite on the distiphallus. It can be differentiated from *P. guatemalensis* by the small yellow patch on posterolateral corner of mesonotum (Fig. 71) (mesonotum described as being all black in *P. guatemalensis*) and the longer distiphallus with the spinose sclerite located a little before midpoint of the distiphallus. The phallus of *P. guatemalensis* is shorter and the spinose sclerite is located near the end of distiphallus. This species was identified as morphospecies *Phytobia* Mit-5 in Boucher & Pollet (2021).

3.5.8 Genus *Phytoliriomyza* Hendel

This genus was originally erected for species having proclinate orbital setulae (in addition to other characters) which made the genus easily differentiated from other similar agromyzid genera, especially *Liriomyza*. But the concept of the genus has changed and now includes species with upright or reclinate orbital setulae and some without orbital setulae. The colour is also variable with some species having the mesonotum and scutellum dark, often greyish while others have the scutellum and sometimes pre-scutellar area yellow as in many *Liriomyza*, but males *Phytoliriomyza* do not have a stridulatory organ in males, unlike *Liriomyza*. Most *Phytoliriomyza* have only 3 orbital bristles including 2 ors and 1 ori (some exceptions, e.g., *P. grandis* (Spencer) from Brazil has 2 ors and 3 ori). The halters are often slightly darkened apically to completely brown and the male genitalia is usually distinctive with conspicuous spines on the surstyli and on inner margin of epandrium. Only two specimens were collected from the Mitaraka massif (Boucher & Pollet 2021) and they represent *Phytoliriomyza jurgensi* Spencer, originally described from Costa Rica (Spencer 1983) and later found to occur in Guadeloupe and St-Christopher (Étienne & Martinez 2003 a, b).

Phytoliriomyza jurgensi Spencer

(Figs. 78-81)

Phytoliriomyza jurgensi Spencer, 1983: 63; Étienne & Martinez 2003a: 261; Étienne & Martinez 2003b: 95; Martinez & Étienne 2002: 20.

Material examined: Guyane: Mitaraka, Sampling site: MIT-E-savane roche 2. 02°13'59.8''N, 54°27'46.5''W, 471 m. Open / partially opened areas. 13- 20.viii.2015, MT(6m). leg. Pierre-Henri Dalens. MITARAKA/230 (sorted by M. Pollet) (1 ♂, 1 ♀: MNHN)

Diagnosis: This species can be recognized by its small size (1.2-1.4 mm), yellow frons, anepisternum and notopleuron yellow, sparse acrostichal setulae in 2 rows, and long pubescence on the first flagellomere.

Distribution: Costa Rica, Guadeloupe, St-Christopher, French Guiana*.

Host: Unknown.

Comments: According to the original description (Spencer 1983), *P. jurgensi* has the first flagellomere and palpus black, and the tibiae and tarsi brownish black, but the Mitaraka specimens are paler, having the first flagellomere and palp yellowish-brown in the male and brown in the female, and the tibiae and tarsi are dark yellow. The Mitaraka specimens are also slightly smaller with a wing length of 1.2 mm in the male and 1.4 mm in the female (1.4 mm in male in original description) and they have 3+2 dc (Fig. 79) (3+1 dc in original description). Externally, *P. jurgensi* is most similar to *P. pilosella* Spencer (Spencer & Stegmaier 1973), but it can be differentiated from this species by a yellow frons (part of frons slightly darker in *P.*

pilosella), a brown or black palpus (yellow in *P. pilosella*), and a yellowish to greyish scutellum (brown in *P. pilosella*). Both species have extremely small and weakly sclerotized genitalia, but the distiphallus of *P. jurgensi* has a cup-shaped or funnel-shaped distal end (Fig. 80) and the tubules have a darker and more sclerotized medial region (Fig. 81). In *P. pilosella*, the phallus has one pair of very long, coiled tubules.

3.6 Discussion

This study was part of a large-scale biodiversity program known as "Our planet reviewed" and contributed significantly to the main objective of this program (the discovery of new species) by adding eight new species of Agromyzidae to the list of 20 species of Diptera already discovered for the region (Touroult et al. 2021). It also offered an important contribution to our knowledge of the Agromyzidae of French Guiana for which only four species were previously reported. The Agromyzidae fauna of French Guiana now includes 14 species: *Agromyza mitarakensis* n.sp., *Calycomyza grenadensis* Zlobin, *Calycomyza inselbergensis* n.sp., *Cerodontha pseudonigrihalterata* n.sp., *Liriomyza huidobrensis* (Blanchard), *Liromyza sativae* Blanchard, *Liriomyza touroulti* n.sp., *Liriomyza trifolii* (Burgess), *Nemorimyza thanatos* n.sp., *Nemorimyza maculosa* (Malloch), *Phytobia dalensi* n.sp., *Phytobia pluviasilvae* n.sp., *Phytobia corona* n.sp. and *Phytoliriomyza jurgensi* Spencer.

The four agromyzid species that were previously recorded in French Guiana (*L. huidobrensis*, *L. sativae*, *L. trifolii* and *N. maculosa*) are best known for the damage they cause to various crops and ornamental plants (Spencer 1973c). These economically important species were reported from Cayenne (CABI 2022), the capital city of French Guiana. Due to their ability to feed on a wide variety of commercial plants, these species are found in many different regions of the world (CABI 2022). For example, *L. huidobrensis*, is highly polyphagous, feeding on hundreds of plant species in 49 families (Weintraub et al. 2017), but until the 1980s, its distribution was restricted to Central and South America. This species has now spread in more than 40 countries around the world, mainly due to commercial shipments of flowers and cultivated food crops (Weintraub et al. 2017). Among the other previously recorded French Guiana agromyzids, *Nemorimyza maculosa*, has a more limited, but expanding distribution. This species is an oligophagous species feeding on multiple genera in the family Asteraceae, including crop plants such as *Lactuca* (lettuce) and ornamental plants such as *Chrysanthemum*, *Aster* and *Dahlia* (Spencer 1973c). This species was, until recently, restricted to the Neotropical and Nearctic region, but has now been reported from Spain, Portugal and the Canary Islands (CABI 2021; Černý et al. 2018). These economically important species were not collected at Mitaraka, which is located at the extreme opposite side of Cayenne (Fig. 1, in Boucher & Pollet 2021), and where a totally different environment exists.

The Agromyzidae so far identified from the Mitaraka massif are represented by previously known Neotropical species (*C. grenadensis* and *P. jurgensi*) with limited distributional data and by new species, currently known only from Mitaraka. Although French Guiana is believed to have few real endemic insect species due to the lack of barriers to neighboring countries (Brûlé & Touroult 2014), recent findings suggest that Mitaraka may represent a center of endemism for many insect species, including those specifically associated with inselbergs (Touroult et al. 2021). Inselbergs are characterized by a distinct plant community, including rare or endemic species in French Guiana (Corinne et al. 2003; Vlasáková & Gustafsson 2011), that are sometimes associated with a unique insect fauna (Touroult & Dalens 2015; Vlasáková et al. 2008). Although it is too early to discuss the distribution patterns of the newly described agromyzid species, some may prove to be endemic to the region, or more specifically to the inselbergs of the Mitaraka massif. Surprisingly, none of the species so far identified from Mitaraka correspond to the known Brazilian agromyzid fauna. Brazil, a country adjacent to French Guiana, is the best studied region in the neotropics, with approximately 115 agromyzid species recorded. Although most of these species are known from the south and southeast region of the country (Monteiro et al. 2019), some were recently reported from the Brazilian Amazon, in the state of Pará, but south of the Amazon River, e.g. *Calycomyza ipomoensis* Esposito (Esposito 1994), *Liriomyza valladaresae* Carvalho-Filho (Carvalho-Filho et al. 2016), and many additional species (Monteiro & Esposito 2015, Monteiro et al. 2019). A little further south, in the state of Mato Grosso and Rondônia, 14 new *Phytobia* species were recently described (Sousa and Couri 2017), although none corresponded to the Mitaraka *Phytobia* species.

Among the newly described *Phytobia* species, two of them are part of the *Phytobia unica* group: *P. dalensi* and *P. corona*. These are closely related to some atypical, partially yellow coloured *Japanagromyza* species (subfamily Agromyzinae), namely *J. maculata* (Spencer) and *J. spadix* (Spencer), that were originally described in a new genus, *Geratomyza* Spencer (Spencer & Stegmaier 1973), but synonymized with *Japanagromyza* a few years later (Spencer 1984). Boucher (2010) erected a species group (*Japanagromyza maculata* group) to accommodate these species in a key to Central American Agromyzidae, along with the *Phytobia unica* group, both keying in the same couplet but differentiated by the simple versus paired tubules of the phallus. Further studies will be necessary to confirm the identity of the species included into these two species groups (so far endemic to the Neotropical region) and to consider the resurrection of the genus *Geratomyza*. These two new species of *Phytobia* belonging to the *P. unica* group, are therefore of special importance for future research.

The other *Phytobia* species, *P. pluviasilvae* is the only newly described species collected from the rainforest habitat in Mitaraka. This species is unusual in having an extra-long and coiled phallus and a long and thin hypandrium, two characters not shared by other Neotropical congeneric species. Many additional new and interesting species are expected in the genus *Melanagromyza* and *Ophiomyia* the two most diverse genera of Agromyzidae collected from the Mitaraka massif (Boucher & Pollet 2021), which are still waiting to be described.

This preliminary list of Agromyzidae species collected during the "Our planet reviewed" Mitaraka massif expedition will provide an important and interesting starting point for the future study of French Guiana Agromyzidae, and more broadly of tropical rainforest and inselbergs. The remote locality and difficulty of access of the Mitaraka massif will make a future expedition unlikely in the short term. Unfortunately, in many years from now, the Agromyzidae fauna may be completely different, as climate change has already had its effect on the rainforest landscape and the plant community present on the inselbergs (Amigo 2020; Fonty et al. 2009).

3.7 Acknowledgments

The material for this study was collected during the "*Our Planet Reviewed*" Guyane-2015 expedition in the Mitaraka range, in the core area of the French Guiana Amazonian Park, organized by the MNHN and Pro-Natura international. The expedition was funded by the European Regional Development Fund (ERDF), the Conseil régional de Guyane, the Conseil général de Guyane, the Direction de l'Environnement, de l'Aménagement et du Logement and by the Ministère de l'Éducation nationale, de l'Enseignement supérieur et de la Recherche. It was conducted in collaboration with the Parc Amazonien de Guyane and the Société entomologique Antilles-Guyane. I would like to thank Marc Pollet (coordinator for the Mitaraka Diptera) for the invitation to study the Agromyzidae collected during this project. Marc participated to this expedition as member of the first team (22 February-11 March 2015), hereby supported financially by MNHN and Pro-Natura international. I would also like to thank Pierre-Henri Dalens and his team for collecting most of the agromyzid material studied. Finally, I would like to thank Nigel Wyatt (NHM) for providing pictures of the holotype of *Agromyza simillima* and Valerie Levesque-Beaudin at the Centre for Biodiversity Genomics for providing a loan of additional Costa Rican specimens of *Nemorimyza* Mit-2 (all females, and therefore not used in this present study). I would also like to thank Christopher Buddle (McGill University) and Jeff Cumming (CNC) for their comments on the manuscript.

3.8 References

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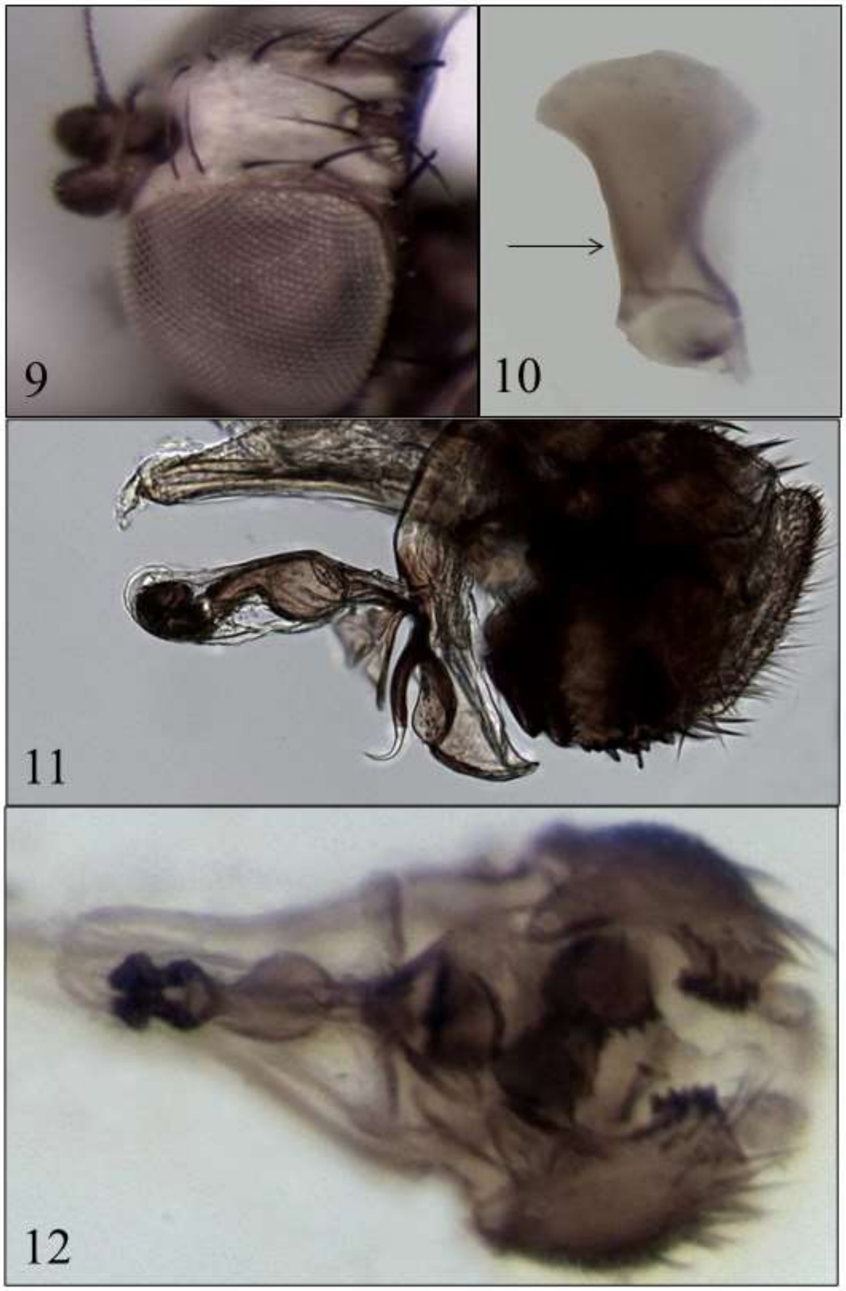
3.9 Figures

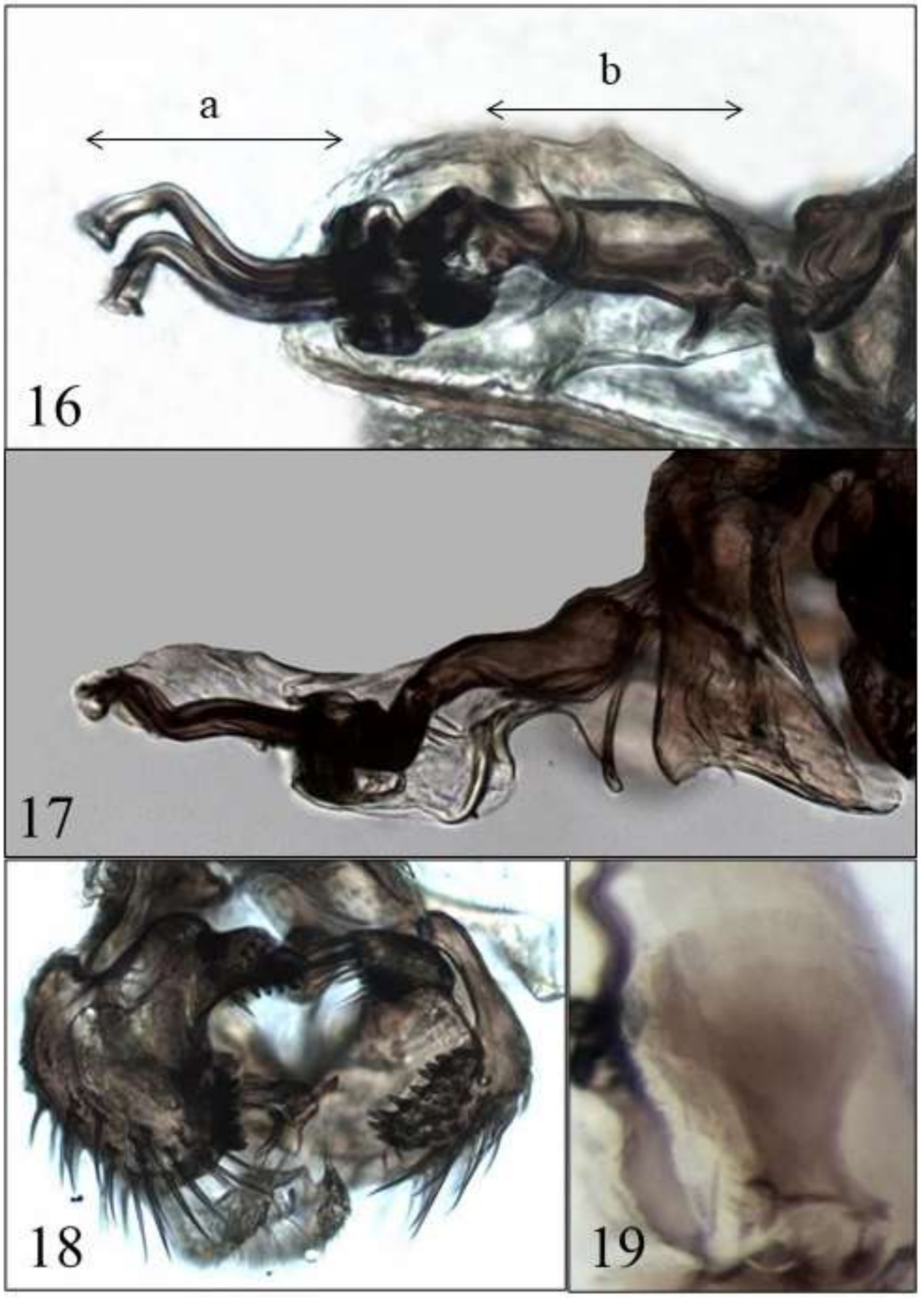
Figures 1-5. *Agromyza mitarakensis* n.sp. 1. head, lateral; 2. head antero-lateral showing clypeus; 3. ejaculatory apodeme; 4. phallus, lateral view; 5. phallus, ventral view.

Figures 6-8. *Agromyza simillima* Spencer. 6. phallus ventral view; 7. habitus lateral view (head missing); 8. head (Photos Nigel Wyatt).

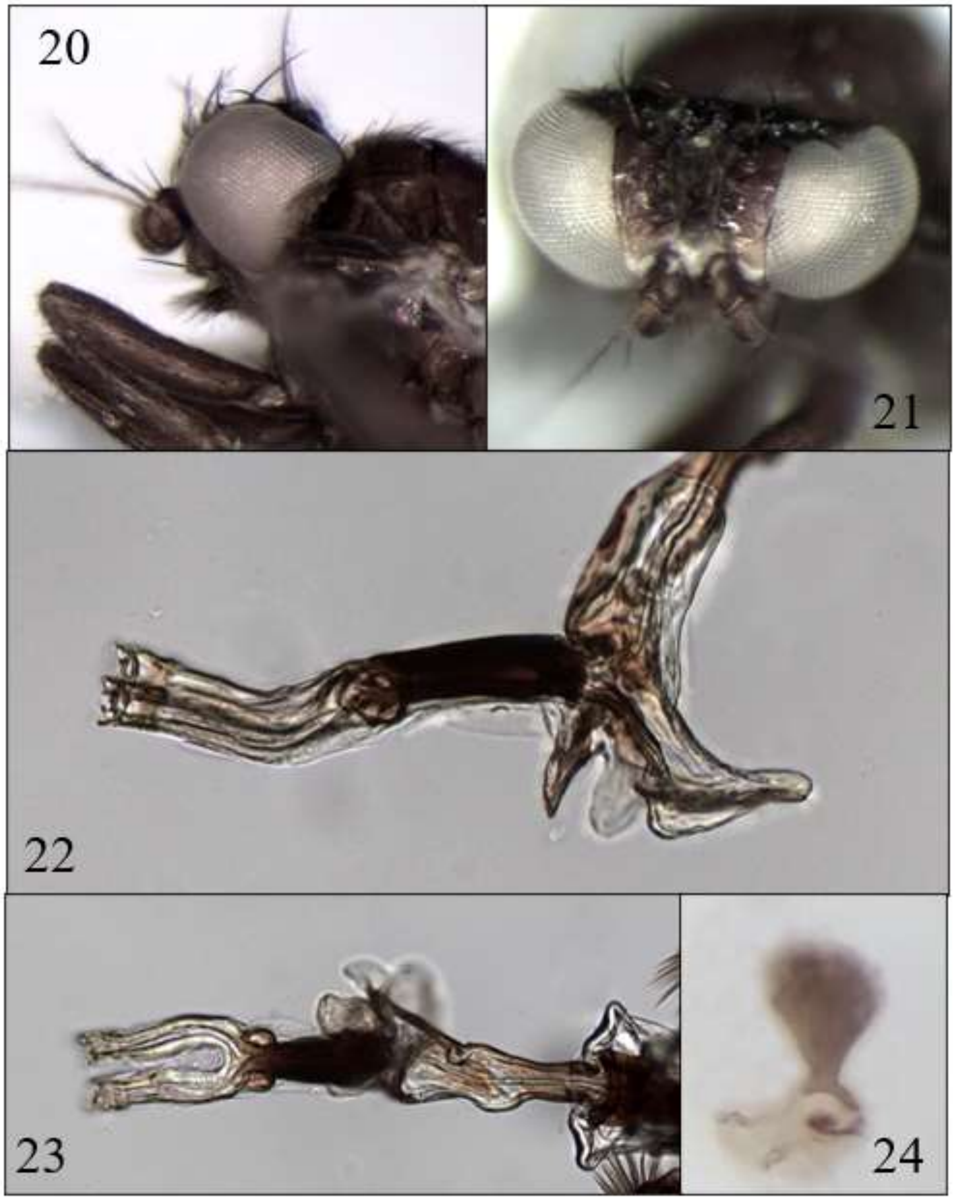


Figures 9-12. *Calycomyza grenadensis* Zlobin. 10. head, dorso-lateral view; 11. ejaculatory apodeme; 12. phallus, lateral view; 13. phallus, ventral view.

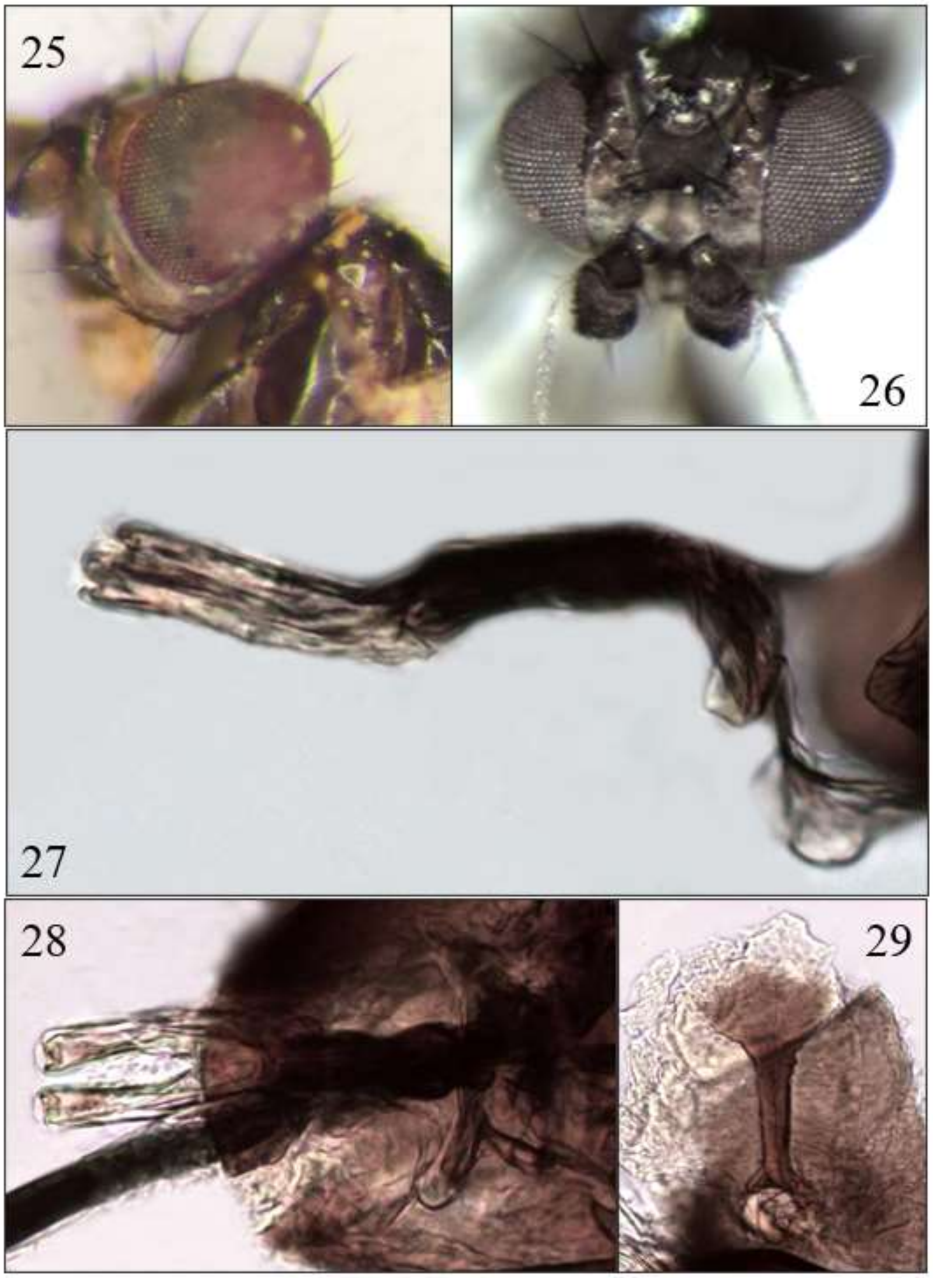
Figures 13-15. *Calycomyza inselbergensis* n. sp. 13. habitus, lateral view; 14. head, anterior view; 15. head, dorsolateral view.



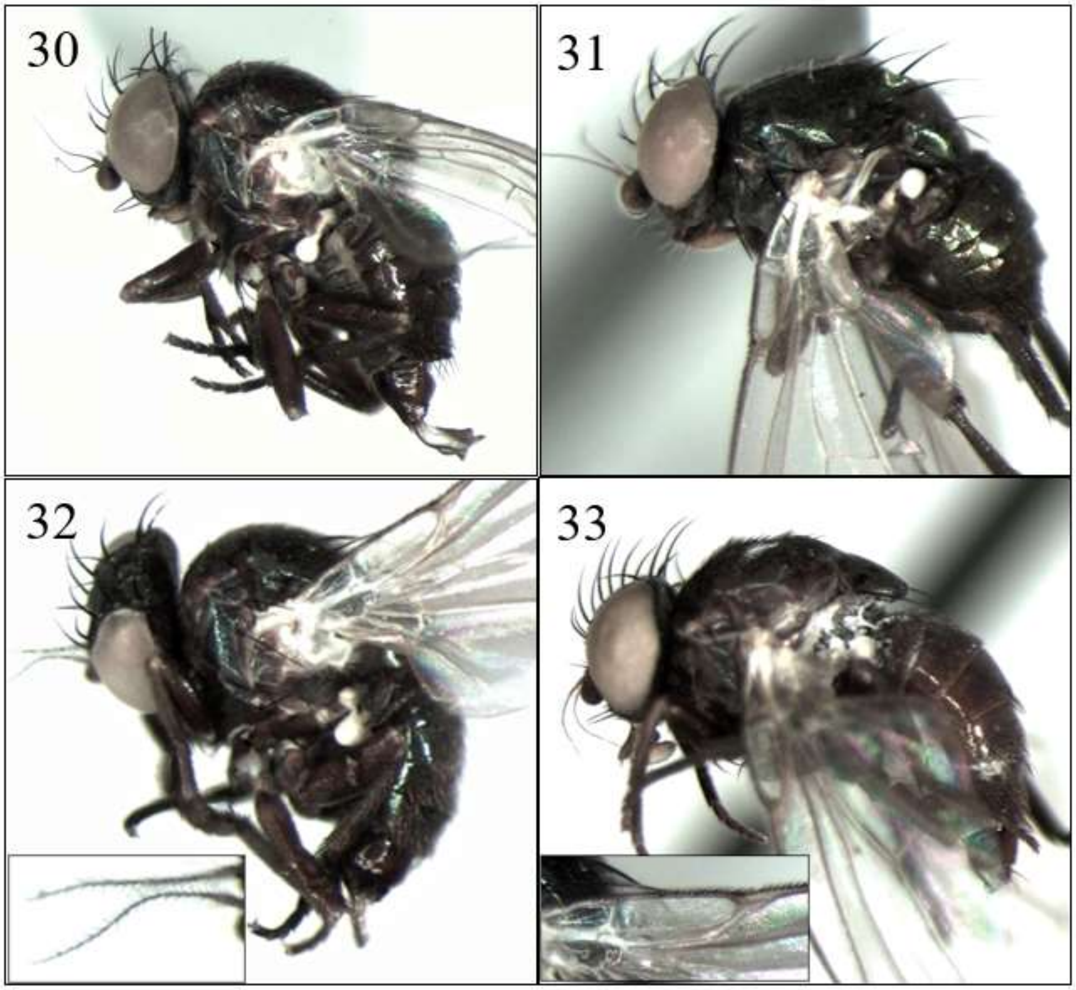
Figures 16-19. *Calycomyza inselbergensis* n.sp. 16. phallus, lateroventral view: arrow a: referring to the length of distal tubules anterior to the ring-like sclerite; arrow b: referring to the length of mesophallus; 17. phallus, lateral view; 18. epandrium, latero-ventral view; 19. ejaculatory apodeme.



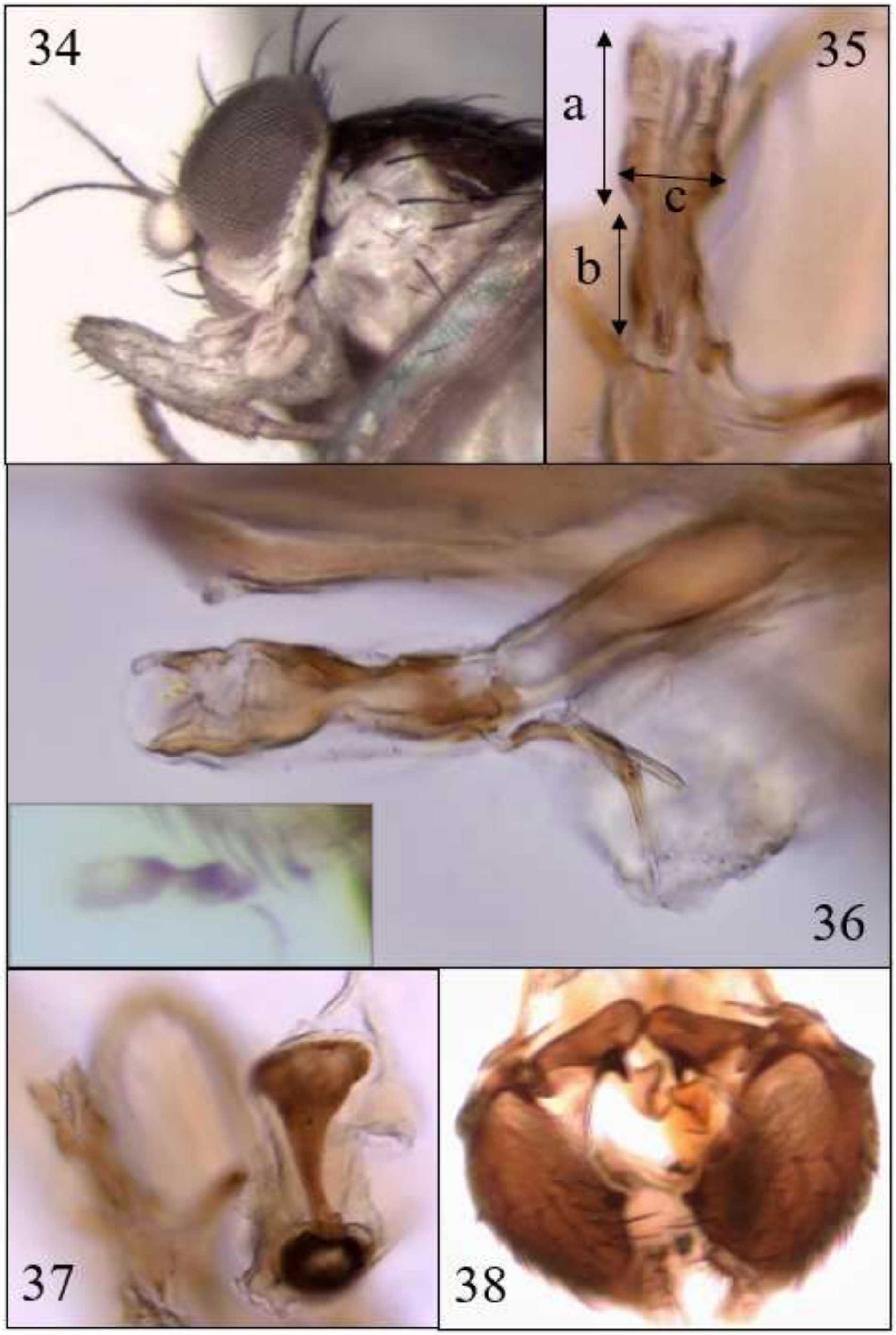
Figures 20-24. *Cerodontha pseudonigrihalterata* n.sp. 20. head, lateral view; 21. head dorsal view; 22. phallus, lateral view; 23. phallus, ventral view; 24. ejaculatory apodeme.



Figures 25-29. *Cerodontha nigrihalterata* n.sp. 25. head, lateral view; 26. head dorsal view; 27. phallus, lateral view; 28. phallus, ventral view; 29. ejaculatory apodeme.

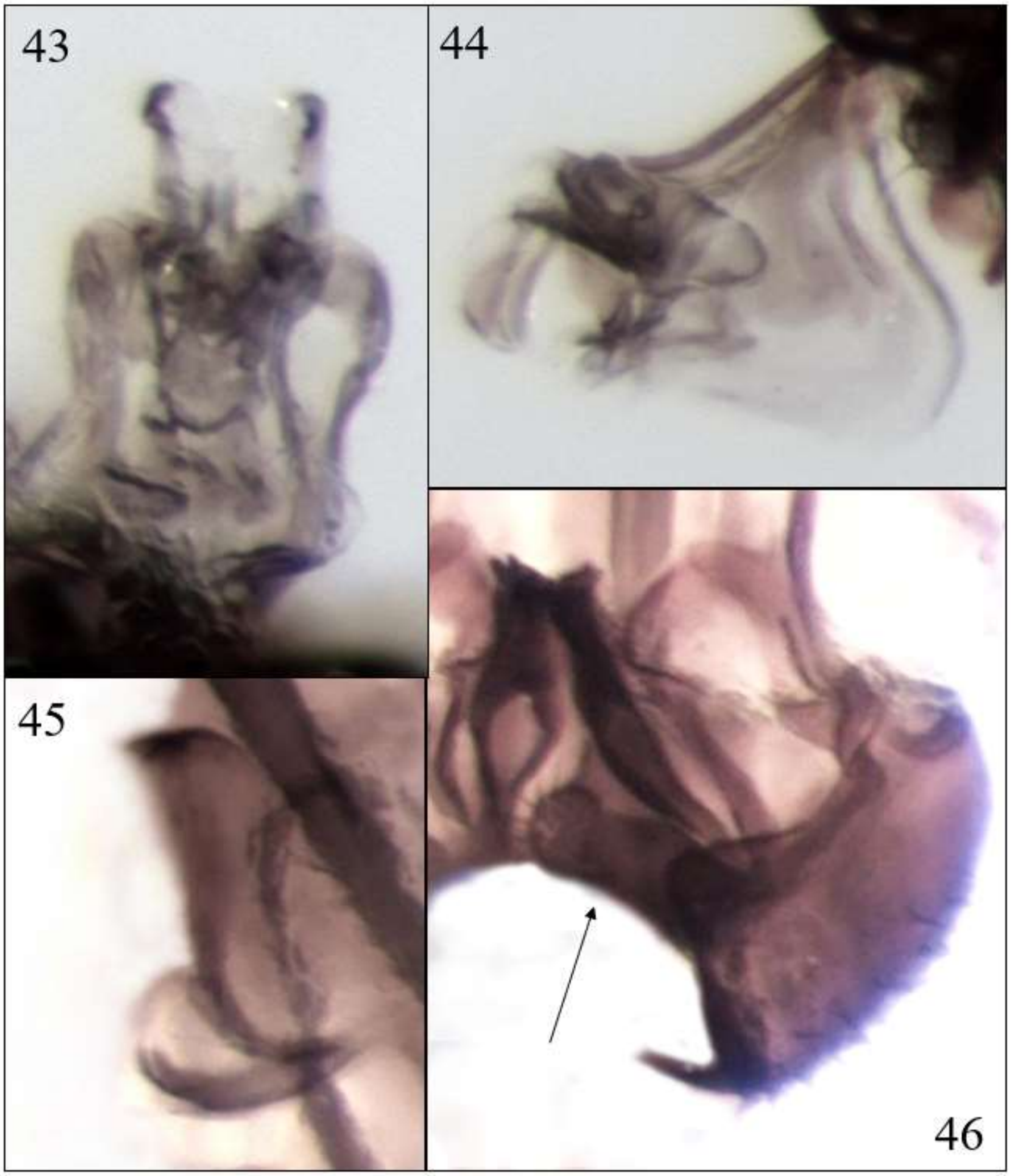


Figures 30-33. *Japanagromyza* morphospecies of Mitaraka, body lateral views. 30. *Japanagromyza* Mit-1; 31. *Japanagromyza* Mit-2; 32. *Japanagromyza* Mit-3, with inset showing arista with long pubescence; 33. *Japanagromyza* Mit-4, with inset showing subcostal vein joining with R_1 and distal margin of R_1 expanded near junction with costa.

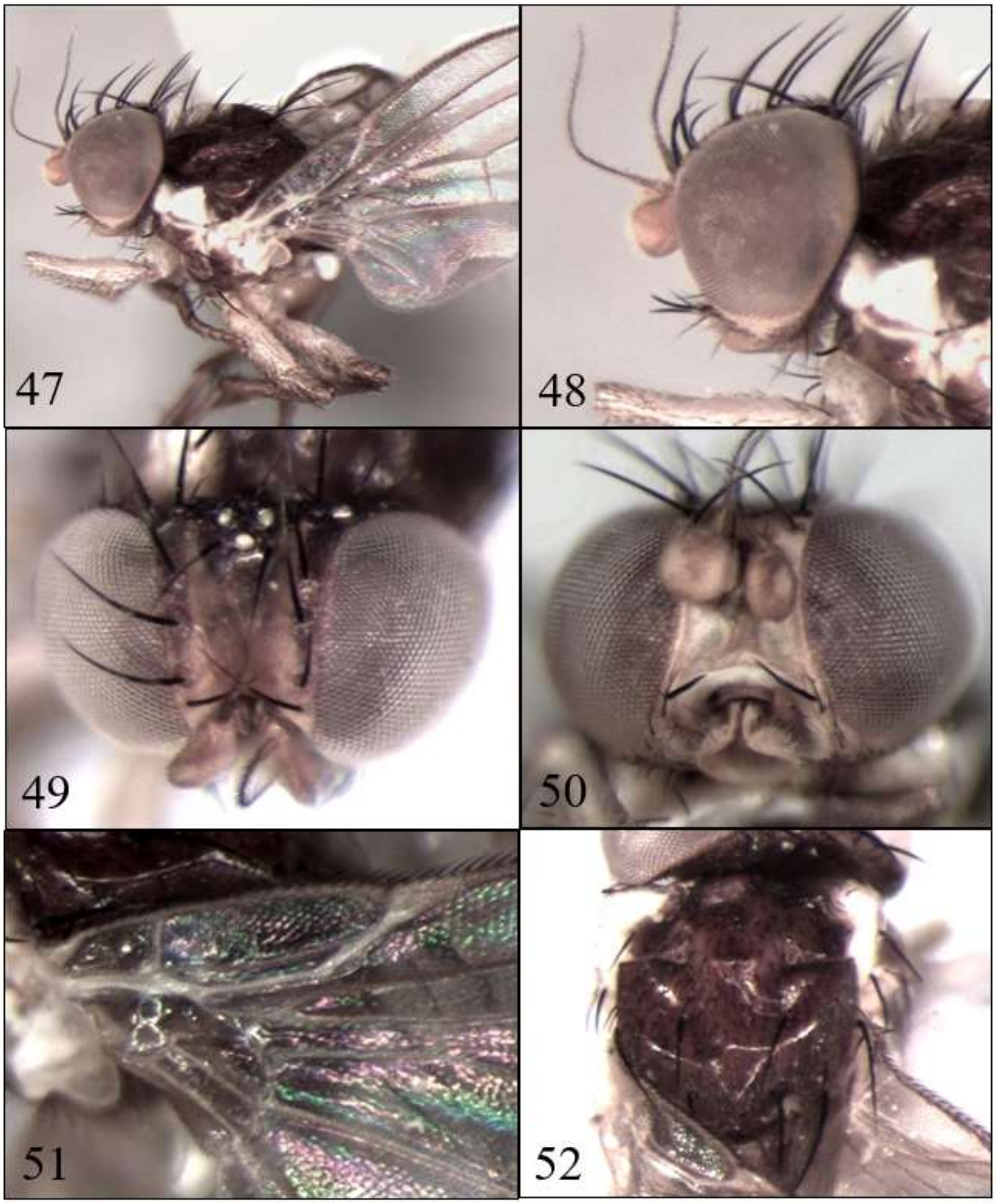


Figures 34-38. *Liriomyza touroulti* n.sp. 34. head in lateral view; 35. phallus, ventral view; 36. phallus, lateral view, inset at lower magnification; 37. ejaculatory apodeme (with phallus in background); 38. epandrium with surstyli.

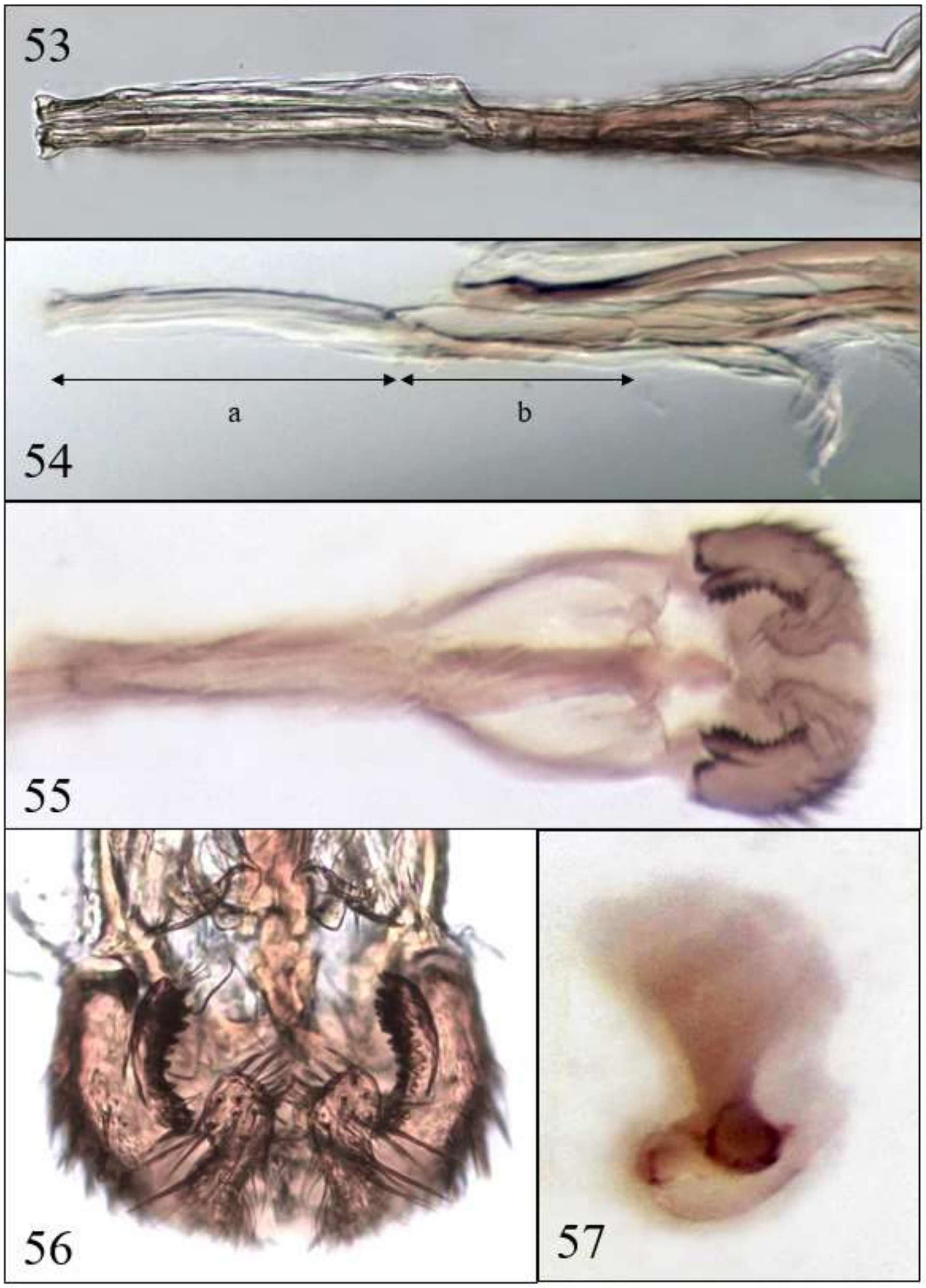
Figures 39-42. *Nemorimyza thanatos* n.sp. 39. head, lateral view; 40. head anterodorsal view; 41. phallus ventral view; 42. phallus anterolateral view.



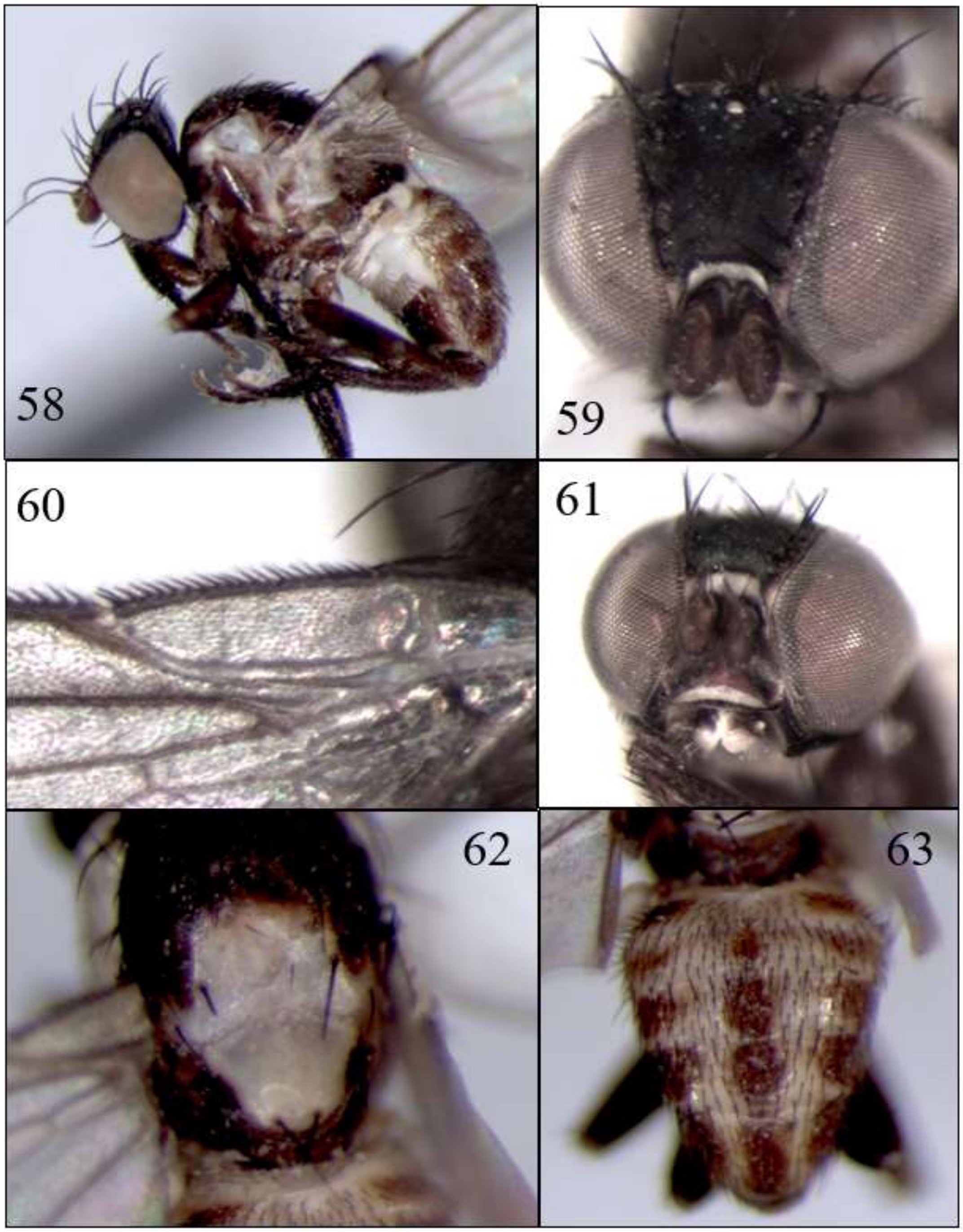
Figures 43-46. *Nemorimyza thanatos* n.sp. 43. phallus ventral view, low resolution; 44. phallus anterolateral view, low resolution; 45. ejaculatory apodeme; 46. epandrium, surstylus (arrow) and postgonites.



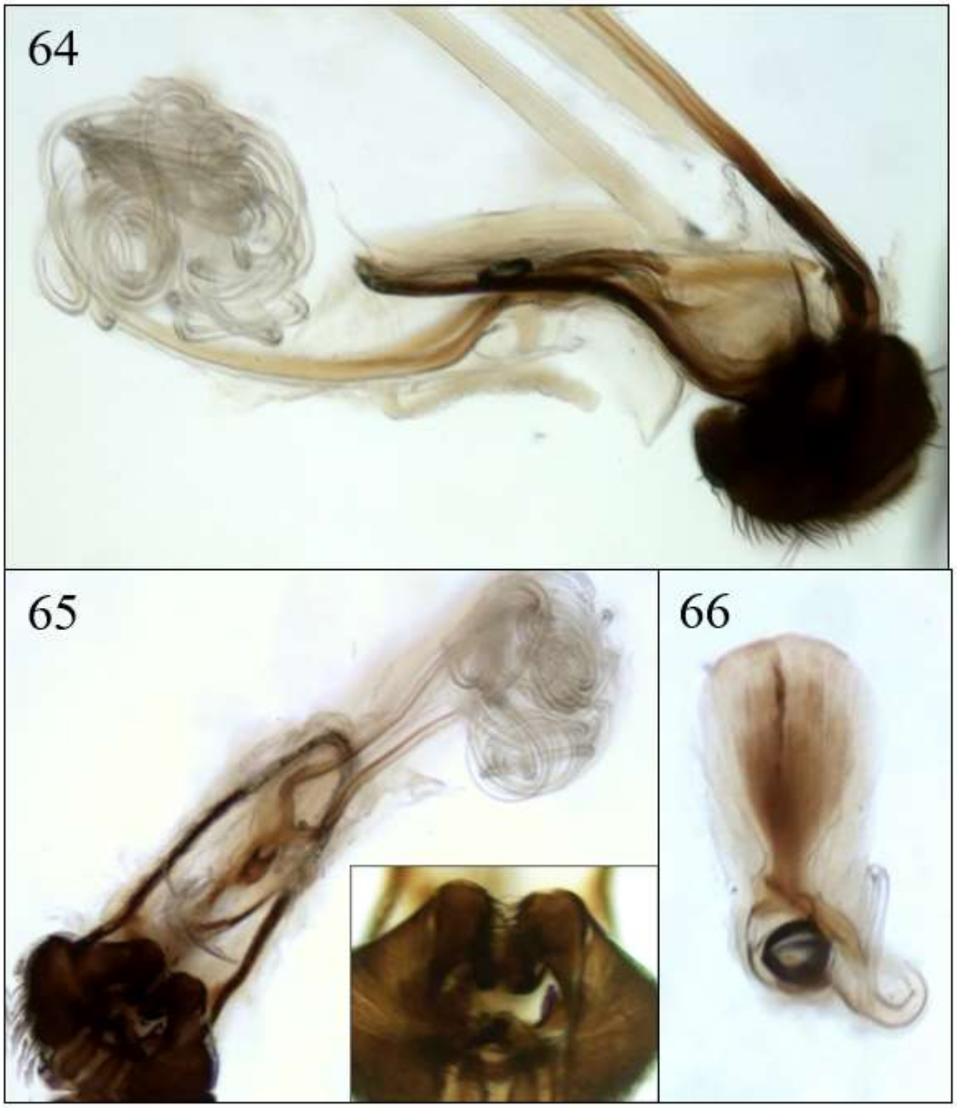
Figures 47-52. *Phytobia dalensi* n.sp. 47. body, lateral; 48. head, lateral view; 49. head, dorsal view; 50. head anterior view; 51. basal section of wing; 52. mesonotum, dorsal view.



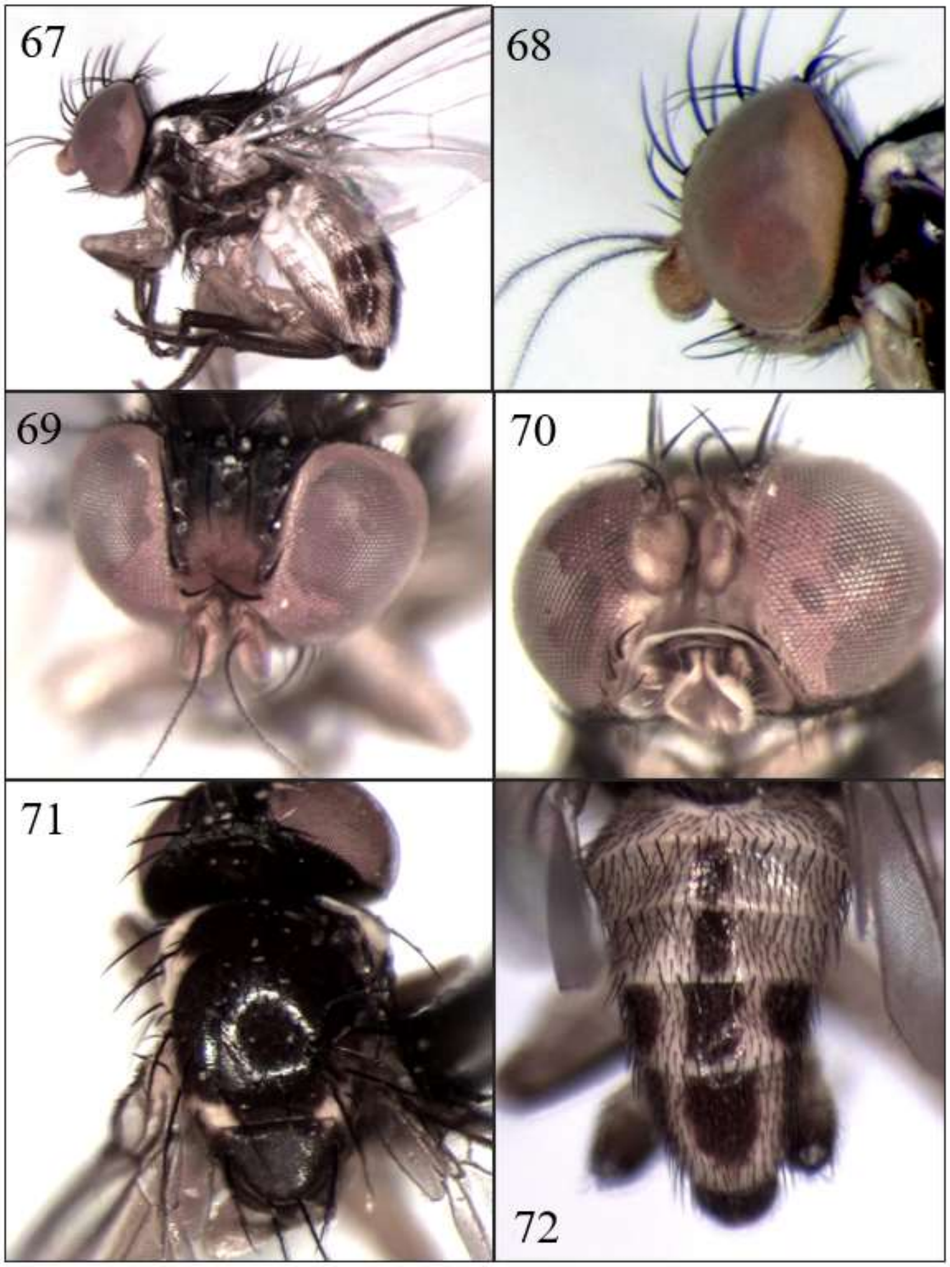
Figures 53-57. *Phytobia dalensi* n.sp. 53. phallus in ventral view; 54. phallus in lateral view (a: distiphallus; b: mesophallus); 55; hypandrium and epandrium; 56. epandrium with surstyli; 57. ejaculatory apodeme.



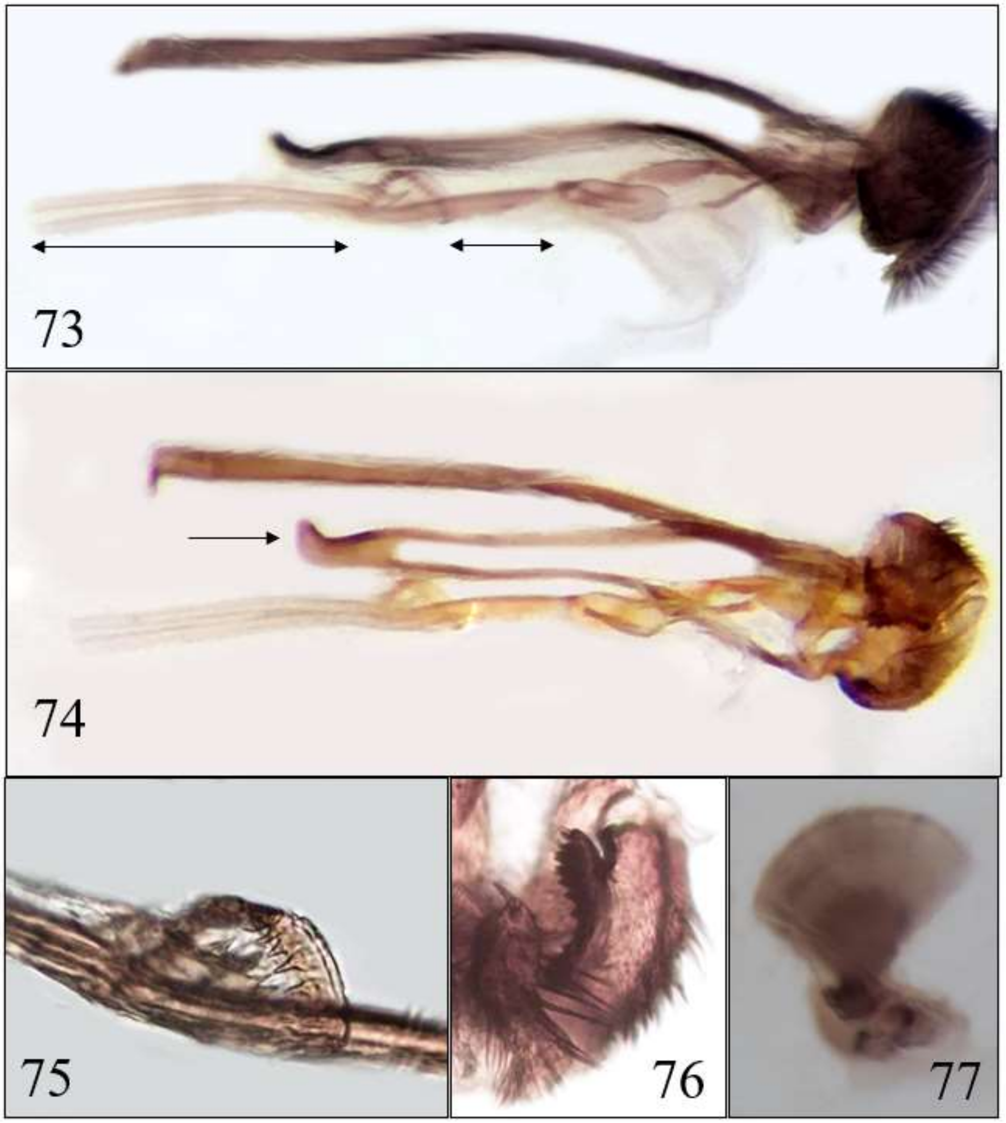
Figures 58-63. *Phytobia pluviasilvae* n.sp. 58. body, lateral; 59. head, dorsal view, showing frons; 60. wing; 61. head, anterior view; 62. mesonotum, posterodorsal view; 63. abdomen, dorsal view.



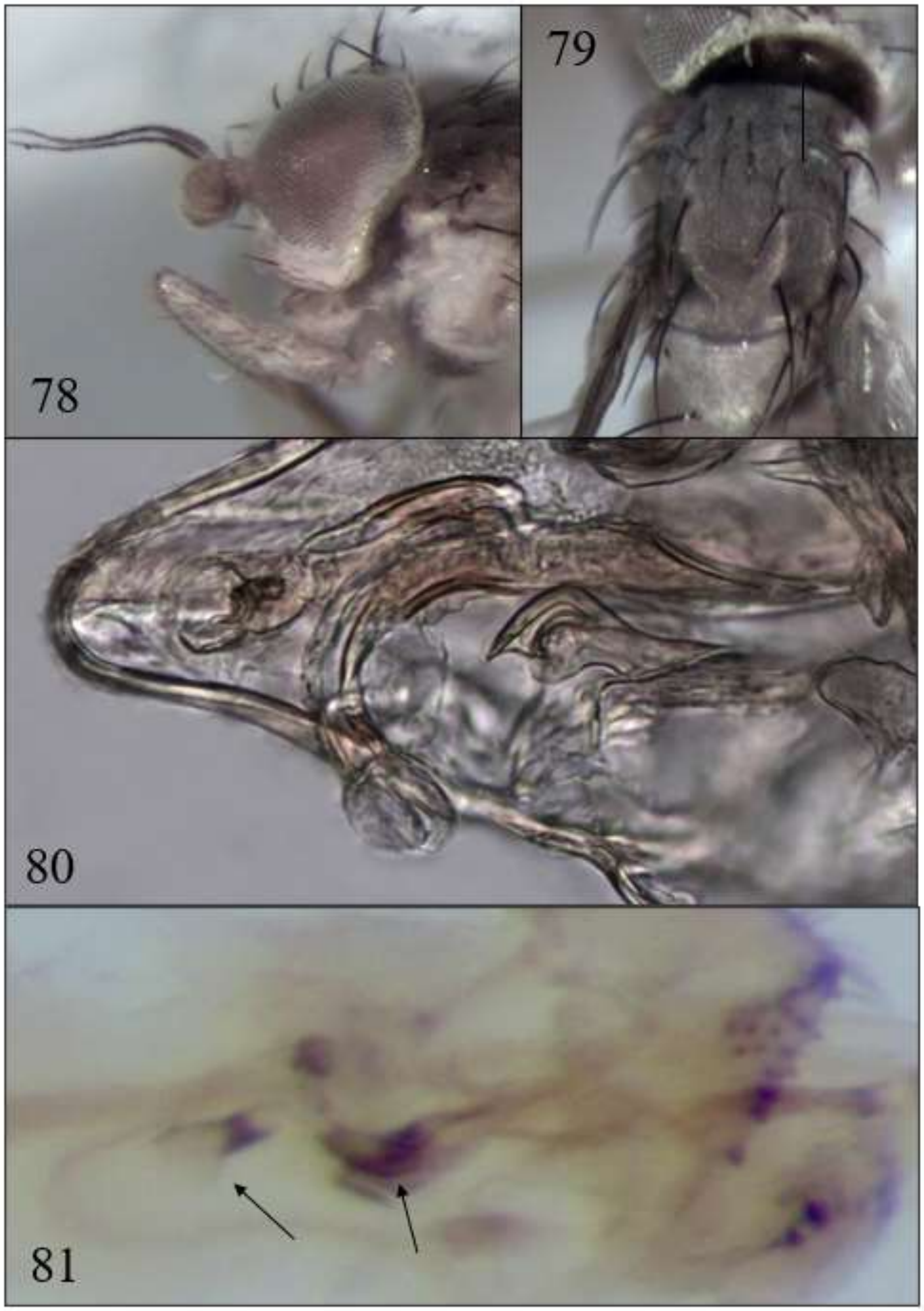
Figures 64-66. *Phytobia pluviasilvae* n.sp. 64. male genitalia with phallus in lateral view; 65. male genitalia showing hypandrium, phallus, and surstyli (inset) in ventral view; 66; ejaculatory apodeme.



Figures 67-72. *Phytobia corona* n.sp. 67. body, lateral; 68. head, lateral view; 69. head dorsal view; 70. head, anterior view; anterior view; 71. mesonotum, dorsal view; 72. abdomen, dorsal view.



Figures 73-77. *Phytobia corona* n.sp. 73. male genitalia in lateral view (apex of distiphallus apparently missing); 74. same, in lateroventral view. Arrow pointing distal end of hypandrium; 75. close-up of ring-like sclerite with teeth. 76. epandrium with surstylus, right side. 77. ejaculatory apodeme.



Figures 78-81. *Phytoliriomyza jurgensi* Spencer, 78. head, lateral view; 79 mesonotum and scutellum, dorsal view; 80. phallus, ventral view. 81. phallus and epandrium, lateroventral view. Arrows pointing funnel-shaped distal end of tubule and darker region of tubule.

CONNECTING STATEMENT

The previous chapters were focused on the agromyzid fauna of Mitaraka, French Guiana, collected in lowland rainforest and on an inselberg as part of “Our planet Reviewed” project. The next chapter is exploring the agromyzid fauna from another intensive biodiversity study: the Zurqui All-Diptera Biodiversity Inventory (ZADBI) project, and this happened in a different environment and locality: in mid-elevation cloud forests in Costa Rica. The opportunity to study the Agromyzidae in these two different environments is of great importance to fill in the gaps in distribution data for Agromyzidae and to learn more about their biology and habitat preference. The cloud forests are also greatly affected by climate change, and it is urgent to study their biodiversity to be able to monitor changes over time and to better protect these habitats.

Chapter 4 is formatted for submission to Zootaxa

CHAPTER 4. THE LEAF-MINER FLIES (DIPTERA: AGROMYZIDAE) OF COSTA RICAN CLOUD FORESTS

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4.1 Abstract

Cloud forests are known as important reservoirs of biodiversity and species endemism, but they are also one of the most threatened tropical ecosystems. This study investigates the leaf-miner flies (Diptera: Agromyzidae) collected over a period of one year at three different cloud forest localities in Costa Rica, as part of the Zurquí All Diptera Biodiversity Inventory (ZADBI). A total of 976 specimens of Agromyzidae was collected, resulting in the identification of 158 species (or morphospecies). This number is surprisingly high, considering that only 65 species of Agromyzidae were previously recorded in Costa Rica. Most of the species were collected from the main site (Zurquí) and only six species were collected at all three sites. Most of the species were collected from a Malaise trap set-up near a forest-edge at the main site (Zurquí), but the more limited sampling at the other sites added some unique species and one new genus record (*Haplopeodes*) for Costa Rica. Of the 14 previously recorded Neotropical genera of Agromyzidae, 12 were sampled at the cloud forest sites, in addition to the genus *Metopomyza* Enderlein, previously unreported from the Neotropical Region. The species richness reported for each of the genera collected at the cloud forest sites, exceeded previously known numbers for Costa Rica. The high diversity of the genus *Phytoliriomyza* Hendel (26 species) at the cloud forest sites is noteworthy as only 27 congeneric species were previously reported for the entire Neotropical region. The presence of two northern temperate genera (*Phytomyza* and *Metopomyza*) at the sites is discussed. Taxonomic treatment, illustrations and geographic distributions are provided for selected species. The high species richness, the presence of many new species, including some possible endemics to the cloud forests, support conservation efforts of these fragile ecosystems.

Key words: Diptera, Agromyzidae, Neotropical, Costa Rica, ZADBI, Zurquí, Cloud forest

4.2 Introduction

The loss of biodiversity is alarming and is of major concern, globally. Documenting species diversity is foundational because it allows a baseline understanding so that prioritization around conservation can be done effectively (Hanson 2004). Costa Rica is a relatively small Neotropical country (51,100 km^2), interesting for its species density (number of species per unit of area) and having probably one of the most diverse faunas relative to its size (Kohlmann et al. 2010; Obando 2002). Among Costa Rica's major terrestrial ecosystems, the cloud forests are well known for their species richness in addition to being considered the most important ecosystems for species endemism in the country (Kappelle 2016b; Obando 2002).

Cloud forests, also known as Tropical Montane Cloud Forests (TMCFs), or tropical montane rainforests, are normally found at altitudes between 1200 m and 2500 m (but can be as low as 500 m or higher than 3500 m) and are characterized by cool temperature, high humidity and a frequent or persistent cloud cover (Boehmer 2011; Jarvis & Mulligan 2011; Kappelle 2016b). Due to their ability to intercept cloud water, these forests serve as an important water reservoir for plants, animals, and stream inhabiting insects of the forests, in addition of providing freshwater to the human population of nearby cities (Bruijnzeel and Proctor 1995; Hostettler 2002; Janzen and Hallwachs 2021). But these forests are better known for their rich and impressive biodiversity, including some rare and endemic species of plants and animals, including visibly stunning species such as the resplendent quetzal (*Pharomachrus mocinno* De la Llave), a well-known bird species restricted to the cloud forests of Central America (Hostettler 2002).

Costa Rican's cloud forests are among the best studied of all TMCFs (Bruijnzeel et al. 2010, Lawton et al. 2016), and although they are known for their rich diversity of plants and animals (Kappelle 2016b; Nadkarni & Wheelwright 2000), there is still a lot to be learned about their insect fauna (Hanson 2014; Kappelle 2016a), especially at mid and high elevations where there is a lack of extensive sampling (Kappelle 2016b). While insects are not as well-known as birds or mammals, they are critical in ecosystems, and among insects, the flies (Diptera) are dominant in terms of diversity and ecological importance (Pape et al. 2009). Major collecting effort investigating the fly diversity in some of these forests, took place in 2013 as part of a large and comprehensive biodiversity project: the Zurquí All-Diptera Biodiversity Inventory (also known as the ZADBI project). With the expertise of 59 taxonomists from around the world, the flies were identified, resulting in an unexpected high diversity of species (Borkent et al. 2018; Brown et al. 2018). Agromyzidae, the leaf-mining flies (identified by the author), was ranked as one of the most species-rich Diptera families of the ZADBI project (Borkent et al. 2018).

The list of agromyzid species/morphospecies in Borkent et al. (2018, appendix 1) was used for quantitative purpose to fulfill the primary objective/question of this project, which was "how many dipteran species are present in a small patch of cloud forest at the primary site Zurquí?", and to compare this number with limited sampling at two other cloud forest sites. No interpretation or discussion of the findings was provided for the family Agromyzidae, although an extremely diverse and interesting fauna was found, in an environment that had never been studied.

Since the publications by Spencer (1973a, 1983), only a few studies have dealt with the Costa Rican fauna of Agromyzidae (Woodley and Janzen, 1995; Boucher, 2004; Boucher, 2005; Boucher, 2010; Boucher and Hanson, 2006; Boucher and Nishida, 2014). Research to date has not specifically studied the species found in mid to high elevation cloud forests of Costa Rica (or elsewhere), although a few agromyzid species are known to occur there, such as the two species of *Liriomyza* mining leaves of *Bocconia frutescens* (Papaveraceae) at 1538 m elevation in Monteverde (Boucher and Nishida 2014). With only 65 species previously described from Costa Rica (Table 2), there is no doubt that many more species await discovery.

The objective of the present work is to provide the faunistic analysis and discussion of the diversity of Agromyzidae found at three Costa Rican cloud forest sites as part of the ZADBI project and to examine this species richness relative to previous knowledge of the agromyzid fauna of Costa Rica and the Neotropical region. Preliminary results on species identification, including taxonomic notes and illustration of selected species are provided, and the presence of some unique species apparently endemic to tropical cloud forests are also discussed.

4.3 Materials and Methods

Diptera were sampled by trained technicians at three different cloud forest sites in Costa Rica: Zurquí de Moravia, San José Province (10.047°N, 84.008°W, 1600 m), Tapantí National Park, Cartago Province (9.720°N, 83.774°W, 1600 m) and Las Alturas, Puntarenas Province (8.951°N, 82.834°W, 1540 m) (Fig. 1) for a little more than one year. The primary study site, Zurquí, consisted of a 150 x 266 m patch of a mid-elevation (1600 m) cloud forest, surrounded by pasture (Fig. 2) and situated on the southwest margin of Braulio Carrillo National Park. This site was sampled continuously with two Malaise traps running from Sept. 12, 2012 to Oct. 18, 2013, with Malaise trap #1 placed near a forest-edge (Fig. 3C) and Malaise trap #2 inside of the forest, near a small permanent creek (Borkent & Brown 2015; Borkent et al. 2018; Brown et al. 2018). Additional sampling methods were used at this site for three full days every month including a variety of light traps, emergence traps over a wide array of terrestrial and aquatic substrates, baiting traps with various attractants (fruit, carrion, human and pig dung), yellow pan traps, a flight-intercept trap, 4 additional Malaise traps (including a dry Malaise trap using potassium cyanide as the killing agent (to capture larger specimens dry) and a canopy Malaise trap) (Borkent & Brown 2015; Borkent et al. 2018). In addition to these traps, hand collecting and/or sweeping was also performed during these 3 days each month. Finally, to increase the number of species obtained, an intensive "Diptera Blitz", with 18 coauthors was organized at Zurquí to collect on-site for a few days (August 5-9, 2013). The other cloud forest sites were sampled with a single Malaise trap placed on the forest edge: Tapantí, approximately 40 km southeast from Zurquí (Fig. 1), was sampled from Oct. 28, 2012 to Oct. 13, 2013 and the other site, Las Alturas, situated at approximately 180 km southeast from Zurquí (Fig. 1), was sampled from Oct. 13, 2012 to Oct. 13, 2013 (Borkent et al. 2018). Malaise traps #1 and #2 at Zurquí and those at the other two sites were emptied weekly. Diptera specimens collected from all three sites were processed and sorted to family level and mounted/pinned by technicians in Costa Rica, except for some specimens, considered redundant, that were kept in ethanol. After preparation, the specimens were sent to appropriate family specialists (Borkent et al. 2018).

The Agromyzidae specimens were received by the author (in 2015), who identified all specimens to genus level using a Central American key to agromyzid genera (Boucher 2010), followed by morphospecies identification based on external morphological characters in addition to male genitalic characters (dissection procedures outline in Boucher & Pollet 2021). The morphospecies (Table 1), were named using the genus name + species number, preceded by the prefix ZADBI. The species numbers are not always following a specific order and are sometimes not in a logical sequence, but they are important for association to specific notes and species database. Some agromyzids were identified to previously named species using the keys/genitalic images to Costa Rican agromyzids (Spencer 1983), Neotropical Agromyzidae (e.g. Monteiro, Carvalho-Fihlo & Esposito 2015, Spencer 1963, Spencer 1973b, Spencer & Stegmaier 1973, Zlobin 1996), and Nearctic Agromyzidae (Spencer & Steyskal 1986).

In addition to the ZADBI material, a few other Neotropical specimens were examined for comparison and for extra distribution data. These specimens are from the following institutions: Lyman Entomological Museum, Ste-Anne-de-Bellevue, Quebec (LEMQ); Canadian National Collection of Insects and Arachnids, Ottawa, Ontario (CNC); Royal Belgian Institute of Natural Sciences (RBINS); and (pictures only) from the National History Museum, London (BMNH). Species distribution is based on published data and on specimens examined. Morphological terminology follows Cumming & Wood (2009). Abbreviations are as follows: dc: dorsocentral setae (numbered starting posteriorly); ors: orbital setae; ori: frontal setae.

The Jaccard's Index (JI) (Jaccard 1901), a well-known measurement of similarity between two sets of patterns (Fletcher & Islam 2018), was used to measure the similarity of species between the different cloud forest sites (calculated by taking the size of the intersection divided by the size of the union of the two sets) with the result multiplied by 100 to obtain a percentage (Fletcher & Islam 2018). This index was selected due to the small number of sets to analyze and for its usefulness for data dealing with presence-absence only (Chung et al. 2019).

Digital images of specimens were taken with a luminera infinity camera 1 mounted on a stereo microscope Leica MZ 12.5 or a compound microscope Leica DMLB. Image stacking was performed using Combine ZP software.

The specimens collected during the ZADBI project will be deposited in el Museo Nacional de Costa Rica, San José, Costa Rica (MNCR) which recently assumed responsibility for the collection previously housed at the Instituto Nacional de Biodiversidad in Costa Rica.

4.4 Results

4.4.1 Generic composition and overall species richness

Out of 976 agromyzid specimens received, 891 specimens could be identified into 158 species or morphospecies (Table 1). The rest of the specimens, consisting of 85 females, in the genera *Phytoliriomyza* and *Melanagromyza* could not be confidently delineated or associated with conspecific males and were left unidentified (excluded from Table 1).

Most of the species collected at the cloud forest sites belonged to the genus *Liriomyza* (31 species), followed closely by *Melanagromyza* (30 species) and *Phytoliriomyza* (26 species). Other genera included *Calycomyza* (16 species), *Cerodontha* (11 species), *Ophiomyia* (10 species), *Phytobia* (7 species), *Metopomyza* (6 species), *Nemorimyza* (5 species), *Phytomyza* (5 species), *Japanagromyza* (4 species), *Agromyza* (4 sp.) and *Haplopeodes* (3 species) (Table 1), with the genus *Metopomyza* and *Haplopeodes* newly recorded in Costa Rica. The species number reported for each of these genera exceeded their previously known diversity for the country (Fig. 4). The genus *Pseudonapomyza* previously reported in Costa Rica, from a single species (Table 2), was not collected at the sites.

Of a total of 14 previously recorded Agromyzidae genera in the Neotropical region, all were present in the cloud forest sites, except for *Pseudonapomyza* and *Amauromyza* (Fig. 5). The genus *Metopomyza*, previously unreported from the Neotropical region, was present at the cloud forest sites (Fig. 5).

The three most species-rich genera at the cloud forest sites (*Liriomyza*, *Melanagromyza* and *Phytoliriomyza*) were also the most abundant in the samples. *Liriomyza*, was by far the most abundant with 424 specimens, due to three common species *Liriomyza* ZADBI-2, *L*. ZADBI-8 and *L*. ZADBI-9 (Table 1) accounting for 58% of the *Liriomyza* specimens.

4.4.2 Agromyzid species richness at the study sites

Species richness was especially high at the main site (Zurquí) with 117 species/morphospecies identified (Table 1). Zurquí, was the most productive when considering the number of species obtained from all sampling methods (117 species) or considering only the results from Malaise trap #1 (112 species), followed by Tapantí (51 species) and Las Alturas (39 species) (Table 1; Fig. 6). Of the 117 species (all sampling methods) identified from Zurquí, 76 species were restricted to this site, compared to 21 species found only at Tapantí and 18 species found only at Las Alturas (Fig. 6). When comparing the agromyzid fauna of the three sites (with Zurquí all sampling methods), Zurquí and Tapantí were the most similar, sharing 28 species, and having a Jaccard Index (JI) of similarity of 20% (Fig. 6). When considering the diversity from Malaise trap #1 only, Zurquí and Tapantí are also the most similar with a JI of 19.8%. Six species were shared by all three sites (Fig. 6): *L. sabaziae* Spencer, *Liriomyza* ZADBI-2, ZADBI-9, L. ZADBI-12, L. ZADBI-17, L. ZADBI-20 and *Melanagromyza* ZADBI-1 (Table 1). Species from most genera were collected at least at two of the sites, except for *Agromyza* species that were collected only at Zurquí, and *Haplopeodes* species that were collected only at Tapantí (Fig. 7).

4.4.3 Collecting methods at Zurquí

Most of the sampling methods used at Zurquí caught at least one Agromyzidae (Fig. 8), except the baited traps and yellow pan traps. The two Malaise traps, #1 and #2, set-up permanently for over 1 year collected 112 and 4 species respectively, while the other Malaise traps set-up for 3 days each month collected a few additional species: Malaise trap #3 (10 sp.); Malaise trap #4 (6 sp.); other Malaise traps (canopy and dry Malaise) (2 sp.). Other trapping methods set-up or performed for 3 days each month included emergence trap over vegetation (2 sp.), emergence trap over water (1 sp.), hand collecting (sweeping) (4 sp.), light traps (bucket and pan light traps) (4 sp.), and flight intercept trap (6 sp.) (Table 1; Fig. 8). Malaise trap #1 collected 88 unique species, while Malaise trap #2 also set-up for a full year, in the forest, did not add any new agromyzid species to the inventory. The other trapping methods collected only 5 unique species (not collected by Malaise trap #1) (Fig. 8). These are *Liriomyza* ZADBI-29 and *Metopomyza* ZADBI-6 (flight intercept trap); *Metopomyza* ZADBI-2 (Malaise trap #3); *Nemorimyza ranchograndensis* (Spencer) (hand collecting) and *Phytoliriomyza* ZADBI-8 (Malaise trap #4) (Table 1).

4.4.4 Species previously recorded from Costa Rica

Based on preliminary results, thirteen species from the cloud forest sites correspond to species already reported from Costa Rica (Table 1). These are as follows: *Agromyza fusca* Spencer (Figs. 9-16) (Annex 1), *Calycomyza devia* Spencer, *Calycomyza meridiana* (Hendel) (Figs. 20-24) (Annex 1), *Cerodontha dorsalis* (Loew), *Liriomyza archboldi* Frost (Figs. 31-34, 53) (Annex 1), *Liriomyza sabaziae* Spencer, *Melanagromyza bidentis* Spencer, *Melanagromyza minima* (Malloch), *Nemorimyza maculosa* (Malloch), *N. posticata* (Meigen), *N. ranchograndensis* (Spencer), *Ophiomyia puntohalterata* (Frost) and *Phytoliriomyza pilosella* Spencer (Table 1).

4.4.5 Species newly recorded in Costa Rica

Based on preliminary results, seven previously named agromyzid species are for the first time recorded in Costa Rica. These are as follows: *Calycomyza dominicensis* Spencer (Fig. 17-19, 54) (Cuba, Dominica, Guadeloupe) (Annex 1), *C. opaca* Zlobin (Dominica, Guadeloupe, Martinique, Saint Lucia), *Cerodontha* (*Butomomyza*) *puertoricensis* Spencer (Puerto Rico), *Japanagromyza sasakawai* Monteiro, Carvalho-Fihlo & Esposito (Figs. 27-30, 55) (Brazil) (Annex 1), *Melanagromyza floridensis* Spencer (Florida, Caribbean, Venezuela), *M. wedeliae* Spencer (Florida, Colombia) (Figs. 40-42) (Annex 1) and *Phytoliriomyza leechi* Spencer (California) (Fig. 47).

4.4.6 Species new to science

The majority of the agromyzids collected from the cloud forests appear to be undescribed and many are already confirmed as new. These include *Cerodontha* (*Diz.*) n.sp. near *Cerodontha* (*Diz.*) *scirpioides* Zlobin (Figs. 25, 26) (Annex 1), *Liriomyza* n.sp. near *L. commelinae* (Frost) (Figs. 35-39) (Annex 1), and multiple species of *Metopomyza*, *Cerodontha* (*Cer.*), *Haplopeodes* and *Phytomyza*, and many more are expected (Figs. 43-46). A conservative estimate is that at least 60% of the species collected from the cloud forest sites are new to science.

4.5 Discussion

An incredible diversity of Agromyzidae resulted from this one-year survey in Costa Rican cloud forests, even surpassing the number of species known for the entire country. The genus *Phytoliriomyza* was especially rich in species, closely matching its known diversity for the whole Neotropical region. Another unexpected finding was the presence of the Holarctic genus *Metopomyza*, with species collected at Zurquí and Tapantí. There were only three Nearctic genera that were still unreported from the neotropics, these included *Metopomyza*, *Aulagromyza* Enderlein and *Euhexomyza* Lonsdale (with most species of Nearctic *Euhexomyza* previously included in *Hexomyza* Enderlein, see Lonsdale 2014). It is expected that the two still missing genera (*Aulagromyza* and *Euhexomyza*) will be eventually found in the neotropics, but probably at mid or high elevation as it was observed for *Metopomyza*. Six new species of *Metopomyza* were collected, and all include at least one male specimen, which will be important for the formal species descriptions and diagnosis. This pattern of distribution of a northern temperate taxon being present (and probably confined) in Neotropical mountains is not unique to *Metopomyza*, for example it was previously reported in the family Mycetophilidae (Diptera) (Kurina & Oliveira 2015). Another mostly north temperate genus (although not restricted to mountains), *Phytomyza* was present at the cloud forest sites with five species, collected mainly at Zurquí, although one species was collected at Tapantí and Las Alturas. The particular species richness of Agromyzidae found in the mid-elevation cloud forests may be explained in part by an overlap of Nearctic and Neotropical faunas as it is observed for the Diptera richness found in high Mexican mountains (Amorim 2009). In addition to this species richness, the cloud forests appear to be home to a unique agromyzid fauna. High species richness and endemism are two characteristics of cloud forests (Kappelle 2016b).

4.5.1 Generic composition and overall species richness

Agromyzid species richness found at the Costa Rican's cloud forest sites (158 species) exceeded the known species richness of the entire country (65 species) (Table 2) by approximately 135% and represented about 30% of the known Neotropical agromyzid diversity (nearly 500 species). These numbers are most likely an underestimation of diversity considering that numerous female specimens could not be confidently identified to morphospecies.

The two most species rich genera at the cloud forest sites: *Liriomyza* and *Melanagromyza* (Table 1) also reflects the most diverse genera in the Neotropical region with approximately 105 and 96 species respectively (Fig. 5), but *Phytoliriomyza* which is in third place for species richness at the cloud forest sites (26 species) is not known to be especially diverse in the Neotropical region with 27 species (Fig. 5). This high number of *Phytoliriomyza* species found at the sites is noteworthy as it almost surpasses the number of species previously known for the entire Neotropical region (27 species). Two of the *Phytoliriomyza* species were identified to previously named species (Table 1), namely *P. leechi* (Fig. 47) and *P. pilosella*, but many of the remaining species are expected to be new. The *Phytoliriomyza* of the cloud forests are typical of the genus, with the phallus ending in elongate paired tubules (Figs. 47-52) and the surstylus and/or epandrium with a characteristic comb of long bristles or fingerlike projections. This high diversity of undescribed species is most probably related to the rich diversity of potential host plants for this genus in the cloud forests. *Phytoliriomyza* is the only confirmed agromyzid genus known to feed on Bryophytes (liverworts) and one of the rare and the dominant genera feeding on ferns (Spencer 1990). Interestingly, montane cloud forests are known to harbour a large diversity of these plants (Glime 2019).

The presence of two northern temperate genera, *Phytomyza* and *Metopomyza* is also of particular interest. *Phytomyza* is the largest agromyzid genus with 774 world species (Lonsdale and von Tschirnhaus 2021) but only 19 species have been reported in the Neotropical region. Spencer (1977) mentioned that *Phytomyza* was a cold adapted genus that was virtually absent from the tropics except at high altitudes. For example, the only two *Phytomyza* species known from Venezuela, were collected in high elevation near Colonia Tovar, Aragua and in Mérida (Spencer 1973b). The finding of five species at the cloud forest sites supports this and suggests that the genus *Phytomyza* may be more diverse in the Neotropical region than expected, but only at higher elevation. Only one *Phytomyza* species was previously known in Costa Rica (Spencer 1983) and this is despite the examination of many hundreds of specimens from the region (Spencer & Stegmaier 1973). The genus *Metopomyza* includes 21 species in the world, mostly restricted to the Nearctic and Palaearctic regions except for one Oriental species. No species were previously recorded in the Neotropical region but 6 new species are now recorded from the Costa Rican cloud forests. Neotropical *Metopomyza* species may be endemic to TMCFs considering the absence of this genus from previous Neotropical taxonomic and faunistic studies on Agromyzidae. This is also supported by an interesting additional specimen examined from a cloud forest in Ecuador (Podocarpus National Park, 1000 m, Marc Pollet & Anja De Braekeleer; 1F, RBINS), which also represents a new species of *Metopomyza* (unfortunately a female). These two north temperate genera, *Phytomyza* and *Metopomyza* were absent from another recent survey in a lowland tropical rainforest in French Guiana (Boucher & Pollet, 2021).

4.5.2 Agromyzid species richness at the study sites

The species richness of Agromyzidae at each study site was impressive when compared to other Diptera families. The Agromyzidae was ranked as one of the most species rich Diptera families at Zurquí (position 13th out of 73 families). The number of species of Agromyzidae found at Zurquí is remarkable given the small area sampled (150 x 266 m). Agromyzidae was in third place (behind Ceratopogonidae and Tachinidae) at Tapantí, and in second place (behind Tachinidae) at Las Alturas (out of 55 families studied at the three sites) (Brown et al. 2018; Borkent et al. 2018).

These two additional families (Tachinidae and Ceratopogonidae) are among the most species-rich families in the world with 8500 and 6267 species respectively (Brown et al. 2018), while Agromyzidae with approximately 3200 world species, is far behind. Considering that Agromyzidae is not included in the top 15 of most species-rich Diptera families (Brown et al. 2018), but among the most species-rich at the cloud forest sites, suggest that the Agromyzidae fauna is probably a lot more diverse than is presently known, especially in the Neotropics where most regions lack diversity inventories.

When comparing the agromyzid fauna of the three sites, Zurquí and Tapantí were the most similar, agreeing with the results found in Borkent et al. (2018) for all Diptera studied at the three sites (55 families). Despite this similarity, the sites showed an important species turnover, especially noticeable at Zurquí where 65% of the species were unique to this site. Although Zurquí and Tapantí are only about 40 km from each other, the isolated nature of TMCFs is known to provide ideal condition for speciation and high species turnover between TMCFs sites is common (Jones et al. 2008; Vázquez-García 1993; Wilson and Rhemtulla 2018).

Some species were present at only one site, and very common. For example, *Liriomyza* ZADBI-8 (81 specimens) was one of the most abundant species at Zurquí, and although a Malaise trap was set-up for an entire year at the other sites, the species was not collected elsewhere. It cannot be explained why this species was not found at the other sites. Although the identity of this species is not yet fully determined, it appears to be a new species closely related to *Liriomyza cordillerana* Sehgal, a northern temperate species occurring in the western Canadian mountains (Sehgal 1968, Spencer 1969), western United States and in the mountains of northeast Mexico (3000 m) (Zlobin 1997). *Liriomyza* ZADBI-8 also shows some similarities with *L. irazui* Spencer, described from Costa Rica at the base of volcán Irazú (3120 m) (Spencer 1983). It is interesting that all these species are known from high elevations. *Liromyza* ZADBI-8 is part of the *flaveola*-group of species, which are known to be Poaceae feeders (Zlobin 2002).

Only a few species were present at all three sites. These are in the genus *Liriomyza*, except for one species in the genus *Melanagromyza*. These widely distributed species across the sampled sites may have a more extensive distribution and may not be restricted to cloud forests. A few species of *Liriomyza* are oligophagous or highly polyphagous (including some important pest species) and are widely distributed. This is the case for one of the species collected from all three sites: *Liriomyza sabaziae* Spencer, (identified as *Liriomyza* ZADBI-9 in Borkent et al. 2018) an oligophage feeding on multiple species of Asteraceae (Spencer 1990). It was originally described from Venezuela and Colombia (Spencer 1963), but was later found in California, Costa Rica and Peru (Korytkowski 2014; Spencer 1981, 1983).

Although the two secondary sites (Tapantí and Las Alturas) were not as productive in terms of species found, they were important in bringing some important additional data for future studies, in addition to reporting for the first time the presence of the genus *Haplopeodes* in Costa Rica. Previously thirteen species of *Haplopeodes* were reported from the Neotropical region (Martinez & Étienne 2002; Korytkowski 2014), all from South America, and mainly from Argentina and Brazil.

4.5.3 Collecting methods at Zurquí

The main site (Zurquí) was the most productive in terms of species number, although the multiple collecting techniques that were used at this site (compared to a single Malaise trap used at each of the other two sites) does not explain the higher diversity found at Zurquí. Indeed, the bulk of the species (95.7%) were collected with a single Malaise trap (Malaise trap #1) set-up permanently for an entire year. The additional sampling methods used at Zurquí were not very productive for Agromyzidae (collecting only 4% of the species), although they were found to be useful for collecting other Diptera families (Borkent et al. 2018). Nevertheless, these additional sampling methods added three new species of Agromyzidae to the survey: *Liriomyza* ZADBI-29 (flight intercept trap), *Metopomyza* ZADBI-6 (flight intercept trap) and *Metopomyza* ZADBI-2 (Malaise trap #3). These specimens will be important for future studies. The *Liriomyza* is an unusual species with brown mesonotum and scutellum and a yellow first flagellomere, similar to

Liriomyza flaviantennata (Spencer) described from Brazil. Surprisingly, Malaise trap #2, which was also set-up for a full year (in the forest at the stream), caught less species (and no unique species) than Malaise trap #3 and #4 that were set-up for only 3 full days per month for one year.

Contrastingly, Malaise trap #2 was useful in the "all Diptera" inventory (Borkent et al. 2018), catching 23% of the species (excluding Phoridae), although this Malaise trap was less productive than Malaise trap #1, which caught half of the Diptera species (50.4%) recorded at Zurquí. The success of Malaise trap #1, set-up permanently at a forest edge (Fig. 3C) is impressive, collecting most of the agromyzid fauna recorded at Zurquí. This high productivity may be explained by the well-known "edge effect" (Ries et al. 2004) in addition to the wind, which could be responsible for bringing a higher number of species to this trap (Borkent et al. 2018). The malaise traps installed at the forest edge at each of the other two sites (Tapantí and Las Alturas) for an entire year were not as productive and this could be attributed to multiple factors that affect the efficiency of Malaise traps in general: temperature, precipitation, wind, trap orientation and placement (e.g. in shaded location or exposed to the sun), as well as height of surrounding vegetation (Matthews and Matthews 1971; Steinke et al 2021).

Among the sampling methods, the emergence trap over water is particularly interesting, as it collected one specimen lumped in with *Phytoliriomyza* ZADBI-6 (all females, and possibly representing more than one species). No Agromyzidae are aquatic in the larval stage, but a few are known to feed on the internal tissues of aquatic plants. These are in the genus *Agromyza*, *Phytomyza* and *Liriomyza* (Spencer 1990). Unfortunately, the specimen is a female and is therefore unidentified.

4.5.4 Species previously recorded from Costa Rica

A few species collected at the cloud forest sites corresponded to previously named species that were already reported in Costa Rica, although slight morphological differences were sometimes present (Annex 1). Some of them are widely distributed in the New World and are also present in the Palaearctic region. These are *Cerodontha dorsalis*, *Nemorimyza maculosa* and *N. posticata* (Meigen) (Table 1), three well known species of some economic importance (Boucher 2002; Černý et al. 2018; Sousa & Couri 2021; Spencer 1973c). Other species, such as *Calycomyza meridiana* and *Liriomyza commelinae* have a more southern distribution and are widespread in the Neotropical region (Annex 1), while a few have a more limited distribution and are currently known from Florida, the Caribbean and Costa Rica (e.g. *Liriomyza archboldi* (Fig. 53) and *Phytoliriomyza pilosella*) (Annex 1), a distribution pattern commonly observed in other Neotropical species (Spencer & Stegmaier 1973).

4.5.5 Species newly recorded in Costa Rica

Among the new Costa Rican records, a few species were previously known only from the Caribbean, including *Calycomyza opaca* and *Calycomyza dominicensis* (Fig. 54) (Annex 1), whereas others show a more important range extension, including *Japanagromyza sasakawai* previously known from northern Brazil (Fig. 55), or *Phytoliriomyza leechi*, previously known from Northern California. This latter species has a distribution pattern similar to *Liriomyza lupini* Spencer, a species originally known from high altitude in the Sierra Nevada, California and later found at 2820 m elevation near Volcán Irazú in Costa Rica (Spencer 1983). Some of these species are further discussed in Annex 1. Many new species and new Costa Rican records are expected from the list of morphospecies (Table 1). For example, 16 species of the genus *Calycomyza* were collected, with four species identified (Table 1). Although the identities of the remaining species are not yet confirmed, they do not correspond to any previously known Costa Rican species. Many of them exhibit a combination of unique characters including some with 3+1 dorsocentral setae (dc), a character observed in only a few Neotropical species (e.g., *C. compositana* Spencer, *C. cruciata* Valladares, *C. cuspidata* Sasakawa, *C. longicauda* (Blanchard)) in addition to other less commonly seen characters such as a brown face (C. ZADBI-8 and C. ZADBI-11), brownish frons (C. ZADBI-1) (similar to *C. devia* and *C. obscura* Spencer) or brown halter (C. ZADBI-7) (similar to *C. palmaris* Spencer and *C. parilis* Spencer, both from Venezuela). Further taxonomic work will confirm if these morphospecies are previously named species newly recorded in Costa Rica, or if they represent new species. The real distribution of these species in Costa Rica is unknown. Further collecting in different habitats in lowlands and highlands will be necessary to confirm these biogeographic patterns.

4.5.6 Species new to science

Based on preliminary results, many species from this study are recognized as new to science. These include multiple aberrant and/or distinctive species included in the genus *Haplopeodes* (with an unusual, elongated halter) (Fig. 44), *Cerodontha (Cer.)* (with an extremely large lunule and projecting frons) (Fig. 43), *Liriomyza* and *Phytomyza* (each with a greatly enlarged flagellomere) (Fig. 35, 45), *Cerodontha* (*Diz.*) (with a reduced lower ors) (Fig. 25) and multiple *Metopomyza* species with very large orbital plates (Fig. 46). It is without any doubt that many additional species will be confirmed as new with further study of the material.

The family Agromyzidae is still poorly studied in Costa Rica, which makes it premature to discuss the broader distribution patterns of individual species. Some of the species collected at the cloud forest sites may have a more widespread distribution in Costa Rica and elsewhere in the Neotropics, but some may be restricted in their distribution. Tropical Mountain Cloud Forests are recognized for their high level of endemism for many groups of organisms, including some insect taxa (Jones et al. 2008; Germann 2012; Montoya et al. 2012; Jocque et al. 2013; Kappelle 2016a). Preliminary results based on specimens examined from the ZADBI project and from other collections, suggest that some agromyzid taxa may be restricted to these habitats. These include a few species of *Cerodontha* (*Dizygomyza*) with a reduced lower upper orbital seta (see discussion in Annex 1, under *Cerodontha* (*Diz.*) n.sp. near *scirpioides*), the Neotropical *Metopomyza* species (discussed previously), and the two unusual *Cerodontha* (*Cerodontha*) species with an enlarged lunule. These aberrant *Cerodontha* (*Cer.*) (*C*. ZADBI-7 and *C*. ZADBI-8) were collected at Zurquí and Tapantí, but an additional specimen with the same morphological characteristics, was examined from a specimen collected in “montane rainforest pasture” in Ecuador (1500 m) in 1987, by L.D. Coote & B.V. Brown (1M: CNC) (O. Lonsdale pers. com).

4.5.7 Host plants

Although none of the species collected as part of the ZADBI project were reared from a host plant, a few identified species had a known host plant recorded in the literature (e.g. *Agromyza fusca* (*Ichnanthus pallens* (Sw.) (Poaceae)); *Calycomyza dominicensis* (*Elephantopus* sp. (Asteraceae)); *Japanagromyza sasakawai* (*Terminalia catappa* L. (Combretaceae)); *Liriomyza archboldi* (*Bidens pilosa* L. (Asteraceae)) (Annex 1). Some of these species may have a different host plant in the Costa Rican cloud forests. For example, *J. sasakawai* has been reared from *Terminalia catappa* L. in Belém (in the state of Pará) (Monteiro et al. 2015). Although the authors mentioned that the species was described from the Brazilian Amazon, it seems that this information is inaccurate, as the specimens emerged from leaves collected at the Universidade Federal do Pará in Belém city. The plant species (*Terminalia catappa*), commonly known as Beach almond, is native to Southeast Asia but has now spread in many tropical regions of the world and lives at low elevation (below 300-400 m) along coastline (Thomson & Evans 2006). It is unlikely that this plant species would occur in the cloud forests of Costa Rica. A host shift is therefore possible, and the small differences noted in the male genitalia (Annex 1) may be a sign of incipient speciation.

4.6 Conclusion

Cloud forests are unique habitats that support biodiversity that is known and unknown, and unfortunately, they are among the most threatened ecosystems in the world, disappearing at a faster rate than any other tropical forest biomes (including lowland rainforests). In the northern Andes, it is estimated that only 10% of the cloud forests remains intact. The major threats to the survival of cloud forests, include the conversion of land for grazing and cultivation, tourism, introduction of alien species and climate change (Hamilton, 1995). The latter is blamed for the rapid decline of the insect community as the rising hot air is evaporating the clouds, exposing the fauna to a warmer and drier climate (Janzen & Hallwacks 2019; 2021). Global warming could therefore lead to the extinction of many species that depend on this unique environment provided by the cloud forest (Hamilton et al. 1995, Ponce-Reyes et al. 2013, Ray et al. 2006). It is a fact that species most at risk of extinction have specific habitat requirement and limited geographic distribution (Costello 2015), which correspond to the many species so far known to be endemic to the cloud forests.

Research on agromyzid flies from these habitats is critically important when considering these broader implications. Flies are considered non-charismatic, and seldom capture the imagination of the general public, but they are vital to all ecosystems, and this research has illustrated how poorly the fauna is known in the neotropics. If we want to conserve, preserve, and protect ecosystems, a first, critical step is knowing what we have: we need a name, a description, and dots on a map.

4.7 Acknowledgments

All the material for this study was collected during the ZADBI project and was funded by National Science Foundation (USA) grant DEB 1145890 to Brian V. Brown and Art Borkent. I would like to thank Brad Sinclair (CNC) for the loan of Agromyzidae specimens, Marc Pollet for sending the specimens from Podocarpus National Park, Owen Lonsdale (CNC) for bringing my attention to the Ecuadorian *Cerodontha*, Daniel Whitmore (NHML) for providing information of the type specimen of *A. fusca*.; Brian Brown-Art Borkent for inviting me to participate in this great project, for answering various questions related to the project and for granting me the permission to use the pictures for Figures 2 and 3. I would also like to thank Christopher Buddle (McGill University) and Jeff Cumming (CNC) and for their comments on the manuscript.

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4.9 Annex 1

Taxonomic treatment and discussion of selected agromyzid species collected from the ZADBI project (from Table 1). The new species are not formally described here. Asterisks in distribution lists indicate new locality records.

4.9.1 *Agromyza fusca* Spencer

(Figs. 9-16)

Agromyza fusca Spencer, 1963: 295; 1967: 2; Spencer & Stegmaier 1973: 139; Martinez & Étienne 2002: 28; Étienne & Martinez 2003: 250; Boucher 2010: 1067; Sousa & Couri 2016: 2022.

Specimens examined. **Costa Rica**. Prov. San José. Moravia. Zurquí de Moravia, Tower path. 1600m. 30 MAR – 6 ABR 2013. Proyecto ZADBI. Malaise trap #1, ZADBI-647. -84:00:57 10:02:58 #106495. INB0004424655 (1F: MNCR); same except 28 JUN – 5 JUL 2013, ZADBI-894. #107115. INB0004433158 (1F: MNCR); same except 28 ABR – 3 MAY 2013, ZADBI-728. #106748. INB0004392009 (1F: MNCR); same except 14 – 21 JUN 2013, ZADBI-859. #107009. INB0004448991 (1F: MNCR); same except 21 – 28 JUN 2013, ZADBI-890. #107111. INB0004410286 (1M: MNCR); same except 7 – 14 JUN 2013, ZADBI-853. #106911. INB0004447900 (1M: MNCR).

Supplementary material examined. **Argentina**: Salta Province. El Rey National Park. Rio La Sala, Malaise FIT. Humid mossy Chaco forest. 900m, 5-15.xii.1987, S & J Peck (1M: LEMQ). **Costa Rica**: Heredia, 3km S Puerto Viejo, OTS- La Selva, 100m, Malaise. Xi.1992, P. Hanson (1F: LEMQ); Cartago, 550m. Turrialba, CATIE. 4.ix.1986 s.s. Reventazon gorge. L. Masner

(1M : CNC); Puntarenas Prov. Monteverde Estacion biologica. 1540-1800m. 21-24 viii.1995. J.M. Cumming (1F: CNC). **Venezuela**: Yacambo. 1200m. 10.v.1981. H.K. Townes (1M: CNC).

Comments. *Agromyza fusca* Spencer is a distinctive *Agromyza* species with brown halter and partially infuscated wing (Figs. 9, 10), that is now widespread in the Neotropical region. The species was originally described from Brazil (Spencer 1963) and later found to occur in Dominica (Spencer & Stegmaier 1973), Guadeloupe (Étienne & Martinez 2003), Argentina and Costa Rica (Boucher, 2010), and reported here for the first time in Venezuela. The specimens examined differ slightly from the original description in Spencer (1963) where *A. fusca* is described as entirely black. Most of the specimens examined from Costa Rica and elsewhere (Argentina, Venezuela), have the first flagellomere yellow to yellowish-brown, except one female specimen from Monteverde cloud forest (Costa Rica) with entirely brown antennae. In the original description, the colour of the first flagellomere is not specifically mentioned. Unfortunately, the holotype at the National History Museum, London (BMNH), is now missing the head (image of habitus available on the BMNH website: (https://data.nhm.ac.uk/dataset/collection-specimens/resource/05ff2255-c38a-40c9-b657-4ccb55ab2feb/record/4149691). Also, the male phallus of the specimens examined has a pale ventral membranous structure, ending in a tubule, better seen in lateral view (Fig. 11, arrow), a character that may have been overlooked in the original description and in the illustration found in Spencer & Stegmaier (1973). This membranous structure is not visible in the holotype (Fig. 16), although a lateral view is not available on the BMNH website (genitalia is fixed in ventral view on a permanent mount). The ejaculatory apodeme of this species has never been described but it is distinctive due to its elongated foot and narrow blade (Fig. 13, 15). This species was collected only at the main site (Zurquí), and only from Malaise trap #1, from March 30th to July 5th.

Host plant. *Ichnanthus pallens* (Sw.) (Poaceae) (Étienne & Martinez 2003).

Distribution. Argentina, Brazil, Costa Rica, Dominica, Guadeloupe, Venezuela*.

4.9.2 *Calycomyza dominicensis* Spencer
(Figs. 17-19)

Calycomyza dominicensis Spencer, Spencer & Stegmaier 1973: 183; Martinez & Étienne 2002: 29; Spencer et a. 1992: 285; Spencer 1990: 269.

Specimens examined. Costa Rica. Prov. Puntarenas. Coto Brus. Z.P. Las Tablas. Est Biol Las Alturas. 1500-1600m. 11-19 MAR 2013. Proyecto ZADBI. Malaise trap, ZADBI-680. -82:50:04 08:57:07 #106671. INB0004442403 (1M: MNCR); same except 5-13 AGO 2013, ZADBI-1270, #107949. INB0004440254 (1F: MNCR); same except #107949. INB0004440228 (1F: MNCR); same except 28 ENE-4 FEB 2013, ZADBI-548, #106219. INB0004450237 (1F: MNCR).

Comments. This species was previously known from the Caribbean region. Although possible mines on *Elephantopus elatus* Bertol in Florida (Spencer & Stegmaier 1973) and mine on *E. mollis* (H.B.K.) in Venezuela (Spencer 1973b) may represent this species (Spencer et al. 1992). *C. dominicensis* can be distinguished from other Neotropical *Calycomyza* by its size (2.2 mm in male to 2.5 mm in female), presence of three dc (3rd dc much reduced), fronto-orbital plate slightly darkened to upper ors and by the distinctive well sclerotized phallus, with a pair of distinct tubular extensions, extending beyond the margin of the distiphallus in lateral view (Fig.

17). The specimens examined correspond to the original description except the male specimen is a little larger, with a wing length of 2.4 mm, and females are smaller with a wing length from 2.0 to 2.3 mm. The epandrium and surstyli with multiple spines (Fig. 19), and ejaculatory apodeme with large stalk and blade slightly expanded (Fig. 18) are for the first time illustrated. This species was only collected at Las Alturas.

Host plant. *Elephantopus* sp. (Asteraceae).

Distribution. Cuba, Costa Rica*, Dominica, Guadeloupe.

4.9.3 *Calycomyza meridiana* (Hendel)

(Figs. 20-24)

Agromyza meridionalis Malloch 1914.

Agromyza meridiana Hendel 1923: 123, *nomen novum* for *Agromyza meridionalis* Malloch, preoccupied by *Agromyza meridionalis* Strobl, 1900.

Phytobia (*Calycomyza*) *meridiana* (Hendel), Frick 1952: 395; Spencer 1963: 348.

Calycomyza meridiana (Hendel), Spencer 1966: 146; 1967: 9; 1973a:153; 1973b: 46; 1983: 60; Spencer & Stegmaier 1973: 185; Sasakawa 1992b: 15; Sasakawa 1992c: 823; Sasakawa 2005: 158; Martinez & Étienne 2002: 30; Korytkowski 2014: 28.

Specimens examined. Costa Rica. Prov. Puntarenas. Coto Brus. Z.P. Las Tablas. Est Biol Las Alturas. 1500-1600m. 7-14 ENE 2013. Proyecto ZADBI. Malaise trap, ZADBI-545. -82:50:04 08:57:07 #106216. INB0004450328 (1M: MNCR); Costa Rica. Prov. San José. Moravia. Zurquí de Moravia, Tower path. 1600m. 16-23 AGO 2013. Proyecto ZADBI. Malaise trap #1, ZADBI-1092. -84:00:57 10:02:58 #107693. INB0004447715 (1F: MNCR).

Comments. This species was originally described from Costa Rica and later found in many Neotropical countries. Its yellow antennae (Fig. 20) differentiate this species from most other Neotropical *Calycomyza*, except *C. punctata* Sasakawa (1992d) from Peru, but this later species has completely yellow fronto-orbital plate (black in *C. meridiana*). Spencer (1963) mentioned that the notopleural area of *C. meridiana* appears brownish, but later described as being yellow (Spencer 1973b; Sasakawa 2005), as it is in the specimens examined (Fig. 20). The ejaculatory apodeme of this species was never illustrated before. It has a short and wide stalk with the blade only slightly wider than its base (Fig. 24).

Host plant. Unknown.

Distribution. Brazil, Colombia, Costa Rica, Dominica, Ecuador, El Salvador, Guyana, Jamaica, Mexico, Peru, Venezuela.

4.9.4 *Cerodontha* (*Dizygomyza*) n.sp. (nr *scirpioides* Zlobin)
(Figs. 25-26)

Material examined. Costa Rica. Prov. Cartago. Paraíso, P.N. Tapantí, 1600m. 20-27 ENE 2013. Proyecto ZADBI. Malaise, ZADBI-505. -83:46:30 09:43:21 #106170. INB0004392883 (1M: MNCR).

Comments. This specimen from Tapantí was identified as *C. (Diz.) scirpioides* Zlobin in the list of species/morphospecies in Borkent et al. (2018), a species previously described from high elevation (2500 m) in western Mexico (near El Salto, Durango), but it is now considered a new species (formal description will be done elsewhere). The species differs from *C.* (*Diz.*) *scirpioides* (Zlobin 1997) by its smaller size (2.6 mm in male not 3.0 mm), fronto-orbital plate yellowish (not blackish); calypter and fringe brown (not yellowish-white), and slight differences in the male phallus, including the shorter curvature at the base of the distiphallus and the small apical enlargement of the mesophallus (arrow, Fig. 26). The study of the *Cerodontha* specimens from Tapantí, has brought my attention to previous specimens that were (temporarily) identified as *C (D.) scirpioides* in Boucher 2005. These specimens were from Cartago (1400m) (Costa Rica) (1 male, LEMQ) (with the distiphallus much less dilated), and San Cristobal, Chiapas (7000' = 2133 m) (Mexico) (1 female, CNC). But it is now becoming clearer that these specimens represent a new species. *C. scirpioides* from Durango (Mexico) (Zlobin 1997), along with the specimens examined from Tapantí (Costa Rica), Cartago (Costa Rica), and Chiapas (Mexico) have in common a reduced lower ors (Fig. 25, arrow), a character rarely observed in Agromyzidae, but also observed in *C.* (*Diz.*) ZABDI-4 (Table 1) and in *C.* (*Diz.*) *impatientis* Sasakawa. The latter species was collected from Parque National Henri Pittier, Rancho Grande, Venezuela (Sasakawa 1992d) but described from a female specimen only (and without any figures). Parque National Henri Pittier is home to a well known cloud forest in South America (Sanchez & Liria 2009) and it is plausible that *Cerodontha* (*Diz.*) *scirpioides*, *C.* (*Diz.*) *impatientis*, the two species of *Cerodontha* (*Dizygomyza*) from ZADBI and the species (wrongly) identified as *C.* (*Diz.*) *scirpioides* in Boucher (2005) represent closely related species that are restricted to mid and high elevation Neotropical cloud forests.

Host plant. Unknown.

Distribution. Costa Rica.

4.9.5 *Japanagromyza sasakawai* Monteiro, Carvalho-Fihlo & Esposito

(Figs. 27-30)

Japanagromyza sasakawai Monteiro, Carvalho-Fihlo & Esposito, 2015: 494.

Specimens examined. Costa Rica. Prov. San José. Moravia. Zurquí de Moravia, North pasture. 1600m. 5 – 12 JUL 2013. Proyecto ZADBI. Malaise trap #3, ZADBI-911. -84:00:57 10:02:58 #107159. INB0004408017 (1M: MNCR); Costa Rica. Prov. San José. Moravia. Zurquí de Moravia, Tower path. 1600m. 5 – 12 JUL 2013. Proyecto ZADBI. Malaise trap #1, ZADBI-909. -84:00:57 10:02:58 #107157. INB0004448985 (1F: MNCR).

Comments. This species was recently described from the State of Pará, in Brazil. The Zurquí specimens agree externally with the original description of the species. *J. sasakawai* is a black species without any metallic reflection. The male genitalia appear to show slight variation including a few more spines on the surstyli (Fig. 28) (apparently 7 not 5) and a slightly bent apex of distiphallus (Fig. 29). The cerci are very large (Fig. 28, arrow) as originally described. This species was identified as morphospecies (*J.* ZADBI-1) in Borkent et al. 2018.

Host plant. *Terminalia catappa* L. (Combretaceae) (Monteiro, Carvalho-Fihlo & Esposito, 2015).

Distribution. Brazil. Costa Rica*.

4.9.6 *Liriomyza archboldi* Frost

(Figs. 31-34)

Liriomyza archboldi Frost, 1962: 51; Spencer 1983: 55; Spencer & Steigmaier 1973: 97; Spencer & Steyskal 1986; Spencer 1990: 317; Martinez & Étienne 2002: 34.

Material examined. Costa Rica. Prov. San José. Moravia. Zurquí de Moravia, Tower path. 1600m. 1-7 JUN 2013. Proyecto ZADBI. Malaise trap #1, ZADBI-810. -84:00:57 10:02:58 #106920. INB0004449118 (1M: MNCR); same except 30 MAR-6 ABR 2013, ZADBI 647; #106495. INB0004424654 (1M: MNCR).

Comments. The two specimens from Zurqui correspond to the original description of the species except for having both vti and vte on dark background (Fig. 31) (as opposed to vti on yellow and vte on black). The distinct pubescence of the first flagellomere of this species is characteristic (Fig. 31). The mention of this species in Spencer 1963 was a mistake and represents *L. trifolii* (Spencer & Stegmaier 1973). This species was overlooked in Spencer's 1967 catalog. It is considered an uncommon species (Spencer 1983), and is so far known from the Bahamas, Florida and Costa Rica. In Costa Rica it was previously reported from low elevation, in San José (Spencer 1983). Its host plant (*Bidens Pilosa* L.) is native to South America but is now cosmopolitan in distribution. It is considered an invasive weed and can grow in many different habitats and up to 3200 m of elevation (GISD 2022).

Host plant. *Bidens pilosa* L. (Asteraceae) (Spencer & Stegmaier 1973; Spencer 1990).

Distribution. Florida. Bahamas. Costa Rica.

4.9.7 *Liriomyza* n.sp. (near *L.* c*ommelinae* (Frost))

(Figs. 35-39)

Material examined. Costa Rica. Prov. San José. Moravia. Zurquí de Moravia, Tower path. 1600m. 11-18 OCT 2013. Proyecto ZADBI. Malaise trap #1, ZADBI-1280. -84:00:57 10:02:58 #107959. INB0004415923 (1M: MNCR); same except 14-21 JUN 2013, ZADBI-859; #107009. INB000432453 (1F: MNCR).

Comments. The specimens from Zurquí were identified as *Liriomyza commelinae* in Borkent et al. (2018), but it is now considered a new species. *L. commelinae* is a widespread Neotropical species, easily recognized by the characteristic enlarged first flagellomere in male, a character rarely seen in *Liriomyza*, but also occurring in *L. robustae*, a species described from the western Andes in Colombia (Spencer, 1984). *L. commelinae* also has a characteristic yellow colouration at the posterior end of the mesonotum. These two characters are identical in the Zurquí specimens (Figs. 35, 36). The abdomen of *L. commelinae* has been described as blackish centrally but variably yellow laterally in Spencer and Steyskal (1986), but the Zurquí specimens have a characteristic yellow first tergite, extending posteriorly into a point (Fig. 37). The phallus of *L. commelinae* has been illustrated by various authors (Spencer 1963, Spencer 1973b; Valladares 1984; Pallacios-Torres et al. 2005) with some important variations between them, which could bring confusion. The phallus illustrated in Valladares (1984) and in Palacios-Torres et al. (2015) has long membranous tubules. These may have been overlooked in ventral view illustrations of Spencer (1963) and Spencer & Stegmaier (1973). The phallus of the Zurquí specimen has a few unique characteristics including a little triangular sclerite at the base of the distiphallus (Fig. 37, arrow), wider tubules with the distal end slightly enlarged, and a distinct curvature in the phallus in lateral view (Fig. 39) (straight in *L. commelinae*). These differences are important and justify the status of a new species. The epandrium and surtylus of the male Zurquí specimen are illustrated in Fig. 36 but has never been illustrated for *L. commelinae* for comparison.

Host plant. Both *L. commelinae* and *L. robusta* feed on Commelinaceae These two *Liriomyza* species with enlarged first flagellomere are considered sister species (Spencer 1990). It is likely that this very similar new species of *Liriomyza* from Zurquí also feed on a host plant in this family.

Distribution. Costa Rica.

4.9.8 *Melanagromyza wedeliae* Spencer

(Figs. 40-42)

Melanagromyza wedeliae Spencer, Spencer & Stegmaier 1973: 54; Spencer 1984: 8; Martinez & Étienne 2002: 40; Spencer & Steyskal 1986: 31; Spencer 1990: 313; Braun et al. 2008: 98.

Specimens examined. Costa Rica. Prov. San José. Moravia. Zurquí de Moravia, Tower path. 1600m. 26 ABR-3 MAY 2013. Proyecto ZADBI. Malaise trap #1, ZADBI-728. -84:00:57 10:02:58 #106748. INB0004392034 (1 ♂: MNCR).

Comments. *Melanagromyza wedeliae* was originally described from southern Florida but later found in Colombia and Brazil. It is characterized by having a narrow gena, orbital setulae in two rows (inner row proclinate and outer row reclinate), eye pilose in male with a patch of white hairs at level of ors and the abdomen with a metallic green reflection. The specimen from Zurquí agrees with the external characteristic of the species. The genitalia is also characteristic with the distiphallus in the form of a short and broad tubule, slightly upcurved (Fig. 40) and a greatly elongated hypandrial apodeme (Fig. 42), not previously illustrated. The ejaculatory apodeme was not described previously but it has no (or short) stalk and the blade is rounded with irregular margin (Fig. 41)

Host plant. *Wedelia paludosa* DC, *Pseudogynoxys chenopodioides (Kunth) (= Senecio confusus* Britten), *Bidens pilosa* L., *Trixis vauthieri* DC (Asteraceae). Larva feeding in flower heads (Braun et al. 2008; Spencer & Stegmaier1973; Spencer 1984; Spencer 1990).

Distribution. Florida, Brazil, Colombia, Costa Rica*.

4.10 Figures and Tables

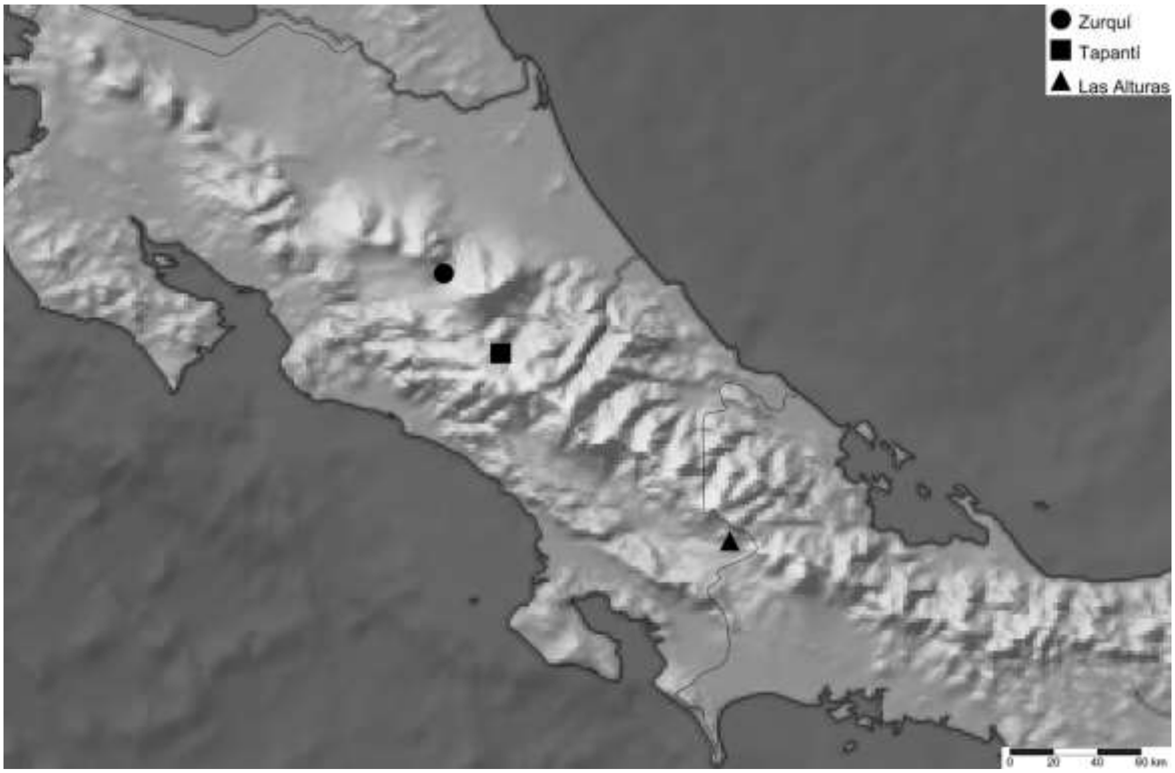


Figure 1. Map of Costa Rica with localities of the three study sites (Legend top right corner).

Figure 2. Main sampling site at Zurquí de Moravia, consisting of primary tropical cloud forest surrounded by pasture (from Borkent and Brown 2015).

Figure 3. Examples of sampling techniques that yield Agromyzidae. A. Emergence trap over vegetation; B. Flight intercept trap; C. Malaise trap at the forest edge (From Borkent and Brown 2015).

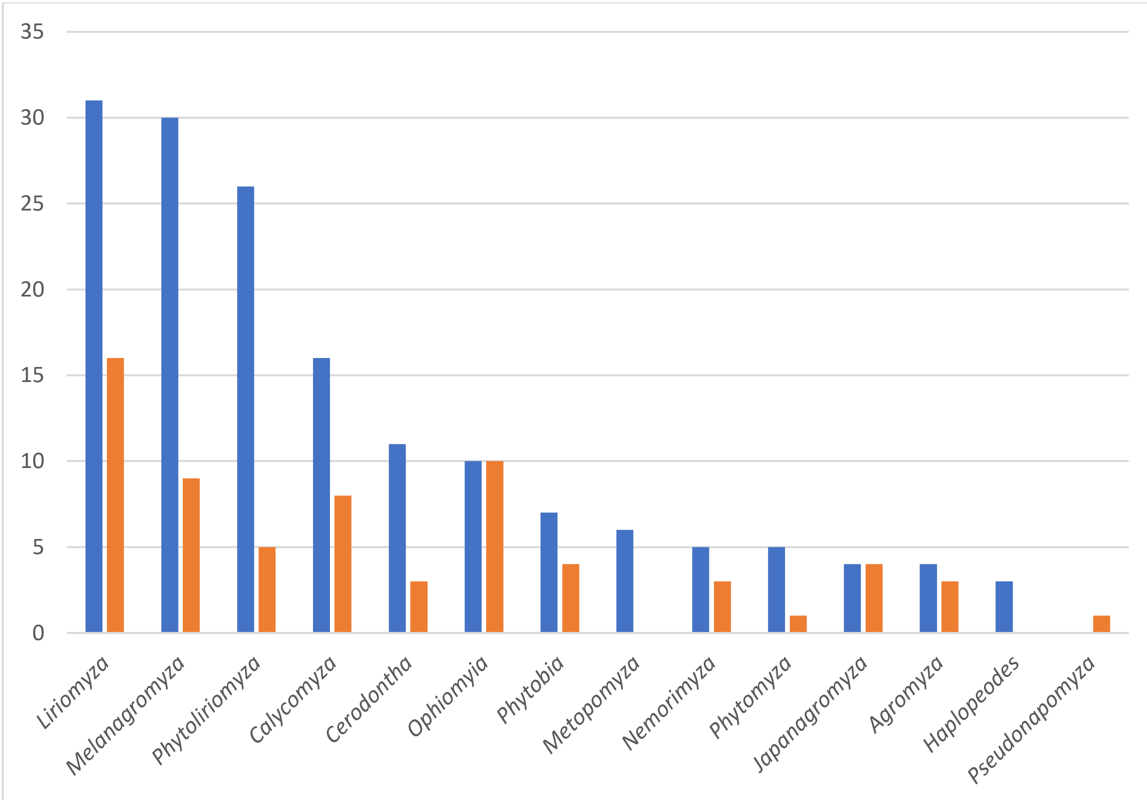


Figure 4. Comparison of species richness per genus at the cloud forest sites (blue) and for Costa Rica (orange). Ranked from the most species-rich at the cloud forests to the least species-rich.

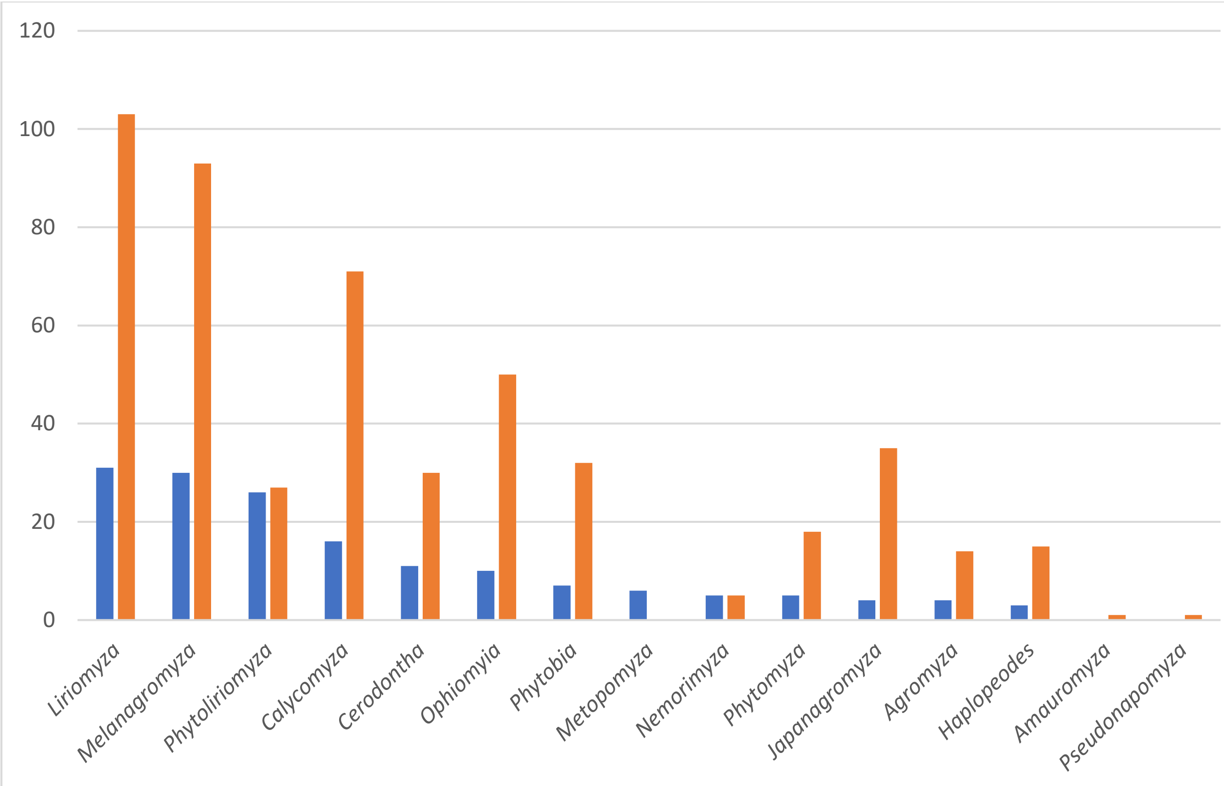


Figure 5. Comparison of species richness per genus at the cloud forest sites (blue) and for the Neotropical region (orange). Ranked from the most species-rich at the cloud forests to the least species-rich.

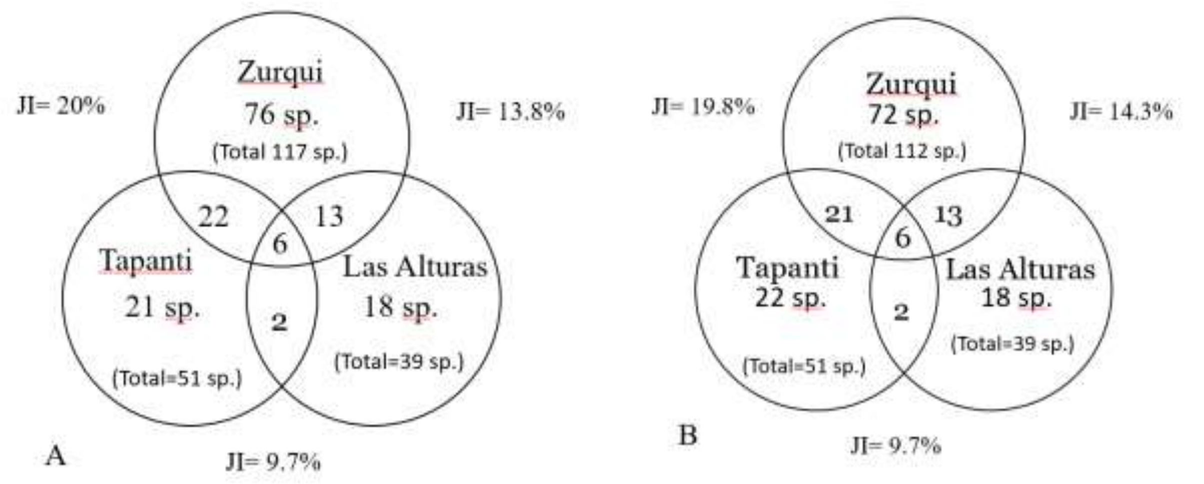


Figure 6. Venn diagram showing number of species collected at each study site: A) Zurqui all sampling methods; B) Zurqui Malaise trap #1 only, with Jaccard Index of similarity (JI) shown for each pair comparison and expressed as a percentage.

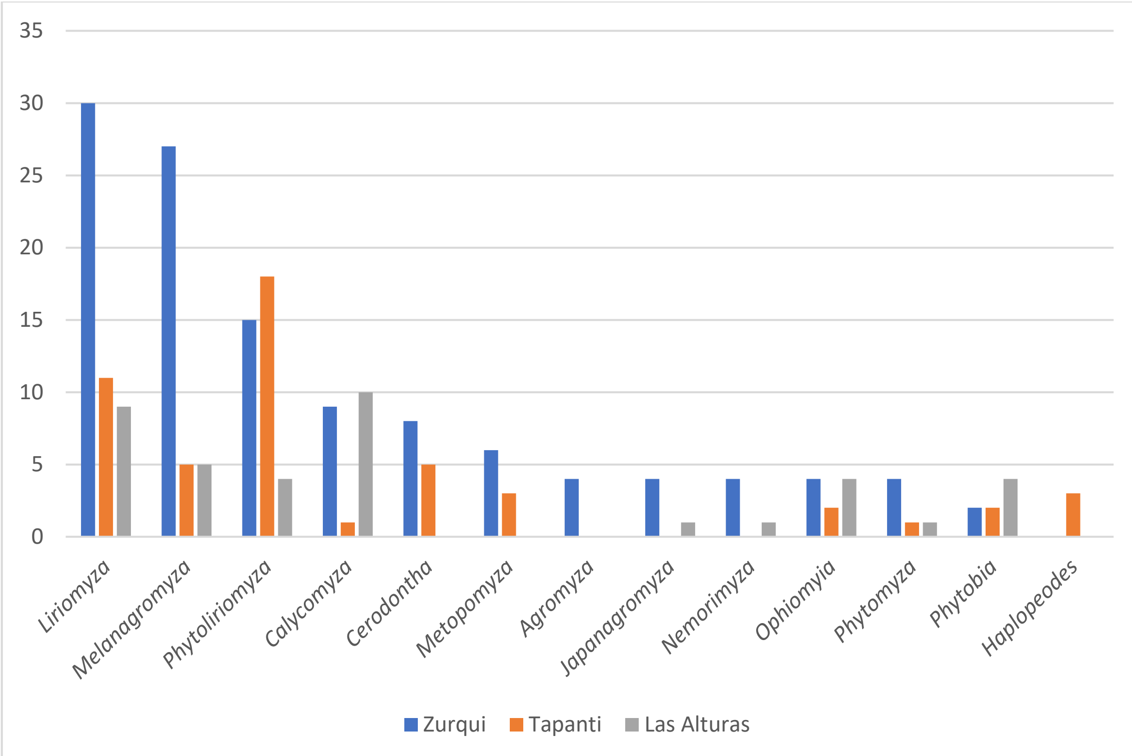


Figure 7. Comparison of species richness per genus for each study site. Ranked from the most species-rich genus at Zurquí to the least species-rich.

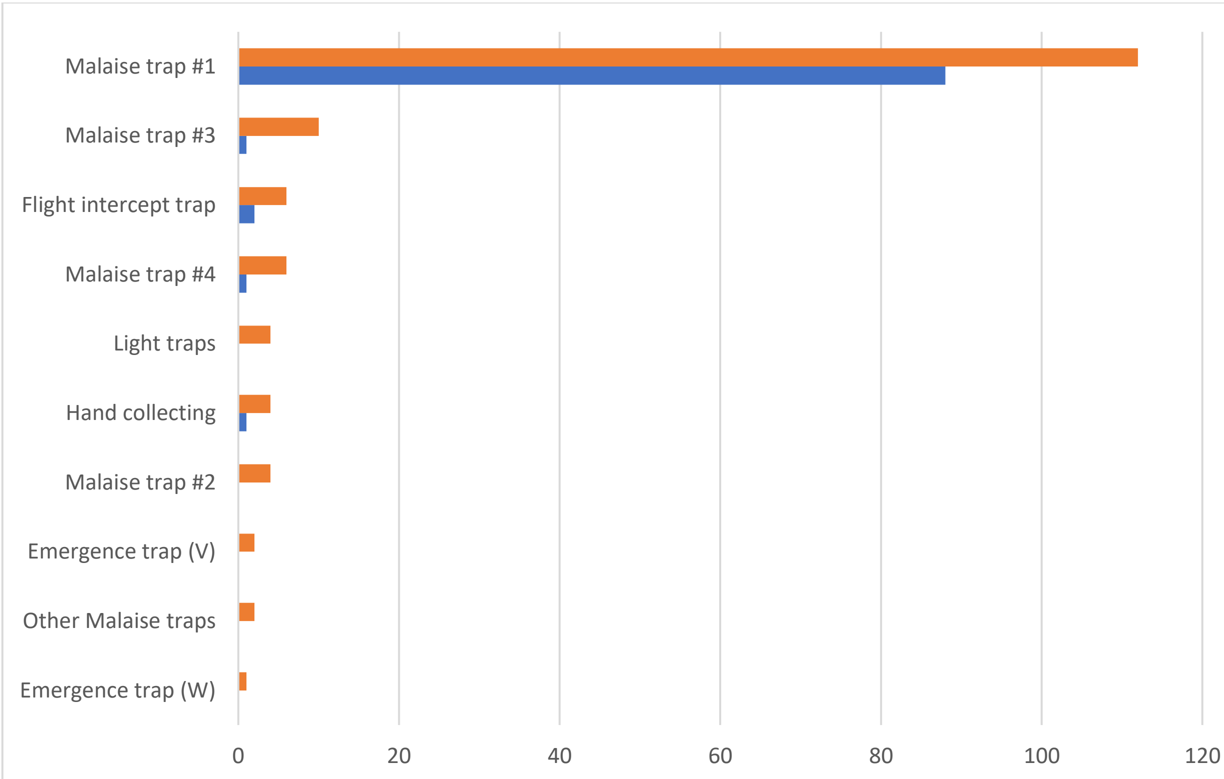
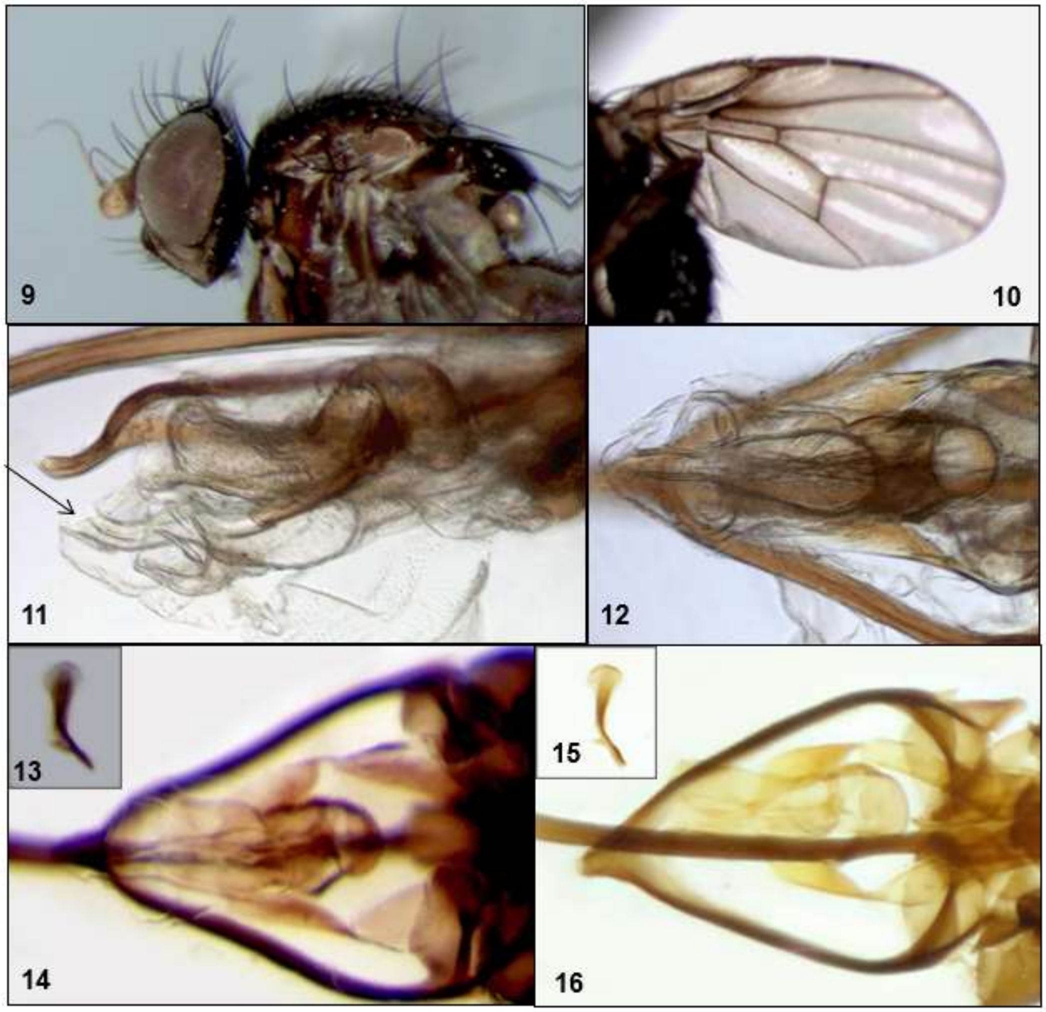
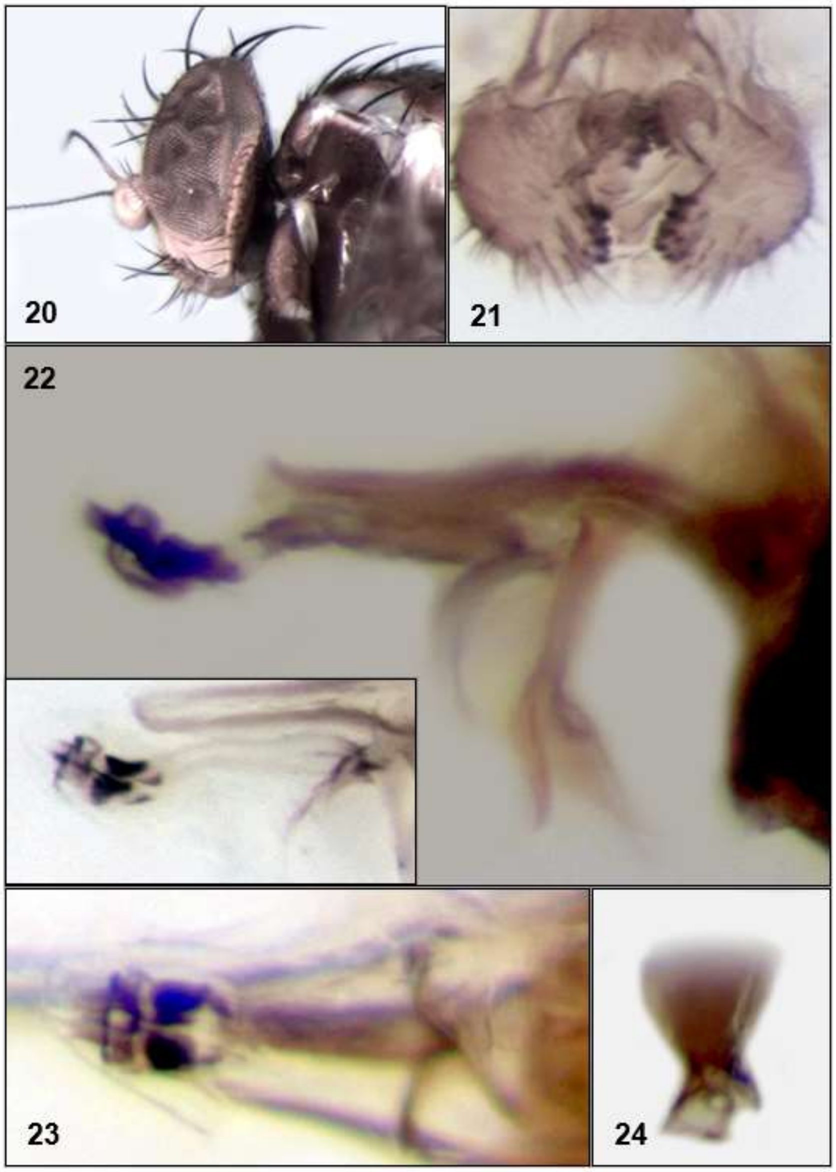


Figure 8. Collecting methods used at Zurquí that successfully caught at least one Agromyzidae with total number of species for each (in orange) and number of unique species (not collected by other methods) (in blue). Ranked from top to bottom from the most productive to the least productive.

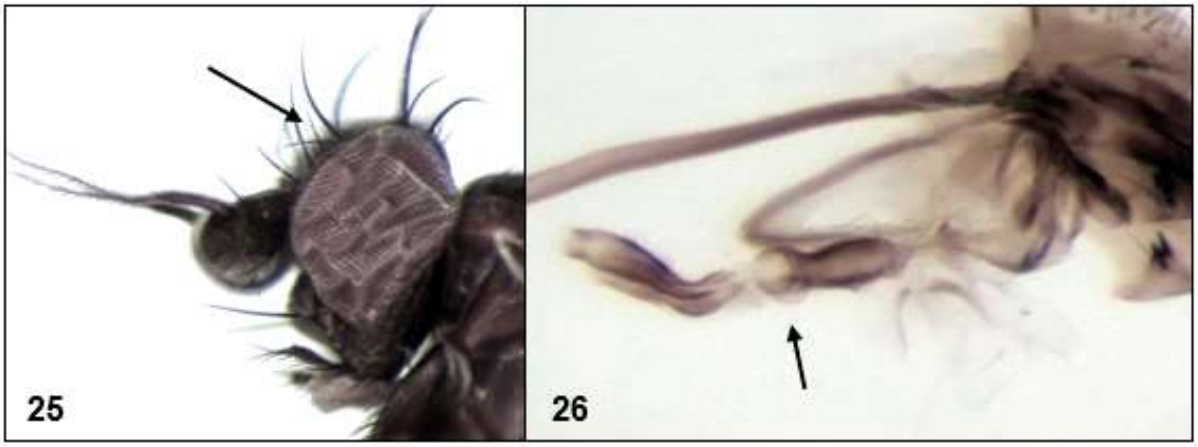


Figures 9-16. *Agromyza fusca* Spencer, 9. head, thorax, lateral view; 10. wing; 11. phallus, lateral view; 12. phallus, ventral view; 13. ejaculatory apodeme; 14. phallus ventral view, low resolution; 15-16. holotype, unique identifier NHMUK012809047, BMNH. 15. Ejaculatory apodeme; 16. phallus ventral view.

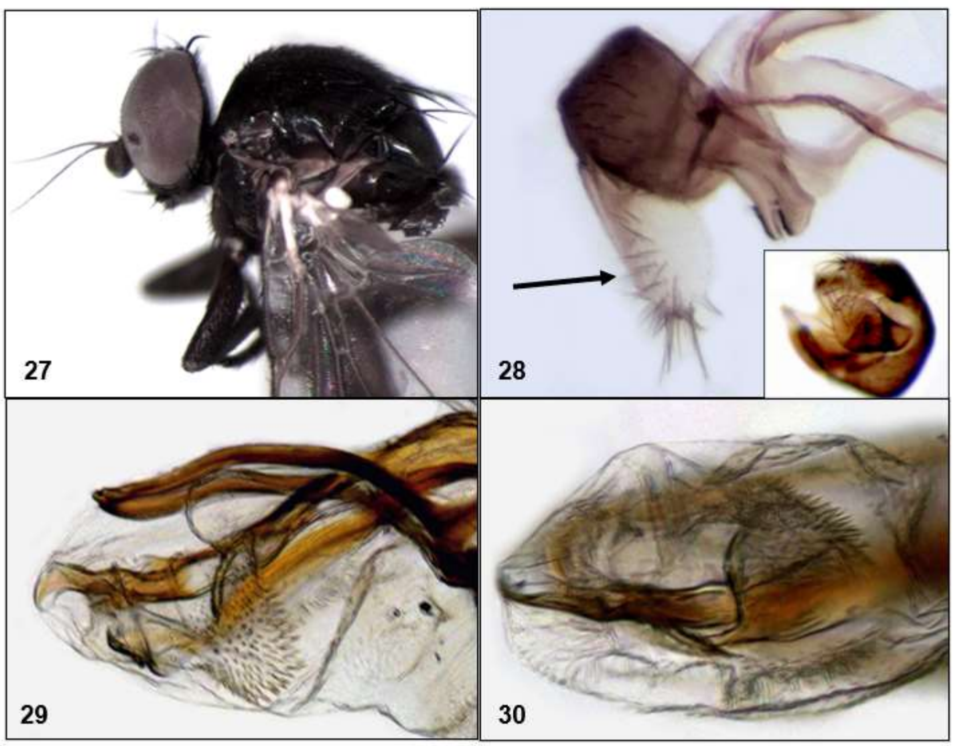
Figures 17-19. *Calycomyza dominicensis* Spencer, 17. phallus, lateral view; 18. ejaculatory apodeme; 19. male genitalia in ventral view.



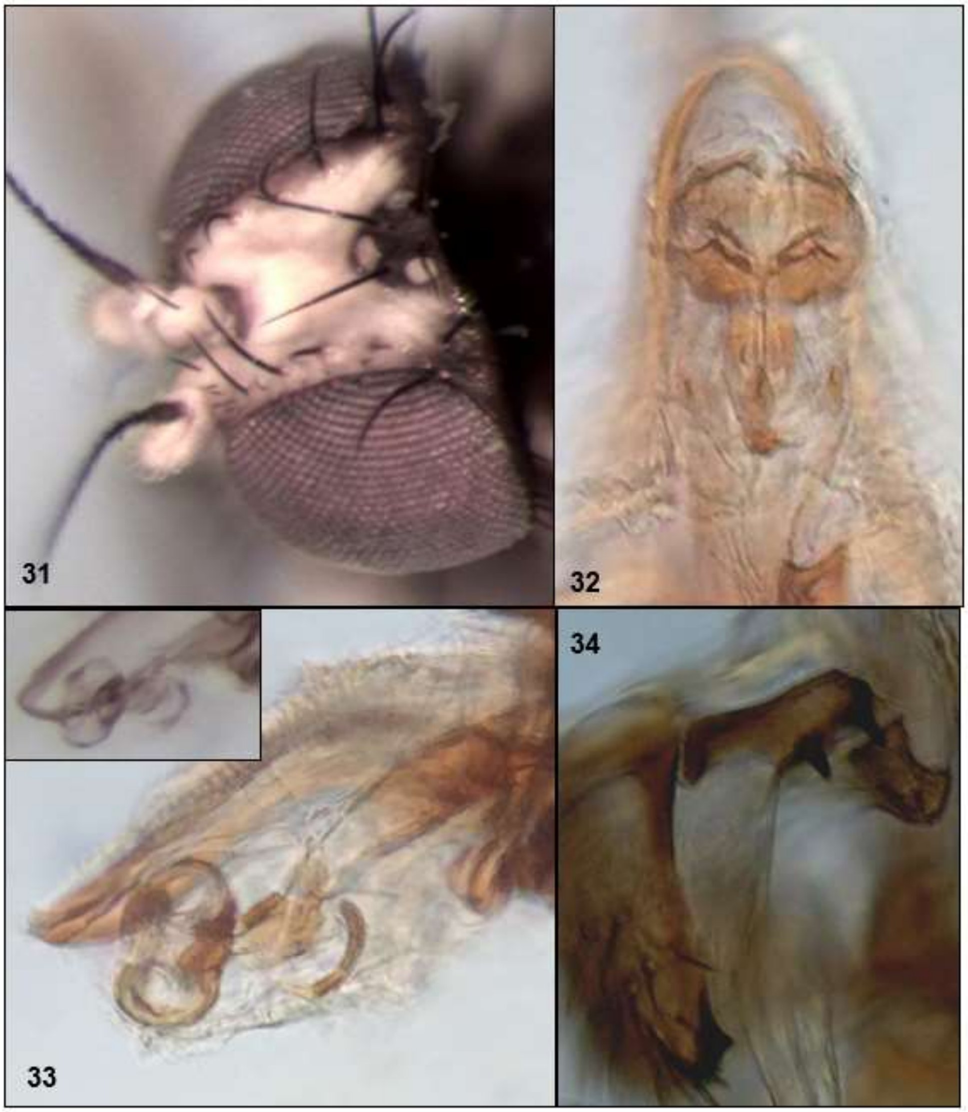
Figures 20-24. *Calycomyza meridiana* (Hendel), 20. head lateral view; 21. epandrium with surstyli; 22. phallus lateral view; 23. phallus ventral view; 24. ejaculatory apodeme.



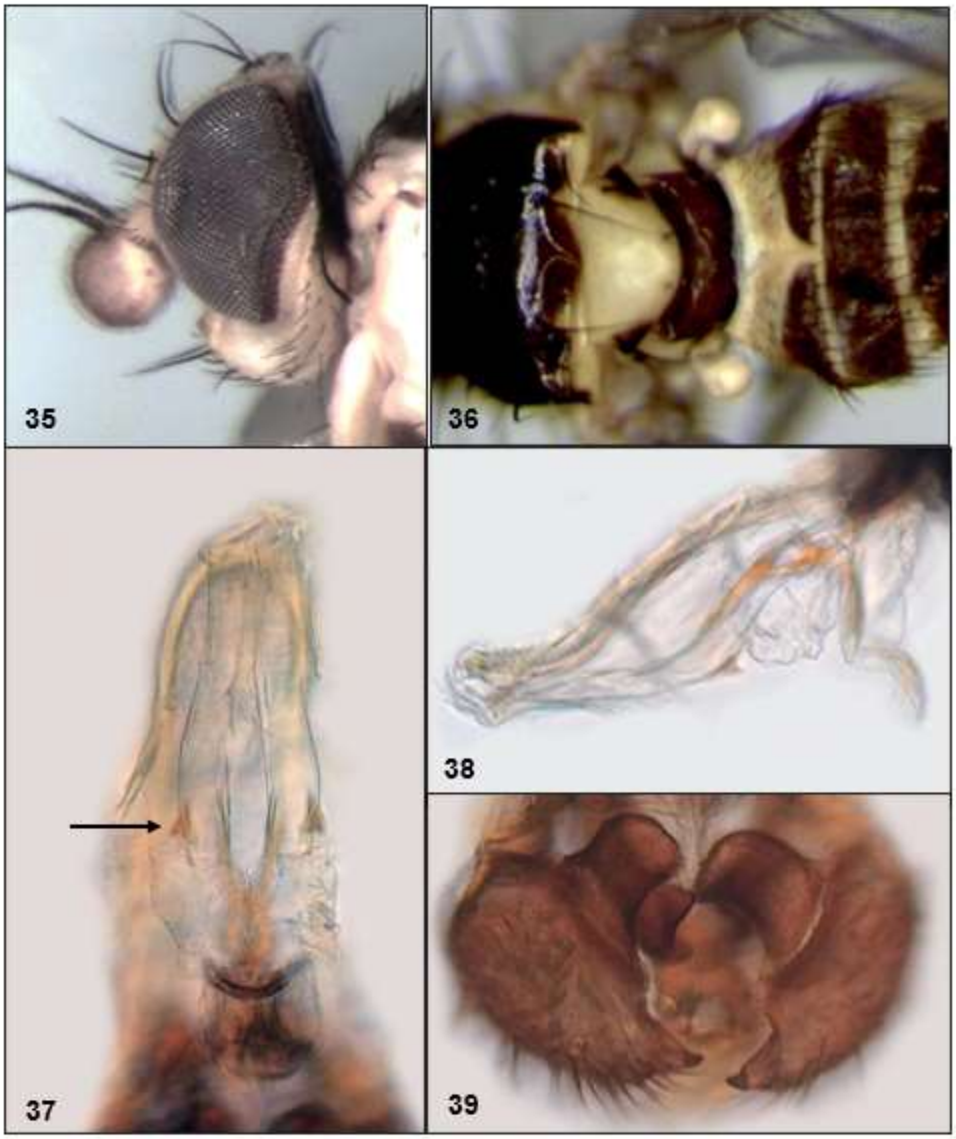
Figures 25-26. *Cerodontha (Diz.) n.sp.* (near *C. scirpioides* Zlobin), 25. Head, lateral; 26. Phallus, lateral.



Figures 27-30. *Japanagromyza sasakawai* Monteiro, Carvalho-Fihlo & Esposito, 27. head lateral view; 28. epandrium with surstyli, lateral (inset, posterior view); 29. phallus lateral view; 30. phallus ventral view.



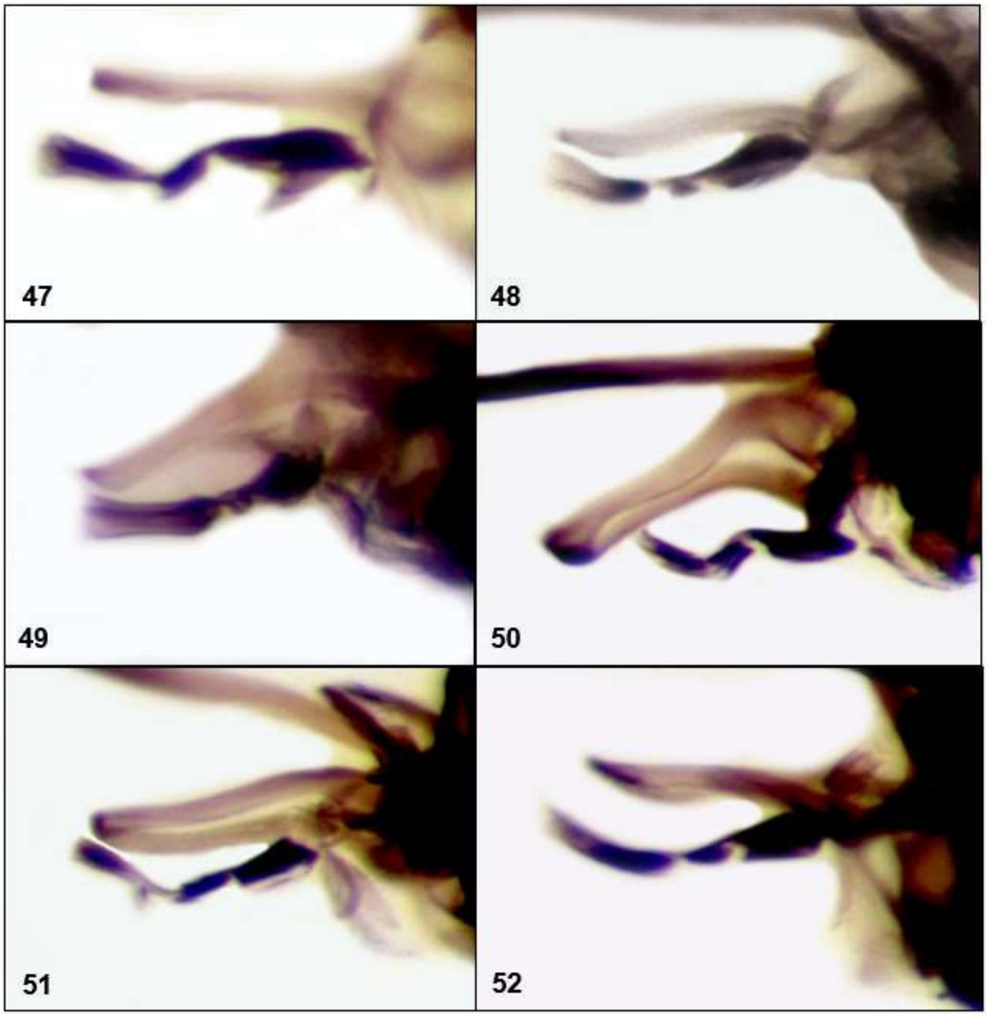
Figures 31-34. *Liriomyza archboldi* Frost, 31. head dorsal view; 32. phallus ventral view; 33. phallus lateral view; 34. epandrium and surstylus (left side), ventral view.



Figures 35-39. *Liriomyza n.sp.* (near *L.commelinae* (Frost)), 35. head latera view; 36. mesonotum and abdomen, dorsal view, male; 37. phallus ventral view; 38. Phallus lateral view; 39. epandrium and surstyli.

Figures 40-42. *Melanagromyza wedeliae* 40. phallus, latera view; 41. ejaculatory apodeme; 42. hypandrium with long apodeme, ventral view.

Figures 43-46. New species of Agromyzidae, 43. *Cerodontha* (*Cer*.) ZADBI-8; 44. *Haplopeodes* ZADBI-1; 45. *Phytomyza* ZADBI-3; 46. *Metopomyza* ZADBI-4.



Figures 47-52. Diversity of *Phytoliriomyza* species from the cloud forest sites. 47. *P.leechi* Spencer; 48. *P*. ZADBI-2; 49. *P*. ZADBI-3; 50. *P*. ZADBI-5; 51. P. ZADBI-1; 52. *P*. ZADBI-16.

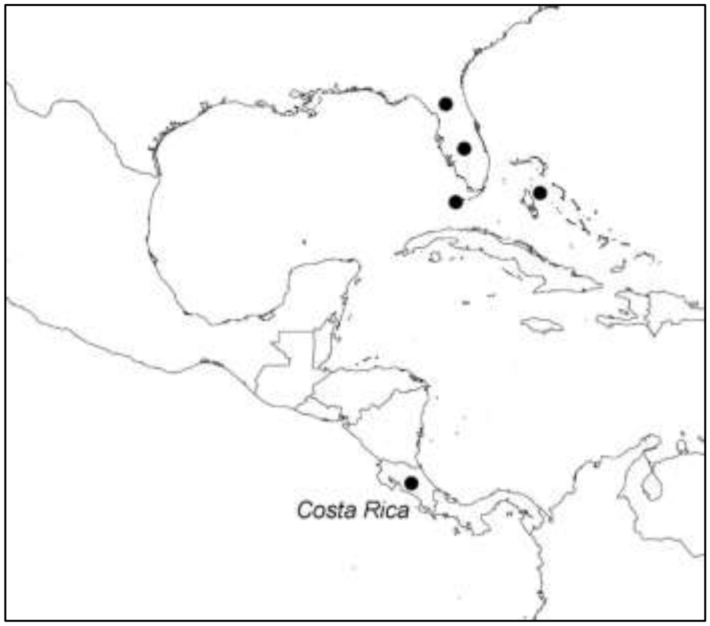


Figure 53. Distribution of *Liriomyza archboldi* Frost, a species previously recorded in Costa Rica.

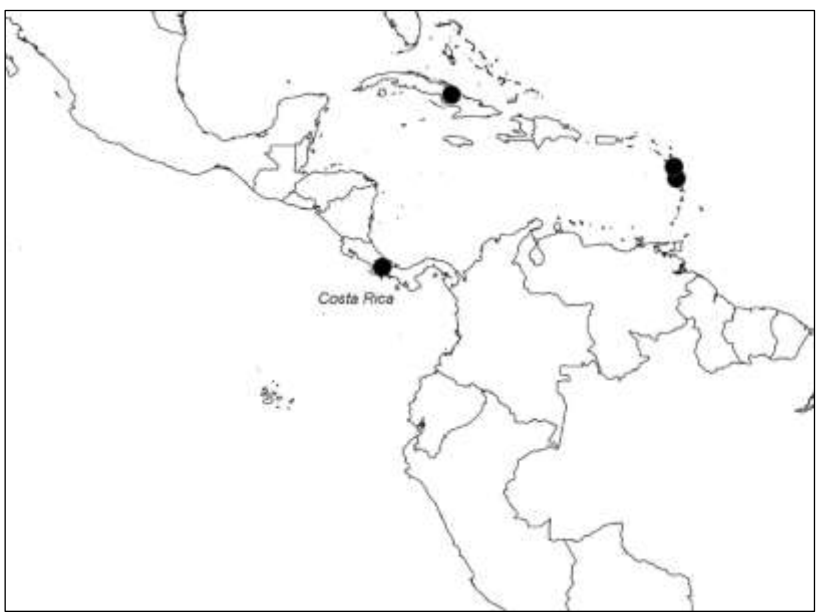


Figure 54. Distribution of *Calycomyza dominicencis* Spencer, a species newly recorded in Costa Rica.

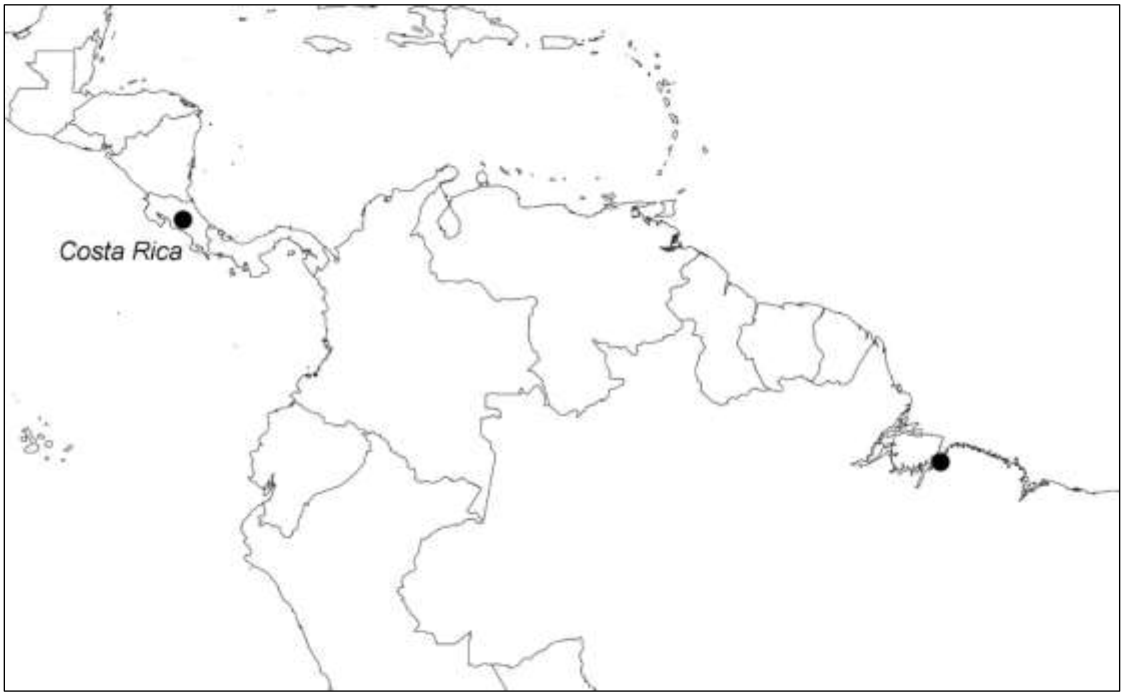


Figure 55. Distribution of *Japanagromyza sasakawai Monteiro, Carvalho-Filhlo*, a species newly recorded in Costa Rica.

Table 1. Preliminary data on Agromyzidae species and morphospecies identified from the three cloud forest sites in Costa Rica (abbreviation: Z = Zurquí; T = Tapantí; L.A. = Las Alturas) (modified from Borkent et al. 2018) with + for presence and blank for absence at the site, and with total number of specimens collected for each species (number in bold means that male(s) are present). Names of species previously reported from Costa Rica are in bold. Details on collecting method for the Zurquí specimens are given with the following abbreviations: M# = Malaise (#1,2,3,4); M-Dry = Dry Malaise; M-Ca = Canopy Malaise; Em-V = Emergence trap over vegetation; Em-W = Emergence trap over water; H = Hand collecting; FIT = Flight intercept trap; Light-P = Pan light trap; Light-B = Bucket light trap.

Species names or morphotypes	Z	Methods at Zurquí	T	L.A.	# specimens
***Agromyza fusca* Spencer**	+	M#1			**6**

Agromyza ZADBI-1	+	M#1			**1**
Agromyza ZADBI-2	+	M#1			1
Agromyza ZADBI-3	+	M#1			1
***Calycomyza devia* Spencer**				+	**4**
Calycomyza dominicensis Spencer				+	**4**
***Calycomyza meridiana* (Hendel)**	+	M#1		+	**2**
Calycomyza opaca Zlobin	+	M#1			**2**
Calycomyza ZADBI-1	+	M#1/M-Dry			1
Calycomyza ZADBI-2	+	M#1		+	**7**
Calycomyza ZADBI-3	+	M#1	+		**2**
Calycomyza ZADBI-4				+	**2**
Calycomyza ZADBI-5				+	**4**
Calycomyza ZADBI-6	+	M#1		+	**6**
Calycomyza ZADBI-7	+	M#1			1
Calycomyza ZADBI-8	+	M#1/Em-V			2
Calycomyza ZADBI-9				+	1
Calycomyza ZADBI-10				+	1
Calycomyza ZADBI-11	+	M#1			1
Calycomyza ZADBI-12				+	**1**

***Cerodontha dorsalis* (Loew)**	+	M#1			1
Cerodontha (Butomomyza) puertoricensis Spencer	+	M#1			**6**
Cerodontha (Butomomyza) ZADBI-3	+	M#1			**12**
Cerodontha (Dizygomyza) n.sp. (near *C. scirpioides* Zlobin)			+		**1**
Cerodontha (Dizygomyza) ZADBI-4	+	M#1	+		**3**
Cerodontha (Poemyza) ZADBI-1	+	M#1			**15**
Cerodontha (Poemyza) ZADBI-2	+	M#1			**2**
Cerodontha ZADBI-5			+		2
Cerodontha ZADBI-6			+		2
Cerodontha (Cer.) ZADBI-7	+	M#1			1
Cerodontha (Cer.) ZADBI-8	+	M#1	+		4
Haplopeodes ZADBI-1			+		4
Haplopeodes ZADBI-2			+		**4**
Haplopeodes ZADBI-3			+		1
Japanagromyza sasakawai Monteiro et al.	+	M#1,3			**2**
Japanagromyza ZADBI-2	+	M#1		+	**5**
Japanagromyza ZADBI-3.	+	M#1			1

Japanagromyza ZADBI-4	+	M#1			**3**
Liriomyza archboldi **Frost**	+	M#1			**2**
Liriomyza n.sp (near *L. commelinae* (Frost))	+	M#1			**2**
Liriomyza sabaziae Spencer	+	M#1/M-Ca/H	+	+	**89**
Liriomyza ZADBI-1	+	M#1			**5**
Liriomyza ZADBI-2	+	M#1,2,3	+	+	**78**
Liriomyza ZADBI-3	+	M#1			**1**
Liriomyza ZADBI-4	+	M#1/Light-P			**30**
Liriomyza ZADBI-5	+	M#1,4		+	**32**
Liriomyza ZADBI-6	+	M#1			2
Liriomyza ZADBI-7	+	M#1	+		4
Liriomyza ZADBI-8	+	M#1/Light-P			**81**
Liriomyza ZADBI-10	+	M#1/FIT			**8**
Liriomyza ZADBI-11	+	M#1/Light-P			**3**
Liriomyza ZADBI-12	+	M#1	+	+	**5**
Liriomyza ZADBI-13			+		1
Liriomyza ZADBI-14	+	M#1,4		+	**6**
Liriomyza ZADBI-15	+	M#1			4

Liriomyza ZADBI-16	+	M#1		+	**4**
Liriomyza ZADBI-17	+	M#1	+	+	**6**
Liriomyza ZADBI-18	+	M#1	+		**2**
Liriomyza ZADBI-19	+	M#1		+	**25**
Liriomyza ZADBI-20	+	M#1/Light-B/H	+	+	**6**
Liriomyza ZADBI-21	+	M#1			**8**
Liriomyza ZADBI-22	+	M#1	+		**4**
Liriomyza ZADBI-23	+	M#1			**3**
Liriomyza ZADBI-24	+	M#1,2	+		**6**
Liriomyza ZADBI-25	+	M#1	+		**5**
Liriomyza ZADBI-26	+	M#1			1
Liriomyza ZADBI-27	+	M#1			**2**
Liriomyza ZADBI-28	+	M#1			2
Liriomyza ZADBI-29	+	FIT			**1**
***Melanagromyza bidentis* Spencer**	+	M#1			**1**
Melanagromyza floridensis Spencer	+	M#1,3	+		**3**
***Melanagromyza minima* (Malloch)**	+	M#1			**2**
Melanagromyza wedeliae Spencer	+	M#1			**1**

Melanagromyza ZADBI-1	+	M#1	+	+	**28**
Melanagromyza ZADBI-3	+	M#1			**1**
Melanagromyza ZADBI-4			+		**1**
Melanagromyza ZADBI-5	+	M#1			1
Melanagromyza ZADBI-6	+	M#1,3/FIT			**5**
Melanagromyza ZADBI-7	+	M#1,3			**5**
Melanagromyza ZADBI-8				+	1
Melanagromyza ZADBI-9	+	M#1			1
Melanagromyza ZADBI-10	+	M#1			2
Melanagromyza ZADBI-11				+	1
Melanagromyza ZADBI-12	+	M#1			1
Melanagromyza ZADBI-13	+	M#1			1
Melanagromyza ZADBI-14	+	M#1		+	2
Melanagromyza ZADBI-15	+	M#1			4
Melanagromyza ZADBI-16	+	M#1,3	+		**7**
Melanagromyza ZADBI-17	+	M#1		+	5
Melanagromyza ZADBI-18	+	M#1			1
Melanagromyza ZADBI-19	+	M#1			**1**
Melanagromyza ZADBI-20	+	M#1			1

Melanagromyza ZADBI-21	+	M#1			**2**
Melanagromyza ZADBI-22	+	M#1			**1**
Melanagromyza ZADBI-23	+	M#1			**1**
Melanagromyza ZADBI-24	+	M#1			**2**
Melanagromyza ZADBI-25	+	M#1	+		2
Melanagromyza ZADBI-26	+	M#1			1
Melanagromyza ZADBI-27	+	M#1			1
Metopomyza ZADBI-1	+	M#1/FIT	+		**12**
Metopomyza ZADBI-2	+	M#3			**1**
Metopomyza ZADBI-3	+	M#1,3/FIT	+		**17**
Metopomyza ZADBI-4	+	M#1			**2**
Metopomyza ZADBI-5	+	M#1	+		**3**
Metopomyza ZADBI-6	+	FIT			**1**
Nemorimyza maculosa **(Malloch)**	+	M#1			2
Nemorimyza posticata **(Meigen)**	+	M#1/H			**2**
Nemorimyza ranchograndensis **(Spencer)**	+	H			**1**
Nemorimyza ZADBI-1	+	M#1			4
Nemorimyza ZADBI-2				+	1

***Ophiomyia punctohalterata* (Frost)**	+	M#1			1
Ophiomyia ZADBI-1				+	**1**
Ophiomyia ZADBI-2	+	M#1			1
Ophiomyia ZADBI-3			+		1
Ophiomyia ZADBI-4	+	M#1			1
Ophiomyia ZADBI-5			+		1
Ophiomyia ZADBI-6				+	1
Ophiomyia ZADBI-7				+	1
Ophiomyia ZADBI-8				+	1
Ophiomyia ZADBI-9	+	M#1			1
Phytobia ZADBI-1	+	M#1			**7**
Phytobia ZADBI-2	+	M#1			**1**
Phytobia ZADBI-3				+	2
Phytobia ZADBI-4				+	1
Phytobia ZADBI-5			+	+	**2**
Phytobia ZADBI-6				+	**1**
Phytobia ZADBI-7			+		1
Phytoliriomyza leechi Spencer	+	M#1	+		**20**
***Phytoliriomyza pilosella* Spencer**	+	M#1		+	**4**

Phytoliriomyza ZADBI-1	+	M#1			**8**
Phytoliriomyza ZADBI-2	+	M#1,4	+		**27**
Phytoliriomyza ZADBI-3	+	M#1,3,4	+		**15**
Phytoliriomyza ZADBI-4	+	M#1,2,3	+		**13**
Phytoliriomyza ZADBI-5	+	M#1	+		**14**
Phytoliriomyza ZADBI-6	+	M#1,2,4/Em-VW		+	14
Phytoliriomyza ZADBI-7			+		**2**
Phytoliriomyza ZADBI-8	+	M#4	+		6
Phytoliriomyza ZADBI-9	+	M#1		+	**11**
Phytoliriomyza ZADBI-10			+		**10**
Phytoliriomyza ZADBI-11	+	M#1			**1**
Phytoliriomyza ZADBI-12	+	M#1	+		**7**
Phytoliriomyza ZADBI-13			+		**3**
Phytoliriomyza ZADBI-14				+	1
Phytoliriomyza ZADBI-15			+		**3**
Phytoliriomyza ZADBI-16			+		**2**
Phytoliriomyza ZADBI-17	+	M#1	+		**5**
Phytoliriomyza ZADBI-18			+		**1**

Phytoliriomyza ZADBI-19			+		**1**
Phytoliriomyza ZADBI-20			+		3
Phytoliriomyza ZADBI-21			+		1
Phytoliriomyza ZADBI-22	+	M#1			1
Phytoliriomyza ZADBI-23			+		1
Phytoliriomyza ZADBI-24	+	M#1			**1**
Phytomyza ZADBI-1	+	M#1			**1**
Phytomyza ZADBI-2	+	M#1			1
Phytomyza ZADBI-3			+	+	**4**
Phytomyza ZADBI-4	+	M#1			**6**
Phytomyza ZADBI-5	+	M#1			4

Table 2. List of Costa Rican species of Agromyzidae. The list is based on distribution records found in Martinez and Étienne (2002) and additional subsequent records (Notes: *Calycomyza obscura* Spencer, *Calycomyza ecliptae* (Spencer), *Phytoliromyza jacarandae* Steyskal & Spencer, listed in Martinez and Étienne (2002); *Cerodontha* (*Diz.*) *scirpioides* Zlobin, listed in Boucher (2005); and *Calycomyza lantanae* (Frick) listed in Monteiro et al. (2019) as present in Costa Rica are invalid and here excluded). New species additions for Costa Rica are in BOLD. The right column is signaling the presence at the cloud forest sites.

Species	**Cloud forest**
Agromyza animata Spencer	
Agromyza fusca Spencer	X
Agromyza venezolana Spencer	
Calycomyza addita Spencer	

Calycomyza caguensis Spencer	
Calycomyza devia Spencer	X
***Calycomyza dominicensis* Spencer**	X
Calycomyza hyptidis Spencer	
Calycomyza meridiana (Hendel)	X
***Calycomyza opaca* Zlobin**	X
Calycomyza sidae Spencer	
Calycomyza verbenivora (Spencer)	
Cerodontha (*But*.) *puertoricensis* Spencer	X
Cerodontha (Cer.) dorsalis (Loew)	X
Cerodontha (Diz.) nigrihalterata Boucher	
Japanagromyza frosti (Frick)	
Japanagromyza lonchocarpi Boucher	
Japanagromyza perpetua Spencer	
Japanagromyza phaseoli Spencer	
***Japanagromyza sasakawai* Monteiro et al.**	X
Liriomyza archboldi Frost	X
Liriomyza commelinae (Frost)	
Liriomyza costaricana Spencer	
Liriomyza huidobrensis (Blanchard)	
Liriomyza insignis Spencer	
Liriomyza irazui Spencer	
Liriomyza lupini Spencer	
Liriomyza marginalis (Malloch)	
Liriomyza microglossae Spencer	
Liriomyza mystica Boucher & Nishida	
Liriomyza prompta Boucher & Nishida	

Liriomyza sabaziae Spencer	X
Liriomyza sativae Blanchard	
Liriomyza schmidti (Aldrich)	
Liriomyza schmidtiana Spencer	
Liriomyza trifolii (Burgess)	
Melanagromyza bidentis Spencer	X
Melanagromyza caerulea (Malloch)	
Melanagromyza chitariensis Spencer	
Melanagromyza cirsiophila Spencer	
***Melanagromyza floridensis* Spencer**	X
Melanagromyza floris Spencer	
Melanagromyza mayi Spencer	
Melanagromyza minima (Malloch)	X
Melanagromyza neotropica Spencer	
Melanagromyza rosales Woodley	
***Melanagromyza wedelia* Spencer**	X
Nemorimyza maculosa (Malloch)	X
Nemorimyza posticata (Meigen)	X
Nemorimyza ranchograndensis (Spencer)	X
Ophiomyia buscki (Frost)	
Ophiomyia caribbea (Spencer)	
Ophiomyia costaricensis Spencer	
Ophiomyia crotonis (Frost)	
Ophiomyia eleutherensis (Spencer)	
Ophiomyia gentilis Spencer	
Ophiomyia lantanae (Froggatt)	
Ophiomyia magna Spencer	

Ophiomyia punctohalterata (Frost)	X
Ophiomyia valida Spencer	
Phytobia dorsocentralis (Frost)	
Phytobia picta (Coquillett)	
Phytobia rabelloi Spencer	
Phytobia xanthophora (Shiner)	
Phytoliriomyza conjunctimontis (Frick)	
Phytoliriomyza costaricencis (Spencer)	
Phytoliriomyza imperfecta (Malloch)	
Phytoliriomyza jurgensi Spencer	
***Phytoliriomyza leechi* Spencer**	X
Phytoliriomyza pilosella Spencer	X
Phytomyza loewii Hendel	
Pseudonapomyza asiatica Spencer	

GENERAL DISCUSSION

This thesis investigated the diversity of leaf-miner flies (Agromyzidae) in largely unexplored tropical habitats of French Guiana and Costa Rica and resulted in the identification of more than 200 species/morphospecies. This species-richness at the sites suggests that the actual number of leaf-miner fly species reported for the Neotropical region must be much higher than previously known. Surprisingly, the family Agromyzidae was not listed in Amorim (2009) as a family with a “huge hidden diversity” in the Neotropical region, but this was before the Mitaraka and the ZADBI project, which provided new important findings on the Neotropical diversity of Agromyzidae.

From other past regional surveys, the family Agromyzidae was not necessarily considered common or species-rich in the Neotropical region. For example, Spencer (1982) in his monograph of Agromyzidae of Chile, mentioned that the family Agromyzidae was characteristically depauperate in this country as it was in other areas of the southern hemisphere. Although there has been some progress since then, Agromyzidae are still less diverse in the Neotropical region than temperate region and could reflect a research bias towards temperate regions of the world. This research bias is common and could be partly explained by the presence of more taxonomists and better funding availabilities in developed countries (Magurran 2017; Titley et al. 2017).

The identification of the Agromyzidae from both surveys was challenging, due to the presence of multiple female specimens in the samples and the high number of apparently undescribed species, and this resulted in most Agromyzidae being identified as morphospecies rather than named species. Considering these challenges associated with morphology-based identification, a different approach, DNA barcoding, was performed on selected French Guiana specimens to test its use in assisting with species identification, species delineation, male/ female associations, and diversity assessment. This was for the first time explored for Neotropical Agromyzidae in a context of a biological inventory, rather than the more traditional use of DNA barcoding for the identification of economically important species (e.g., Scheffer et al. 2006). DNA barcoding was found particularly useful to resolve some taxonomic issues and ambiguities arising from traditional morphological identification but was not helpful in providing species identification, due to the lack of a comprehensive barcode reference library for Neotropical Agromyzidae. This emphasizes the need of traditional taxonomic work to build such a library.

Although most of the specimens were identified as morphospecies only (and that the sampling effort is not comparable between the two localities), there are still some interesting patterns noticeable in the generic composition found in lowland/inselberg of Mitaraka, French Guiana versus the mid-elevation cloud forests of Costa Rica (Fig. 1). For example, the genus *Melanagromyza* was dominant at both localities (the most species-rich at Mitaraka and the second most species-rich at the cloud forests). *Melanagromyza* is known to be a predominantly tropical genus, with a little more species reported in the neotropics than the Nearctic region. The number of species is known to decrease rapidly at high elevations and high latitudes (Spencer 1982, 1984). The species-richness of *Melanagromyza* found at the cloud forests, especially at the main site (Zurquí) seems unusual considering the elevation. It would be interesting to sample at higher elevations, in other Costa Rican cloud forests, for example in the adjacent Braulio Carrillo National Park, to investigate the presence of this genus. The genus *Liriomyza* is normally considered a north temperate genus (Lonsdale & Tschirnhaus 2021; Spencer 1972; Spencer and Steyskal 1986) although it is the most species-rich genus in the Neotropical region. The sampling in French Guiana resulted in only 3 *Liriomyza* species, all from the inselberg and none from the lowland rainforest, compared to a high species richness at the cloud forests (*Liriomyza* was the dominant genus at Zurquí and Tapantí), apparently supporting a preference for temperate climate, although more sampling in the lowland rainforest would be necessary to reinforce this pattern. This could also bring further information on two temperate genera, *Phytomyza* and *Metopomyza*, both present at the cloud forest but absent from the lowland rainforest and inselbergs.

There are no recent Neotropical surveys that include Agromyzidae that could be used as comparison, or rather: there are no recent published surveys that include Agromyzidae. One of the problems is that Agromyzidae are often excluded from the results in biodiversity and ecological surveys (e.g. Amorim et al. 2022). This is often due to the taxonomic impediment, where the shortage of expertise and identification tools and the large number of undescribed species prevent fine taxonomic work (Borkent 2021; Giangrande 2003). Other insect surveys, often too ambitious, remain unfinished projects (Borkent & Brown 2015). For example, 200,000 Diptera specimens were collected in Brazil from Malaise traps by the Museu de Zoologia da USP but the results are unpublished (Brown 2005). No list of Diptera is available for other large Neotropical biological surveys such as The Colombia Arthropod Project (Brown 2005). The success of the ZABBI project in getting the full data for All Diptera at Zurquí is discussed in Borkent & Brown (2015).

Although leaf-miner flies are not difficult to catch, the results of the two surveys suggests that not all sampling methods were equally productive, as previously seen for the family Agromyzidae (Scheirs et al. 1997). Overall, the malaise traps caught most of the species of Agromyzidae in both surveys, but not all Malaise traps could be considered successful, considering that Malaise trap #2 set-up for a full year at Zurquí resulted in only 4 species of Agromyzidae (and no unique species). Although possible reasons for this were discussed, it would be interesting to investigate further the reason for this lack of productivity.

Figure

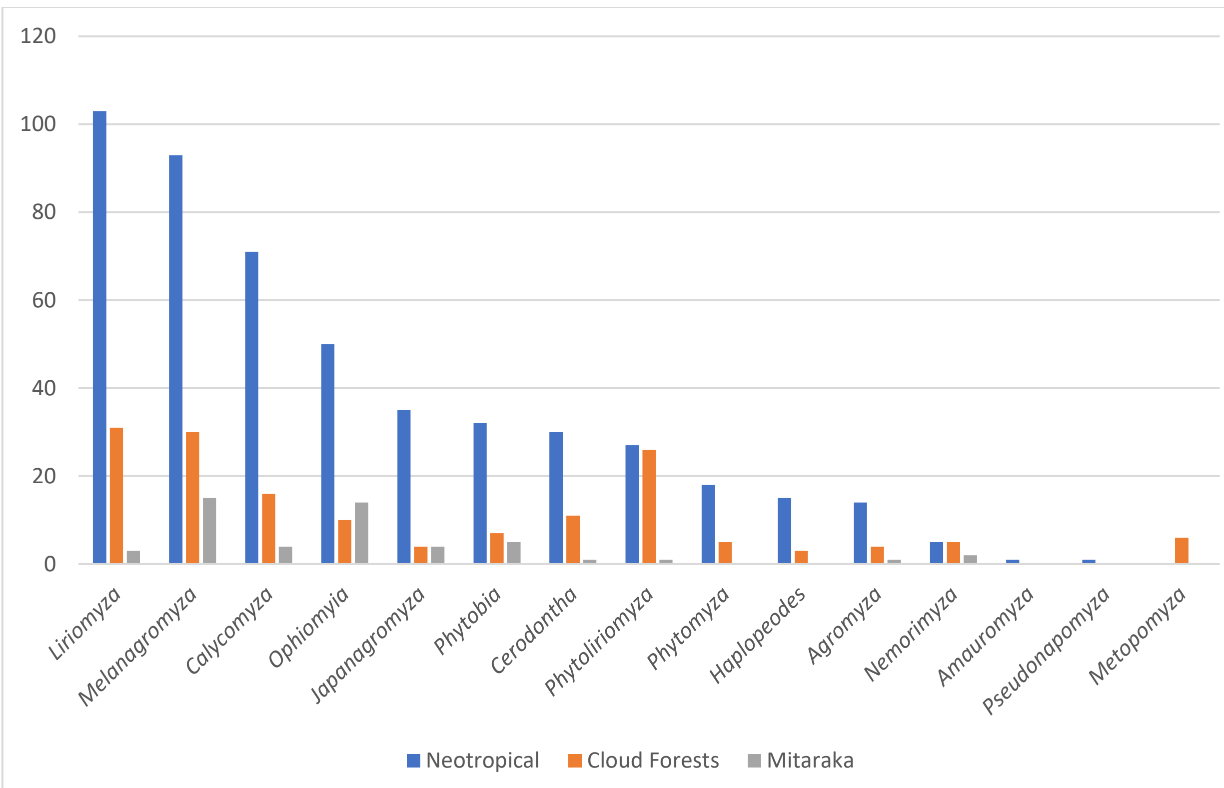


Figure 1. Comparison of species richness per genera for the Neotropical region, the cloud forest sites, and Mitaraka. Ranked from the most species-rich in the Neotropical region to the least species-rich.

CONCLUSION AND FUTURE DIRECTIONS

This study is an important contribution to our knowledge of the Neotropical fauna of Agromyzidae and will serve as a foundation for many subsequent studies. It principally showed the need to build a reference barcode library of Neotropical Agromyzidae which will be important in assisting morphological identification and accelerate the discovery of new species. The high species richness and possibly high percentage of endemic species in some endangered habitats of the neotropics showed the potential of Agromyzidae as an indicator taxon for conservation studies. To use them in their full potential, future work is needed to complete the taxonomic and systematic treatment of these Agromyzidae. My plan for future research is as follow:

DNA barcoding and taxonomy

There are a little more than 2000 specimens of Neotropical Agromyzidae on BOLD (Barcode of Life Database) mostly from the area of Guanacaste, Costa Rica that were collected by D. Janzen and W. Hallwachs (as part of the BioAlfa project (Janzen & Hallwack 2019)). These specimens are included into 398 BINs and are just awaiting to be identified/described. I have agreed to identify this material, which will serve as an extremely valuable resources for future molecular identification, for data sharing, taxonomic revisions and much more.

Host plants

Although no Agromyzidae were reared at the cloud forest sites, ongoing collaboration (e.g. Boucher & Nishida 2014) with naturalist/entomologist Kenji Nishida (research associate, Universidad de Costa Rica) who is actively rearing agromyzid flies in Monteverde cloud forest, and elsewhere in Costa Rica will certainly bring new host records for the material collected during the ZADBI project. One new host record has already been discovered for a new species of

Liriomyza forming mines on *Iochroma arborescens* Schltdl. (Solanaceae) (Fig. 1), and successfully reared by K. Nishida in San Isidro de Coronado (1420 m), in Costa Rica. This species corresponds to *L*. ZADBI-3, a species for which a single male had been collected at the main site (Zurquí) from Malaise trap #1. This will be the first record of an agromyzid developing on this plant. Description of the species with information on its biology will be completed in a future publication. It is to be hope that a similar future collaboration with botanists in French Guiana, especially those specializing on the flora of inselbergs will be established to discover some of the interesting host relationships of the leaf-miners of inselbergs.

Taxonomic revisions

Many new species will be described in future publications, and these will be finished in a larger context of revisionary work that will include keys to species to facilitate their identification. This work will include keys and descriptions of the numerous *Ophiomyia* and *Melanagromyza* from the inselberg in Mitaraka, with many of them having their corresponding DNA barcode and the numerous *Liriomyza*, *Melanagromyza* and *Phytoliriomyza* species of the cloud forests. A new key to genera of the Neotropical Agromyzidae will be written to accommodate the new genus record *Metopomyza*, with a key and description of all the new species.

With the new advancement in molecular technologies, morphological taxonomy has lost popularity, and like the world's biodiversity, it is suffering from an important decline that may lead to the extinction of this branch of science. Taxonomy is at the base of ecological, evolutionary, and biogeographical studies and provides fundamental work needed for species monitoring and conservation decisions. Agromyzidae are part of an important ecosystem and have a variety of relationships with plants, parasitoids and predators. Biodiversity includes all the life that exists, and this diversity is crucial to maintaining healthy ecosystems. Each organism, as big or small as it can be, plays its role and should be all treated equally. In the cloud forests of Costa Rica, this includes the well known and charismatic resplendent Quetzal (Fig. 2), but also a variety of smaller life forms like the jewel beetle, the leaf-cutter ants and of course the leaf-miner flies (Figs. 3-6).

I am hoping that this work on the Neotropical fauna of Agromyzidae will stimulate further research in the neotropics, not only on Agromyzidae but any small organisms that have been neglected but that constitute the bulk of the unknown biodiversity.

Figures

Figure 1. A leaf-mine on *Iochroma arborescens* Schltdl. (Solanaceae) caused by a new species of *Liriomyza* in San Isidro de Coronado, Costa Rica. (Photo. K. Nishida).

Figure 2. The Resplendent Quetzal (*Pharomachrus mocinno* La Llave) is a well-known bird endemic to Neotropical Cloud Forests. (Photo. ©2010-2019 Kenji Nishida / Web National Geographic Japan (©Nikkei National Geographic Inc.).

Figures 3-6. Examples of biodiversity of the cloud forests. (3) walking stick, (4) leaf-cutting ants, (5) jewel beetle, (6) leaf-miner fly (Photos S. Boucher, except Fig. 6, K. Nishida).

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