Diversidad Criptica, Sistemática y Biogeografía Histórica del género *Manerebia* Staudinger, 1897 (Satyrinae: Pronophilina) en el Neotrópico

**Cryptic Diversity, Systematic and Historical Biogeography of the genus *Manerebia* Staudinger, 1897 (Satyrinae: Pronophilina) in the Neotropics**

By

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In memoriam to

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Resumen

Las mariposas Pronophilina Reuter, es una de las subtribus de la tribu Satyrini, son reconocidas como uno de los grupos de mariposas más diversificados en ambientes montañosos y presentan altos niveles de endemismo. Sin embargo, la determinación taxonómica precisa de las especies en muchos géneros de Pronophilina se ha visto afectada por la diversidad criptica y taxones taxonómicamente confusos como es el caso del género Manerebia Staudinger. Este género es un grupo de mariposas andinas, que se distribuye desde el norte de Argentina hasta Venezuela, y presenta una alta diversidad criptica y una variación fenotípica alta (polimorfismos). Se han descrito varias especies nuevas durante las últimas décadas, y otras aún esperan ser descritas. No obstante, la ubicación de Manerebia dentro de la subtribu Pronophilina debe considerarse provisional porque no hay un análisis filogenético y su monofilia aún no se ha evaluado. Además, aún se desconocen las relaciones filogenéticas de las especies dentro del género. Aunque, el género es de interés desde las perspectivas ecológica, evolutiva, biogeográfica y de conservación, pero como base necesaria para tales estudios se necesita un conocimiento sólido que ayude a comprender e inferir los patrones filogenéticos y biogeográficos sobre la historia evolutiva del género Manerebia en el Neotrópico. Por lo tanto, evaluamos la monofilia del género Manerebia, determinamos su posición taxonómica y las relaciones filogenéticas dentro de la tribu Satyrini, y proporcionamos una mejor comprensión de las relaciones a nivel de subtribu dentro de Satyrini. Encontramos a Manerebia como un grupo monofilético en Pronophilina y aclaramos sus relaciones filogenéticas. Descubrimos que el uso de un muestreo taxonómico más grande puede ayudar a mejorar los problemas al usar genes individuales y permite construir relaciones sistemáticas más sólidas. Con base en nuestros análisis, encontramos 48 especies distintas de nuestras 24 especies nominales muestreadas, de las cuales 14 son especies nuevas. Por lo tanto, de acuerdo con nuestra propuesta sistemática, el género Manerebia comprendería 58 especies nominales, pero por el momento algunas permanecen sin describir. Los análisis filogenéticos, junto con los métodos de delimitación de especies y los caracteres morfológicos, permitieron evaluar la alta diversidad criptica dentro del género. Además, nuestro análisis destaca la importancia de emplear el marco de taxonomía integradora para la detección de diversidad criptica en regiones como el Neotrópico. Generamos la primera hipótesis filogenética para el género Manerebia basada en datos de secuencias mitocondriales (COI) y utilizando herramientas filogenéticas. Se proponen nueve clados para el género...
Manerebia a lo largo de los Andes Central y del Norte, siendo el Norte de los Andes la zona con mayor riqueza para el género. Nuestros análisis nos permitieron aclarar algunas de las relaciones filogenéticas dentro del género a nivel de especie. Finalmente, nuestro estudio exploró la historia biogeográfica del género Manerebia estimando tiempos y tasas de diversificación de sus linajes y empleando un análisis biogeográfico para reconstruir su historia evolutiva. Nuestros resultados nos permitieron inferir que el tiempo de divergencia de Manerebia fue entre el Mioceno tardío y el Plioceno, y la mayoría de los linajes existentes ya habían aparecido en el Pleistoceno. El género tuvo un estallido temprano general en el límite del Mioceno tardío / Plioceno temprano seguido de una desaceleración debido a una disminución en la especiación a lo largo del Pleistoceno, y este patrón se refleja para todos los clados en Manerebia. Los eventos de dispersión fue posiblemente el proceso biogeográficos más común dentro del género, y nuestros resultados nos permiten confirmar el papel de la geomorfología andina en la evolución de la biodiversidad Neotropical.

**Palabras claves.** Delimitación de especies, código de barras, Pleistoceno, especiación y diversificación, tiempos de divergencia, áreas ancestrales, Satyrini.
Abstract

The Pronophilina Reuter butterflies, one of the subtribes of the tribe Satyrini, are recognized as one of the most diversified groups of butterflies in mountain environments and present high levels of endemism. However, the accurate taxonomic determination of species in many genera of Pronophilina has been affected by the cryptic diversity and taxonomically confusing taxa as is the case of the genus *Manerebia* Staudinger. This genus is an Andean butterflies group, which is distributed from northern Argentina to Venezuela, and it presents a high cryptic diversity and a phenotypic variation (polymorphisms). Several new species have been described during the last few decades, and others still await description. Nevertheless, the placement of *Manerebia* within the subtribe Pronophilina is to be considered tentative because there isn't a phylogenetic analysis, and its monophyly is not evaluated yet. In addition, the species phylogenetic relationships within the genus are unknown yet. However, the genus is of interest from ecological, evolutionary, biogeographic, and conservation perspectives, but as a necessary base for such studies a robust knowledge is needed to help to understand and infer the phylogenetic and biogeographic patterns about the genus *Manerebia* evolutionary history in the Neotropics. Hence, we evaluated the monophyly of the genus *Manerebia*, determined its taxonomic position and phylogenetic relationships within the tribe Satyrini, and provided a better understanding of the at the subtribe level relationships within the Satyrini. We found *Manerebia* as a monophyletic group into Pronophilina and clarified its phylogenetic relationships. We found that using larger taxonomic sampling may help to improve the problems when using individual genes and it allows to build systematic relationships more robust. Based on our analyses we found 48 distinct species from our sampled 24 nominal species, where 14 are new species. Therefore, according to our systematic proposal, the genus *Manerebia* would comprise 58 nominal species, but for the moment some remain undescribed. The phylogenetic analyses, together with the species delimitation methods and the morphological characters, allowed us to evaluate the high cryptic diversity within the genus. In addition, our analysis highlights the importance of employe the integrative taxonomy framework for the detection of cryptic diversity in regions such as the Neotropics. We generated the first phylogenetic hypothesis for the genus *Manerebia* based on sequence data from mitochondrial (COI) using phylogenetic tools. Nine clades are proposed for the *Manerebia* along the Central and Northern Andes being the
Northern Andes the zone with the most richness. Our analyses permitted us to clarify some of the phylogenetic relationships within the genus to species-level. Finally, our study explored the biogeographical history of the genus *Manerebia* estimating times and rates of diversification for its lineages and employing a biogeographical analysis in order to reconstruct its evolutionary history. Our results allowed us to infer that the divergence time of *Manerebia* was between the late Miocene and Pliocene, and most extant lineages had already appeared in the Pleistocene. The genus had an overall early burst in the late Miocene / early Pliocene boundary followed by deceleration due to a decrease in speciation along to Pleistocene, and this pattern is reflected for all clades in *Manerebia*. Dispersal events are possibly the most common process within the genus, and our results confirm the role of the Andean geomorphological in the evolution of Neotropical biodiversity.

**Keywords.** Species delimitation, barcode, Pleistocene, speciation and diversification, divergence times, ancestral areas, Satyrini.
List of papers and author’s contribution

The thesis is based on the following papers (listed thematically):

I. Evaluating the monophyly and the subtribal position of the Andean genus *Manerebia* Staudinger with remarks on phylogenetic relationships within the tribe *Satyrini* (Nymphalidae, Satyrinae)

Oscar Mahecha-J. & Tomasz W. Pyrcz

Manuscript
[OM and TP collected molecular and ecological data, performed of data analyses and wrote the manuscript].

II. Solving the cryptic diversity of the genus *Manerebia* Staudinger in the Neotrop: description of new species and taxonomic considerations (Nymphalidae: Satyrinae: Pronophilina)

Oscar Mahecha-J., Klaudia Florczyk, Keith Willmott, José Cerdeña, Anna Zubek, Pierre Boyer, Jackie Farfán, Dorota Lachowska-Cierlik, Paola Triviño, M. Gonzalo Andrade-C & Tomasz W. Pyrcz.

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[OM, TP, and KW conceived the study, OM, TP, PB, JC, JF, AZ and PT collected molecular and ecological data, performed majority of data analyses and wrote the manuscript, KF, DLC and GAC contributed to data analysis and revisions of the manuscript, KW and JC provided samples and contributed by deep knowledge of species ecology and distribution].

III. A contribution elucidation of the phylogenetic relationships of the Andean genus *Manerebia* Staudinger (Nymphalidae: Satyrinae: Pronophilina) based on a molecular phylogeny

Manuscript

[OM and TP conceived the study, OM, KW, TP, ME, and PB coordinated the collection of field data, analysed data, KW and TP participated in writing the manuscript, DLC, CG, ME, and DA contributed to data analysis and revisions of the manuscript, KW provided samples and contributed by deep knowledge of species ecology and distribution].

IV. An exploration of the complex biogeographical history of the Neotropical butterfly Manerebia Staudinger (Nymphalidae: Satyrinae: Pronophilina)

Oscar Mahecha-J., Marianne Espeland & Tomasz W. Pyrcz

Manuscript

[OM, ME and TP collected molecular and ecological data, performed of data analyses and wrote the manuscript].

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Oscar Mahecha-J.
# CONTENTS

## INTRODUCTION

References

## SUMMARY

## THEORETICAL AND CONCEPTUAL OVERVIEW

- Species concept
- Speciation
- Cryptic Diversity: What are cryptic species?
- Phylogeny and Times of Divergence
- Historical biogeography
- Species Delimitation
- The genus *Manerebia* Staudinger, 1897

References

## REASONING

References

## OBJECTIVES OF THE THESIS

- General
- Specific

## RESEARCH QUESTIONS AND HYPOTHESIS

- Chapter I.
- Chapter II.
- Chapter III.
- Chapter IV.
CHAPTER I.  
Evaluating the monophyly and the subtribal position of the Andean genus *Manerebia* Staudinger with remarks on phylogenetic relationships within the tribe Satyrini (Nymphalidae, Satyrinae). Oscar Mahecha-J. & Tomasz W. Pyrcz.

CHAPTER II.  
Oscar Mahecha-J., Klaudia Florczyk, Keith Willmott, José Cerdeña, Anna Zubek, Pierre Boyer, Jackie Farfán, Dorota Lachowska-Cierlik, Paola Triviño, M. Gonzalo Andrade-C & Tomasz W. Pyrcz.

CHAPTER III.  
A contribution elucidation of the phylogenetic relationships of the Andean genus *Manerebia* Staudinger (Nymphalidae: Satyrinae: Pronophilina) based on a molecular phylogeny

CHAPTER IV.  
An exploration of the complex biogeographical history of the Neotropical butterfly *Manerebia* Staudinger (Nymphalidae: Satyrinae: Pronophilina)
Oscar Mahecha-J., Marianne Espeland & Tomasz W. Pyrcz.

CONCLUSIONS 206
RESEARCH PERSPECTIVES 208
LIST OF PUBLICATIONS 210
LIST OF ATTENDANCE EVENTS 211
SUPPLEMENTARY MATERIAL

Chapter I. 212

Chapter II. 212

Chapter III. 212

Chapter IV. 212
Introduction

The Neotropical region includes the American tropics, from northern Mexico to central Argentina (Morrone 2001). It is the most species-rich biogeographic region in the world (Chazot et al. 2016). Within the Neotropics, species diversity often spans many taxonomic groups, such as plants (Myers et al. 2000), vertebrates (Haddad & Prado 2005), and arthropods (Devries & Walla 2001; Rosser et al. 2012; Chazot et al. 2016).

The Andean orogeny followed an uplift pattern from south to north, with episodic periods of intense mountain building (Garzione et al. 2008; Hoorn et al. 2010; Chazot et al. 2016). Geological evidence shows that the central Andes rose between 1.5-2.5 km during a period of rapid uplift between 10 and 6 million years ago, followed by another period of accelerated uplift in the Northern Andes between 5 and 2 million years ago (Chazot et al. 2016; Mahecha et al. 2019). This rapid rise is one of the main events in the geological history of the South American continent and probably participated in the formation of the modern Amazon basin (Hoorn et al. 2010; Chazot et al. 2016) and large mountain habitats such as páramos, snow-capped mountains, Andean forests, among others, perhaps affecting the origin of the current Neotropical biota (Chazot et al. 2016).

The biota of the Andes tend to be, from a phylogenetic point of view, more recent than the biota of other biogeographic regions of the world. It is very common to find series of species with high morphological similarity because they can be under the same selection forces which leads to evolutionary convergences (Coyne & Orr 2004), and makes taxonomic determination difficult. This morphological similarity between different species is currently known as cryptic diversity. Cryptic divergence can be driven by random processes (for example, genetic drift, mutations) or by divergent selection (Coyne & Orr 2004; Costello et al. 2013; Espíndola et al. 2016), so longer isolation periods are expected to increase ecological divergence (Feder et al. 2013), a supposition demonstrated in several taxa (e.g. in Lepidoptera) (Coyne & Orr 2004; Espíndola et al. 2016).

The discovery of biodiversity may be impeded to some extent by the presence of cryptic diversity (Bickford et al. 2007; Espíndola et al. 2016), when genetic divergence within a nominal species is present but is not accompanied by morphological
differences between sets of populations. Several studies have highlighted the presence and the importance of cryptic diversity, which could represent a substantial proportion of natural capital (Dincă et al. 2011).

Several factors have stimulated a growing literature that discusses the importance of cryptic diversity (Espíndola et al. 2016). In the first place, the prioritization of areas for conservation is often determined by the richness and endemism of the species present in these areas, in which species with high phenotypic similarity can occur, causing errors in taxonomic determination. Therefore, the study of cryptic diversity is necessary to estimate these diversity parameters (Fusinatto et al. 2013; Espíndola et al. 2016). Second, the discovery of cryptic diversity establishes evolutionarily significant units for conservation (Beheregaray & Caccone 2007; Demos et al. 2014; Espíndola et al. 2016). Third, the discovery of cryptic diversity is essential for understanding biotic and anthropogenic changes, invasions, and the state of ecosystems, among others (Pérez-Portela et al. 2013; Espíndola et al. 2016). Thus, cryptic diversity becomes a vital component of biological diversity (Meleg et al. 2013; Espíndola et al. 2016).

In turn, delimiting and identifying independent lineages is critical not only for taxonomy, but also for understanding the processes leading to diversification, the definition of conservation strategies, and communication between scientists and non-scientific communities (Costello et al. 2013; Espíndola et al. 2016). Likewise, given the global biodiversity crisis, the cataloguing of species has become a race against time. An estimate of the diversity of cryptic species is important to better understand the evolutionary processes and patterns of ecosystem functioning, while at the same time having profound implications in conservation biology for different taxonomic groups (Bickford et al. 2007; Esteban & Finlay 2010).

On the other hand, the increasing number of new species of butterflies has been overshadowed by many synonyms that have invalidated several of these species, since several of these species present a great morphological variation as occurs in the genus Pedaliodes Butler, 1867 or on the contrary, when several species present a great morphological similarity that led to the inclusion of different species within the same species or cataloguing several subspecies, which causes inflation and taxonomic underestimation (Viloria 2001). Another case is the genus Manerebia Staudinger, 1897, a group of butterflies distributed throughout the tropical Andes, which presents a high
cryptic diversity, making taxonomic determination difficult (Pyrcz et al. 2006; Mahecha-J. et al. 2021).

Additionally, there is a lack of a comprehensive phylogenetic hypothesis which prevents inferring their historical and evolutionary biogeographic patterns, and the lack of knowledge of natural histories of this group of butterflies (Pyrcz et al. 2006; Mahecha-J. et al. 2021). Therefore, this thesis was focused on evaluating cryptic diversity in the genus Manerebia, on generating a phylogenetic hypothesis and on producing an approximation of the historical biogeographic patterns of this genus in the Neotropical region.

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the Andes (Lepidoptera: Nymphalidae, Satyrinae, Pronophilina). *Zootaxa*, 4970 (2), 293–302. https://doi.org/10.11646/zootaxa.4970.2.3


Summary

This thesis expands the general knowledge of cryptic diversity, systematics, phylogeny, and historical biogeography of the genus *Manerebia* Staudinger, 1897, a group of mountain butterflies belonging to the Neotropical satyrine subtribe Pronophilina. It comprises four chapters:

**Chapter I** details the evaluation of the monophyly of the genus *Manerebia* and its phylogenetic relationship with other genera of the subtribe Pronophilina, and some remarks about the phylogenetic relationships within the tribe Satyrini (objective I).

**Chapter II** explains the phylogenetic tools used to delimit species within the genus *Manerebia*. Moreover, it details the evaluation of the cryptic diversity of the genus, and as a result, includes taxonomic descriptions of some new species and subspecies from *Manerebia*. Finally, it describes a proposal of the systematic arrangement of the genus (objective II).

**Chapter III** describes the phylogeny of the genus *Manerebia* based on molecular characters (COI), and explains the proposition of clustering the species of *Manerebia* into nine clades, and defines the geographic distribution for all of them (objective III).

Finally, **Chapter IV** includes an approximation on the historical biogeography of the genus *Manerebia* by providing the timing of divergence, the diversification patterns, and the identification of hypothetical ancestral areas, and possible speciation processes that might occur in the genus (objective IV).
Theoretical and conceptual overview

Species concept

Since ancient times, biologists have tried to classify the different taxonomic groups using a variety of tools for this purpose, such as morphological comparisons, phylogenetic analysis, and geometric morphometry, among others. In this way, different classification systems are proposed for living beings, from Linnaeus' Systema Nature (1758) to the taxonomic proposals based on the cladistics for each taxonomic group in particular (Torretti 2010). However, the use of any methodology for the taxonomic determination of species has a rooted problem from the epistemological point of view of Biology, which is to define what is a biological species? This question has become a great debate in the field of Evolution and Systematics in recent years (Wheeler & Meier 2000; Cadena 2003).

The critical point of the debate is based on the fact that a species must function as the unit of evolutionary theory and, in turn, be the basis for describing the different historical patterns of taxonomic diversity and these must be reflected in the different biological classifications (Cracraft 1989; Cadena, 2003). Following this order of ideas, it has been emphasized the need to be able to distinguish between the question of what is a species? And what are the operational criteria used to recognize the various biological species? (De Queiroz, 1999). To this end, various species concepts have been proposed throughout history, which are solid speaking from a philosophical perspective but leave many questions for biologists, especially taxonomists, on how to assign or define the species rank (Cadena 2003).

Despite all the proposed concepts of species, reaching a general and satisfactory definition for all seems to be quite unlikely because there will always be some subjectivity to define to what extent a species can be considered (Helbig et al. 2002; Cadena 2003; Mallet 2013) and even more so knowing all the variations of types of species that are managed in biology (e.g. cryptic species, endemic species, tourist species, among others).

Conflicts between species definitions appear basically when defining operational criteria to identify these taxa. On the one hand, we have what we could call taxonomic concepts, which would be those whose objective is to classify and that does not assume
any type of hypothesis about the speciation process. In front of these are the evolutionary concepts, in which the species are defined as evolutionary units. Proponents of evolutionary species concepts are more interested in establishing phylogenies, identifying monophyletic groups, and determining patterns and processes of speciation than in the classification of species alone. Within evolutionary definitions, it is possible to differentiate between clade concepts and contemporary species. Among the former would be grouped phylogenetic concepts (more related to ancestry-descent and cladogenesis patterns). For their part, contemporary concepts base their definitions on criteria that can be applied to current species. In turn, here we could encompass concepts based on reproduction (such as the biology of species or recognition) or on cohesion (the cohesive or evolutionary concept) (Perfectti 2002).

In turn, Mallet (2013) highlights this problem with the use of the concept of species, since it is a concept that is currently used not only by taxonomists but also by many other branches of Biology, such as Ecology, Conservation, Biogeography, etc., where the species is the important unit for their decisions, and the drawback lies in how the species is being defined, and as something more philosophical or something more practical, still involving no number of questions and debates, because it could be said that the definition of species is somewhat more dependent on the type of biological issue that is being worked on and is not considered as a unified concept in Biology, that is, the word species is more used in practice than as a biological concept, which maximizes the problem of the concept of species.

However, there are proposed species concepts that are more accepted and therefore more used in the scientific community. Concepts such as the Biological Species (BSC) of Dobzhansky (1937) and Mayr (1942; 1963) which at the time was widely accepted and is one of the most used, define that a species are those individuals of the same population that are they can cross with each other and leave a fertile offspring over time, that is, there is a genetic flow between them and there is no presence of pre-and postzygotic barriers (Cadena 2003; Torretti 2010). However, it is a concept that has many drawbacks, among which are highlighted, that it is a concept only applicable to sexual organisms, it is not replicable for asexuals such as Protozoa and Bacteria; does not apply to hybrids, such as the Heliconius heurippa butterfly (Nymphalidae: Heliconiinae), which is a product of the natural hybridization of the Heliconius cydno x
Heliconius melpomene species and which is considered an example of hybridization speciation (Salazar et al. 2003; Mallet et al. 2007).

The BSC concept really has more use in zoology than in botany, since in plants hybridization is very common (John Lynch, personal comment), due to polyploidy processes that easily change the number of chromosomes from one generation to another generating a genetic incompatibility (genetic imbalance). In addition, it is very subjective when it comes to allopatric populations such as the case of Cistothorus apolinari and Cistothorus hernandezi, two species of birds, from the Cundiboyacense area, for some ornithologists they are considered under the BSC concept as only one species: C. apolinari, with two subspecies C. a. apolinari and C. a. hernandezi, and it is still a debate for ornithologists of how they should handle the taxonomic status if as a single species or two different species (Cadena 2003). Therefore, it is not a concept that is applicable to all of Biology but it is the most used concept in practice to delimit species, especially in zoology.

On the other hand, another widely used concept is the Phylogenetic Species Concept (PSC), which proposes that a species is an irreducible group of individuals diagnostically different from other groups and that it exhibits a parental pattern of ancestry and descent (Nelson & Platnick 1981; Cracraft 1989; Wheeler & Meier 2000; Cadena 2003). With this concept, the shortcomings presented by the Biological Species concept can be mitigated, since it is a theoretically very broad and robust concept, because it is possible to use it in both sexual organisms (the smallest aggregation of a population) and asexual (lineages) (Nixon & Wheeler 1990).

This concept, in turn, helps to solve the taxonomic difficulties of allopatric populations, such as the case of C. apolinari and C. hernandezi named above, where according to the PSC concept, there are two allopatric species and they are not considered subspecies, despite that no differences have been found in the song, which from the point of view of the BSC concept, is a factor that can generate speciation processes in birds; The differences lie more in the population genetic distances that occur in these two species, that is, there is a genetic incompatibility despite presenting similar phenotypes and songs, which leads us to use the term cryptic species, rather than the BSC light are not recognizable and are handled as polytypic species.
(subspecies), generating a taxonomic underestimation in the species range, which can be solved using the PSC concept (Cadena 2003).

Also, the PSC allows solving the discussions about the allopatric sister species that come from the same ancestor in common, for some researchers called superspecies (Futuyma 2005), very difficult to treat and explain with the BSC but very easy with the PSC. On the other hand, the PSC is very useful in systematics since it allows the use of terms based on Evolutionary Biology based on the ancestry of the species, allowing kinship relationships; For example, it is possible to use terms such as monophyletic, paraphyletic, and polyphyletic group, which depend on the degree of ancestry-descendants, managing to group taxonomic groups according to their evolutionary history, mediated by ancestral characters (Plesiomorphic) and derivatives (Apomorphic: Sinapomorphic and Autapomorphic). For these reasons, the PSC represents a unified species concept and phylogenetic species are at the same time the basic units of Scientific Nomenclature, Linnaean classification, and organic evolution (Wheeler & Meier 2000).

Additionally, the use of the concept of phylogenetic species is potentially more easily testable because it is based on observable characters and is more compatible with phylogenetic theory since speciation events are marked by character transformations as well as providing a context within which they can be carried out explain changing gene frequencies without losing the unique historical information imparted by character transformation (Wheeler & Meier 2000). For example, the spine is found throughout the terminal lineage that we call Vertebrata, but this does not mean that the spine of the cat and the elephant are identical, but to say why the spine of the elephant looks so different (Transformation), it becomes a character that is not shared by the cat's spine, as it presents some modifications inherent to the taxonomic group, which may lead to denying the evolutionary history and exclude our ability to recover that history of these groups that share a certain degree of ancestry, which does not happen when using the PSC, since the transformation characteristics is evidence of speciation processes, since characters are modifications of preexisting characters, and such transformations result in recoverable and hierarchical sets of nested attributes (Wheeler & Meier 2000).

Another advantage of the PSC is with respect to geographic isolation, if the populations are allopatric, parapatric or sympatric, which is quite critical for a concept
that is based on interbreeding because in practice it is difficult to check if there is still gene flow as it happens. For example, with the BSC concept, phylogenetic species are compatible with any mode of speciation that is not in conflict with the observed character distributions or with the results of the cladistic analysis (Wheeler & Meier 2000). An example can be seen in the populations of *Eurytemora affinis*, which is a copepod (Crustacea) that was considered cosmopolitan worldwide according to the BSC concept, but when using the phylogenetic concept and the various cladistic analyzes, it was found that it was a complex of cryptic species (17 spp.) and that there were actually different species of this copepod, where speciation processes such as allopatry and sympatry can be evidenced (Eunmi 2000).

The previous examples are what some authors call phylogenetic diversity and cryptic diversity that is based on the PSC concept, which become an excellent alternative to measure the diversity of species in an area from the identification of monophyletic groups, thus being an objective way to enumerate the number of real species in each zone, without falling into the masking of cryptic species (Wheeler & Meier 2000; Bickford *et al.* 2007), and in this way to propose with greater reliability priority areas for conservation.

Consider, for example, two areas A and B with an equal number of species, if the species in area A all members are closely related within a single monophyletic group, while those in area B represent a large number of related monophyletic groups. Then the conservation of area B results in saving more biological diversity in terms of the types and differences of attributes of the species (Wheeler 1995a; Wheeler & Meier 2000). Therefore, we can say that the phylogenetic species, based on the PSC, are formulated as possible hypotheses. These, in turn, can be proven by means of comparable observations. Furthermore, phylogenetic species are independent of the assumptions of evolutionary processes and modes of speciation, which provide a rationale that these mechanisms can be evaluated or studied. Phylogenetic species are based on characters (Transformations), making them theoretically sound and easily applicable in practice (Wheeler & Meier 2000).

Similarly, phylogenetic species provide an objective basis for measuring and comparing biological diversity as mentioned above. Therefore, phylogenetic species are fully consistent with phylogenetic theory but are formulated in a way that is
independent of cladistic analysis (Wheeler & Meier 2000), becoming a good species concept, regardless of the gaps that it presents. The PSC, but compared to other species concepts, in my opinion, is the one that presents the least subjectivity, in addition to all the advantages that it offers to biological systematics and the ease of being used in other types of studies, such as Ecology, Biogeography, Conservation, among others, for the ease of distinguishing between the different types of characters, for its explanatory power and for being totally verifiable, in addition, to being in agreement with the phylogenetic and evolutionary theory.

**Speciation**

The formation of new species can be considered as a temporary process by which some populations differentiate and achieve evolutionary independence. Harrison (1998) has proposed that various degrees of differentiation are achieved during the speciation process, which is reflected by different species concepts. Once the populations interrupt the gene flow between them, different alleles can be fixed in each one of the populations, with which they would have their own diagnostic characteristics, and would arrive at the phylogenetic species. Subsequently, the differences may be accentuated by the appearance of barriers to genetic exchange or new cohesion mechanisms. At this point, one could already speak of species under the cohesive concept or under the concepts of reproductive isolation. Finally, species achieve that name under the genealogical concept when exclusivity is achieved, the property by which species present exclusive genomes with their own unique characteristics. This process is the most common in the case of allopatric speciation. If speciation occurs in sympatry, the route to follow may be slightly different, implying that populations achieve species status under the concepts of isolation/cohesive and phylogenetic at the same time (Perfectti 2002; Coyne & Orr 2004).

For some species concepts, the impediment of gene flow between populations directly implies the existence of speciation. Reproductive isolation can be produced by various types of reproductive barriers that can be basically classified as prezygotic and postzygotic, depending on when they act. Prezygotic barriers imply impediments to zygote formation. The post-zygotic barriers result from all those situations in which the zygotes, and the adult individuals if they come to complete their development, have practically nullified their biological efficacy. Within the prezygotic barriers, we have all
those situations that imply that the two species cannot mate. Included in this section would be temporary isolation, isolation by habitat or resource differentiation; and ethological isolation. Furthermore, mechanical or physiological incompatibilities between the reproductive apparatus of both species would also be part of the prezygotic barriers. Within the postzygotic barriers, we find the mortality of the zygotes, the non-viability of the hybrids, and the sterility of the hybrids. Hybrid zygotes may not develop and abort shortly after their formation or during a later phase of development/metamorphosis (Perfectti 2002; Coyne & Orr 2004).

There are various criteria to differentiate the types of speciation, the most widely used being spatial. Depending on the geographic separation patterns that diverging populations present, four basic types of speciation can be distinguished: allopatric, parapatric, stasipatric, and sympatric speciation, although stasipatric speciation can be considered as a variant of speciation parapatric (Perfectti 2002; Coyne & Orr 2004; Futuyma 2005).

**Cryptic diversity: what are cryptic species?**

The literature is full of definitions for the concept of cryptic species. Some authors consider it to be a synonym for sister species (Saez & Lozano 2005), although for others the concept of "brother" is related to a more recent common ancestry than "cryptic", which implies a sister-species relationship (Knowlton 1986; Bickford *et al.* 2007). Other authors defend that sister species are included in the category of pseudo-sister species if once diagnostic characters are found for each species and only a slight phenotypic similarity is presented (Knowlton 1993; Saez & Lozano 2005). Adding to the confusion of these concepts, some authors refer to camouflaged species or secret species as "cryptic species" (Claridge *et al.* 2005; Bickford *et al.* 2007), and another view is to consider that two or more species are "cryptic" if they are, or have been, classified as a single nominal species because they are at least superficially morphologically indistinguishable (Bickford *et al.* 2007).

Some authors postulated that the species designated as "cryptic" should diverge recently, and may be separable only with molecular data, in addition, they must be in sympatry, or be reproductively isolated (Bickford *et al.* 2007). In addition, adding the problem that there is no single species concept (Mallet 2001), therefore leaves a weakness in certain characteristics to define a cryptic species if, for example, we only
base ourselves on the fact that there must be a barrier to the genetic flow, which could lead to reproductive isolation and therefore a speciation process (Bickford et al. 2007).

For this reason, the most accepted definition of a cryptic species suggests that they are several species that present a high morphological similarity and are indistinguishable at first glance by means of traditional morphological characters and that they are classified under the same species being totally different species (Bickford et al. 2007; Trontelj & Fišer 2009).

Besides, cryptic diversity is an issue that in recent years has taken on great importance from the point of view of Phylogenetic Systematics and Conservation Biology since new species of various taxa have been reported worldwide, which has increased the species richness of various areas of the world (Bickford et al. 2007). The discovery of cryptic diversity, defined as two or more distinct species that were classified as one due to morphological similarity (Bickford et al. 2007; Pfenninger & Schwenk 2007) is believed to be a potentially important factor influencing future conservation decisions (Witt et al. 2006). Bickford et al. (2007) predicted that "the discovery of cryptic species is likely not random with respect to taxon and biome and therefore could have profound implications for evolutionary theory, biogeography, and conservation". They based their prognosis on data taken from different studies focused on Systematics and Quantitative Biology (Trontelj & Fiser 2009). On the contrary, Pfenninger & Schwenk (2007), inspired by this prediction, carried out a quantitative meta-analysis of 771,931 documents containing 2207 reports on species cryptics in various rows of Metazoa. They unexpectedly concluded that the proportion of species is distributed almost evenly among the major metazoan taxa and biogeographic regions when correcting for species richness and study intensity. In a recent review on cryptic biodiversity, Beheregaray & Caccone (2007) took a radical position towards this point of view, despite the impact it could have on the assessment and conservation of biodiversity (Trontelj & Fiser 2009).

With regard to cryptic diversity in butterflies, the study by Hebert et al. (2004) in which they deciphered by means of the bar code technique (barcoding) several cryptic species that made up the group *Astraptes fulgerator* Walch, 1775 (Hesperiidae), where the authors report a complex of at least 10 species in this group *A. fulgerator*. Largely sympatric, these taxa have mostly different host plants, resulting in caterpillar variation,
and with different ecosystem preferences, but there is no marked genital differentiation in adults. Furthermore, the results show that cryptic species are prevalent in tropical regions, a critical issue in efforts to document species richness globally. They also illustrate the value of the DNA barcode, especially when combined with traditional taxonomic tools, by revealing the hidden diversity in different groups of species.

Burns et al. (2007) studied the butterfly *Perichares philetis* (Gmelin, [1790]) (Hesperiidae), which according to the barcode is divided into a complex of four species in the Guanacaste Conservation Area (ACG) in northwestern Costa Rica. Three of the species are new, and all four are described, where the caterpillars, pupae, and host plants, according to the authors, offer better distinctive characters than the adults, whose differences are, for the most part, subtle and diffuse characters due to the intraspecific variation that exists in the group. The caterpillars of the two species are generalist herbivores; of the other two, they are palm specialists, of which each one feeds on different genera of palm, observing a specialization of these cryptic species in their diet. The four GCA taxa proposed by the authors belong to a panneotropic complex of at least eight species. It is likely that this complex includes even more species, the delimitation of which will require further molecular studies.

Haubrich & Schmitt (2007) studied the influence of cyclical climatic fluctuations and their impact on high altitude species, where they investigated the genetic structure of cold-adapted animals and their coherence with geographic distributions throughout the late Quaternary. They analyzed an endemic species in the Swiss Alps, *Erebia melampus* (Füssli, 1775) (Nymphalidae), by allozyme electrophoresis to detect its intraspecific differentiation, and detected a strong differentiation in three lineages by means of the genetic distances between the two groups of *E. melampus*. The average genetic distance between these three groups was 0.17. These results give evidence of the existence of a complex of species within the *E. melampus/ E. sudetica* group and indicate a discontinuous distribution within this group during at least the last ice age. One of them, *E. sudetica inalpina*, is found in the northern Alps and most likely had its refuge in the glacial Würm north of the Alps. The western group of *E. melampus* could have had a refuge in the southwestern margin of the Alps, the eastern group in the lower altitudes of the southeastern and/or eastern Alps. In the latter, a further subdivision is possible within this relic area.
For their part, Dincă et al. (2011) studied the European white wood butterfly *Leptidea sinapis* (Linnaeus, 1758) (Pieridae), which have become a model to study speciation in Europe. The authors showed that the so-called *Leptidea* group actually consists of three species. The new species is proposed based on the study of DNA or karyological data. Such a discovery challenges the knowledge we have about biodiversity since the study carried out by the authors exemplifies how a cosmopolitan species can go unnoticed even as a model species intensively studied for evolutionary studies such as speciation.

Lukhtanov et al. (2015) used molecular characters and reorganized the taxonomy of butterfly species close to *Polyommatus* (Agrodiaetus) *ripartii* (Freyer, 1830) (Lycaenidae). The subgenus (Agrodiaetus) is a model system in evolutionary research, but its taxonomy is poorly elaborated because, as a general rule, most of its species are morphologically undifferentiated. The taxon *P. (A.) valiabadi* was supposed to be one of the few exceptions to this rule due to its exactly distinguishable wing pattern, but it was realized that, in fact, *P. valiabadi* is a triplet of cryptic species, strongly differentiated by their mitochondrial karyotypes and haplotypes but not by morphology.

Lavinia et al. (2017) focused on studying the butterfly fauna of the Atlantic Forest, an access point to biodiversity in Argentina. They tested the efficacy of genomic libraries for specimen identification, used it to assess the frequency of cryptic species, and examined geographic patterns of genetic variation, making this study the first large-scale genetic evaluation of southern butterflies. South America. The authors found three new butterfly records for the country (*Eurema agave*, *Mithras hannelore*, and *Melanis hillapana*). In summary, this study not only supported the usefulness of the DNA barcode for the identification of Argentine butterfly species but also highlighted several cases of deep, intraspecific, and superficial interspecific divergence that should be studied in more detail in the species of butterflies in the Neotropics.

Pyrcz et al. (2006) and Mahecha-J et al. (2021) indicated a high cryptic diversity in the subtribe Pronophilina, but pointed out that has not been well-studied yet (e.g. the genera *Manerebia*, *Pedaliodes* Butler, 1867, *Eretris* Thieme, 1905). For example, Marín et al. (2017) found in the *Pedaliodes obstructa* complex many putative species based on molecular analyses of COI barcodes, while Padrón et al. (2021) reported several cryptic
species within the genus *Altopedaliodes* Foster, 1964 according to morphological and molecular analyses.

** Phylogeny and times of divergence **

The evolutionary history of life includes two primary components: phylogeny and timescale (times of divergence). Phylogeny refers to the branching order (relationships) of species or other taxa within a group, crucial for understanding the inheritance of traits and for constructing classifications. However, a time scale is equally important because it provides a way to compare phylogeny directly with the evolution of other organisms and with geological history, climate, and environmental impacts, among others. Temporal information can come from both the fossil record (Benton 1993; Blair *et al.* 2006) and molecular clocks, which are often calibrated using the fossil record (Hedges & Kumar 2003; Kumar 2005; Wray 2001; Blair *et al.* 2006).

The molecular clock hypothesis is a consequence of the neutral theory of evolution which postulates that for any DNA sequence, mutations accumulate at relatively constant rates as long as the DNA sequence retains its original function (Gojoborl *et al.* 1990; Chávez *et al.* 2004). The difference between the nucleotide sequences of a DNA or protein segment of two species can be proportional to the time that has elapsed since these two species diverged from a common ancestor (coalescence time). This time is measured in arbitrary units and can then be calibrated in thousands of years for a given sequence if there are fossil records of the species that can be measured by chemical or biological dating methods (Chávez *et al.* 2004).

** Historical biogeography **

Historical Biogeography tries to explain the current distribution of organisms through processes that occur on a large scale of time, e.g. millions of years. According to Crisci *et al.* (2003), the reconstruction in the historical biogeography of the biogeographic events of the past can be done from three different perspectives that are related to three different aims: a) The reconstruction of the history of the distribution of individual groups (biogeography of the taxon); b) The reconstruction of the history of the areas of endemism (search for relationships between the areas, or biogeography of the area), and c). Reconstruction of the history of the distribution of biota (search for spatial homology).
Similarly, two major fields of this discipline are often recognized: historical biogeography and ecological biogeography. The first is interested in the study of the causes that have operated in the past affecting the organic distribution, encompassing scales and broad hierarchies (taxonomic, geographical, and chronological). It relies primarily on systematics, Earth science, and paleontology to postulate explanations for current biogeographic patterns, particularly supraspecific details. Ecological biogeography, on the other hand, has as its basic interest to investigate the influence of ecogeographic and biotic factors on organisms on a rather local scale (Brown & Gibson 1983; Bueno & Llorente-Bousquets 2000).

However, macroecology and the study of the distribution of biomes worldwide, implies broad geographical scales, although not larger phylogenetic hierarchies, its approach is ecological, not historical. It turns out that biogeography includes different sets of concepts, methods, and strategies, according to the spatial scale, and timing of the processes and patterns that it investigates. It is clear that there are substantial differences between studying the patterns and causes of the distribution of organisms over a short period of time and in a particular area, or studying them in terms of lineages and ancestry on a continental or global scale, spanning geological periods. In the first case, the interest is centered on the effect of ecological factors on populations and communities, while in the second it makes sense to investigate the historical interrelationships between the areas of endemism based on the taxa that constitute them (Bueno & Llorente-Bousquets 2000).

There are different processes that affect the distribution of organisms both in space and in time. Such interactive processes of different kinds, be they geographical (eustatic, tectonic, climatic, and oceanographic), evolutionary (adaptation, speciation, divergence, and extinction), and ecological (immigration, emigration, and biotic interactions), intervene in the formation of patterns on different scales of time and space (Bueno & Llorente-Bousquets 2000). Thus, it becomes pertinent to carry out comprehensive analyzes of organic distribution, which include both ecological mechanisms and historical 'disturbances', to better understand the causes of the spatial and temporal distribution of life and its different components. However, a bridge must be built that links ecological and historical biogeography, and that makes it possible to delineate the relative importance of ecological and historical causes on the distribution of life on Earth (Vuilleumier 1999; Bueno & Llorente-Bousquets 2000). Historical
biogeography has had a permanent interest in recognizing biotic areas as units where allopatric speciation has been generated and establishing relationships between these areas. There have been basically two criteria that have been used to establish relationships between areas: 1) phenetic, which addresses the similarity of shared biotic elements, and 2) genealogical, which addresses the history of fragmentation of the areas and their biota (Bueno & Llorente-Bousquets 2000).

On the other hand, in biogeography, there are several hypotheses focused on explaining the different patterns of the geographic distribution of the different animal and plant species (Brown & Lomolino 1998; Cardoso da Silva et al. 2004). One of these hypotheses refers to the fact that the geographical distributions of the different taxonomic groups are limited by ecological and historical factors, which means that many taxa are restricted to certain geographical regions, called endemic species. Another hypothesis describes that endemic species are not distributed randomly, but they are grouped in some specific geographical areas, which is known as provincialism (Cardoso da Silva et al. 2004), where the regions or geographical areas that present the less two taxa are called areas of endemism (Cracraft 1985; Cardoso da Silva et al. 2004). In turn, the areas of endemism are of great importance in historical biogeography because they represent the essential geographic units for the analysis in its different methods (Cardoso da Silva et al. 2004). The areas of endemism can be defined by the delimitation of the congruent distribution of two or more species that present a restricted range (Morrone 1994). The identification of endemic zones allows proposing primary homologies between geographic areas (Morrone 1994; 1995; Cardoso da Silva et al. 2004) in order to establish priority zones for the conservation of biodiversity (Myers et al. 2000; Huang et al. 2008).

Therefore, inferring the ancestral distribution area for a clade of organisms is one of the objectives of historical biogeography (Brown & Lomolino 1998; Díaz 2011), and is part of the natural history of organisms. The importance of vicariance and dispersion in the distribution of a group of organisms and their mechanism of speciation is an important issue in the reconstruction of the ancestral ranges of distribution of the analyzed taxa (Díaz 2011). The main assumption of the approach of the ancestral area is that the ancestral area of a taxon can be deduced from the information provided by the topology of the area cladogram (Hausdorf 1998; Díaz 2011), given the assumptions that: (1) the plesiomorphic areas in a cladogram they are more likely part of the
ancestral area than apomorphic areas, and (2) areas represented in more than one branch have a greater probability of being part of the ancestral area than less represented areas (Diaz 2011).

**Species delimitation**

A fundamental question of systematics is how to delimit species, especially when they belong to the same lineage? (Sites & Marshall 2004). A part of the problem of the delimitation of species in such a case is that morphology has some limitations (for example, when the same species presents a high degree of polymorphism between its populations or when two or more species present convergence in their morphology). Traditional taxonomy usually based almost exclusively on characters of external or internal morphology only produces morpho-species (species established exclusively on the basis of morphology). Several new methods have been developed over the past 15 years to delineate species and test species hypotheses (Sites & Marshall 2003; Medina et al. 2013), and many scientists have obtained strongly supported results combining these methods with traditional taxonomy. Given that the complexity of the biology of a species, the limits of species have to be studied from multiple and complementary perspectives, so a comprehensive approach to taxonomy should become a generalized practice (Padial & De la Riva 2007; Medina et al. 2013).

In recent years, this type of approach has been widely adopted and developed, with the aim of integrating the basic concepts and methods of traditional taxonomy with new concepts and methodologies. It is called Integrated Taxonomy or Integrative Taxonomy (Dayrat 2005; Medina et al. 2013). Integrated taxonomy is a conceptual framework in which species are hypotheses, and independent character groups are used to build stable species hypotheses (Padial & De la Riva 2007). Furthermore, the level of confidence in the limits of species supported by different types of data is much higher than those supported by a single type of evidence (Dayrat 2005). Some of the problems of current taxonomy do not only arise from the methods used, but also from the never-ending debate about what a species is. Although, a generalized adoption among taxonomists of a species concept based on lineage could sometimes lead to taxonomic inflation in certain cases, however in most cases detailed and comprehensive studies contribute to a better understanding of what species are (Padial & De la Riva 2007; Medina et al. 2013).
The genus *Manerebia* Staudinger, 1897

Lamas & Viloria (2004) recognize a total of 45 species of *Manerebia*, including those described posteriorly by Pyrcz *et al.* (2006). The genus is exclusively tropical Andean, including the peripheral ranges of the Sierra Nevada de Santa Marta and the Cordillera Venezuelana de la Costa. Some species of *Manerebia* are found in premontane forests such as low as 800 m (*Manerebia mycalesoides* (C. & R. Felder, 1867), *Manerebia magnifica* Pyrcz & Willmott, 2006 and some Bolivian species), however, the genus is the most diverse at medium and high elevations, and in cloud forests between 2300-3000m. Some species are found in the moorland habitats up to 4000 m (*Manerebia levana* (Godman, 1905), *Manerebia ignilineata* (Dognin, 1896), *Manerebia seducta* Pyrcz & Willmott, 2006). Several species of *Manerebia* are confined to narrow ecological zones, such as the paramo-forest ecotone (*Manerebia interrupta* (Brown, 1944)), while other species of *Manerebia* are usually found in dense cloud forests (Pyrcz *et al.* 2006).

The name *Manerebia* was initially proposed by Staudinger (1897) for five new species from Peru and Bolivia, apparently closely related (*Manerebia cyclopina*, *Manerebia cyclopella*, *Manerebia cyclops*, *Manerebia typhlops*, and *Manerebia thyphlopsella*). Subsequently, other taxa in the genus were described, mostly coming from the southern tropical Andes (Forster, 1964), with the exception of *Manerebia nevadensis* Krüger, 1925, for Colombia and an Ecuadorian species *Manerebia keradialeuka* (Hayward, 1968). Brown (1944) introduced the generic name *Penrosada* for a group of mainly northern Andes species formerly placed in the genus *Lymanopoda* Westwood, 1851, including *Penrosada leaena* (Hewitson), *Penrosada apiculata* (C. & R. Felder), *Penrosada lanassa* (C. and R. Felder), *Penrosada lisa* (Weymer, 1911), and *Penrosada keithi* (Dyar, 1913), a synonym of *Manerebia satura* (Pyrcz *et al.* 2006).

Adams & Bernard (1979, 1981) and Adams (1986) described four species of *Penrosada* for Colombia and Venezuela. All subsequent authors considered that both *Manerebia* and *Penrosada* are valid, separate genera, without further exploring their relationships at the infraspecific level (Forster 1964; Miller 1968; Adams & Bernard 1977; 1979; 1981; Adams 1985; 1986; D’Abrera 1988; Racheli & Racheli 2001). Pyrcz *et al.* (2006) examined the head, thorax, wing venation, coloration patterns, and male genitalia of all the species included in *Penrosada* Brown and *Manerebia* did not find
autapomorphies that distinguish the two genera, therefore, following the initial proposition of Lamas & Viloria (2004), considered *Penrosada* as a junior synonym of *Manerebia*. It has to be pointed out that Brown's (1944) original description of *Penrosada* made no reference to *Manerebia*, and his morphological diagnosis of the genus fully applies to the species treated for *Manerebia* by Staudinger (1897). For example the vein M1-M2 of the hindwing is characteristically short (shorter than in *Lymanopoda* Westwood) and gently curved; the root of the vein M3 is much closer to the vein Cu1 than to M2; the wing ocellus in the cell Cu2-1A/1B of both fore and hindwing is generally fully developed. The straight yellow or whitish band on the hindwing underside is present in all the species of *Penrosada* identified by Brown and absent in all five species of *Manerebia* as specified originally by Staudinger but this characters almost certainly does not define a monophyletic group, being highly variable between and even within populations. Some species of *Manerebia*, for example, *M. trimaculata*, *M. ignilineata*, *M. interrupta*, and *M. apiculata*, are polymorphic, with shortened, discontinuous, or even entirely absent bands. Other typical characteristics of the genus *Manerebia* include male genitalia characterized by the elongated uncus, sharp subuncus, and slender valves with a strongly serrated dorsal edge (Pyrcz et al. 2006).

Also, Lamas & Viloria (2004) and Pyrcz et al. (2006), consider the monobasic genus *Posteuptychia* Forster (1964) as a junior synonym of *Manerebia*. Forster proposed it for *Pronopila mycalesoides* C. & R. Felder based on the very unusual genital morphology of the male. In fact, the genitalia of *M. mycalesoides* are closely similar to *M. nevadensis*, one of the few northern Andean species originally described within *Manerebia* (Pyrcz et al. 2006).

Miller (1968) included *Manerebia* in the tribe Pronophilini as identified by Reuter (1896), a Neotropical section of the, by then, family Satyridae. Adams & Bernard (1977, 1979, 1981), Adams (1986), and Pyrcz (1999) did not question this decision, but Viloria (2001) suggested that *Manerebia* does not belong to Pronophilina, instead to the Holarctic subtribe Erebiina (following the subtribal systematic arrangement proposed by Harvey (1991)). Viloria (2001) listed three possible synapomorphies of the Pronophilina absent in *Manerebia*: (1) setose eyes, (2) hindwing cross vein M1-M2 curved basad into the discal cell (3) hindwing discal-cell is equal to or less than half the maximum length of wing (Pyrcz et al. 2006). The absence of setae on the eyes occurs, among others, in another group of the Satyrinae, the subtribe Satyrina distributed mostly in the Palearctic,
but also in two species of the genus *Quilaphoteosus* (Pyrcz et al. 2006; Hodór, unpubl.; Pyrcz 2010). Some preliminary molecular data were published by Peña et al. (2011) which contradict the hypothesis advanced by Viloria (2001) pointing out that the genus *Manerebia* forms an internal clade within the Pronophilina, sister to the genus *Lymanopoda*. On the other hand, Pyrcz et al. (2006) and Viloria (2001) suggest *Tamania* Pyrcz or *Idioneurula* Strand as possible sister genera of *Manerebia*. The systematics at the species-level of *Manerebia* remains very complex, also due to some identification errors or descriptions of synonymous taxa made by Brown (1944), Forster (1964), D’Abrera (1988), and Adams (1986) (see: Pyrcz et al. 2006).

In addition to describing the genus *Penrosada*, Brown (1944) also referred to all the Ecuadorian species of this, although he seemed to be unaware of two species already described at the time from Ecuador: *M. trimaculata* and *M. ignilineata*. He redescribed the latter as a form of *Penrosada lanassa*. He also misapplied the names *leaena, lanassa,* and *apiculata* to various species and described several infra-specific forms that differ in the expression of the posterior dorsal band as new taxa. In the absence of a good number of specimens, Brown's taxa identification was largely based on sketchy genital illustrations of single individuals. Forster (1964) clarified, to some extent, the classification of species and illustrated the poorly known taxa described by Staudinger (1897). Unfortunately, he did not examine the genitalia of *Penrosada sensu* Brown, and therefore did not notice that *Penrosada* and *Posteuptchia* were morphologically similar to other *Manerebia*. Finally, Adams (1986) referred to most of the species of *Manerebia* (under the name *Penrosada*) from Colombia and Venezuela, providing valuable distribution data, and description of several new species but also made some further identification errors, similarly to previous authors. Lamas & Viloria (2004) and Pyrcz et al. (2006) provided a synonymous checklist for the entire genus, thus correcting some taxonomic errors. Currently recognized species of *Manerebia* in the northern Andes, identified based on morphological characters, ere illustrated by Pyrcz et al. (2006).

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**Reasoning**

Increased destruction and disturbance of ecosystems are leading to a decrease in diversity at the local and regional levels (Brook 2006). Since most species are not described or could not be described, efforts to catalogue and explain biodiversity have increased in recent years. Taxonomy has been developed based mainly on morphological characters which has led to serious difficulties in the determination of species-level taxa, since some characters tend to be homoplasic or very subjective, generating a problem in the delimitation of species in some systematic groups in particular (Medina et al. 2013).

In turn, the taxonomic challenge posed by the existence of cryptic species has caused a greater problem in whose morphological characters are not very informative. It is, therefore, very easy to fall into the dilemma of taxonomic inflation or underestimation (Tattersall 2007). This problem occurs in several groups of butterflies (e.g.: *Pedaliodes* (Pyrcz et al. 2013), *Manerebia* (Pyrcz et al. 2006), *Astraptes fulgerator* Walch, 1775 (Hebert et al. 2004), *Mechanitis* Fabricius, 1807 (S-Brown 1977; Giraldo 2015), among others), causing serious problems in their taxonomic determination, and hampering the development of the knowledge of their species richness and natural history (Hebert et al. 2004; Giraldo 2015).

As mentioned above, this is, in particular, the case of the genus *Manerebia* Staudinger. It is estimated, on the one hand, that taxonomic inflation may occur to some extent while, on the other, that some species-level taxonomic underestimation may also take place, due to the far reaching morphological similarity between the species (cryptic diversity) (Mahecha et al. 2019). In addition, there is no hypothesis about the phylogenetic relationships within the group (Pyrcz et al. 2006), causing a lack of knowledge of the possible evolutionary and biogeographic mechanisms that have led to the diversification of the genus *Manerebia* throughout the Andes mountains.

**References**

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Objectives of the thesis

**General**

To assess cryptic diversity, systematic relationships, and historical biogeography of the genus *Manerebia* Staudinger, 1897 in the Neotropics.

Evaluar la diversidad críptica, las relaciones sistemáticas y la biogeografía histórica del género *Manerebia* Staudinger, 1897 en el Neotrópico.

**Specific**

- Validate the taxonomic status of the species of the genus *Manerebia* until now in order to propose a taxonomic classification of the group (Chapter I-II).

  Validar el estatus taxonómico de las especies descritas hasta la fecha del género *Manerebia* para proponer una clasificación taxonómica del grupo (Capítulo II).

- Determine the phylogenetic relationships of the genus *Manerebia* and its taxonomic status within of the subtribe Pronophilina (Chapter I-III).

  Determinar las relaciones filogenéticas del género *Manerebia* y su estatus taxonómico dentro de la subtribu Pronophilina (Capítulo I-III).

- Infer the ancestral areas, the areas of endemism, and the time of divergence of the species of the genus *Manerebia* (Chapter IV).

  Inferir las áreas ancestrales, áreas de endemismo y tiempos de divergencia de las especies del género *Manerebia* (Capítulo IV).

- Determine possible mechanisms of speciation that have occurred within the genus *Manerebia* (Chapter IV).

  Determinar los posibles mecanismos de especiación que se han presentado en el género *Manerebia* (Capítulo IV).
Research questions and hypothesis

Chapter I. What is the systematic position of the genus *Manerebia* within the tribe Satyrini?

_Hypothesis:_

According to different authors, the systematic position of the genus *Manerebia* is uncertain as its phylogenetic relationships have not been sufficiently researched, and it might either belong in the subtribe Erebiina, according to Viloria (2007), Lamas & Viloria (2004) or to Pronophilina (Peña _et al._ 2006; 2011) of the tribe Satyrini. Its sister status relative to the genera *Lymanopoda, Idioneurula, Diaphanos*, and *Ianussiusa* was proposed by Pyrcz _et al._ (2006). Also, its monophyly has not been convincingly demonstrated despite some morphology based evidence presented by Pyrcz _et al._ (2006). Therefore, testing the monophyly of the genus, and performing a phylogenetic analysis would allow us to clarify the tribal status of *Manerebia* and to test previous hypothesis on its monophyly and sister group/s.

Chapter II. What are the effects of cryptic diversity on the systematics of the genus *Manerebia*?

_Hypothesis:_

The high phenotypic similarity affects the alpha-taxonomy of the genus *Manerebia*, by underestimating the total number of species richness of the genus, suggesting the existence of a number of unrecognized new species. Therefore, the use of phylogenetic tools for species delimitation would allow us to identify several putative species, and to assess the overall cryptic diversity in the genus and, eventually, to enable us to produce a sound taxonomic arrangement.

Chapter III. What are the phylogenetic relationships within the genus *Manerebia*?

_Hypothesis:_

As there is no phylogenetic hypothesis of the genus *Manerebia*, it is not possible to know what the phylogenetic relationships are between its species. Therefore, by carrying out a molecular analysis, it will be possible to establish possible systematic
relationships within the genus and to corroborate it with the taxonomic proposals made by Pyrcz (2004) and Pyrcz et al. (2006) based on morphological characters.

Chapter IV. How did historical biogeographic processes influence the evolutionary history of the genus *Manerebia* in the Neotropics?

*Hypothesis:*

Current biogeographical patterns of the genus *Manerebia* are the result of historical processes such as vicariance and dispersal that have occurred during the divergence and diversification processes of the genus. Main divergence of the genus *Manerebia* might have happened rather recently (during or near the Pleistocene), and a high diversification rate due to the orogenic processes of the Andes mountain range and subsequent different climatic fluctuations that occurred during the last pleistocenic glaciation.
CHAPTER I

Evaluating the monophyly and the subtribal position of the Andean genus Manerebia Staudinger with remarks on phylogenetic relationships within the tribe Satyrini (Nymphalidae, Satyrinae)

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Abstract

The Pronophilina butterflies, one of the subtribes of the Satyrini, are recognized as one of the most diversified groups of butterflies in mountain environments and present high levels of endemism. However, the accurate taxonomic determination of species in many genera of Pronophilina has been affected by high cryptic diversity and taxonomically confusing taxa as is the case of the genus Manerebia. Nevertheless, the placement of Manerebia within the subtribe Pronophilina is to be considered as tentative as until now no phylogenetic analysis has yet been carried out, and its phylogenetic relationships have not been previously rigorously evaluated. We utilized molecular phylogenetic approaches and a large taxonomic sampling for several species of Satyrini included new sequences of Manerebia. We evaluated the monophyly of the genus Manerebia, determined its taxonomic position and phylogenetic relationships within the tribe Satyrini, and provided a better understanding of the at the subtribe level relationships within the Satyrini. We found Manerebia as a monophyletic group into Pronophilina and clarified its phylogenetic relationships, being Manerebia the sister of the clade formed by (Lymanopoda + Ianussiusa) + (Idioneurula + Diaphanos). Besides, we found that using larger taxonomic sampling may help to improve the problems when using individual genes and it allows to build systematic relationships more robust. Finally, we did some taxonomic remarks and proposed a new subtribe for Satyrini:
Cyllopsisina subtrib. n. Mahecha-J. & Pyrcz.

Key words. Pronophilina, butterfly systematic, phylogenetic methods, taxonomy.

Introduction

The butterfly tribe Satyrini Boisduval, 1833, is the most diverse and species-rich group of the nymphalid subfamily Satyrinae. Currently, it comprises approximately 2200 extant species, with many more being described each year (Peña & Wahlberg 2008; Peña et al. 2011; Toussaint et al. 2012; Yang & Zhang 2015). The tribe is distributed worldwide with the highest diversity in the Oriental and Neotropical realms (Ackery et al. 1999; Peña et al. 2011; Yang & Zhang 2015). Many species of the Satyrini have been designated as model organisms in various research fields such as conservation biology and ecology (e.g. Schmitt & Haubrich 2008; Mahecha-Jiménez et al. 2011; Slamova et al. 2012; Restrepo & Halffter 2013; Santos et al. 2020), evolutionary biology (Balmer et al. 2018; Nokelainen et al. 2018; Halali et al. 2020), systematicand biogeography (e.g. Kodandaramaiah et al. 2010; Peña et al. 2011; Neild et al. 2014; Matos-Maraví et al. 2014; Freitas et al. 2016; Marín et al. 2017b; Pyrcz et al. 2017; Mahecha et al. 2019; Espeland et al. 2019; Yang et al. 2020; Pyrcz et al. 2021; Usami et al. 2021; Zhang et al. 2021), and developmental biology (e.g., Ross 2003; Oliver et al. 2012; Freitas et al. 2016).

According to the results of various systematic studies 13 subtribes have been identified grouped within two clades: 1) Euptychiina + Ypthimina + (Melanargiina + Satyrina) + Maniolina + Pronophilina + Erebiina, and 2) Coenonymphina + Ragadiina + Eritina + [Parargina + (Mycalesina + Lethina)] (Peña et al. 2006; Penz 2007; Peña & Wahlberg 2008; Wahlberg et al. 2009; Kodandaramaiah et al. 2010; Marín et al. 2011; Peña et al. 2011; Yang & Zhang 2015). Recently, a new systematic arrangement for the Satyrini was proposed, including three new subtribes: Gyrocheilina, Calleberiina and Calistina, and a total of 14 subtribes (Zhang et al. 2021). Despite the above studies, it is fair to assume that phylogenetic relationships within the tribe taxonomic status of several subtribes remain unresolved (Marín et al. 2011; Peña et al. 2011; Yang & Zhang 2015). For example, Peña et al. (2011) conducted a comprehensive phylogenetic study on Satyrini, validating the taxonomic status of 12 subtribes, based on five mitochondrial and nuclear genetic markers (COI, EF-1α, GAPDH, RpS5, and wingless) and recognized that the relationships of different subtribes were incongrucent, and with the
basal nodes weakly supported. Marín et al. (2011) defined the 13 subtribes of the Satyrini but stated too that the relationships of different subtribes were too complex and incongruent. Yang & Zhang (2015) used nine mitochondrial, nuclear, and ribosomal genetic markers (COI, COII, Cytb, EF-1α, GAPDH, RpS5, wingless, 28s rDNA, and 18s rDNA), but studied only 11 subtribes (they did not include the Pronophilina and Ragadiina subtribes, only the Chinese Satyrini), finding that the relationships of different subtribes were a somewhat incongruent in comparison to the results of Peña et al. (2011), which was probably due to the limited number of taxa used in this study. Zhang et al. (2021) reorganized the Satyrini tribe into 14 subtribes based on genome analysis, which presented a good branches support: The Satyrini crown group composed by Satyrina + (Euptychiina + Calistina) + (Pronophilina + Gyrocheilina + ((Ypthimina + Calleberiina) + Erebiina) + Coenonymphina + ((Parargina + Lethina) + Mycalesina) + (Ragadiina + Eritina). However, Zhang et al. (2021) used a limited number of species in some taxa (e.g. Calisto Hübner, [1823] and Euptychia Hübner, 1818). For that reason, there is a need to do further phylogenetic studies that involve large taxonomic sampling and employing a large number of molecular characters, and whenever possible combining molecular with morphological characters. Moreover, assessing of the monophyly of the subtribes of the Satyrini remains a priority to obtain more robust phylogenetic proposals for the group (Marín et al. 2011; Zhang et al. 2021).

The Pronophilina butterflies, one of the subtribes of the Satyrini, are recognized as one of the most diversified group of butterflies in mountain environments, distributed within narrow altitude ranges and presenting high levels of endemism (Adams 1985; Pyrcz et al. 2006; Pyrcz & Víloria 2007; Mahecha-Jiménez et al. 2011; Marín et al. 2014; Marin et al. 2017a; Pyrcz et al. 2016; Álvarez-Hincapié et al. 2017; Mahecha et al. 2019). The highest diversity of Pronophilina is reported in cloud forests, between 2500 and 2900m (Adams 1985; 1986; Pyrcz & Garlacz 2012; Mahecha et al. 2019). Although, the accurate taxonomic determination of species in many genera of Pronophilina has been affected by high cryptic diversity and taxonomically confusing taxa, currently the state of knowledge of their alpha taxonomy is remarkably solid (Pyrcz et al. 2006; Marín et al. 2017a; Mahecha et al. 2019; Mahecha-J et al. 2021). High species-diversity and endemism in the Andes makes the systematics and evolution of several genera belonging to this group particularly interesting, such as is the case of
Steromapedaliodes Forster, 1964 (Pyrcz et al. 2017), Lymanopoda Westwood, 1851 (Casner & Pyrcz 2010; Pyrcz et al. 2018), or indeed of the genus Manerebia Staudinger, 1897 (Pyrcz et al. 2006; Mahecha-J et al. 2021). It has to be said that the placement of Manerebia within the subtribe Pronophilina is to be considered as tentative as until now no phylogenetic analysis has yet been carried out, and only partial molecular data were available for some of species (Peña et al. 2006; Mahecha-J. et al. 2021). It has even been suggested that the genus might represent the predominantly Holarctic tribe Erebiina (Viloria 2007), and thus the tribal position and monophyly of the genus, and its affinities with other Neotropical Satyrinae remain unclear (Pyrcz et al. 2006; Mahecha-J. et al. 2021).

In the present study, we reconstruct the phylogeny of Satyrini employing large taxonomic sampling and a large number of molecular characters, such as ribosomal, mitochondrial, and nuclear genes. Our aims are to (1) evaluate the monophyly of the genus Manerebia; (2) determine its taxonomic position and phylogenetic relationships within the tribe Satyrini; and (3) provide a better understanding of the at the subtribe level relationships within the Satyrini, all the before based on molecular evidence. The phylogenetic hypotheses raised were (1): the genus Manerebia is a monophyletic group based on proposed by Pyrcz et al. (2006) and Mahecha-J. et al. (2021), where the species that conform to the genus have morphological synapomorphies and for this reason can share an evolutionary history that may be reaffirmed by molecular characters; (2) based on the proposed by Pyrcz et al. (2006) the genus Manerebia is part of the Pronophilina subtribe and it is phylogenetically related to the clade formed, based on Peña et al. (2011), by the genera Idioneurula Strand, 1932 + Diaphanos Adams & Bernard, 1981 + Lymanopoda + Ianussiusa Pyrcz & Viloria, 2004; (3) The Pronophilina subtribe is a monophyletic group within the Satyrini crown group, corroborating the results of Peña et al. (2011), and Zhang et al. (2021).

Material and methods

Taxa sampling

A total of 231 species were sampled representing fourteen subtribes of Satyrini according to Zhang et al. (2021), and 88 Satyrini genera (out of 209) following the classifications of Peña et al. (2006), Peña et al, 2011, Yang & Zhang (2015), and Zhang et al. (2021). Caligo atreus Kollar, 1850 (Brassolini), Morpho sulkowskyi (Kollar,
1850) (Morphini), and *Pierella helvina* (Hewitson, 1859) (Haeterini) representing three tribes of Satyrinae were chosen as outgroups in line with the articles of Peña *et al.* (2006) and Peña & Wahlberg (2008).

**Acquisition of sequence data**

The genetic sequences were mainly collated from published sources and downloaded from NCBI GenBank (https://www.ncbi.nlm.nih.gov/nuccore). Moreover, new sequences were generated for this study. These include several species that did not have any available published sequence especially of the genus *Manerebia* (13 new sequences were included in the study). Five molecular markers were used to reconstruct the phylogeny from Satyrini: one mitochondrial gene, cytochrome c oxidase subunit I (COI: 1458 bp), was available for all species in the study, and four nuclear genes were included when available: ribosomal protein S5 (Rps5: 683), wingless (400 bp), glyceraldehyde-3-phosphate dehydrogenase (GAPDH: 691 bp), and elongation factor-1α (EF-1α: 1238 bp). These genes have been employing in phylogenetic studies in Nymphalidae butterflies (e.g. Monteiro & Pierce 2001, Peña *et al.* 2006, Wahlberg *et al.* 2009, Casner & Pyrcz 2010, Peña *et al.* 2010; Marín *et al.* 2011, Kodandaramaiah *et al.* 2010, Peña *et al.* 2011, Matos-Maraví *et al.* 2014, Yang & Zhang 2015, Matz & Brower 2016, Pyrcz *et al.* 2016, Marín *et al.* 2017b, Pyrcz *et al.* 2017, Nattier *et al.* 2017, Kodandaramaiah *et al.* 2018, Pyrcz *et al.* 2021). Some species are represented only by the COI gene. These species represented by only the COI tend to be closely related to species with more genes obtained (Supplementary material 1), minimizing the potential bias these samples could have in our analyses (Wiemers *et al.* 2020).

For the new sequences, at the CEPUJ, DNA was isolated from a pair of legs, dry or ethanol-preserved, using a NucleoSpin® Tissue extraction kit (Macherey-Nagel Düren, Germany) and following the manufacturer's protocol. Amplification of part of the mitochondrial gene Cytochrome Oxidase I (COI) was performed using HybLCO and HybHCO primers with universal primer tails, respectively T7 Promoter(F) and T3(R) (Folmer *et al.* 1994, Wahlberg & Wheat 2008), with standard PCR protocol. PCR reactions were performed in a 20 µl reaction volume in Eppendorf Mastercycler® nexus thermocycler. The PCR cycling profile comprised an initial denaturation at 95°C for 5 min followed by 40 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 1 min, and a final extension period of 72°C for 10 min. Sequences of the Glyceraldehyde-3-Phosphate
Dehydrogenase gene (GAPDH) were amplified using primers HybFrigga and HybBurre. PCR reactions were performed as above, but with an annealing temperature of 55°C. Amplification products were checked by electrophoresis on 1% agarose gels stained with Midori Green (NIPPON Genetics). Finally, samples were sent to Macrogen Europe (Amsterdam, Netherlands) for purification and sequencing. DNA extraction, PCR and sequencing at the FLMNH followed methods described in Willmott et al. (2018).

**Phylogenetic Analyses**

The Chromatograms and sequences from NCBI Genbank obtained were edited and base calls checked using Geneious v. 4.8.5 (Drummond et al. 2009). We searched for reading frame errors and unexpected stop codons by translating the nucleotide sequences to peptides using Geneious v. 4.8.5 (Drummond et al. 2009). Nucleotide sequences from each genetic marker were aligned up separately using MAFFT v. 7.245 (Katoh & Standley 2014), then refined and concatenated in Geneious v. 4.8.5. (Drummond et al. 2009). To investigate potential conflict between the partitions of the mitochondrial and nuclear datasets, the Congruence Among Distance Matrices (CADM) test (Campbell et al. 2011, Pyrcz et al. 2017, Quan et al. 2020) was performed using the function ‘CADM’, global’ implemented in the R-package ‘ape’ (Paradis et al. 2004) and using pairwise distances calculated with the Maximum Composite Likelihood model (MCL) (Tamura et al. 2004, Pyrcz et al. 2017) in MegaX (Kumar et al 2018). A Kendall’s coefficient of concordance W value of 0 means complete incongruence among genes, while a value of 1 indicates complete concordance (Campbell et al. 2011, Cardoso et al. 2021). The null hypothesis of complete incongruence among DNA sequence distance matrices was tested with 999 permutations (Quan et al. 2020, Cardoso et al. 2021).

The concatenated matrix, partitioned by gene, was used to perform two phylogenetic reconstruction methods: Maximum Likelihood (ML) and Bayesian Inference (BI) (Cardoso et al. 2021, Pola et al. 2021). The best partition scheme option and the best-fit model for nucleotide substitution was selected using ModelFinder function (Kalyaanamoorthy et al. 2017) as implemented in IQ-TREE version 2.1.3 (Minh et al. 2020) under the Bayesian Information Criterion (BIC) (Pan et al. 2019). Therefore, the best nucleotide substitution model for each gene was GTR+F+I+G4. For
the best partition scheme ModelFinder estimated the GTR+F+I+G4 model for the first partition (COI), SYM+R4 for the second (EF-1α and GAPDH), and third partition (Rps5), and TN+F+I+G4 for the fourth partition (wingless).

For the ML analysis was performed in IQ-TREE version 2.1.3 (Minh et al. 2020), based on the best partition scheme inferred by ModelFinder. The analysis parameters were obtained from the ModelFinder function at the time of estimating the best substitution model and the best partition scheme, for which a heterogeneity rate was used: Gamma, with an alpha of 0.2 and a number of rate categories of 4. The branches support was evaluated using the UltraFast Bootstrap (UFBoot) (Hoang et al. 2018), and Shimodaira-Hasegawa (SH-aLRT) (Guindon et al 2010) algorithms, both with 1000 repetitions (Pola et al. 2021).

BI analyses were conducted in BEAST2 v. 2.5.2 (Bouckaert et al. 2019) applying the estimated nucleotide substitution model for each gene by ModelFinder. Markov Monte Carlo Chains (MCMC) were run for 60 million generations and sampled every 5000 generations. A strict molecular clock was used for each gen (Opgenoorth et al. 2020, Pola et al. 2021, Rubinoff et al. 2021), and the mitochondrial gene COI was assigned a genetic ploidy of 0.5, and the nuclear genes of 2.0 (BEAST Developers 2017; Núñez et al. 2020, Matos-Maraví et al. 2019). The Yule model (Pure-birth Yule model) (Yule 1925) was used, carrying out four independent analyses, which were later combined in LogCombiner v. 2.5.2 (Bouckaert et al. 2019). The combined trees were then summarized in TreeAnnotator v. 2.5.2 (Bouckaert et al. 2019) with a burn-in of 25% to build the tree of maximum credibility (Maurin 2020; Pola et al. 2021). MCMC convergence was further evaluated with Tracer v.1.6 (Rambaut & Drummond 2014) to confirm an appropriate effective sample size (ESS> 200) (BEAST Developers 2017 Maurin 2020). Clades that obtained an UFBoot ≥ 95, SH-aLRT ≥ 80, and Bayesian posterior probability (pp) ≥ 0.95 were considered strongly supported (Pola et al. 2021).

Finally, the phylogenetic trees obtained from both methods were edited in Geneious v.4.8.5 (Drummond et al. 2009) and then refined in Inkscape 0.92.3 (2405546, 2021-06-09) (https://www.inkscape.org).
Results

The final concatenated alignment comprised 5750 bp., including five molecular markers. The concatenated data set has only 1.3% of missing character data. The matrix of mitochondrial and nuclear genes was found to be congruent based on the CADM test (W = 0.785, p = 0.005). Hence, the results of the CADM test indicated that all genes could be combined in a single phylogenetic analysis. ML and BI analyses resulted in basically the same tree topology, allowed us to prove the monophyly of the genus *Manerebia*, and its position in the subtribe Pronophilina (SH-aLRT: 97 / UFBoot: 100 / pp: 1) (Figs.1, 2). *Manerebia* appears as a sister group to the clade conformed by the genera (*Idioneurula + Diaphanos*) + (*Lymanopoda + Ianussiusa*) (CL.1). In turn, CL.1 is the sister group of the clade formed by the so-called south temperate Pronophilina (CL.2): *Faunula* + (*Elina + Neomaenas*) + (*Cosmosatyrus + Auca*) + (*Argyrophorus + Pampasatyrus*), and the genus *Eretris* (CL.3). Finally, CL.1, CL.2, and CL.3 are associated with the genera (*Steremnia + Steroma*) (CL.4). All the aforementioned clades are strongly supported by branch values in both phylogenetic methods (Figs.1, 2).

At the same time, according to our results, the clades CL.1, CL.2, CL.3, and CL.4 are part of one of the two proposed and defined phylogenetic groups within the subtribe Pronophilina, the second group being the so-called –large pronophilines‖ which includes the other genera of Pronophilina sensu lato, and in turn, it is divided into two subclades the ‘Pedaliodes’ sensu lato (CL.5) and ‘Pronophila’ sensu lato (CL.6) (Figs.1, 2). Additionally, we reaffirmed the monophyly of Pronophilina within the Satyrini with strong branch support in both phylogenetic analyses (SH-aLRT: 97.3 / UFBoot: 100 / pp: 1) (Figs.1, 2).

On the other hand, it is interesting to note that the phylogenetic analysis carried out allow establishing and clarifying some of the systematic relationships among the Satyrini, in addition to corroborating the monophyly of the tribe Satyrini within the subfamily Satyrinae (SH-aLRT: 91.9 / UFBoot: 99 / pp: 0.99). Our results allowed to verify the Satyrini crown-group conformed by (*Euptychiina + Calistina*) + *Cyllopsisina subtrib. n.* + *Gyrocheilina* + (*Erebina + Maniolina*) + (*Ypthimina + (Callerebiina + Satyrina*) + Pronophilina (Figs.1, 2). Based on our analyses, we propose a new subtribe: *Cyllopsisina subtrib. n.* Mahecha-J. & Pyrcz, a well-supported large clade in the phylogenetic trees (SH-aLRT: 99 / UFBoot: 100 / pp: 1). Therefore, all the genera that
were part of the subtribe Euptychiina as previously recognized are transferred to this new subtribe, except the genus *Euptychia sensu stricto* which remains in the subtribe Euptychiina *sensu novum*. The new subtribe Cyllopsisina *subtrib. n.* appears as the sister group of the clade Calistina + Euptychiina (SH-aLRT: 65.1 / UFBoot: 69 / pp: 0.72), with the two subtribes being closely associated with a good support (SH-aLRT: 95.4 / UFBoot: 80 / pp: 0.87) (Figs.1, 2). Thus, following the nomenclatural rules, *Euptychia sensu stricto* remains in the Euptychiina *sensu novum* as the type-genus, and the genus *Cyllopsis* R. Felder, 1869 is selected as the type-genus of the new subtribe Cyllopsisina *subtrib. n.*

Otherwise, Erebiina is the sister group of the Maniolina with a good support value (SH-aLRT: 75.6 / UFBoot: 91 / pp: 0.84), being both subtribes validated. Satyrina and Callerebiina are related but are supported with a moderate branch value (SH-aLRT: 73.1 / UFBoot: 76 / pp: 0.79). Gyrocheilina, the subtribe formed by the monotypic genus *Gyrocheilus* Butler, 1867, appears related to (Erebiina + Maniolina) + (Satyrina + Callerebiina) + Ypthimina but with rather low branch support, allowing to validate the status of this subtribe. Also, the clade (Erebiina + Maniolina) formsa clade with (Callerebiina + Satyrina) + Ypthimina, but with a low support value (SH-aLRT: 68.3 / UFBoot: 68 / pp: 0.63) (Figs.1, 2). In turn, the relationship of (Parargina + Lethina) + Mycalesina is validated, as well the association between (Coenonymphina + Eritina). Finally, both phylogenetic analyses support with a strong branch value the phylogenetic position of Ragadiina as a sister group from all other subtribes along Satyrini (SH-aLRT: 96 / UFBoot: 97 / pp: 0.98) (Figs.1, 2).
**FIGURE 1.** Maximum likelihood tree (ML) reconstructed from five molecular markers. SHaLRT / UFBoot support values indicated on nodes.
FIGURE 1. Continuation: Maximum likelihood tree (ML) reconstructed from five molecular markers. SHaLRT / UFBoot support values indicated on nodes.
FIGURE 1. Continuation: Maximum likelihood tree (ML) reconstructed from five molecular markers. SHaLRT / UFBoot support values indicated on nodes.
FIGURE 2. Bayesian Inference tree (BI) reconstructed from five molecular. Posterior probability values indicated on nodes.
FIGURE 2. Continuation: Bayesian Inference tree (BI) reconstructed from five molecular. Posterior probability values indicated on nodes.
FIGURE 2. Continuation: Bayesian Inference tree (BI) reconstructed from five molecular. Posterior probability values indicated on nodes.
Discussion

In this study, we involve the largest species-level taxonomic sampling for the Satyrini, to date. The recovery of identical topologies under ML and BI methods based on five molecular markers, with branches supported with high Bootstrap, SH-aLRT and pp values, suggests that the phylogenetic relationships have been accurately reconstructed. Moreover, based on molecular evidence our phylogenetic hypotheses raised are considered to be solid.

Our phylogenetic results show that the monophyly of the genus Manerebia is confirmed by molecular characters, as is its position within the subtribe Pronophilina. These results make it possible to definitely rule out the hypothesis of Viloria (1998; 2003; 2007), who transferred some genera (e.g. Manerebia) from Pronophilina into Erebiina, and these genera are actually more closely related to Pronophilina genera, and are distantly related to the Palearctic Erebiina, a result before evidenced by Peña et al. (2006), but our analyses confirmed with more robust this result. Miller (1968) based on morphological characters, proposed three putative synapomorphies of Pronophilina absent in Manerebia, and also in Idioneurula and Ianussiusa, namely: setose eyes, the base of the M1-M2 vein curved or angled in the disc cell of the hind wing, and the length of the disc cell of the hind wing equal to or shorter than the maximum length of the hind wing. However, Pyrcz et al. (2006) argue that these morphological synapomorphies are too weak to support the inclusion of Manerebia within Erebiina, for example, the Erebiina also does not have setose eyes, similarly to the Holarctic subtribe Satyrina (Pyrcz et al. 2006), even some south temperate Pronophilina may or may not have setose eyes, for instance, Quilaphoetosus monachus monachus (Blanchard, 1852), Quilaphoetosus monachus valdiviae (C. Felder & R. Felder, 1867) and Quilaphoetosus janiroides (Blanchard, 1852) present setose eyes, but Argyrophorus argenteus Blanchard, 1852, Argyrophorus chiliensis (Guérin-Méneville, [1830]), Cosmosatyrus leptoneuroides C. Felder & R. Felder, 1867, among others, show no eye hair among the ommatidia (Hódor & Pyrcz 2013).

Furthermore, the monophyly of Manerebia based on molecular evidence is supported by morphological synapomorphies such as: fine dark lines on the wing in the ventral part in the postdiscal and submarginal area, occasional submarginal ocelli in the CuA1-CuA2 veins in the forewing, the ocellus in space CuA2-1A/1B of the forewing
and hindwing is usually fully developed, the hindwing vein M1-M2 is characteristically short (shorter than in Lymanopoda) and gently curved, the root of vein M3 is much closer to vein CuA1 than M2, the hindwing is slightly incised near the anal angle, walking legs are yellowish, uncus is strongly arched, curved or straight, the subunci short or long, the ‘teeth’ at the distal tip of the valva often extending anteriorly along the dorsal edge, and the dorsal base of the valva armed with a projection with numerous small ‘teeth’ or the ‘teeth’ confined to the distal tip of the valva, and the dorsal base of the valva armed with a simple projection only (Pyrcz et al. 2006; Mahecha-J. et al. 2021a; Mahecha-J. et al. 2021b).

Peña et al. (2006) found Manerebia as the sister group of ((Lymanopoda + Ianussiusa) + Idioneurula) (including Tamania Pyrcz, 1995 as a synonym of Idioneurula proposed by Huertas & Arias (2007)) but presented low branches support values and they did not include Diaphanos in the study. Peña et al. (2011) reported to Manerebia as the sister-genus of (Lymanopoda + Diaphanos) with good support but they did not include Idioneurula in the analysis. Matz & Brower (2016) found Manerebia to be a sister-genus of Idioneurula, conforming a clade to (Lymanopoda + Ianussiusa), and in turn, this clade being the sister group of (Diaphanos + (Steremnia + Steroma)), although, their phylogenetic relationships presented a rather low support value. Therefore, our phylogenetic results allow clarifying the phylogenetic relationships of Manerebia along Pronophilina with strong support in both analyses.

Moreover, some morphological characters share between Lymanopoda, Diaphanos, Manerebia, Ianussiusa, and Idioneurula might help to support our result, such as: the hindwing cross-vein M1-M2 entirely straight or only very slightly curved (almost imperceptible), naked eyes, and hindwing discal cell short, equal to half the total length of the hindwing or less, the hindwing vein M1-M2 is characteristically short, small sizes (14 -18 mm approx.), wing shape, male genitalia high-domed (almost globular) tegumen; a very well-defined suture between the tegumen and the uncus (generally a constriction), the long, curved, and usually stylized uncus, the well-developed subunci, always much shorter than the uncus (rarely rudimentary), with little basal expansion; the saccus semi-globular; the valvae generally dorsally ornamented (with teeth or toothed processes), and the aedeagus straight or slightly curved (sometimes with lateral, dorsal or apical tooth-like processes) (Viloria 1994; Pyrcz et al. 2006; Viloria 2007). Nevertheless, some of these morphological characters are shared
with other genera outside of Pronophilina, as the Holarctic genus *Erebia* Dalman, 1816 and others Erebiina, and might be considered as homoplasies. Recently, in the check-list of Colombian butterflies Garwood *et al.* (2021) arbitrarily (perhaps by a simple mistake) transferred these four genera to the subtribe Euptyihiina (now Cyllopsisina *subtrib. n.*), and our phylogenetic analyses do not support such a move.

On the other hand, our data corroborate the monophyly of Pronophilina in agreement with Wahlberg *et al.* (2009), Peña *et al.* (2011), and Matz & Brower (2016). Miller (1968) considered that the Pronophilina (originally Pronophilini) are most closely related to the Satyrina (originally Satyrini) based on morphological characters. Based on molecular characters Wahlberg *et al.* (2009) found the Pronophilina as a sister group of a clade made up by Erebiina + Maniolina + Melanargiina + Calistina + Satyrina + Ypthimina, while Peña *et al.* (2011) established the Pronophilina as sister to Erebiina + Maniolina, but both studies presented a low support. Espeland *et al.* (2019) found the Pronophilina as the sister group of the Euptyihiina (including *Euptychia sensu stricto*) with high support, although they only included a representative of Pronophilina (*Manerebia undulata* Pyrcz & J. Hall, 2006). In our study we found the Pronophilina to be the sister group of the clade constituted by Gyrocheilina + Erebiina + Maniolina + Callerebiina + Satyrina (including Melanargiina) + Ypthimina with a good support value. Our results marginally differ from Zhang *et al.* (2021) who found the Pronophilina as the sister group of a clade including Gyrocheilina + Erebiina + Maniolina + Callerebiina + Ypthimina, but without Satyrina (including Melanargiina), which appears as a sister clade of the rest of Satyrini crown group. Despite of this, our hypothesis on phylogenetic relationships of the Pronophilina can be considered as robust.

Our results support the new subtribe Cyllopsisina *subtrib. n.* and corroborate the results of Matos-Maraví *et al.* (2014) who found the genus *Euptychia* outside of Euptyihiina, and Zhang *et al.* (2021) who pointed out low support between *Euptychia* and the other genera traditionally considered as ‘euptyihiines’, and their suggestion that *Euptychia sensu stricto* may belong to another subtribe of the Satyrini. Following Espeland *et al.* (2019) who pointed out that *Cyllops* is part of the called ‘Cyllopsis’ clade, and now this clade is the sister of the rest ‘cyllopsisines’. Though, Espeland *et al.* (op. cit.) found good support for the monophyly of Euptyihiina and proposed nine clades for this subtribe, with the clade *Euptychia* as sister of the
remaining ‘euptychiines’, but according our results, Euptychiina sensu novum. is the sister group of Calistina. It has to be pointed out, however, that Espeland et al. (2019) study did not involve a large taxonomic sampling (only 106 species including the outgroups), for example, they included only two species of Euptychia and only one of Calisto (Calistina), and involving larger taxonomic sampling is more advantageous for building systematic relationships (Tsang et al. 2014; Wang et al. 2020a; Wang et al. 2020b), in particular in such diverse groups as the Satyrini (Marin et al. 2011; Zhang et al. 2021).

Earlier molecular studies have found Euptychiina (with or without Euptychia) to be the sister group to a larger clade consisting of many arrangements of Erebiina + Maniolina + Melanargiina + Pronophilina + Satyrina + Ypthimina, but with a low support (Peña et al. 2011; 2006; Wahlberg et al. 2009; Matos-Maraví et al. 2014; Espeland et al. 2019), but according to our phylogenetic data, the enigmatic Caribbean subtribe Calistina was found to be the sister group to Euptychiina s. nov. (that is including exclusively the genus Euptychia sensu stricto) with a good support. Wahlberg et al. (2009), on the other hand, found Calisto close to Melanargiina, although with low support. Matos-Maraví et al. (2014) found the genus Calisto was not recovered within any valid Satyrini subtribes, supporting the validity of the tribe Calistina, and it was placed as sister to the genus Euptychia and of the sampled subtribes Ypthimina, Melanargiina, Satyrina, Erebiina, Maniolina, and Pronophilina, except for Euptychiina with low support values, whereas Espeland et al. (2019) found Calisto as the sister group to the African and Asian Ypthimina with a good support, but their data included only one species of Calistina and Ypthimina, and we included 12 and 11 species respectively in our study which is much more robust in the taxonomic sampling compared to theirs.

The relation of Erebiina + Maniolina was robust in our analyses and it supports previous studies of Peña et al. (2011), Wahlberg et al. (2009), Espeland et al. (2019), and Zhang et al. (2021). Nonetheless, Zhang et al. (2021) made a systematic arrangement according to which Maniolina were transferred to Erebiina based on high support value of the two clades. However, from a morphological, biogeographical and ecological point of view, these two subtribes are very different and extremely well defined (Erebiina are boreal and montane cold habitat adapted, humid grassland with the centre of diversity in the Alps, Maniolina are mostly lowland, warm and dry
habitat adapted with the centre of diversity in Central Asia). Therefore, despite a high support of their monophyly in genomic data, an integrative approach should be applied here, allowing obtaining a more comprehensive insight when nomenclatural changes are proposed (Cadena & Zapata 2021, Escalona et al. 2021). We, thus, maintain the validity of Erebiina and Maniolina as separate subtribes. Our phylogenetic results allow us to corroborate the taxonomic arrangement made by Zhang et al. (2021) into the genus Erebia, where the species Boeberia parmenio Böber, 1809 was transferred to the genus Erebia and the validity of the three subgenera (Magda) Grishin, 2021, (Erebia) Dalman, 1816, and (Boeberia) Prout, 1901. However, we do not accept the transfer of the genera Pyronia Hübner, [1819] and Aphantopus Wallengren, 1853 to Maniola Schrank, 1801, and the genus Hyponephle Muschamp, 1915 and Ereminephele to Cercyonis Scudder, 1875, due to the high support values for each genus, and the clear phylogenetic relationships found by us and Wiemers et al. (2020). The clade obtained of Mycalesina + (Parargina + Lethina) is corroborated by the results of Wahlberg et al. (2009), Peña et al. (2011), and Zhang et al. (2021), supporting the robustness of our data.

Finally, the systematic proposal made by Zhang et al. (2021) with genomic data, to create three new subtribes for Satyrini (Callerebiina, Calistina, and Gyrocheilina), the transfer of Melanargiina to Satyrina, and the genus Paralasa Moore, 1893 to Ypthimina is corroborated by our analyses with the use of individual genes. However, the position of Satyrina as the sister of the Callerebiina differs from that proposed by Espeland et al. (2019) and Zhang et al. (2021) who found Satyrina as the sister of the remaining Satyrini crown group. Moreover, the clade of Eritina + Coenonymphina is not supporting by Espeland et al. (2019) and Zhang et al. (2021) because they found Coenonymphina as sister of the Satyrini crown group, and Eritina as subtribe sister of the Ragadiina, but, on turn, our phylogenetic relationships agrees with Wahlberg et al. (2009) and Peña et al. (2011). These differences are caused by the type of data used in each study, since the use of genomic data may provide more information than the use of some selected genes that can generate possible polytomies in phylogenies (Espeland et al. 2019, Zhang et al. 2021). However, using larger taxonomic sampling may help to improve these problems and allow to build systematic relationships more robust (Marin et al. 2011, Tsang et al. 2014, Wang et al. 2020a, Wang et al. 2020b), and be able to generate results similar to genomic data as is the case of our study which the majority of the phylogenetic relationships found by Espeland et al. (2019) and Zhang et al. (2021) with genomic data, were recovered by us.
Final Taxonomic Remarks

We combined again the species *Ypthimomorpha itonia* (Hewitson, 1865) (Ypthimina) with the genus *Ypthima* Hübner, 1818, as initially proposed by Hewitson (1865), and transferred to the genus *Ypthimomorpha n. syn.* by Van Son (1955) (*Ypthimaitonia* Hewitson, 1865 **comb. reinst.**). In turn, the monobasic genus *Dangond* Adams & Bernard, 1979, whose representative *D. dangondi* Adams & Bernard, 1979, an endemic species of the Serranía del Perijá on the Colombian-Venezuelan border (Pyrcz & Rodríguez 2007) and the species *Altopedaliodes reissi* (Weymer, 1890) are transferred to the genus *Pedaliodes* Butler, 1867, given the strong branch support and its nesting within the clade *Pedaliodes* in our phylogenetic analyses (*Pedaliodes dangondi* (Adams & Bernard, 1979 **comb. nov.**, and *Pedaliodes reissi* Weymer, 1890 **comb. reinst.**). In addition, it is possible that the genus *Altopedaliodes* Forster, 1964 might be polyphyletic (Padrón et al. 2021).

Matz & Brower (2016) transferred some species of the genus *Argyrophorus* Blanchard, 1852 to the genus *Punargentus* Heimlich, 1963 based on three molecular markers (COI, wingless, and EF-1α) (e.g. *A. chiliensis* (Guérin-Méneville, [1830]) and *A. monticolens* (A. Butler, 1881)), and separated these species from *A. argenteus* Blanchard, 1852, although, according to our phylogenetic analyses and based on Pyrcz et al. (2019) the genus *Punargentus* is a junior synonym to the genus *Argyrophorus*. Finally, according to our molecular data *Euptychia jesia* A. Butler, 1869 is a junior synonym of *Euptychia westwoodi* A. Butler, 1867 due to the shorter branch length that they are presenting.

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CHAPTER II

Solving the cryptic diversity of the genus *Manerebia* Staudinger in the Neotrop: description of new species and taxonomic considerations (Nymphalidae: Satyrinae: Pronophilina)

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Abstract

The genus *Manerebia* Staudinger contains 34 currently recognized species ranging through out the Neotropical region. However, their systematic remain poorly understood and have been presented some taxonomic conflicts. Taxonomic uncertainty regarding names, suggestions of polytypic species complexes, and undescribed cryptic diversity all contribute to the current. However, their systematic remain poorly understood and have been presented some taxonomic conflicts. To gain insights into the
systematics of this group, we inferred single locus phylogenies and conducted species delimitation analyses using COI mitochondrial gene. Moreover, we used morphological data and genetic distances to help to make taxonomic decisions. Based on our analyses we found 48 distinct species from our sampled 24 nominal species, where 14 are new species, of which 8 are described in this paper. Therefore, according to our systematic proposal, the genus *Manerebia* would comprise 58 nominal species, but for the moment some remaining undescribed in this paper. The phylogenetic analyses, together with the species delimitation methods and the morphological characters, allowed us to evaluate the high cryptic diversity within the genus *Manerebia*. Hence, our analysis highlights the importance of employe the integrative taxonomy framework for the detection of cryptic diversity in regions such as the Neotropics.

**Key words:** Cryptic diversity; molecular systematics; integrative taxonomic; species delimitation.

**Introduction**

Species constitute fundamental ecological and historical units in biological systems (Coyne & Orr 2004; Vitecek *et al.* 2017; Demos *et al.* 2018) and it has important implications for the understanding and conservation of biodiversity, as well as for how we mobilize efforts and allocate resources to develop conservation strategies (Freitas *et al.* 2020). Over the years, different criteria and tools have been used to define species, leading to a succession of species concepts that resulted in extended controversy within the research community (de Queiroz 2007; Freitas *et al.* 2020). Moreover, the relationship between species richness and geography can be greatly biased if the number of species is underestimated (Hortal *et al.* 2015; Demos *et al.* 2018). These knowledge gaps in species-level taxonomy, the so-called ‘Linnean shortfall’, are particularly prevalent in tropical species (Hughes *et al.* 2017; Demos *et al.* 2018); they also characterize recently diverged and morphologically conservative clades where cryptic species are present (Demos *et al.* 2018; Fišer *et al.* 2018).

In parallel, a variety of methods for recognizing new species or testing species hypotheses was developed, usually referred to as ‘Species delimitation tools’ (Wiens 2007; O’Meara 2010; Vitecek *et al.* 2017; Matos-Maravi *et al.* 2019). Likewise, despite the demonstrated informativeness of molecular taxon delimitation to test species hypotheses, not all studies that successfully employ molecular taxonomic taxa
delimitation tools follow through to describe these new taxonomic entities (Pante et al. 2015; Steiner et al. 2015; Vitecek et al. 2017). Remarkably, this has been related to the complex taxonomic procedures associated with the formal description of new species (Pante et al. 2015; Vitecek et al. 2017). ‘Species delimitations’ by these methods constitute statements of population structure, and hence of independent evolutionary lineages (Demos et al. 2018). Integrative taxonomic assessments using multiple independent datasets from voucher specimens are needed before the resulting groups warrant taxonomic recognition as species (Demos et al. 2018; Freitas et al. 2020).

Independent data, such as: morphology, vocalizations, etology ecology, and geographic distribution, may be used to evaluate the relationships of groups once they are delimited (Demos et al. 2018; Freitas et al. 2020; Escalona et al. 2021). The results of delimitation studies can provide a strong foundation for subsequent integrative taxonomic revisions and should be especially valuable in cases involving taxonomic confusion or uncertainty of the samples (Demos et al. 2018; Matos-Mavarí et al. 2019).

The Pronophilina subtribe (Nymphalidae, Satyrinae) has been recognized as one of the most diversified group of butterflies in mountain environments, distributed within narrow altitude ranges and presenting high levels of endemism (Adams 1985; Pyrcz et al. 2006; Pyrcz & Viloria 2007; Mahecha-Jiménez et al. 2011; Marín et al. 2014; Marin et al. 2017; Pyrcz et al. 2016; Álvarez-Hincapié et al. 2017; Mahecha et al. 2019). The highest diversity of Pronophilina is reported in cloud forests, between 2500 and 2900m (Adams 1985; 1986; Pyrcz & Garlacz 2012; Mahecha et al. 2019; Mahecha-J et al. 2021). However, the correct identification of species in many genera of Neotropical has been affected by high cryptic diversity and taxonomically confusing taxa (Pyrcz et al. 2006; Marín et al. 2017; Mahecha et al. 2019; Mahecha-J. et al. 2021). Moreover, the high level of diversity and endemism in the Andes makes the systematics and evolution of its fauna active, as is the case of the genus Manerebia Staudinger, 1897 (Pyrcz et al. 2006).

The genus comprises 34 described species, with several more identified but for the moment remaining undescribed (Pyrcz et al. 2006; Mahecha-J et al. 2021). Miller (1968) placed the genus of Manerebia in the tribe Pronophilini (Pyrcz et al. 2006; Pyrcz & Viloria 2007). Brown (1944) introduced the generic name Penrosada for some Manerebia of the northern Andes. Adams & Bernard (1979; 1981) and Adams (1986) continued to use both Manerebia and Penrosada as distinct genera, without any
discussion about their systematic relationships, until Lamas et al. (2004), and Lamas & Viloria (2004) considered Penrosada Brown as a subjective junior synonym of Manerebia base on morphological similarities as the male genitalia (Pyrcz et al. 2006). Pyrcz (1995) suggested its closer relationship with north Andean pronophiline genera Idioneurula Strand. Viloria (2002) pointed out that all three genera share common characters atypical of Pronophilina, namely naked eyes and the cross-cell vein M1-M2 on the HW either only slightly incurved or not at all, and suggested they should all be removed to the tribe Erebiini or sub-tribe Erebiina (Pyrcz 2004; Pyrcz & Viloria 2007). Recently, Mahecha-J. et al. (in prep.) found, based on molecular characters, that Manerebia is the sister group of the clade conformed by ((Lymanopoda + Ianussiua) + (Diaphanos + Idioneurula)).

The wing shape of Manerebia are characterized by the anal margin of the hindwing slightly incised near tornus; generally, a fully developed ocellus in CuA2-Ia of both the forewing and hindwing; the cross-vein M1-M2 shorter than Lymanopoda Westwood, and straight or gently curved, M3 much closer to CuAI than to M2; short and yellowish legs; on the FWV, sometimes showing on the upperside as well; and ventral surface has a faint pattern of postdiscal and submarginal lines and fully developed submarginal ocelli, the largest of which is invariably in cell Cu1-Cu2 (Pyrcz 2004; Pyrcz et al. 2006; Pyrcz & Viloria 2007). Male genitalia are characterised by a long, arched uncus, fully developed gnathos, and slender valvae with a strongly dentate ampulla (Pyrcz 2004). A remarkable feature of the genus Manerebia is the external similarity of several species which can only be identified with certainty through genitalia dissection and sometimes only with molecular data, which has led to considerable confusion in literature and resulted in underestimating their true taxonomic diversity (Pyrcz et al. 2006). Additionally, some species of Manerebia can be polymorphic and depending on the morphotype can have a band shortened, discontinuous or even completely absent, for example in M. ignilineata (Dognin, 1896), M. apiculata (C. Felder & R. Felder, 1867), and M. trimaculata (Hewitson, 1870). For that reason, this character has only an infrasubspecific value (Pyrcz et al. 2006; Pyrcz & Viloria 2007).

The most recent contributions to the taxonomy of Manerebia are Pyrcz (2004), who described several new species from northern Peru (Chachapoyas), and the revision of North Andean Manerebia (Pyrcz et al. 2006), which identified 23 species found from
extreme northern Peru, across Ecuador, Colombia and Venezuela, and included the descriptions of 10 new species and 13 new subspecies. Pyrcz et al. (2006) also pointed out the presence of an undescribed subspecies of *M. prattorum* in Lambayeque department on the western slopes of the Andes in Peru, but refrained from naming it as it exceeded the geographical scope of that paper. Further new species have been collected during recent explorations of the north Peruvian, during ongoing field work in southern Ecuador, and through recent expeditions in Colombia. Nevertheless, the genus *Manerebia* needs to be revised due to high cryptic diversity (Pyrcz et al. 2006; Mahecha-J. et al. 2021). Therefore, this paper aims to examine the current knowledge of systematics of the genus *Manerebia* based on morphological and molecular data and apply delimitation species methods for evaluating the cryptic diversity into the genus. We expected the use of different morphological characters (e.g. wing coloration, and genitalia shape) together with molecular information and species delimitation methods, might favour narrower or broader species definitions in the genus *Manerebia*, allowing us to discover the cryptic species that might be within the genus and make an arrangement systematic according to our results.

**Material and methods**

**Material**

The material examined in this study was obtained by TP, JC, JF, and PB during field work in Cajamarca and Piura (2018), by JC and JF during field-work in the Tabaconas Namballe Sanctuary (2010–2014), PB and TP (2000–2018), PT, CP, and OM during field-work in Colombia (2016-2020), and by KW and collaborators (2000–2019) during field work in southern Ecuador. Comparative material has been studied in the collections of CEPUJ (Nature Education Centre, formerly Zoological Museum, Jagiellonian University, Kraków, Poland), including the collection of Pierre Boyer, the FLMNH (McGuire Centre for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, USA), INABIO (Instituto Nacional de Biodiversidad, Quito, Ecuador), MUSA (Museo de Historia Natural, Universidad Nacional de San Agustín, Arequipa, Perú) and MUSM (Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Perú), Instituto de Ciencias Naturales of the Universidad Nacional de Colombia (ICN-MHN-L), Museo de Historia Natural of the Universidad de Caldas (MHN-UC), Museo Pontificia
Universidad Javeriana (MPUJ), Colección entomológica of the Universidad Nacional de Colombia, Sede Medellín (UNAL-MED), E. Schmidt Mumm’s personal collection at Instituto Alexander von Humboldt, Jean F. LeCrom’s personal collection (C-JFL), and Carlos Prieto’s personal collection (P-CP).

**Morphology Dissections**

Terminal parts of male and female abdomens were removed and soaked in 10% KOH solution for 5–10 minutes. Subsequently, these were preliminarily cleaned out of soft tissue in water and stained in chlorazole black to better visualize soft genital parts. Dissected genitalia were cleaned of water using ethanol 90% and 95% solutions. Genitalia were photographed using Nikon digital camera DS–Fi1 and Olympus SZX9 stereomicroscope, and images were then processed in Combine ZP and Corel PHOTO–PAINT X3 programs to enhance focus and improve clarity. Genital dissections were kept in glycerol vials pinned under corresponding specimens. Genital terminology follows largely Razowski (1996) and Klots (1956). Adults were photographed with a Minolta E–500 digital camera and colour plates were composed using Adobe PhotoShop version 8. The following abbreviations are used in the text: FW: forewing; HW: hindwing; D: dorsum; V: venter; HDP: hindwing dorsal median patch.

**Molecular Data Collection**

At the CEPUI, DNA was isolated from a pair of legs, dry or ethanol-preserved, using a NucleoSpin® Tissue extraction kit (Macherey-Nagel Düren, Germany) and following the manufacturer’s protocol. Amplification of part of the mitochondrial gene *Cytochrome Oxidase I (COI)* was performed using HybLCO and HybHCO primers with universal primer tails, respectively T7 Promoter(F) and T3(R) (Folmer *et al*.1994, Wahlberg & Wheat 2008), with standard PCR protocol. PCR reactions were performed in a 20 µl reaction volume in Eppendorf Mastercycler® nexus thermocycler. The PCR cycling profile comprised an initial denaturation at 95°C for 5 min followed by 40 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 1 min, and a final extension period of 72°C for 10 min. Amplification products were checked by electrophoresis on 1% agarose gels stained with Midori Green (NIPPN Genetics). Finally, samples were sent to Macrogen Europe (Amsterdam, Netherlands) for purification and sequencing. DNA
Phylogenetic Analyses

Some available COI sequences were extracted from the Barcode of Life Data System (BOLD) (Ratnasingham & Hebert 2007) and NCBI (https://www.ncbi.nlm.nih.gov/nuccore), and used to reconstruct the phylogenetic tree (Supplementary material 1). The Chromatograms, and sequences from NCBI Genbank and BOLD obtained were edited and base calls checked using Geneious v. 4.8.5 (Drummond et al. 2009). We searched for reading frame errors and unexpected stop codons by translating the nucleotide sequences to peptides using Geneious v. 4.8.5. COI sequences were aligned using MAFFT v. 7.245 (Katoh & Standley 2014) and then refined with Geneious v. 4.8.5.

The best-fit DNA sequence evolution model of our dataset was estimated to ModelFinder (Kalyaanamoorthy et al. 2017) which is included in IQ-TREE version 2.1.3 (Minh et al. 2020) and using the Bayesian Information Criterion (BIC) to choose the most suitable model (Pan et al. 2019). The GTR+I+G evolution model was selected to implement in the phylogenetic analyses. The first phylogenetic analysis was conducted using Bayesian inference (BI) with BEAST2 suite v. 2.5.2 (Bouckaert et al. 2019). The MCMC chains were run for 30 million generations and sampled once every 5000. A strict molecular clock was used (Opgenoorth et al. 2020; Pola et al. 2021; Rubinoff et al. 2021), and mitochondrial COI locus a gene ploidy of 0.5 was assigned (BEAST Developers 2017; Matos-Maraví et al. 2019; Núñez et al. 2020). The Birth-Death model (Gernhard 2008; Maurin 2020) was used, and four independent runs were started and combined with LogCombiner v. 2.5.2 (Bouckaert et al. 2019).

The combined sampled trees from the analysis were then summarized in TreeAnnotator v. 2.5.2 (Bouckaert et al. 2019) with a selected burn-in of 25% to build the maximum clade credibility tree (Maurin 2020; Pola et al. 2021). Chain convergence was further assessed with Tracer v.1.6 (Rambaut et al. 2014) to confirm sufficient effective sampling size (ESS > 200) (BEAST Developers 2017; Maurin 2020). A second phylogenetic analysis was inferred using the Maximum Likelihood (ML) in IQ-TREE v. 2.1.3 (Minh et al. 2020) with performed 10000 ultrafast bootstrap approximation
algorithm replicates to explore branches support across the topology (Minh et al. 2013; Hoang et al. 2017).

Species delimitation analyses

Our taxonomy is largely based on the biological species concept developed by Dobzhansky (1935) and further elaborated by Mayr (1957). Accordingly, a species is a group of individuals that can successfully interbreed in natural conditions and produce fertile offspring, and whose species' integrity is maintained by prezygotic or postzygotic reproductive barriers between individuals of different species. The outcomes of such reproductive barriers are heritable traits that can be identified and used to delimit species. Subjective decisions must necessarily be made in the case of allopatric taxa, and typically we use the kinds of divergence seen in closely related sympatric species as a yardstick to make decisions for allopatric taxa.

Species and subspecies delimitation were performed here using four main sets of data: wing shape and colour pattern, genitalia, DNA barcodes, and ecological traits such as habitat and elevational range. External characters, which are subject to interspecific differences within Manerebia, such as wing size and shape, especially the outlier of the FW distal margin and apex, FWD male androconial patches, and colour patterns, are presumed to play an important role in species recognition. The colour patterns of Manerebia are very simple and but comprise several characters considered taxonomically valuable by virtue of their variation in sympatric close relatives, in particular the most conspicuous traits such HWD orange or yellow patches, FW and HW submarginal ocelli, and finally the HWV median yellow patches. It is important to emphasize that in some species, as pointed out before (Pyrcz et al. 2006), the expression of the latter character is subject to important individual variation within given populations (M. apiculata, M. ignilineata and M. interrupta), whereas in others (M. germaniae, M. inderena, M. franciscae, M. haywardi) they are exceptionally stable, so this peculiarity of the genus was taken into account in considering the expression of the HWV median band. Genital structures that vary between sympatric species and species groups were also taken into consideration. In particular, the male genitalia tend to be more variable between closely related species and are better documented (females of some species are rare or unknown), and since they play a mechanical role in mating they are thus thought to participate in species isolation systems, by helping the female to
grasp and position the abdomen of the female. Important genitalic characters in *Manerebia* include the length and shape of the uncus, the shape of terminal parts of the valva, including the size of the processes, their number and direction, and shape and length of the aedeagus. Ecological preferences that were taken into account include the occurrence of species within particular elevational bands which correspond to specific types of cloud forest habitats.

On the other hand, DNA barcode data, when resulting in strongly supported tree topologies and marked genetic distances, were considered as decisive in species delimitation. However, for some taxa DNA sequences were available for only single individuals, and thus their relationships as inferred from genetic data might need confirmation. Where adults showed significant differences in external morphology, colour traits presumed to play an important role in mating strategies, and habitats, such as occurring within mutually exclusive bands of altitude, they were treated as separate species, as in previous papers (Pyrcz 2004; Pyrcz et al. 2006; Mahecha-J et al. 2021), even if DNA barcodes were not as distinct. Conversely, in some cases, DNA barcode divergence was more pronounced than morphological divergence, underlining the fact that complete agreement between all data types is unlikely, and indeed is the rationale for the integrative taxonomic approach. Finally, we consider the allopatric taxa which exhibit noticeable differences in colour patterns but occur in the same kind of habitat and at similar elevations with no, or only marginal differences in COI sequences, as subspecies.

To help guide taxonomic decisions, tree-based species-delimitation methods were used: The Bayesian implementation of the Poisson Tree Processes (bPTP) (Zhang et al. 2013) using the parameters of the bPTP analysis set as follows: 500,000 generations, a thinning of 500 and burn-in of 10% (Pan et al. 2019). To employ the bPTP method, the ML tree as input data was used (García-Melo et al. 2019). Another delimitation method was the generalized mixed Yule-coalescent GMYC method (Pons et al. 2006) allows determining the point of transition from species level to population level combining models of stochastic lineage growth with coalescent theory in a robust maximum-likelihood generating an ultrametric guide tree (Shen et al. 2019; García- Melo et al. 2019). To conduct the GMYC method, the ultrametric tree estimated in BI analysis was used as input data. Both methods were calculated using the online server (http://species.h-its.org/). The inter–intra specific genetic distances of the samples were
estimated based on the Kimura-2 Parameter (K2P) model of evolution (Kimura 1980) in MEGA X (Kumar et al. 2018).

Additionally, we used STACEYv.1.2.4 package (Jones 2017) available in BEAST2 suite v. 2.5.2 (Bouckaert et al. 2019). STACEY uses a Bayesian approach to infer species delimitation and species phylogeny, based on the multispecies coalescent model (MSCM) (Jones 2017; Tomasello 2018; Ito et al. 2019; Matos-Maraví et al. 2019). It incorporates a model for the per-branch population parameters, and the ‘birth-death-collapse’ model from the species delimitation method DISSECT (Jones et al. 2015). STACEY has been proved to be faster than BEAST (Heled & Drummond 2010) reaching convergence while inferring the right species tree phylogeny, and for species delimitation purpose, faster than DISSECT (Jones 2017). In addition, the priors on the nucleotide substitution and on the clock models can be defined in STACEY (Tomasello 2018). We used a strict molecular clock (Opgenoorth et al. 2020; Pola et al. 2021; Rubinoff et al. 2021), and the birth–death–collapse model following Jones et al. (2015) and Matos-Maraví et al. (2019) with GrowthRate prior as log-normal (M = 5, S = 2) and relativeDeathRate as uniform in [0, 1], while the popMean prior was set to log-normal (M = −7, S = 2). We assigned to mitochondrial COI locus a gene ploidy of 0.5 (BEAST Developers 2017; Matos-Maraví et al. 2019; Núñez et al. 2020).

The analyses were run four independent times for 60 million generations each, with parameters sampled every 2000 generations. Convergence of the stationary distribution and ESS values (>200) were checked in Tracer v.1.6 (Rambaut & Drummond 2014). Output MCMC samples of the species trees were combined with LogCombiner v. 2.5.2 (Bouckaert et al. 2019) after discharging the 25% of the analyses as burn-in. The obtained files were processed in SpeciesDelimitationAnalyser v.1.8.0 (Jones et al. 2015; Matos-Maraví et al. 2019) with a $1 \times 10^{-4}$ ‘collapse height’ and asimilarity cut-off of 1.0. The combined STACEY output files were additionally used to produce maximum clade credibility trees in TreeAnnotator suite v. 2.5.2 (Bouckaert et al. 2019). We chose the clustering with the highest posterior probability (counts) as the working species hypothesis.

For all analyses, Lymanopoda nevada Krüger, Idioneurula erebioides (C.Felder & R.Felder, 1867), and Ianussiusa maso (Godman, 1905) were used as outgroup taxa. Geneious v. 4.8.5 (Drummond et al. 2009) was used to format the resulting trees of each
analysis, and then were refined in Inkscape 0.92.3 (2405546, 2021-06-09) (https://www.inkscape.org).

Results

A total of 832 individuals were examined, and 52 male and 14 female genital dissections were made. The COI mitochondrial dataset consisted of 140 aligned sequences with a length between 500-680 bp for 24 nominal species of Manerebia. The phylogenetic analyses, together with the species delimitation methods and the morphological characters, allowed us to verify the high cryptic diversity within the genus Manerebia, and based on them we suggested the existence of 48 distinct species from our sampled 24 nominal species, where 14 are new species, of which 8 are described as below, and the other taxonomic descriptions will be done in future articles due to we need to revise more samples and acceded to the holotypes. Although, according to our analyses they may be cataloged as valid new species (Fig.1). Moreover, several subspecies were transferred to species level, such as: M. inderena clara, M. inderena antioquiana, M. inderena similis, M. inderena clara, M. inderena ssp., M. satura pauperata, M. satura lamasi, M. rufanalis fernandina, M. undulata milaena, M. trirufa ssp., among other.

In fact, the results found by the delimitation species analyses did not display substantial differences and were similar to the total number of nominal species found, but there were differences in some species clustering (Fig.1; Supplementary material 3, 4), for example, M. trimaculata is a single species in bPTP analysis, but in GYCM and STACEY are three different species, and a similar case is with M. apiculata from Casanare-Colombia that appears as a different species from the other samples of M. apiculata from Bogotá and Guasca, both from Colombia, in the STACEY and GYCM analyses, but all samples are a single species in bPTP. A special case is M. ronda n. sp. and M. pauperata n. stat. that according to delimitation species methods and genetic distances are a single species but ecologically and morphologically are such different which may be considered as different species. Consequently, incongruent of these species clustering was further investigated by referring to the genetic distances (see Supplementary material 2) and morphological characters.
FIGURE 1. Phylogenetic analyses (ML-BI) and species delimitation methods based on COI mitochondrial gene. Node support: low/weak (UFB <50, PP <0.50), moderate (UFB = 50–84, PP = 0.50–0.84), high (UFB = 85–94, PP = 0.85–0.94) or strong (UFB ≥ 95, PP ≥0.95) – Photo by Kertell Ken ©.
(Note: For viewing the FIGURE 1 with quality better, see the file within Supplementary material: Chapter II in the Drive below link, please).

https://drive.google.com/drive/folders/1cBCUG1WJsTaAcRcfQaWM_HgLkAN3P_o4?usp=sharing
New species and subspecies descriptions

*Manerebia bernito* Triviño & Mahecha-J. n. sp. (Figs. 4, 5, 6)

**Type locality:** Colombia, Boyacá, Pisba, Vda. Miraflores, Sabana El Crisol

**Diagnosis:** *M. bernito* n. sp. is in many aspects similar to *M. levana*. The male and female have submarginal small ocelli only in the VFW-VHW (between Cu2-A1 veins), but the ocelli of the females are just bigger than of the male. In the males, the ocelli are reduced to black dots. *M. bernito* n. sp. differ from *M. levana*, in the submarginal ocelli in the DFW-DHW and VFW-VHW of both sexes bigger than *M. bernito* n. sp. The wing pattern of both sexes of *M. bernito* n. sp. is similar to *M. clarita* n. sp. (see below), *M. levana* and the female of *M. pervaga*; however, *M. bernito* n. sp. has a wider postdiscal yellow-brown line on the Cu1 vein than *M. clarita* n. sp., *M. levana* and the female of *M. pervaga*. Both sexes in *M. bernito* n. sp. are darker brown in discal cell, the postdiscal dark yellowish-brown line is composed of lunular streaks incurved basally in cells M1-M2 to A1-Cu2, while in *M. levana*, the VHW postdiscal band is yellowish-orange, indistinct, oblique and marked at its distal edge by a thin, dentate and dark brown line, dividing the wing into a yellowish-orange area basally and a chestnut area distally, and in the female of *M. pervaga*, the VHW is greyish brown, darker brown on the discal cell, and the postdiscal dark yellowish-brown line is composed of lunular streaks incurved basally in cells M1-M2 to A1-Cu2, approximately parallel to distal margin (Pyrcz et al. 2006). Additional differences are found in male genitalia (Fig. 5A).

**Description:** MALE (Fig. 4A): **Head:** antennae ventrally white with yellow brownish clubs, dorsally brown; eyes dark with yellow-brown hairs covering the back base of the eye; labial palp two times longer than head and dorsally covered with abundant yellow-brown and ventrally yellow scales. **Thorax:** dorsally black covered with golden hairy scales, which that cover the patagium, tegulae, and prothorax, ventrally brown; legs dorsally white with short red spines on the tarsus, ventrally of white color to the last three tarsi, and the latter in turn have many short spines of silver color. **Abdomen:** dorsally brown, hairy, ventrally densely hairy, first abdominal segment white, median segments yellow-brown and last segments light brown. **Wings:** Forewing rounded triangular (length: 18.4-19.2mm; mean: 19.6mm; n=5), hindwing rounded, tornus rounded; anal margin very slightly excavated near the angle; dorsal surface of both wings hairy in basal half and along anal margin. Dorsal surface ground color coffee-brown. DFW uniformly brown, large-sized androconial patch uniforms until to
the submarginal area, completely covering the discal cell. VFW ground color brown, slightly darker at the base, costal margin lighter, it presents a thin orange-brown band between the apical and marginal areas extending towards the costal margin of the subapical, as well as the base of the costal margin area. There are three submarginal small white points between Rs-M3 veins near to the alar margin or termen; there is a submarginal smaller ocellus in Cu2-A1 cells. DHW is entirely dark brown. VHW ochreous-brown; dark brown at the base of discal cell; with a thin red-brown band that begins from upper part of the basal area in the discal cell bordering the entire median, post-median, terminal, tornal, and coming back again to down part of the basal zone of discal cell, located between the Rs-A1 veins, but in some specimens, this line is not as pronounced; a thick yellowish-brown band from the base of the discal cell to CuA1 vein extending to the terminal zone; a very fine line is presents from postdiscal to submarginal area yellow-brown in the Cu2 vein, and a thicker line from discal to submarginal area yellow-brown at the beginning of the A1 vein extends to the tornal-terminal part, decreasing its color and hue. These postdiscal lines are ‘V’ shaped in cells CuA1 to A1 and approximately parallel to the distal marginal, similar to M. levana, M. pervaga female and M. clarita n. sp.; a band orange-brown to long of costal margin is presents; there is a submarginal smaller ocellus in CuA2-A1 cells. Male genitalia (Fig. 5A): Uncus long and arched with length similar to tegumen; at distal tip to valva with ‘five teeth’, which are very near it one to each other, similar to M. levana (Fig. 5B); with many hairs along to the valva, extending anteriorly; aedeagus’s length like the valva, elongate similar to M. levana, M. clarita n. sp., and M. pervaga (Fig. 5A–D); with two dorso-lateral patches of spines in middle of posterior section but in M. bernito n. sp. these spines are smaller than M. clarita n. sp., and M. pervaga although similar to M. levana; the apical part of the aedeagus of the subsequent process is rounded in shape; saccus long. FEMALE (Fig. 4B): it is a little big than a male, similar to the wing-color patterns to males. Female genitalia were not examined.

Molecular data: BI, ML and the species delimitation methods (Fig.1) and genetics distances (Supplementary material 2) indicate that M. bernito n. sp. is a distinct species, and related to M. pervaga.


Etymology: This species is dedicated to the second author’s grandfather: Bernabé Cruz, who bequeathed her his love for nature, the respect and deep admiration for the role of women in the society, the territory defense, his love for trees, wood, and the service to the community; bernito is used in apposition.

Remarks: This species is similar to *M. levana*, *M. pervaga* and *M. clarita* n. sp. *M. bernito* n. sp. presents valid taxonomic characters at the species level, such as the male genitalia and the wing pattern which allows differentiate it from other species. Nevertheless, a molecular analysis should be done to recognize their phylogenetic relationships between them. *M. bernito* n. sp. was found flying in the paramo over *Chusquea tessellata* plants in a buffer area on the eastern slope in the Pisba National Natural Park (Fig. 6A–B). *M. levana* has been observed flying too over *C. tessellata* in the Monas paramo near to Guasca-Cundinamarca (Fig. 6C–D). In the Pisba National Natural Park several threats to the natural habitat were identified, for example intensive grazing of livestock and frequent burning of the vegetation by the local inhabitants. The paramo of Pisba was categorized by Triviño et al. (2017) as a critically endangered (CR). This is in risk of ecosystem collapse, mainly due to intensive mining activity. Until now, the eastern slope of the paramo area of Pisba is the only known locality for *M. bernito* n. sp., for that reason, conservation plans are necessary to preserve this species and its habitat.

*Manerebia clarita* Mahecha-J. & Triviño n. sp. (Figs. 4, 5, 6)

Type locality: Colombia, Casanare, Municipio La Salina, Vda. Chinibaque, Sabana Páramo de la Colorada-Sierra Nevada del Cocuy.

Diagnosis: This species is externally most similar to *M. pervaga* Pyrcz & Viloria, 2006, from the Paramo El Táma on the Venezuela—Colombia border, and the Cerro Oroque, Norte de Santander in Colombia. Both species lack any tornal ocelli on both fore and hindwings, as compared to *M. levana* and *M. bernito* n. sp. which have tornal ocelli in both sexes, although *M. bernito* n. sp. has a tornal ocellus only on the VFW.
The male of *M. clarita* n. sp. differs from the males of *M. pervaga* and *M. levana*, because of the presences of a thick greyish white line on the base of the discal cell to Cu1 vein; a thinner line postdiscal greyish white extends over the Cu2 vein, being thicker in the terminal-termen part; in the A1 cell there is a thin postdiscal grayish white line that widens towards to the margin of the tornus. The male of *M. bernito* n. sp. is similar to male of *M. clarita* n. sp. in the wing pattern, but *M. bernito* n. sp. has a more prominent, wider postdiscal yellowish-brown line along the CuA1 vein than *M. clarita* n. sp. Further differences are found in male genitalia (Fig. 5C).

**Description:** MALE (Fig. 4E): **Head:** antennae ventrally white with yellow-brown clubs, dorsally brown, with club twice as broad as shaft; eyes dark with yellow-brown hairs covering the back base of the eye; labial palp two times longer than head and covered with abundant light brown and black hairy scales. **Thorax:** dorsally blackish-brown, ventrally brown, densely hairy, yellow, covering the patagium, tegulae and prothorax; legs dorsally white with short red spines on the tarsus, ventrally white till last three tarsi which have many short silver spines. **Abdomen:** dorsally dark brown hairy, ventrally densely hairy, lighter on ventral surface, especially at the posterior tip, first abdominal segment white and last segments light brown. **Wings:** Forewing triangular (length: 17-20.2mm; mean: 19.6mm; n=2), hindwing rounded, tornus rounded, anal margin straight; dorsal surface of both wings hairy in basal half and along anal margin. Dorsal surface ground color coffee-brown; diffuse darker orange patch on DHW tornus. DFW uniform brown color, large-sized androconial patch uniform to the submarginal area, completely covering the discal cell. VFW ground color brown, slightly darkened at the base, costal margin lighter, distal margin, apical and subapical region ochraceous-brown, as well as the base of costal margin. VHW darker orange brown; darker brown at the base of discal cell; a thick greyish white line from the base of the discal cell to Cu1 vein extending to the termen, a thinner line from discal to submarginal zone greyish white extends over the vein Cu2, being thicker in the terminal part up to the termen, and in the cell A1 there is a thin postdiscal grayish white line that widen towards to the margin of the tornus near to anal angle. These postdiscal lines ‘V’ shaped in cells Cu1 to A1, and approximately parallel to the distal marginal. **Male genitalia** (Fig. 5C): Uncus long and arched with length similar to tegumen; at distal tipto valva ‘five teeth’, which are a little apart from each other, and more prominent than *M. pervaga* (Fig. 5D); it has dense hair from sacculus towards apex; aedeagus as long as valva, elongate, similar to *M. bernito* n. sp., *M. pervaga* and *M. levana*; with
two dorso-lateral patches of spines in middle of posterior section, but in *M. clarita* n. sp. these spines are more robust than the others; the apical part of the aedeagus of the subsequent process is rectangular in shape; saccus shorter and breadth. FEMALE: unknown.

**Molecular data:** Not available.


**Etymology:** The specific name is a dedication to Clara Inés Jiménez Moreno, mother of the first author, Mahecha-J. She has been the inspiration and support for him throughout his life. Thank you for your dedication to being the best mom and grandmother.

**Remarks:** This species is externally most similar to *M. levana*, *M. pervaga* and *M. bernito* n. sp. This species may be designated as vulnerable, because of the loss of natural habitats where it occurs. In this kind of habitat, many anthropic activities take place, such as livestock and mining. Ecological studies are necessary to evaluate the status of this species.
FIGURE 4. Adults of the Manerebia “levana” group.
A: M. bernito n. sp., holotype;
B: M. bernito n. sp., allotype;
C: M. levana male;
D: M. levana female;
E: M. clarita n. sp., holotype male;
F: M. pervaga male;
G: M. pervaga female.
FIGURE 5. Male genitalia. A: *M. bernito* n. sp., holotype; B: *M. levana*; C: *M. clarita* n. sp., holotype; D: *M. pervaga*.
Manerebia ronda Pyrcz & Boyer, n. sp. (Figs. 7, 12, 14, 15)

**Type locality:** Peru, Cajamarca, NE Bambamarca, Laguna Salahuindo

**Diagnosis:** This species superficially resembles a number of congeners, including *M. nderena* (Adams, 1986), *M. leaena* (Hewitson, 1861), *M. undulata* Pyrcz & Hall, 2006 and *M. germaniae*, all of which have wide HWV yellow median bands, but only some of them do not have any ventral ocelli, in particular *M. nderena leaeniva* Pyrcz & Willmott, 2006, *M. similis* n. stat. Pyrcz & Willmott, 2006, and *M. germaniae*. The latter species has a series of minute HWV submarginal yellowish dots, absent in *M. ronda* n. sp. The most similar taxon in colour pattern is *M. similis* n. stat. (Fig. 15B), whose HWV submarginal line is smooth and parallel to the outer margin, as opposed to the more undulating line in *M. ronda* n. sp. In terms of male genitalia morphology, the most similar species is *M. pauperata* n. stat., which differs in the larger tegumen which is apparent in lateral view, and thinner subuncus (Fig. 15). The nominate subspecies of
*M. ronda* n. sp. differs from *M. ronda amplia* n. ssp. by the more prominent teeth on the apical part of the valva (Fig. 12A, B).

**Description:** MALE (Fig. 7A): **Head:** eyes chocolate brown, naked, lustrous; labial palpi two times length of head, covered with black hair-like scales, longer ventrally, and some sparse yellow scales; antennae slender, 2/5th length of costa, mostly naked, dorsally brown, ventrally milky white, club formed gradually. **Thorax:** black, naked; legs with femur covered with chestnut scales, tibia and tarsus with sandy yellow scales. **Wings:** FW length 18–19 mm, mean: 18.4 mm, n=7; FWD uniform chocolate brown, lustrous, with a barely noticeable submarginal darker smooth line parallel to distal margin. HWD with long hair-like scales in median half, uniform chocolate brown, with a barely noticeable undulating darker submarginal line and yellow median band slightly translucent from venter. FWV dark brown, paler along costal and particularly distal margin due to thin overcast of whitish scales, with a well-marked blackish-brown, undulating and rather irregular submarginal line, a narrow barely visible marginal line, and a faint, dark brown straight, postdiscal line. HWV dark brown, lighter along distal margin where dusted with thin whitish scales, with a zigzagging dark brown submarginal line, a barely visible, narrow marginal line, and a wide, straight, yellow (slightly more intensely coloured towards anal margin) median band, of nearly same width throughout, and a hardly visible arched black discal line angled at almost 90°. **Abdomen:** Covered with dense, mostly brown scales dorsally and laterally, and sparse golden brown scales ventrally. **Genitalia** (Fig. 12A): Uncus arched and 1.5 times longer than tegumen shoulder, gnathos half-length of uncus, strongly uplifted with a short apex; pedunculus with a massive base and apex curved downwards; saccus short, bulbous; valva with a massive basal half ending in massive mid-dorsal process terminated by several short teeth, and a narrow apical half with a dorsal crest made up of five prominent teeth; aedeagus sinuate, shorter than valva. FEMALE (Fig. 7B): Sexual dimorphism marginal, female slightly lighter and duller brown on both upper and venter. Its HWV median band is sandy yellow. **Genitalia** (Fig. 14A): Anal papillae prominent, covered with rather sparse setae of varying length, with a strongly sclerotized basal plate, projecting basally and dorsally into short, sharp apophysis-like tips; membrane below papillae with a moderately sclerotized flange, postvaginal lamella sclerotized produced into two prominent lateral, folded flaps with smooth edges; antevaginal lamella slightly sclerotized, pocket-like; antrum strongly sclerotized with two protruberances; ductus bursae wide and short, opening gradually into a large, oval
corpus bursae, with two parallel wide, dentate signa, running close to each other in ventral position extending over half of bursa length.

**Molecular data:** BI, ML, and species delimitation methods (Fig.1) and genetic distances (Supplementary material 2) do not support the separate specific status of *M. ronda n. sp.*, which is placed within the *Manerebia pauperata n. stat.* clade with zero or close to zero genetic distance.

**Type material:** Holotype ♂: Peru, Cajamarca, NE Bambamarca, Laguna Salahuindo, 2700- S06°36'897 W78°26'159, 2750 m, 11.vi.2018, T. Pyrcz leg., CEP-MZUJ (to be deposited in MUSM); Paratypes (17 ♂ and 2 ♀): 7 ♂: same data as the holotype, CEP-MZUJ; 3 ♂: Cajamarca, ouest de Laguna Salahuindo, nord Bambamarca, S06°36'897 W78°26'159, 2700 m, 11.vi.2018, P. Boyer leg., PBF; 7 ♂ and 2 ♀: Cajamarca, Bambamarca, La Ramada, 06°36'53''S/78°26'09'W, 11.vi.2018. 2715 m, leg. J. Cerdeña and J. Farfán, MUSA.

**Etymology:** This species is named for a local political institution, a peasant patrol or meeting of local authorities in rural Peru, which was particularly active in the Bambamarca area during the insurgency of the Sendero Luminoso in the 1980s. It is treated as a feminine noun in apposition.

**Remarks:** This species is externally most similar to *M. inderena mirena* but its male genitalia and COI barcode data (Fig.1) indicate its close affinities to *M. pauperata n. stat.* (Fig. 10E), which occurs at lower elevations and is externally markedly different, characterized by much large size, no HWV median yellow band, and large submarginal ocelli. Therefore, the two a considered as specifically distinct. Another species which has similar genitalia and somewhat similar colour patterns is *M. germaniae*, which occurs throughout Ecuador and Colombia, which can be recognized by the shorter distance between the base of the valva and the tip of the dorsal process, and by the shorter teeth on the apical part of the valva, as well as by the presence of HWV submarginal yellow dots, which are totally lacking in *M. ronda n. sp.*

So far, the nominate subspecies of *M. ronda n. sp.* has been collected only in the north-central part of the department of Cajamarca, NW of Bambamarca, within the watersheds of western tributaries of the Río Marañón.

**Manerebia ronda amplia** Pyrcz & Boyer, n. ssp. (Figs. 7, 12, 15)

**Type locality:** Peru, Lambayeque Department, Kañaris
**Diagnosis:** This subspecies of *M. ronda n. sp.* is externally most similar to *M. milaena n. stat.* (Fig. 15D), from the western slopes of the Andes in the extreme south of Ecuador. Both are characterized by a milk-white and rather wide HWV median band gradually broadening from the costa to the anal margin, no trace of ocelli and rather smaller size compared to most other similar congeners such as *M. germaniae* Pyrcz & Hall, 2006, or *M. inderena* (Adams, 1986). *M. milaena n. stat.* has a magenta suffusion on both the FWV and HWV, not apparent in *M. ronda amplia n. ssp.* in addition, the submarginal dark brown line of the HWV of *M. ronda amplia n.ssp.* is more undulating than in *M. milaena n. stat.*, and the two differ markedly in male genitalia as described below. The subspecific status of *M. ronda amplia n. ssp.* and *M. ronda ronda* is strongly supported by COI data (Fig.1), and male genitalia (Fig. 12).

**Description:** MALE (Fig. 7C): **Head:** eyes chocolate brown, naked, lustrous; labial palpi two times length of head, covered with black hair-like scales, longer ventrally, and with some sparse yellow scales; antennae slender, 2/5th length of costa, dorsally brown, ventrally somewhat orange, club formed gradually. **Thorax:** black, dorsally mostly naked, with just some sparse hair-like scales along sides; femora sparsely covered with dark-brown scales, tibiae and tarsi densely with milky-white scales. **Wings:** FW length 17.5 mm, n=2. FW apex subacute, outer margin straight. HW oval with smooth outer margin. FWD uniform medium brown. HWD uniform medium brown with white median band slightly translucent from venter. FWV blackish brown gradually turning medium brown towards distal margin, with an undulating dark brown submarginal line. HWV chestnut, pale brown along distal margin, with a nearly straight milky white to pale yellow medium band, gradually broadening from costal to anal margin, and a delicately wavy submarginal dark brown line. **Abdomen:** dorsally and laterally covered with black, ventrally with grey-brown scales. **Genitalia** (Fig. 12B): Uncus arched and 1.5 times as long as tegumen shoulder, gnathos half-length of uncus, strongly uplifted with a short apex; pedunculus with a massive base and apex curved downwards; saccus short, bulbous; valva with a massive basal half ended with a massive mid-dorsal process terminated by several short teeth, and a narrow apical half with a dorsal crest made up of several (5-6) prominent teeth; aedeagus sinuate, shorter than valva. FEMALE: Unknown.

**Molecular data:** BI, ML, and species delimitation methods (Fig.1) do not support the separate specific status of *M. ronda amplia n. ssp.*, which is placed alongside *M.
*ronda* n. **sp.** within the *M. pauperata* n. **stat.** clade with zero or close to zero genetic distance (Supplementary material 2).


**Etymology:** The subspecies name is a feminine Latin adjective meaning ‘wide’, in reference to the HWV median pale yellow band.

**Remarks:** This subspecies is known so far only from a handful of specimens collected in the upper Chamaya river valley in an area where several other new species of Pronophilina have been discovered (within the genera *Pronophila* Doubleday, [1849], *Lasiophila* C. Felder & R. Felder, 1859, *Pedaliodes* Butler, 1867). When first collected, it was identified as *M. milaena* n. **stat.**, which is known from southernmost Ecuador, because of its extremely similar appearance, in particular the shape of the HWV median band. However, its male genitalia are very distinct from those of *M. milaena* n. **stat.**, bearing a close resemblance to those of *M. pauperata* n. **stat.**, with an arched uncus, the basal projection of the valva and multiple crown-like teeth on apex. COI barcode data confirm their close affinity, as *M. ronda amplia* n. **ssp.**, falls within the *M. pauperata* n. **stat.** clade. Externally, however, *M. pauperata* n. **stat.**, is markedly different, being considerably larger, with prominent ventral ocelli and no yellow median band (Fig. 15E). So far, *M. ronda amplia* n. **ssp.** is known from two localities, in the western Peruvian Andes in the departments of Lambayeque and Cajamarca, separated by some 100 km.
FIGURE 7. Adults (left—dorsum, right—venter)
A. *M. ronda* n. sp. ♂ Holotype, Peru, Laguna Salahuindo
B. *M. ronda* n. sp. ♂ Paratype, Peru, Laguna Salahuindo
C. *M. ronda amplia* n. ssp. ♂ Holotype, Peru, Kañaris
D. *M. rufanalis* (?) ♂, Peru, Hacienda Udima
**Manerebia prattorum udima** Pyrcz & Boyer, n. ssp. (Figs. 8, 12, 13, 16)

**Type locality:** Peru, Cajamarca Department, 7 km S Hacienda Udima

**Diagnosis:** The new subspecies differs from nominate *M. prattorum* in the paler, orange instead of reddish-orange, and consistently wider HWD median band, whose outer edges are diffused and extend distally; it is also slightly larger than the nominate subspecies.

**Description:** MALE (Figs. 8A, 16B): *Head:* eyes chocolate brown, naked, lustrous; labial palpi two times length of head, covered with black hair-like scales, longer ventrally, and some sparse yellow basal scales; antennae slender, 2/5th length of costa, mostly naked, dorsally brown, ventrally chestnut, club formed gradually, ventrally sandy yellow. *Thorax:* black, mostly naked; legs covered with lustrous, brown and sandy yellow scales becoming dominant on tibiae and tarsi. *Wings:* FW length 15.5–17.5 mm, n=15, mean: 16.7 mm. FWD uniform lustrous dark brown. HWD lustrous dark brown, with long hair-like scales in basal part, crossed by an oblique, nearly straight, 3 mm wide, rich orange median band which is slightly wider in middle section and has a sharp inner and somewhat diffused distal edge, FWV dull, dark brown, slightly lighter distally, with a faint, irregular dark brown submarginal line. HWV dull dark brown, a shade lighter than forewing, with a dark brown cross-cell narrow band, a faint, sinuate dark brown submarginal line, and a pale yellow median band shaped as on dorsum. *Abdomen:* Dorsally and laterally covered with black, ventrally with grey brown scales. *Genitalia* (Fig. 12D): Uncus long, aligned with tegumen dorsum, with a blunt tip slightly curved downwards, gnathos short, one-third length of uncus, stout at base with a sharp tip; pedunculus prominent, stout, curved downwards; valva elongated with a prominent, blunt dorsal process in middle, and a narrow apical half, ending with four teeth pointing distally; saccus medium deep, narrow; aedeagus s-shaped, short, with a massive apical part. FEMALE (Figs. 8B, 16A): Sexual dimorphism is expressed in larger size of female (FW length: 18-19 mm, mean: 18.5 mm, n=4), and lighter ventral colour with prominent whitish and magenta distal suffusion. *Genitalia* (Fig. 13C): Anal papillae prominent, covered with long setae, with a strongly sclerotized basal plate, projecting basally and dorsally into short, sharp apophysis-like tips; membrane below papillae with a moderately sclerotized flange, postvaginal lamella moderately sclerotized and wide, produced into two prominent lateral, folded flaps; antevaginal lamella strongly sclerotized, arched, pocket-like; antrum strongly sclerotized; ductus
bursae wide and short, opening gradually into a large, oval corpus bursae, with two parallel signa in dorsal position extending over two-thirds of bursa length.

**Molecular data:** BI, ML, and species delimitation methods (Fig.1), and genetic distances (Suplementary material 2) support the separate specific status of *M. prattorum udima* n. ssp. in relation to *M. clara* n. stat., *M. inderena* ssp., and *M. antioquianna* n. stat. The nominotypical subspecies of *M. prattorum* has not yet been barcoded.


**Etymology:** This subspecies is named after the village of Hacienda Udima, situated just above the type locality. The name is treated as a feminine noun in apposition.

**Remarks:** We consider *M. prattorum* and *M. inderena* as specifically different, with the latter being most likely a complex of allopatric species, despite the fact that they cluster together in the phylogenetic analyses because of their highly different colour patterns, that of *M. prattorum* in both the nominate and the new subspecies is marked by a wide HWD orange band, which is extremely conspicuous when the butterfly is on the wings, and must play an important signaling role in mating systems. Also, the HWV median band of *M. prattorum* is two times as wide as in any subspecies of *M. inderena*. Finally, *M. prattorum* flies at lower or considerably elevations than *M. inderena*, occurring at some 1800-2000m, whereas most subspecies of *M. inderena* occur at 2200-2600 m, and the nominate is found even as high as 2800m.

*M. prattorum udima* n. ssp. has been reported so far exclusively from the upper valley of the Río Zaña (Fig. 16). It is possible that it is actually endemic to that area, since it is found at approximately 2000 m above sea level and there are virtually no cloud forests northwards from the type locality on the western slopes of the Andes, as far as the area of Las Minas north of the La Porculla Pass, where the type locality of *M. prattorum prattorum* is situated.
Manerebia inducta Pyrcz & Willmott, n. sp. (Figs. 8, 13, 14)

Type locality: Peru, Cajamarca, Santuario Nacional Tabaconas-Namballe, Lagunas Arrebiatadas

Diagnosis: This new species is distinguished from most of its congeners by the combination of two characters: a very narrow, continuous HWV white median band and an acute FW apex. Only two species of Manerebia possess the above features, M. seducta Pyrcz & Willmott, 2006, and M. apiculata (C. Felder & R. Felder, 1867), but the latter is apparently highly polymorphic with individual forms having a broken HWV pale band or no band at all. Furthermore, in both species the HWV marginal line is displaced further from the hindwing costa, and in M. apiculata it is very undulate. M. seducta can also be recognized from M. inducta n. sp. by possessing a minute single black ocellus with a white pupil in both FWV and HWV cell CuA1-CuA2, which is represented in M. inducta n. sp. by a tiny white dot on the FWV only, and absent in M. apiculata. The shape of the HWV white median band is also slightly different in M. inducta n. sp. and M. seducta, in the former being slightly indented basally in cell M3-M2 and tapering noticeably at the costa. Finally, the FWV of M. inducta is distinctly blackish in the basal two-thirds, with a somewhat clear transition to a paler ground colour in the posterior half of the wing, whereas in M. seducta the basal area is not so dark, and the ground colour changes more uniformly from dark to pale towards the distal margin. Further differences are found in male genitalia, in particular in the valves, such as the presence of a prominent protuberance on the ampulla of M. inducta n. sp., or its much more massive apical part, terminated by a single stout process, which is bifurcate in M. seducta. Although no molecular data are available, external and genital morphology indicate that M. seducta is the likely sister-species of M. inducta n. sp.

Description: MALE (Fig. 8C): Head: frons with a tuft of brown hair-like scales; eyes chocolate brown, naked; labial palpi two and a half times length of head, covered with medium brown hair-like scales, ventrally long, dorsally short; antennae dorsally brown with blackish clubs, ventrally chestnut. Thorax: dorsally blackish, sparsely covered with brown hair-like scales, legs orange brown, tibia and femur covered with dense medium brown scales. Wings: FW length: 17.5 mm; triangular with an acute apex, straight outer margin. HW oval with straight outer margin and smoothly rounded tornus. FWD uniform medium brown. HWD almost entirely uniform medium brown except for some orange scaling along outer margin. FWV chocolate brown with a reddish sheen in basal half, progressively turning lighter, chestnut brown from
postdiscal area, and along costal margin, a series of four, faint postdiscal milky white dots from M1-M2 to CuA1-CuA2 aligned parallel to outer margin, a faint, medium brown, slightly wavy submarginal line. HWV almost uniform chestnut brown with a delicate golden sheen, with a straight, narrow transverse milky white postdiscal line with sharp outer and slightly diffuse inner margin, running from costal to anal margin, a faint, darker brown, slightly irregular submarginal line, running close to distal margin, marginal area dusted with grey. *Abdomen*: dorsally blackish, laterally and ventrally chestnut. *Genitalia* (Fig. 13A): Tegumen slender, with a slightly arched dorsum, uncus similar in length to tegumen and stout, more prominently arched with a blunt tip, gnathos half width and length of uncus, curved dorsally, sharply tipped, pedunculus short and blunt, valva stout, sharply thinning at middle with an elongated apical part sharply terminated and curved dorsally with two dorsal teeth-like processes pointing inwards, aedeagus long, thin and smooth, with a prominent ‘collar’ at junction of anterior and posterior part. FEMALE (Fig. 8D): As described and illustrated in Pyrcz et al. (2006) as female of *M. seducta*. In this species, sexual dimorphism is particularly slight. *Genitalia* (Fig. 14B): Anal papillae prominent, covered with long setae, with a strongly sclerotized basal plate, irregularly rounded anteriorly without apophysis; postvaginal lamella moderately sclerotized and forming a broad plate, with a stepped, tube-like protrusion just posterior of ostium bursae; antevaginal lamella forming two deep, rounded ‘pockets’ with thin, broad flanges dorsally on each side that curve dorsally at their inner edge and fuse anteriorly to form a rounded, inwardly directed ‘keel’ just ventral of ostium bursae; antrum very broad, tapering, strongly sclerotized ventrally; ductus seminalis origin at anterior tip of antrum; ductus bursae wide and short, opening into an oval corpus bursae, with two parallel signa in dorsal position extending over one-half of bursa length.

**Molecular data:** Not available.

**Type material:** Holotype ♂: Peru, Cajamarca, Santuario Nacional Tabaconas-Namballe, Lagunas Arrebiatadas, 05°14'05”S, 79°17'18”W, 3122 m, 07.x.2009, Eric Huamaní leg., MUSA, to be deposited in MUSM. Paratypes (2 ♀): 1 ♀ (Allotype of *Manerebia seducta* Pyrcz & Willmott, 2006): Ecuador, Loja, Km 20 Jimbura – San Andrés road, 4°42'50”S, 79°26'16”W, 3300 m, 24.ix.1997, K. Willmott leg., FLMNH; 1 ♀: Peru, Cajamarca, Santuario Nacional Tabaconas-Namballe, Lagunas Arrebiatadas, 05°14'05”S, 79°17'18”W, 3122 m, 07.x.2009, Eric Huamaní leg., MUSA.
**Etymology:** This species name is a feminine Latin adjective meaning ‘induced’ or ‘exhibited’, and is an allusion to the confusion with the externally similar *M. seducta*.

**Remarks:** This new species was formerly confused with *M. seducta*; whose type locality is Abiseo National Park in the north-central part of the Peruvian Andes (Pyrcz et al. 2006), but the two taxa differ sufficiently externally and in genitalia to be considered as distinct species. In particular, the male of *M. seducta* has visible, although small, single ocelli on the FWV and HWV, while no ocelli are present in *M. inducta n. sp.*, and the HWV submarginal line of *M. seducta* is much farther away from distal margin than in *M. inducta n. sp.* The male genitalia, although presenting several common features, differ in the morphology of the valva, in particular the grooved dorsal surface in the basal half in *M. seducta*, which is smooth in *M. inducta n. sp.*, and in the apical part, which is thinner and longer in *M. seducta* and terminating in three prominent ‘teeth’ (although only 2 are visible on the original figure in Pyrcz et al. 2006), whereas only a single ‘tooth’ is present in *M. inducta n. sp.* and it is located more basally. *M. inducta* is found along the Peru–Ecuador border in uppermost forest and in shrubby gulleys in the paramo where there is *Chusquea*, flying with *M. ignilineata*. It is notable that the type locality of *M. inducta n. sp.*, the Lagunas Arrebiatadas area in the Tabaconas-Namballe sanctuary, also contains another endemic Pronophilina species, *Pedaliodes namballe* Pyrcz & Cerdeña (Pyrcz et al. 2013). Finally, the discovery of *M. inducta n. sp.* is a reminder of the potential pitfalls of designating paratypes from distant geographical localities, even if the specimens appear similar, if not identical, in external morphology.
FIGURE 8. Adults (left—dorsum, right—venter)

A. *M. prattorum udima n. ssp.* ♂ Paratype, Peru, vía Hacienda Udima
B. *M. prattorum udima n. ssp.* ♀ Paratype, Peru, vía Hacienda Udima
C. *M. inducta n. sp.* ♂ Holotype, Peru, Lagunas Arrebiatadas
D. *M. inducta n. sp.* ♀ Paratype, Ecuador, Jimbura — San Andrés road
Manerebia huamanii Cerdeña & Pyrce, n. sp. (Figs. 9, 13)

**Type locality:** Peru, Cajamarca Department, Tres Ríos

**Diagnosis:** This species might be confused, at first sight, only with the Colombian M. apiculata, which is of approximately the same size and shape in terms of the FW apex, and, to some extent, with the Ecuadorian M. interrupta. Both of these species apparently have individual forms with median half-moon shaped yellow patches. However, these patches in M. huamanii n. sp. are situated basally in relation to the postdiscal line, and distally in M. apiculata and M. interrupta, which makes the new species unmistakable. Considerable differences are apparent in the male genitalia, which show some similarities among M. huamanii n. sp., M. seducta and M. ignilineata, including, among others, the stout uncus and massive basal parts of the valva. Molecular data are not available for M. huamanii n. sp. and no sister-species can be immediately identified, but its most closely related species should probably be looked for among those with the most similar genitalia.

**Description:** MALE (Fig. 9C, D): Head: Eyes chocolate brown, naked; antennae naked, reaching 2/5th length of costa, slender with club formed gradually, dorsally chestnut, ventrally sandy yellow, labial palpi two and a half times length of head, covered dorsally with short, ventrally with long, dense brown and sparse yellow hair-like scales. Thorax: Black, dorsally naked, legs brown, tibia covered with brown scales, femur and tarsus with sandy yellow scales. Wings: FW length: 18 mm (n=2); apex acute, distal margin slightly truncate below apex. HW oval with a slightly produced apex. FWD almost uniform medium brown, with a slightly darker basal half. FWD medium brown. FWV medium brown, a shade lighter in distal half, a faint reddish suffusion in submarginal area. HWV reddish brown, with a magenta overcast along outer margin distal to a wavy brown submarginal line, basally edged with grey; a median series of four, half-moon shaped sandy yellow patches with sharp outer edges contiguous to a faint postdiscal line. Abdomen: Dorsally covered with medium brown, ventrally with grey brown scales. Genitalia (Fig. 13B): Uncus long and arched; gnathos one third length of uncus, with a stout base and sharp apex; pedunculus small, directed downwards, saccus medium long, flattened dorso-ventrally; valva stout in basal half with a blunt dorsal process, sharply narrowing into an elongated apical one-third ending with a series of three sharp processes pointing inwards; aedeagus nearly straight, prominently widened in middle, slightly flattened dorso-ventrally, similar in length to valva. FEMALE: Unknown.
Molecular data: Not available.

Type material: Holotype ♂: Peru, Cajamarca, Tres Ríos, 6°56′41″S 78°52′40″ W, 3163 m, 20.viii.2010, E. Huamaní leg., MHN-USA; Paratype ♂: PERU, Piura, entre Las Minas y El Tambo, 2600–2900 m, 10-13.iv.1981, G. Lamas leg., MUSM.

Etymology: This new species is dedicated to Erick Huamaní Villalobos, the collector of the holotype and a member of the Museo de Historia Natural Universidad Nacional de San Agustín de Arequipa (MUSA), in recognition of his invaluable contributions to different projects of the MUSA. The name is treated as a Latinized masculine noun in the genitive case.

Remarks: The wing pattern characters, in particular the yellow patches situated basally in relation to the postdiscal line, and male genitalia, make this species unmistakable. The type locality lies on the western slopes of the Andes in the department of Cajamarca, half-way between two other localities where new species of Manerebia were discovered during this study, Abra de Porculla and Hacienda Udima. The second known specimen, which is associated with this species, comes from a more northerly locality in Piura, some 250 km away, also situated on the western slopes of the Andes. We were, unfortunately, unable to examine its genitalia. Its size, wing shape and colour pattern match those of M. huamanii n. sp. except for the lack of any HWV yellow patches. The presence or absence of HWV median patches or bands is a common, highly variable individual character in the genus Manerebia. Such variation apparently occurs, for example, in M. interrupta, M. apiculata, M. ignilineata (Dognin, 1896) and M. trimaculata (Hewitson, 1870), among other species.

Manerebia punku Pyrcz & Farfán, n. sp. (Figs. 9, 12)

Type locality: Peru, Piura Department, Abra de Porculla.

Diagnosis: This species is immediately recognized from its congeners by the presence of prominent HWV postdiscal yellow spots, and the absence of any median band. No closest relative can be identified at this time.

Description: MALE (Fig. 9A, B): Head: eyes chocolate brown, naked, lustrous; labial palpi two times length of head, covered with black hair-like scales, longer ventrally but overall shorter than in other congeners; antennae slender, 2/5 length of costa, naked, dorsally and ventrally brown, except for ventral side of gradually formed club, which is sandy yellow. Thorax: black; legs dull brown, tibia covered with brown, femur and tarsus with sandy yellow scales. Wings: FW length: 17.5 mm (n=2). FWD
almost uniform medium brown, with a slightly darker basal half. FWD medium brown, with hair-like scales in basal half. FWV medium brown, a shade lighter in distal half, with two, faint postdiscal and submarginal regular lines, and four small postdiscal, pale yellow dots, apparently varying individually with one individual lacking these dots (Fig. 9B). HWV medium brown, a shade lighter in distal half, with dark brown postbasal, postdiscal and submarginal lines, all rather irregular, with latter fainter than others, and a series of 5 postdiscal pale yellow dots, three central ones larger than extremal ones. **Abdomen:** dorsally covered with black scales, laterally and ventrally with grey brown scales. **Genitalia** (Fig. 12C): Uncus long and arched; gnathos one-third length of uncus, with a stout base and sharp apex; pedunculus prominent, directed downwards, saccus short, bulbous; valva slender, with a gradually narrowing apical part ending with a sharp tip turned inwards, with an irregular dorsum; aedeagus stout, sinuate, short. **FEMALE:** Unknown.

**Molecular data:** BI, ML, and species delimitation methods (Fig.1), and genetics distances (Supplementary material 2) indicate that *M. punku n. sp.* is a distinct species. However, based on ML analysis, *M. punku n. sp.* is relating to the clade formed by *M. germaniae*, *M. apiculata*, *M. leaena + M. inderena mirena complex + M. golondrina*, *M. interrupta*, *M. similis n. stat.*, *M. fina n. stat.* clade + *M. trimaculata*, *M. undulata* complex + *M. clara n. stat.*, *M. antioquiana n. stat.*, *M. prattorum udima n. ssp.* clade, and according to BI analysis, it is relating the *M. mycalesoides* and *M. nevadensis* but both analyses showed high branch support of the clades containing it.


**Etymology:** ‘punku’ is a word in Quechua language (spoken currently in the Peruvian Andes) that means door, entry, access, portal, alluding to the Porculla pass that connects the two sides of the Andes at the type locality.

**Remarks:** This species is easily distinguished from other congeners by its small size, rounded wings and, in the typical form, prominent HWV rounded yellow dots. The genitalia are also quite distinctive, being most similar to those of *M. rufanalis* Pyrcz &
Hall, 2006 from southern Ecuador, which is otherwise externally very different. It is the only species of *Manerebia* known so far from the Abra de Porculla (Fig. 16C), where it occurs on the western slopes only, with the eastern slopes being extremely arid with no cloud forest vegetation. The geographic range of this species is incompletely known, and it can only be speculated that it extends both north and southwards where similar habitat is found. In the trees generated based on COI sequences this species is sister to *M. apiculata*, *M. leaena*, and *M. germaniae*, all externally different species occurring at higher elevations in the northern Andes.

*Manerebia granatus* Willmott, Radford & Pyrcz, *n*. *sp*. (Figs. 10, 11)

**Type locality:** Ecuador, Zamora-Chinchipe Department, Cordillera del Cóndor, Destacamento Paquisha Alto

**Diagnosis:** This species superficially resembles a number of congeners, including its sister-species *M. placida* *n*. *sp.*, with which it may be broadly sympatric. It differs from *M. placida* *n*. *sp.* as follows (characters in the latter species in parentheses): the VHW is uniform reddish brown in the distal half, including distally of the submarginal ocelli and white dots (rather than just basally of these markings); the VHW ocellus in cell CuA2-CuA1 is very small, with no distinct ocellar ring (larger with distinct yellowish orange ocellar ring); the VHW ocellus in M2-M1 is not developed (developed, with black centre and ocellar ring); the VHW submarginal red-brown line is more undulate (less undulate); the VHW has virtually no trace of a pale postdiscal line (variable but distinct line present). The species is also similar to other *Manerebia* that lack a VHW pale postdiscal line (e.g., *Manerebia lamasi* *n*. *stat.* Pyrcz & Willmott, 2006, *Manerebia pauperata* *n*. *stat.*), but may be distinguished from them by the much reduced VHW submarginal ocelli, especially that in cell CuA2-CuA1. Two males each of *M. granatus* *n*. *sp.* and *M. placida* *n*. *sp.* were dissected and they showed consistent differences between the two species, in particular the arms of gnathos parallel in dorsal view in *M. granatus* *n*. *sp.* but directed inwards in *M. placida* *n*. *sp.*, and the spines at distal tip of valvae extending further basally along the inner edge in *M. granatus* *n*. *sp.*, but confined to the distal tip in *M. placida* *n*. *sp.* The male genitalia of both species may be distinguished from the otherwise similar species *M. benigni* Pyrcz, 2004 as described below under *M. placida* *n*. *sp.*

**Description:** MALE (Fig10. A, B): *Head*: eyes chocolate brown, naked, lustrous; labial palpi two times length of head, covered with black hair-like scales, longer
ventrally; antennae slender, 2/5th length of costa, 38 segments, mostly naked, dorsally brown, ventrally paler brown, club formed gradually of terminal 12 segments. Thorax: black, with long black hair-like scales; legs dorsally dark brown, ventrally pale yellowish brown. Wings: FW length 20-21 mm, mean: 20.4 mm, n=8; FWD uniform chocolate brown, except for patch of dense, black, elongate rectangular androconial scales in basal half to third of cells 2A-M1 and extending into adjacent posterior half of discal cell. HWD with hair-like scales in median half, uniform chocolate brown, with scattered reddish brown scaling in tornus. FWV dark brown, darker blackish brown in areas with dorsal androconial scales, with a well-marked dark reddish brown, undulating and rather irregular submarginal line, and a narrow reddish brown marginal line; series of five white submarginal dots in cells Cu2-Cu1 to M1-R5, first of these in centre of a small black spot surrounded by a reddish brown ring. HWV dark blackish brown (similar to basal half of FWV) basal to an indistinct, straight, dark brown postdiscal line, distally of this line reddish brown (also extending along tornus) up to a dark reddish brown, undulate submarginal line, then dark brown, with dark reddish brown marginal line; series of seven white submarginal dots in cells 2A-CuA2 (two dots), CuA2-CuA1 to M1-Rs, first three of these in centre of small black spots. Abdomen: Covered with dense, dark brown scales dorsally and laterally, and slightly paler greyish brown scales ventrally, with long dark hair-like scales increasing in density anteriorly. Genitalia (Fig. 11A): Uncus slightly curving and 1.5 times longer than tegumen shoulder, gnathos half-length of uncus, slightly curving upwards and pointed; pedunculus with a massive base and apex curved downwards; saccus short, bulbous; valva with a broad basal half ending in squared-off mid-dorsal process, and a narrower apical half with a distal series of 8 or so squat 'teeth' oriented sub-horizontally; aedeagus curving evenly upwards, tapering anteriorly, shorter than valva, smooth, no visible cornuti. FEMALE (Fig. 10C): Similar to male except: slightly larger (FW length 22 mm); wings slightly paler brown, no FWD androconial patch; reddish brown scales in distal part of HWV scattered, less dense than in male; HWV with narrow, even, straight white postdiscal band; submarginal ocelli larger in cells Cu2-CuA1 of FWV and HWV, and submarginal white spot in HWV cell surrounded by a small black spot. Genitalia (not illustrated): Anal papillae prominent, covered with setae of varying length, with a strongly sclerotized, amorphous basal plate; membrane below papillae a lightly sclerotized, grooved plate terminating in a point posteriorly, bordered anteriorly by a band of fine spine-like protrusions; postvaginal lamella sclerotized and forming a
broad curved plate, produced into two prominent lateral, folded flaps with smooth edges; antevaginal lamella slightly sclerotized, pocket-like and folded; antrum sclerotized; ductus bursae wide and short, opening into a large, oval corpus bursae, with two narrow bands of dorsal signa that converge slightly anteriorly and extend over half of bursa length.

**Molecular data:** BI, ML, and species delimitation methods (Fig.1), and genetic distances (Supplementary material 2) indicate that *M. granatus n. sp.* is a distinct species, and on both trees it clusters with *M. placida n. sp.* and a new, undescribed species from Central Peru, AZ-650 *Manerebia* sp., the sister-species to *M. granatus n. sp.*


Other specimens examined (not considered paratypes): ECUADOR:1 ♀: Zamora-Chinchipe, km 4.3 San Andrés-Jimbura rd., [4°47′59″S,79°18′18″W], 2020 m, 13.x.2010, K. R. Willmott leg., [FLMNH-MGCL-145842; dissection KW-20-022], FLMNH.
**Etymology:** The species name is the Latin word for garnet, in reference to the deep reddish brown colours of this species, and it is treated as a masculine noun in apposition.

**Remarks:** This species is externally most similar to *M. placida n. sp.*, although there are a number of wing pattern differences as mentioned in the diagnosis that are consistent in all examined specimens. Nevertheless, we initially considered that the two taxa might represent subspecies of a single species, given that no males have been collected in sympatry, and the lack of substantial differences in the dorsal androconial scales or male genitalia. However, the DNA barcode of a single female from the Jimbura-San Andrés road in southern Ecuador grouped with those of males of *M. granatus n. sp.* from the Cordillera del Cóndor, based on which it is tentatively considered as belonging to this species, although we exclude it from the type series. This female specimen also has reddish brown surrounding the HWV black ocellus in Cu2-Cu1, as in males of *M. granatus n. sp.*, but in contrast to the yellowish brown ring around the ocellus in *M. placida n. sp.* and *M. benigni*. Assuming this female is conspecific with males from the Cordillera del Cóndor, *M. granatus n. sp.* may be relatively widespread in southern Ecuador, and perhaps even sympatric or locally elevationally parapatric with *M. placida n. sp.* However, so far no males are known from the area where the putative female of *M. granatus n. sp.* was collected. In addition, aside from the rather remarkable differences in wing pattern exhibited between the pierid *Catasticta poujadei condor* Radford & Willmott, 2013, from the Cordillera del Cóndor, and the nominate subspecies in the adjacent Andes, there are few examples of butterfly species with different subspecies in these two regions. *M. granatus n. sp.* was also detected in northern Peru, in the highlands of Chachapoyas, where it was originally mistaken for *M. benigni*, and three among the known Peruvian specimens were actually included as paratypes of that species (Pyrcz 2004). However, their genitalia match the specimens from the type locality of *M. granatus n. sp.* Finally, the divergence in DNA barcode between *M. granatus n. sp.* and *M. placida n. sp.* is comparable or even higher than that between other related *Manerebia* species. In summary, we consider that *M. granatus n. sp.* and *M. placida n. sp.* represent two distinct species.

Most known individuals of *M. granatus n. sp.* were collected at the type locality, a sandstonetepui in south-eastern Ecuador, along a steeply climbing trail through cloud
forest from 2010-2100 m. A single individual was collected on the top of the tepui in stunted elfin forest near 2300 m. A single female that may also represent this species (discussed above) was collected flying 1 m above the ground along the edge of a dirt road through cloud forest. Other congeners present at the type locality included *M. benigni tessmanni* Pyrcz, 2004, *M. pauperata* n. stat., and *M. trimaculata*.

**FIGURE 9.** Adults (left—dorsum, right—venter)

A. *M. punku* n. sp. ♂ Holotype, Peru, Abra de Porculla
B. *M. punku* n. sp. ♂ form, Paratype, Peru, Abra de Porculla
C. *M. huamanii* n. sp. ♂ Holotype, Peru, Tres Ríos
D. *M. huamanii* n. sp. ♂ form Paratype, between Las Minas and El Tambo
**Manerebia placida** Willmott & Pyrcz, *n.* *sp.* (Figs. 10, 11)

**Type locality:** Ecuador, Zamora-Chinchipe Province, km 24 Loja-Zamora road, Quebrada San Francisco

**Diagnosis:** This species superficially resembles a number of congeners, including *M. granatus* *n.* *sp.*, as well as *M. benigni*. Diagnostic characters that distinguish it are discussed under the Diagnosis of that species. From *M. benigni*, *M. placida* *n.* *sp.* may be distinguished by not having a distinct darker postdiscal line immediately bordering the distal edge of the pale postdiscal line, and by the rings surrounding the HWV ocelli being paler yellowish brown, rather than dark reddish brown. The male genitalia of *M. placida* *n.* *sp.* differ from those of *M. benigni* as follows: its gnathos is shorter and more closely parallel to the uncus; the distal half of the valva is more elongate and narrower; the distal 'teeth' of the valva are arranged more horizontally and within the same plane, whereas in *M. benigni* they point in different directions, are arranged more vertically and are less clustered together; and the aedeagus is more slender, less curved, and lacks a small median dorsal projection.

**Description:** MALE (Fig. 10D): Head: eyes chocolate brown, naked, lustrous; labial palpi two times length of head, covered with black hair-like scales, longer ventrally; antennae slender, 2/5th length of costa, 38 segments, mostly naked, dorsally brown, ventrally paler brown, club formed gradually of terminal 12 segments. Thorax: black, with long black hair-like scales; legs dorsally dark brown, ventrally pale yellowish brown. Wings: FW length 19–21 mm, mean: 20.4 mm, n=12; FWD uniform chocolate brown, except for patch of dense, black, elongate rectangular androconial scales in basal half to third of cells 2A-M1 and extending into adjacent posterior half of discal cell. HWD with hair-like scales in median half, uniform chocolate brown, with scattered reddish brown scaling in tornus. FWV dark brown, darker blackish brown in areas with dorsal androconial scales, with a well-marked dark reddish brown, undulating and rather irregular submarginal line, and a narrow reddish brown marginal line; series of five white submarginal dots in cells CuA2-CuA1 to M1-R5, first of these in centre of a small black spot surrounded by a pale yellowish brown ring. HWV dark blackish brown (similar to basal half of FWV) basal to a very narrow, approximately straight, white to cream postdiscal line, distally of this line reddish brown (also extending along tornus) up to series of submarginal white spots and ocelli (as described below), then dark brown, undulate dark reddish brown submarginal line and dark reddish brown marginal line; series of up to seven white submarginal dots in cells 2A-CuA2 (two dots), CuA2-
Molecular data: BI, ML, and species delimitation methods (Fig. 1), and genetic distances (Supplementary material 2) supports *M. placida n. sp.* as a valid species, and as sister species to *M. granatus n. sp.* These two species are also related to *M. lamasi n. stat.* and *M. navarrae.*


**Etymology:** The species name is a feminine Latin adjective in the nominative singular, *placidus,* meaning calm or gentle, partly in reference to the similarity of this
species to \textit{M. benigni}; although that species is named for the Peruvian collector Benigno Calderón, the root of the name is the Latin adjective \textit{benignus}, meaning kind. In addition, the name alludes to the gently undulating VHW submarginal line which somewhat distinguishes this species from \textit{M. benigni}, in which the line is more strongly undulate.

\textbf{Remarks:} \textit{M. placida} \textit{n. sp.} is closely related to \textit{M. granatus} \textit{n. sp.}, and we discuss under that species our decision to recognize these two taxa as distinct species. This species is known to date only from the type locality in southeastern Ecuador, where it occurs in cloud forest from 2000-2100 m. Despite continuous sampling by hand-netting and trapping at that locality from 16 September to 6 December in 2006, the species was only recorded from 10 October to 14 November, during which time it was not uncommon. Males were found puddling along open as well as shady streams from 10:00-12:00, as well as flying up until 14:15 within 1 m of the ground in the forest understorey along trails near streams. Two males were collected in traps baited with rotting fish, one in the understorey and one in the canopy, both near streams. Other congener present at the type locality included \textit{M. rufanalis}, \textit{M. pauperata \textit{n. stat.}}, and \textit{M. trimaculata}.
FIGURE 10. Adults (left—dorsum, right—venter)
A. *M. granatus n. sp.* ♂ Holotype, Ecuador, Paquisha Alto
B. *M. granatus n. sp.* ♂ Paratype, Peru, Alto Río Nieva
C. *M. granatus n. sp.* ♀, Ecuador, San Andrés
D. *M. placida n. sp.* ♂ Holotype, Ecuador, Zamora - Arcoiris
FIGURE 11. Male genitalia (top—lateral view, middle—plan view, bottom—aedeagus extracted, lateral view)
A. *M. granatus* n. sp. Peru, Rodríguez de Mendoza, prep. genit. KF-2736
B. *M. placida* n. sp. Ecuador, Zamora, prep. genit. KW-20-021
C. *M. benigni tessmanni*, Peru, Alto Río Nieva, prep. genit. KF-H_241
D. *M. benigni tessmanni*, Ecuador, Río Troya, prep. genit. KF-6282
FIGURE 12. Male genitalia (top—lateral view, middle—plan view, bottom—aedeagus extracted, lateral view)

A. *M. ronda* n. sp., Peru, Salhuindo, prep. genit. KF-1571
B. *M. ronda amplia* n. ssp., Peru, Pucara, prep. genit. KF-1572
C. *M. punku* n. sp., Peru, Abra de Porculla, prep. genit. KF-1613
D. *M. prattorum udima* n. ssp., Peru, La Florida, prep. genit. KF-1472
FIGURE 13. Male genitalia (A, B) (top—lateral view, middle—plan view, bottom—aedeagus extracted, lateral view) C. Female genitalia (top—lateral view; bottom—ventral view, detail)
A. *M. inducta* n. sp., Peru, Lagunas Arrebiatadas, prep. genit. MUSA_015149
B. *M. huamanii* n. sp., Peru, Tres Ríos, prep. genit. MUSA_015150
C. *M. prattorum udina* n. ssp., Peru, La Florida, prep. genit. KF-1473
FIGURE 14. Female genitalia (top—lateral view; bottom—ventral view, detail)

A. *M. ronda* n. sp., Peru, Laguna Salahuido, prep. genit. MUSA_015151
B. *M. inducta* n. sp., Peru, Lagunas Arrebiatadas, prep. genit. MUSA_015152
FIGURE 15. Male genitalia x adults comparison indicating morphological similarities and genetic affinities (outer—genitalia in lateral view, aedeagus omitted, middle—wings venter)

A. *M. ronda n. sp.*, Peru, Laguna Salahuindo, prep. genit. 1571
B. *M. similis n. stat.*, Peru, Balzapamba, prep. genit. H_161
C. *M. ronda amplia n. ssp.*, Peru, Abra de Porculla, prep. genit. 1572
D. *M. milaeona n. stat.*, Peru, Jimbura, prep. genit. H_175
E. *M. pauperata n. stat.*, Peru, Zamora, prep. genit. H_246
FIGURE 16. Adults live and habitats
A. *M. prattorum udima n. ssp.* female, Peru, La Florida —via La Udima. Photo P. Boyer
B. *M. prattorum udima n. ssp.* male, Peru, La Florida —via La Udima. Photo P. Boyer
C. Abra de Porculla, view towards Pacific coast, type locality of *M. punku*. Photo T. Pyrcz
Taxonomic changes and remarks

**Manerebia lamasi** Pyrcz & Willmott, 2006, n. stat. (Fig. 17)

*Manerebia satura lamasi* Pyrcz & Willmott, 2006: 47. Type locality: Alfonso Ugarte, Cordillera del Cóndor, Amazonas, Peru. Holotype male: MUSM [examined].

**Remarks:** BI, ML, and species delimitation methods (Fig.1), and genetics distances analysis (Supplementary material 2) indicate that the sequences of this taxon cluster in a single well-differentiated, long branch, and the sister species to *M. navarræ* Adams & Bernard, 1979, from the Serrrania de Perija in northern Colombia. Based on it to it rises to a specific rank. *Manerebia lamasi* n. stat. was originally described as a subspecies of *Manerebia satura* based on similar male genitalia and colour patterns. *M. lamasi* n. stat. is known so far exclusively from the Sierra del Condór.

**Manerebia pauperata** Pyrcz & Willmott, 2006, n. stat. (Figs. 15, 17)


**Remarks:** In the phylogenetic analyses *M. pauperata* n. stat. and *M. satura* are situated in distant branches of the tree and cluster in highly resolved clades with other congeners. Also, our rationale for considering them as specifically distinct is the fact that Jamie Radford and colleagues collected both *M. lamasi* n. stat., and *M. pauperata* n. stat. in sympatry in the Cordillera del Cóndor in southern Ecuador. Additionally, the two taxa also consistently differ in wing colour patterns (Fig.11C, D) and male genitalia. We therefore formally recognize *M. lamasi* n. stat. as a distinct species. Despite an overall similar colour pattern in comparison with *M. satura* from central and southern Peru, with the exception of the absence of the HWV yellow median band, *M. pauperata* n. stat. shows some genitalic differences. Moreover, the species delimitation methods (Fig.1) and genetic distances (Supplementary material 2) clearly support treating the two taxa as separate species.

**Manerebia benigni tessmanni** Pyrcz, 2004 (Figs. 11, 17)


**Other specimens examined:** ECUADOR: 1 ♂: Zamora-Chinchipe, Jimbura — Zumba, Río Troya, 2100 m, 15.viii.2017, P. Boyer leg., PBF; 1 ♀: Zamora-

Remarks. *M. benigni tessmanni* was described from the northern part of the highlands of Chachapoyas, from the Abra Pardo Miguel area specifically, and during the course of subsequent field work it has also been recorded in several localities in the southern Ecuadorian provinces of Morona-Santiago and Zamora-Chinchipe. In the phylogenetic analyses *M. benigni* sequences clustered in a highly resolved clade (Fig.1); even if the only sampled specimen of *M. benigni tessmanni* from the type locality clusters in an external position in an internal clade of nominate *M. benigni*. This, in our opinion shows that the more northerly populations of *M. benigni tessmanni* are better differentiated on the molecular level from the nominate, which is logical from a geographical point of view, although they do not differ in colour patterns from the topotypical specimens of this subspecies. We illustrate two specimens from Río Troya and Jimbura, male and female respectively (Fig. 17A, B), associated with this taxon based on male genitalia (Fig. 11C, D), and the fact that the two have matching HWV colour patterns, that are different from both *M. granatus* n. sp. and *M. placida* n. sp. Such a distribution pattern is not unfrequent among pronophiline butterflies at the subspecific level, as exemplified by *Eretris porphyria transmaraniona* Pyrcz 2004, which is also found in the two areas on the opposite sides of the Río Chamaya valley (Pyrcz 2004).
FIGURE 17. Adults (left—dorsum, right—venter)
A. *M. benigni tessmanni* ♂ Ecuador, Río Troya
B. *M. benigni tessmanni* ♀, Ecuador, Jimbura—San Andrés
C. *M. pauperata n. stat.* ♂ Ecuador, Zamora—Chinchipe
D. *M. lamasi n. stat.* ♂, Ecuador, Morona—Santiago

*Manerebia inderena* (Adams, 1986)

*Penrosada inderena* Adams, 1986: 305. Type locality: Colombia, Tolima Department, S above Cajamarca. Holotype: NHML [examined].
Remarks: *Manerebia inderena* has so far been considered as a widely distributed polytypic species. Our preliminary molecular results suggest that at least some of its subspecies should be raised to specific status. The most outstanding case is *M. inderena* *antioquiana* Pyrcz & Willmott, 2006, from the northern portion of the Colombian Central Cordillera, which is highly divergent genetically from other subspecies of *M. inderena*. Also, *M. inderena clara* Pyrcz & Willmott, 2006, from the eastern slopes in central Ecuador, does not segregate with other subspecies of *M. inderena*. Additionally, *M. inderena fina* Pyrcz & Willmott, 2006, from the western slopes in Ecuador, unexpectedly forms a highly supported clade with *M. interrupta* Brown, 1944, and *M. golondrina* Pyrcz & Willmott, 2006. Finally, *M. inderena mirena* Pyrcz & Willmott, 2006, in south-eastern Ecuador is most probably a complex of at least two cryptic species, and further work using a more comprehensive set of samples is underway to clarify the taxonomy of this complex (Fig.1).


*Manerebia inderena antioquiana* Pyrcz & Willmott, 2006: 49. Type locality: Las Antenas, San Felix, Antioquia, Colombia. Holotype male: CEPUJ [examined].

Remarks: BI, ML, and species delimitation methods (Fig.1), and genetic distances (Supplementary material 2) showed that *M. antioquiana* *n. stat.* and the rest of the *M. inderena* situate in distant branches of the tree and cluster in highly resolved clades with other congeners. *M. antioquiana* *n. stat.* is associating with *M. prattorum* and a new species from Serrania del Paramillo in Colombia.


*Manerebia inderena fina* Pyrcz & Willmott, 2006: 49. Type locality: Sector Los Alpes Km. 18, Aloag-Tandapi, Pichincha, Ecuador. Holotype male: CEPUJ [examined].

Remarks: BI, ML, and species delimitation methods (Fig.1), and genetic distances (Supplementary material 2) showed that *M. fina* *n. stat.* and the rest of the *M. inderena* situate in distant branches of the tree and cluster in highly resolved clades with other congeners, being to valid species. *M. fina* *n. stat.* is associating with *M. interrupta*.


*Manerebia inderena clara* Pyrcz & Willmott, 2006: 52. Type locality: Río Horimyacu, Baeza, Napo, Ecuador. Holotype male: CEPUJ [examined].
Remarks: The phylogenetic analyses (Fig.1), and genetic distances (Supplementary material 2) demonstrated that *M. clara* n. stat. is a valid species. Moreover, the rest of the *M. inderena* situate in distant branches of the tree and cluster in highly resolved clades with other congeners. *M. clara* n. stat. is the sister of the a new putative species that was cataloged as a subspecie of *M. inderena*.

*Manerebia similis* Pyrcz & Willmott, 2006, n. stat. (Fig. 15)

*Manerebia inderena similis* Pyrcz & Willmott, 2006: 52. Type locality: arriba de Santa Lucia, Balzapampa, Bolívar, Ecuador. Holotype male: CEPUJ [examined].

Remarks: BI, ML, and species delimitation methods (Fig.1), and genetic distances (Supplementary material 2) showed that *M. similis* n. stat. and the rest of the *M. inderena* situate in distant branches of the tree and cluster in highly resolved clades with other congeners, being to valid species.

*Manerebia milaena* Pyrcz & Willmott, 2006, n. stat. (Fig. 15)


Remarks: The phylogenetic analyses (Fig. 1), and genetic distances (Supplementary material 2) demonstrated that *M. milaena* n. stat. is a valid species. *M. milaena* n. stat., and *M. undulata undulata* situate in distant branches of the tree and cluster in highly resolved clades with other congeners. *M. milaena* n. stat. is associating with the *M. trimaculata* clade.


Remarks: BI, ML, and species delimitation methods (Fig.1), and genetic distances (Supplementary material 2) showed that *M. fernandina* n. stat. is a valid species, and the sister of the *M. rufanalis*.

*Manerebia trimaculata* (Hewitson, 1870)

*Lymanopoda trimaculata* Hewitson, 1870: 159. Type locality: Ecuador, Morona-Santiago Province, St. Rosario. Syntype male: NHML [examined].
**Remarks:** Various sequenced individuals identified as *M. trimaculata* form a widely polytomic clade among morphologically noticeably different subspecies of *M. inderena* and *M. undulata* Pyrcz & Hall, 2006. According to BI and GMYC methods (Fig.1), the *M. trimaculata* complex contains three putative species that are morphologically different, and indeed some are sympatric, but based on ML and bPTP analyses (Fig.1), the complex was not resolved and it might represent a single species with high phenotypic variation, and the genetic distances are not high (between 1-3%). However, since there are still a number of questions to be resolved, we refrain at this stage from making any taxonomic changes regarding these taxa until more data are available. This issue will be discussed alongside the status of the subspecies of *M. inderena* in a forthcoming paper.

**Final Taxonomic Remarks**

For making the taxonomic descriptions of some of the new species of *Manerebia* that were found by our phylogenetics analyses (Fig.1; Supplementary material 3, 4), and genetic distances (Supplementary material 2), we have to revise the holotypes and get more samples to have a good sampling for can correctly describe them in future papers. However, next, we list the samples that are cataloged as new species:

- KW-071009-10, KW-071009-12, and LEP-10185 are *Manerebia n. sp.1* from Morona, Ecuador. This species was considered as *M. inderena mirena*, but it and the rest of the *M. inderena mirena* situate in distant branches of the tree and cluster in highly resolved clades with other congeners, being to valid species. It's possible this species might retain the nominal name of *M. mirena n. stat.* due to these samples are from the type locality in where was described of *M. inderena mirena*.

- LEP-01755, LEP-05249, LEP-04587, and LEP-05247 are considering as *Manerebia n. sp.2* from Ecuador, and as *Manerebia n. sp.1*, was considered as *M. inderena mirena*, but it and the rest of the *M. inderena mirena* situate in distant branches of the tree and cluster in highly resolved clades with other congeners.

- DL-50 *Manerebia n. sp.3* from Zamora, Ecuador was part of the group of *M. inderena mirena*, but it situates in distant branches of the tree and clusters in
highly resolved clades with other congeners, and not with the others *M. inderena mirena*. It is associating with *M. pauperata* n. stat. and *M. ronda* n. sp.

- DL-42 *Manerebia* n. sp.4 from Jerusalen, Peru, with our results it is a valid species, and morphologically different to *M. satura*.

- CFCD00213 and DL-494 are *Manerebia* n. sp.5 another new species from Huanuco, Peru. These samples were cataloged as subspecie of the *M. trirufa*, but there is a high genetic distance between them (Supplementary material 2).

- CFCD00223, DL-50, and CP04-23 are *Manerebia* n. sp.6 from Huanuco, Peru. It is associating with *M. haywardi*, *M. rubescens*, and *Manerebia* n. sp.7 from Peru.

- DL-498 *Manerebia* n. sp.7 from Cusco, Peru. It is associating with *M. haywardi*, *M. rubescens*, and *Manerebia* n. sp.6.

- WOTT685-18 *Manerebia* n. sp.8 from Serranía del Paramillo, Antioquia, Colombia. It is morphologically similar to *M. antioquiana* n. stat. but they present a high genetic distance, being a valid species (Supplementary material 2).

- DL-18, DL-33, and DL-265 are *Manerebia* n. sp.9 from Munchique, Colombia. Before it was considered a subspecie of the *M. inderena*, but with our results it is a valid species, associate with *M. clara* n. stat. but morphologically different.

- AZ-649 *Manerebia* n. sp.10 from Jujuy, Argentina. It is the sister of the *M. cyclops*, and they are in the same clade formed by *M. cyclopina* and *M. typhlops*.

- LEP-82788 *Manerebia* n. sp.11 from Paucartambo, Peru. It is the sister of the *M. trirufa* and *Manerebia* n. sp.5. clade from Peru.

Finally, according to our results, we propose a systematic arrangement in where the genus *Manerebia* would comprise 58 nominal species, and with several more identified but for the moment remaining undescribed (Mahecha-J. et al. in prep.).

**Discussion**

With our results, we managed to evaluate the high cryptic diversity within the genus *Manerebia* allowing us to accept our expectations propose and corroborate the proposed by Pyrcz et al. (2006). Also, our phylogenetic analyses indicate that *Manerebia* taxonomic diversity in the Neotropical region remains underestimated, despite taxonomic descriptions made by Pyrcz et al. (2006). Additionally, lack of morphological
differentiation does not necessarily imply shared evolutionary histories, as is the case in cryptic species that were found within *Manerebia* (e.g. *M. inderena mirena* complex) (Bickford *et al.* 2007; Kaliontzopoulou *et al.* 2011; Ghielmi *et al.* 2016; Freitas *et al.* 2020) or taxa displaying convergent evolution (Freitas *et al.* 2020). Hence, COI data prove very useful in identifying possible cryptic species in Pronophilina butterflies (Marín *et al.* 2015; Pyrcz *et al.* 2016; Marín *et al.*2017; Pyrcz *et al.* 2018; Pyrcz *et al.* 2021), and others groups of butterflies (e.g. Lavinia *et al.* 2017; Nneji *et al.* 2020).

For the above, the genus *Manerebia* is arguably one of the most demanding taxonomically, certainly among all Satyrinae, and perhaps even all butterflies, in terms of its alpha-taxonomy, because the adults belonging to different and not even closely related species are often virtually impossible to identify based on external morphology (Pyrcz *et al.* 2006). This is because colour patterns are very simple, and the few striking characters, in particular the HWV median yellow bands or patches, have apparently evolved convergently in various lineages, and are not always diagnostic, as explained in species delimitation methods. Such traits may reflect an adaptive role in similar high elevation environments, resulting in cryptic, syntopic species which can be easily misidentified. Even more frequently, allopatric populations of loosely related species can be incorrectly considered as conspecific because of confusingly similar external appearance. This has lead in the past to a systematic arrangement in which a number of polytypic, widespread species were identified (Pyrcz *et al.* 2006). Such a situation occurs, to some extent, in other groups of Neotropical montane satyrines, for example in the extremely species-rich genus *Pedaliodes*, with 300+ species recognized (Marín *et al.* 2017). However, in *Pedaliodes* the configuration of androconial patches on the FWD is often very helpful in distinguishing between otherwise similarly patterned sympatric species, whereas this sexual character is of little relevance in the alpha-taxonomy of *Manerebia*. Perhaps the only genus of Neotropical montane Satyrinae that is equally difficult taxonomically is *Forsterinaria* Gray, 1973 (Zubek *et al.* 2014), and *Altopedaliodes* Forster, 1964 (Padrón *et al.* 2021). In these cases, confident identification of specimens may be dependent on examination of the genitalia, in particular of the males, which proves very helpful not only in identifying cryptic, sympatric species, but also in indicating their intrageneric relationships. Even though the male genitalia of *Manerebia* are not particularly complex, the configuration of uncus

126
and valva provide a number of taxonomically useful and phylogenetically informative characters.

In the case of several genera of montane Satyrinae, in particular Pedaliodes, DNA barcoding has proved to be highly effective in confirming previous level identifications of local cryptic species based on external morphology. For example, Marín et al. (2017) found that a 'barcode gap' was present at both local and, in most cases, on a wider geographical scale. In that study, however, only two sympatric species of Manerebia were examined, *M. inderena* and *M. germaniae*. Here, barcode results are somewhat incongruent, especially in the case of *M. ronda n. sp*. On the one hand, they clearly show that simple external morphological characters are not reliable in associating some allopatric populations of *Manerebia* within single species on a wider geographical scale. The most compelling example of this is *M. inderena*, whose so-called subspecies cluster within distant clades of the phylogenetic analyses and likely represents different species. Their status needs to be revised, but such an action will require more sampling from Ecuador and Colombia, and is beyond the scope of this paper.

On the other hand, however, preliminary results based on COI sometimes appear to conflict with morphological, ecological and geographic species-level differences. This is particularly striking in the case of *M. ronda n. sp.*, which shows zero or close to zero genetic distance in comparison with other members of the *M. pauperata n. stat.* clade. *M. pauperata n. stat.* is, in fact, not only morphologically, but also ecologically a completely different species, found at lower elevations, approximately 600–1000 m below the elevations at which *M. ronda n. sp.* occurs. In these examples, the use of COI data on its own would not have provided evidence to justify their existence as separate specific entities.

The *M. ronda n. sp.* represents an empirical example regarding false negatives behind genetic thresholds for species delimitation (see: Escalona *et al.* 2021), which underlines the value employment of integrative taxonomic approaches (e.g. morphology, ecology, etc.). Another example is the new species of Neotropical treefrog *Boana platanera*, which was considered as *Boana xerophylla* before, and despite the low genetic p-distances observed (16S rRNA: 0.7–2.2 %), using morphological and acoustic data permitted to identify them as different species (Escalona *et al.* 2021).

Moreover, Cadena & Zapata (2021) comment that species delimitation using genetic data is often not straightforward, even with some genome-wide data failing to recover
distinct clades corresponding to phenotypically well-defined species (e.g. some groups of birds, plants, other invertebrates, and vertebrates). Besides, rigorous analyses of phenotypic variation remain crucial for species delimitation in the genomics epoch because phenotypes uniquely inform us about the role of selection in maintaining the cohesion of evolutionary lineages in the presence of gene exchange. In addition, the evolutionary theory describes the roles of genetic drift, gene flow, and natural (including sexual) selection in the origin and maintenance of species for integration of genomics with phenomics in species delimitation (Cadena & Zapata 2021). Therefore, if gene flow was the only force setting the boundaries of evolutionary lineages then we could affirm that there would be a single species with a high phenotypic variation: *Manerebia pauperata n. stat.* (including *M. ronda n. sp.*), but with our morphological and ecological evidence, we may consider *M. ronda n. sp.* as different species to *M. pauperata n. stat.*, hypothesizing that there may be other evolutionary mechanisms that are acting in these species and maybe directing their speciation ways.

In summary, we consider *M. pauperata n. stat.* and *M. ronda n. sp.* as genetically cryptic species and a clear empirical example that suggest that false negatives using genetic thresholds can be more common than thought, and possible explanations behind false negatives when looking for cryptic species with genetic data include recent speciation events, coupled with evolutionary mechanisms leading to phenotypic character fixation (e.g. strong positive selection, introgression across species, inadequate sampling, or a combination of them) (Escalona et al. 2021).

Besides, is possible that all these species of *Manerebia* diverged recently in the process of allopatric speciation, as did many other species of Pronophilina butterflies (Adams 1985; Pyrcz & Wojtusiak 2002; Pyrcz et al. 2006; Pyrczet et al. 2016; Álvarez-Hincapié et al. 2017; Mahecha et al. 2019; Mahecha-J. et al. 2021). It is also possible that the diversification of these *Manerebia* has taken place through peripatric speciation, by colonizing of some isolated ‘island’ mountains froma larger ‘continental’ block (Adams 1985; Sanmartín et al. 2010; Mahecha et al. 2019). Finally, our results are strongly suggestive that there are still many undescribed species in the Andes, not only within the genus *Manerebia* but among Pronophilina in general, due to a lack of collecting efforts at high elevation sites. Also, based on our information on the morphological variability of currently recognized species and checking the criteria used to determine them we found that our analyses allowed making for more robust species delimitation, which will aid the advancement towards a more informed and coherent
taxonomy for this group of butterflies. Hence, our analyses highlight the importance of employing the integrative taxonomic approach for the detection of cryptic diversity and clarifying the systematics of some genera within Pronophilina based on external morphology data only (e.g. Altopedaliodes Forster, 1964, Panyapedaliodes (Forster, 1964), and Pedaliodes) using comparative analysis of genitalia, molecular data, and information on elevation and habitat preferences (Padrón et al. 2021).

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134


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CHAPTER III

A contribution elucidation of the phylogenetic relationships of the Andean genus *Manerebia* Staudinger (Nymphalidae: Satyrinae: Pronophilina) based on a molecular phylogeny

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Abstract

The genus *Manerebia* Staudinger is an Andean butterflies group (Satyrinae, Pronophilina); it is distributed from northern Argentina to Venezuela. The genus can be polymorphic and presents a high diversity cryptic. Hence, many new species have been described during the last few decades, and others still await description. It is a monophyletic group, but the species phylogenetic relationships within the genus are unknown yet. The genus is of interest from ecological, evolutionary, biogeographic, and conservation perspectives, but as a necessary base for such studies a robust systematic is needed. For that reason, the aim of this study was to generate the first phylogenetic
hypothesis for the genus *Manerebia* based on sequence data from mitochondrial (COI) using phylogenetic tools. The monophyly of the genus is corroborated with previous molecular hypotheses. Nine clades are proposed for the *Manerebia* along the Central and Northern Andes being the Northern Andes the zone with the most richness. Finally, our results permitted us to present the first *Manerebia* molecular phylogeny and to clarify some of the phylogenetic relationships within the genus to species-level.

**Key words:** Cryptic diversity; molecular systematics; integrative taxonomic; species delimitation.

**Introduction**

The Pronophilina subtribe (Nymphalidae, Satyrinae) has been recognized as one of the most diversified groups of butterflies in mountain environments, distributed within narrow altitude ranges and presenting high levels of endemism (Adams 1985; Pyrcz et al. 2006; Pyrcz & Viloria 2007; Mahecha-Jiménez et al. 2011; Marín et al. 2014; Marin et al. 2017; Pyrcz et al. 2016; Álvarez-Hincapié et al. 2017; Mahecha et al. 2019; Padrón et al. 2021). Almost 90% of all recognized species of Pronophilina occur in the tropical Andes, from Venezuela to northern Argentina, and they are the best-represented group of butterflies in terms of diversity in mountain habitats, such as cloud forests and paramo, being reported the highest diversity levels of Pronophilina in cloud forests between 2500 and 2900m (Adams 1985; 1986; Pyrcz 2004a; 2004b; Pyrcz & Garlacz 2012; Mahecha et al. 2019; Mahecha-J. et al. 2021; Padrón et al. 2021). It is in these habitats that the montane bamboo genus *Chusquea* (Poaceae) are particularly abundant, and known or presumed to be their larval host plants (Viloria 2004; Mahecha-Jiménez et al. 2011; Mahecha et al. 2019; Padrón et al. 2021). Even though the immature stage biology of the Pronophilina remains largely unexplored, it appears that their larvae are oligophagous (Pyrcz 2004b; Greeney et al. 2009; Greeney et al. 2010; Montero & Ortiz 2014; Padrón et al. 2021). Besides, some species of Pronophilina apparently also use other Poaceae, such as *Sipta* and *Calamagrostis* grasses, woody cane (DeVries 1997), and *Guadua* bamboo (DeVries 1987; Pyrcz 2004b; Padrón et al. 2021). Furthermore, the pronophilines have a strong relationship with altitude, and most species are distributed in well-defined, and sometimes very narrow, altitudinal bands (Adams 1985; 1986; Pyrcz & Wojtusiak 1999; 2002; Pyrcz 2004a; 2004b; Pyrcz & Garlacz 2012; Mahecha et al. 2019; Padrón et al. 2021), favoring that the pronophilines
butterflies present a high percentage of endemic taxa along the Andean mountain ranges (Pyrcz 2004; Mahecha et al. 2019; Mahecha-J et al. 2021; Padrón et al. 2021). Hence, many new species have been described during the last few decades, and others still await description (Mahecha-J et al. 2021; Padrón et al. 2021). These two traits of narrow altitudinal range and endemism are features that are observed in the genus *Manerebia* Staudinger, 1897.

The genus comprises 36 described species (Pyrcz et al. 2006; Mahecha-J et al. 2021), but according to species delimitation results obtained by Mahecha-J et al. (in prep.), there would be 58 nominal species that includes the genus *Manerebia*, and with several more identified but for the moment remaining undescribed (Pyrcz et al. in prep.). A remarkable feature of the genus *Manerebia* is the external similarity of several species which can only be identified with certainty through genitalia dissection and sometimes only with molecular data, which has led to considerable confusion in the literature and resulted in underestimating their true taxonomic diversity (Pyrcz et al. 2006; Mahecha-J. et al. 2021). Additionally, some species of *Manerebia* can be polymorphic and depending on the morphotype can have a band shortened, discontinuous or even completely absent, for example in *M. ignilineata* (Dognin, 1896), *M. apiculata* (C. Felder & R. Felder, 1867), and *M. trimaculata* (Hewitson, 1870) (Pyrcz 2004; Pyrcz et al. 2006). For that reason, this character has only an infrasubspecific value (Pyrcz et al. 2006; Pyrcz & Viloria 2007). Moreover, the male genitalia are characterized by a long, arched uncus, fully developed gnathos, and slender valvae with a strongly dentate ampulla (Pyrcz 2004).

The genus *Manerebia* is distributed between Venezuela and northern Argentina, and it is restricted to the cloud forests, paramo, and adjacent grasslands along to the Andes, and because of the inaccessibility of these areas in some places, and their small size too, *Manerebia* are both difficult to collect and often overlooked, and are thus rather poorly represented in most collections. Some species apparently have very restricted geographic ranges and little is known about their natural history (Pyrcz et al. 2006; Mahecha-J. et al. 2021). The most recent contributions to the taxonomy of *Manerebia* were done by Pyrcz (2004), Pyrcz et al. (2006), and Mahecha-J. et al. (2021). The genus is of interest from ecological, evolutionary, biogeographic, and conservation perspectives, but as a necessary base for such studies a robust systematic is needed, due to high cryptic diversity within the genus (Pyrcz et al. 2006; Mahecha-J. et
al. 2021), and to date, there isn’t a phylogenetic study about the genus *Manerebia* that permits understands their phylogenetic relationships. Therefore, we inferred a species-level phylogeny for the genus based on molecular characters, to help clarify the phylogenetic relationships within the genus to species-level.

**Material and methods**

**Specimen Acquisition**

The material examined in this study was obtained by TWP and collaborators during field-work in Colombia, Peru, Argentina, Bolivia, Ecuador (2010-2019), by PB (2000–2018), Carlos Prieto and OMJ during field-work in Colombia (2013-2020), and by KW and collaborators (2000–2019) during field work in southern Ecuador. Comparative material has been studied in the collections of CEPUJ (Nature Education Centre, formerly Zoological Museum, Jagiellonian University, Kraków, Poland), including the collection of Pierre Boyer, the FLMNH (McGuire Centre for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, USA), INABIO (Instituto Nacional de Biodiversidad, Quito, Ecuador), MUSA (Museo de Historia Natural, Universidad Nacional de San Agustín, Arequipa, Perú) and MUSM (Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Perú), Instituto de Ciencias Naturales of the Universidad Nacional de Colombia (ICN-MHN-L), Museo de Historia Natural of the Universidad de Caldas (MHN-UC), Museo Pontificia Universidad Javeriana (MPUJ), Colección entomológica of the Universidad Nacional de Colombia, Sede Medellín (UNAL-MED), E. Schmidt Mumm´s personal collection at Instituto Alexander von Humboldt, Jean F. LeCrom’s personal collection (C-JFL), and Carlos Prieto’s personal collection (P-CP). In total, 140 specimens of *Manerebia*, representing 48 of the 58 nominal species previously placed in the genus were used (Mahecha-J. *et al.* in prep) (Supplementary material 1).

**Extraction and Amplification of DNA**

At the CEPUJ, DNA was isolated from a pair of legs, dry or ethanol-preserved, using a NucleoSpin® Tissue extraction kit (Macherey-Nagel Düren, Germany) and following the manufacturer's protocol. Amplification of part of the mitochondrial gene Cytochrome Oxidase I (COI) was performed using HybLCO and HybHCO primers with
universal primer tails, respectively T7 Promoter(F) and T3(R) (Folmer et al. 1994, Wahlberg & Wheat 2008), with standard PCR protocol. PCR reactions were performed in a 20 µl reaction volume in Eppendorf Mastercycler® nexus thermocycler. The PCR cycling profile comprised an initial denaturation at 95°C for 5 min followed by 40 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 1 min, and a final extension period of 72°C for 10 min. Amplification products were checked by electrophoresis on 1% agarose gels stained with Midori Green (NIPPON Genetics). Finally, samples were sent to Macrogen Europe (Amsterdam, Netherlands) for purification and sequencing. DNA extraction, PCR and sequencing at the FLMNH followed methods described in Willmott et al. (2018).

**Phylogenetic analysis**

The COI mitochondrial dataset consisted of 140 sequences with a length between 500-680 bp for 47 nominal species of *Manerebia*. Some available COI sequences were extracted from the Barcode of Life Data System (BOLD) (Ratnasingham & Hebert 2007) and NCBI (https://www.ncbi.nlm.nih.gov/nuccore), and used to reconstruct the phylogenetic tree (Supplementary material 1). The Chromatograms, and sequences from NCBI Genbank and BOLD obtained were edited and base calls checked using Geneious v. 4.8.5 (Drummond et al. 2009). We searched for reading frame errors and unexpected stop codons by translating the nucleotide sequences to peptides using Geneious v. 4.8.5 (Drummond et al. 2009). COI sequences were aligned using MAFFT v. 7.245 (Katoh & Standley 2014) and then refined with Geneious v. 4.8.5 (Drummond et al. 2009). The best-fit DNA sequence evolution model of our dataset was estimated to ModelFinder (Kalyaanamoorthy et al. 2017) which is included in IQ-TREE version 2.1.3 (Minh et al. 2020) and using the Bayesian Information Criterion (BIC) to choose the most suitable model (Pan et al. 2019). The GTR+I+G evolution model was selected to implement in the phylogenetic analyses (BI and ML). For all analyses, *Lymanopoda nevada* Krüger, *Idioneurula erebioides* (C. Felder & R. Felder, 1867), *Ianussiusa maso* (Godman, 1905), and *Diaphanos* were used as outgroup taxa according to results obtained by Mahecha-J. et al. (in prep.). Geneious v. 4.8.5 was used to format the resulting trees of each analysis, and then were refined in Inkscape 0.92.3 (2405546, 2021-06-09) (https://www.inkscape.org).
The first phylogenetic analysis was conducted using Bayesian inference (BI) with BEAST2 suite v. 2.5.2 (Bouckaert et al. 2019). The MCMC chains were run for 40 million generations and sampled once every 5000. An uncorrelated relaxed-clock was used (Matos-Maraví et al. 2019), and mitochondrial COI locus a gene ploidy of 0.5 was assigned (BEAST Developers 2017; Matos-Maraví et al. 2019; Núñez et al. 2020). The Birth-Death model (Gernhard 2008; Maurin 2020) was used, with GrowthRate prior as log-normal (M=5, S =2) and relative DeathRate as uniform in [0, 1], while the popMean prior was set to log-normal (M=−7, S =2) (Jones et al. 2015; Matos-Maraví et al. 2019). Four independent runs were started and combined with LogCombiner v. 2.5.2 (Bouckaert et al. 2019). The combined sampled trees from the analysis were then summarized in TreeAnnotator v. 2.5.2 (Bouckaert et al. 2019) with a selected burn-in of 25% to build the maximum clade credibility tree (Maurin 2020; Pola et al. 2021). Chain convergence was further assessed with Tracer v.1.6 (Rambaut et al. 2014) to confirm sufficient effective sampling size (ESS > 200) (BEAST Developers 2017; Maurin 2020).

A second phylogenetic analysis was inferred using the Maximum Likelihood (ML) in IQ-TREE v. 2.1.3 (Minh et al. 2020) with performed 10000 ultrafast bootstrap approximation algorithm replicates to explore branches support across the topology (Minh et al. 2013; Hoang et al. 2017), with the ‘bnni’ approach to reduce the risk of overestimating support values (Hoang et al. 2018) and an increased value of maximum number of interations to stop (−nm 10 000) (Kamiński et al. 2021). In discussing support for obtained relationships, the following abbreviations are used: UFB, ultrafast bootstrap; PP, Bayesian posterior probability. Node support is defined as low/weak (UFB <50, PP <0.50), moderate (UFB = 50–84, PP = 0.50–0.84), high (UFB = 85–94, PP = 0.85–0.94) or strong (UFB ≥ 95, PP ≥0.95).

Results

Both phylogenetic methods (BI, and ML) yielded similar topologies (Supplementary material 2 and 3) with respect to specific-level relationships except for some internal nodes near to root. A summary tree was produced in order to draw the phylogenetic relationships, taking as the backbone the BI phylogenetic tree (Fig.1). This tree contains 48 taxa, with each taxon represented by nominal species across Manerebia. The COI gene analysis confirms the monophyly of the Manerebia, and the relationship as the
sister group of the genera ((Lymanopoda + Ianussiusa) + (Diaphanos + Idioneurula)) with strong nodes support (UFB=100, PP=1) (Fig. 1).

**FIGURE 1.** Molecular phylogeny of the genus *Manerebia*. Node support: low/weak (UFB <50, PP <0.50), moderate (UFB = 50–84, PP = 0.50–0.84), high (UFB = 85–94, PP = 0.85–0.94) or strong (UFB ≥ 95, PP ≥0.95) – Photo by Kertell Ken ©.
According to our results, the genus *Manerebia* can be divided into nine clades with strong UFB and PP support (Fig.1); we refer to these as the ‘*navarre*’, ‘*cyclopina*’, ‘*pauperata*’, ‘*satura*’, ‘*leaena*’, ‘*levana*’, ‘*rufanalis*’, ‘*prattorum*’, and ‘*trimaculata*’ clades. However, the phylogenetic position of *M. punku* (from northernmost Peru), and *M. ignilineata* (from Ecuador and northern Peru) is uncertain because both phylogenetic methods appear to associate with different clades with weak UFB and PP nodes support (Supplementary Material 2 and 3), and cannot be associated with any clade. The ‘*navarre*’ clade includes (*M. navarre*: Colombia, (*M. lamasi*: Peru, (*M. placida*: Ecuador, (*M. granatus*: Peru-Ecuador, *M. reducta*: Peru))). This clade is distributed from the Andes of central Peru to northern Andes of Ecuador and Colombia, with only one species reported from Colombia (Fig.2). The ‘*cyclopina*’ clade includes (*M. mycalesoides*: Colombia-Venezuela, (*M. nevadensis*: Colombia, (*M. typhlops*: Peru-Bolivia, (*M. cyclopina*: Peru-Bolivia, (*M. cyclops*: Peru-Bolivia, *M. n.sp.10*: Argentina)))). This clade is distributed along to Eastern in Central and Northern Andes, and in Colombia is found in all three cordilleras including the Sierra Nevada de Santa Marta (SNSM) (Fig.3).

The ‘*pauperata*’ clade contains (*M. n.sp.3*: Ecuador, (*M. pauperata*: Ecuador, *M. ronda*: Peru)). This clade is distributed from Andes of central in Peru to the Amotape-Huancabamba zone in the south of Ecuador (Fig.4). The ‘*satura*’ clade includes (*M. benigni*: Peru-Ecuador, ((*M. satura*: Peru, *M. n.sp.4*: Peru), (*M. rubescens*: Peru, (*M. n.sp.7*: Peru, (*M. haywardi*: Peru, *M. n.sp.6*: Peru)))), and is possible that the ‘*pauperata*’ clade would be the sister group of the ‘*satura*’ clade with high node support (UFB: 87, PP: 0.85) (Fig.1). The ‘*satura*’ clade is distributed from Northern Andes in the Amotape-Huancabamba zone in the south of Ecuador to the central part of the Central Andes in Peru (Fig.5).

The ‘*leaena*’ clade contains (*M. apiculata*: Colombia, (*M. leaena*: Colombia-Venezuela, *M. germaniae*: Colombia-Ecuador)). This clade is distributed in the Northern Andes from the Amotape-Huancabamba zone to central and eastern cordillera in Colombia including the boundary of the Tama National park in between Colombia-Venezuela (Fig.6). The ‘*levana*’ clade includes (*M. bernito*: Colombia, *M. pervaga*: Colombia-Venezuela), and it’s distributed only in the eastern cordillera in Colombia in the Northern Andes (Fig.7). The ‘*rufanalis*’ clade contains (*M. fernandina*: Ecuador, *M.
rufanalis: Ecuador), (M. n. sp.11: Peru, (M. n.sp.5: Peru, M. trirufa: Peru)), and this clade is distributed from north of the Central Andes in Peru till the Northern Andes in Ecuador (Fig.8).

The ‘prattorum’ clade includes ((M. n.sp.9: Colombia, M. clara: Ecuador), (M. prattorum: Peru, (M. antioquiana: Colombia, M. n.sp.8: Colombia))). This clade is distributed from the Central Andes of Peru, the Amotape-Huancabamba zone in Ecuador-Peru, to Central and Western cordillera in Colombia (Fig.9). The ‘trimaculata’ clade contains ((M. mirena: Ecuador, (M. n.sp.1: Ecuador, ((M. undulata: Ecuador, (M. similis: Ecuador, M. n.sp.2: Ecuador)))))), ((M. milaena: Ecuador, M. trimaculata: Ecuador-Peru), (M. golondrina: Ecuador, (M.fina: Ecuador, M. interrupta: Ecuador-Peru))). Phylogenetic relationships of M. undulata + (M. similis + M. n.sp.2) with (M. milaena +M. trimaculata) + M. golondrina + (M. fina + M. interrupta) are well-defined with a high node support (UFB: 89, PP: 0.88). Additionally, the ‘trimaculata’ clade could be associated with the ‘prattorum’ clade with high node support (UFB: 93, PP: 0.92) (Fig.1). The ‘trimaculata’ clade is distributed only from the Amotape-Huancabamba zone in Ecuador-Peru to the boundary between Ecuador and Colombia (Fig.10), and it is the clade most speciose within the Manerebia genus.
FIGURE 2. The species that be part of the 'navarre' clade and their geographical distribution are observed.
FIGURE 3. The species that be part of the ‘cyclopina’ clade and their geographical distribution are observed.
FIGURE 4. The species that be part of the ‘*pauperata*’ clade and their geographical distribution are observed.
FIGURE 5. The species that be part of the ‘satura’ clade and their geographical distribution are observed.
FIGURE 6. The species that be part of the ‘leaena’ clade and their geographical distribution are observed.
FIGURE 7. The species that be part of the ‘levana’ clade and their geographical distribution are observed.
FIGURE 8. The species that be part of the ‘rufanalis’ clade and their geographical distribution are observed.
FIGURE 9. The species that be part of the ‘prattorum’ clade and their geographical distribution are observed.
FIGURE 10. The species that be part of the ‘trimaculata’ clade and their geographical distribution are observed.
Discussion

Our results allowed us to corroborate the monophyly of the *Manerebia* and its phylogenetic relationships with the ‘Lymanopoda’ group, supporting to propose by Pyrcz *et al.* 2006, Mahecha-J. *et al.* 2021, and Mahecha-J. *et al.* (in prep.), who were based on morphological and molecular characters. Some internal nodes near to root were unresolved in the BI and ML phylogenetic trees, indicating the need to use larger taxonomic sampling or include other molecular and morphological characters (e.g. genomic data) that may help to improve these problems, and allow to build systematic relationships more robust within the genus (Marín *et al.* 2011; Tsang *et al.* 2014; Espeland *et al.* 2019; Wang *et al.* 2020a; Wang *et al.* 2020b; Mahecha-J. *et al.* 2021; Padrón *et al.* 2021; Zhanget *et al.* 2021). Nevertheless, both phylogenetic analyses were yielded similar topologies with respect to specific-level relationships with branches values strong, allowing us to propose the nine clades that compound to *Manerebia*, and some of our phylogenetic relationships are supporting the proposal by Pyrcz (2004), and Pyrcz *et al.* (2006) using morphological characters, and Mahecha-J. *et al.* (in. press) employing molecular (COI) characters. Although, they didn’t propose any clade for *Manerebia*, only suggested some associations specific-level.

‘navarre’ clade

According to Pyrcz *et al.* (2006) *M. navarre* is realting to *M. satura, M. franciscae*, and *M. mammuthus*. These species are unique among north Andean *Manerebia* in having a distinct dark brown patch of androconial scales on the DFW in the posterior third of the discal cell and basal part of cells 1A-Cu2 to M2-M, and the male genitalia are also similar, with an arched uncus, relatively long subunci and valva with a spiny dorsal process near the base, similar to *M. leaena, M. apiculata* and *M. germaniae* (Pyrcz *et al.* 2006). However, based on our results, *M. navarre* is more related to *M. lamasi* than *M. satura*, and possibly neither to *M. franciscae*, and *M. mammuthus*, although, we need to include these species into the molecular phylogenetic analyses to able to be verified. The same case happened with *M. lamasi* and *M. pauperata*, in where, based on Pyrcz *et al.* (2006) these taxa were related but our study showed that *M. lamasi* is associated with *M. navarre, M. granatus, M. placida*, and *M. reducta*, conforming the‘navarre’ clade, a result supported by Mahecha-J. *et al.* (in. press). It’s
necessary to include *M. franciscae*, and *M. mammuthus* in the systematic analyses for knowing the phylogenetic position of these species.

'cyclopina' clade

This clade is the only one that is distributed from northern-eastern Argentina to Cordillera de Merida in Venezuela (Pyrcz 2004; Pyrcz et al. 2006). Based on Pyrcz et al. (2006) *M. mycalesoides* are related to *M. magnifica* and *M. nevadensis* according to the male genitalia which is distinctive in the elongated distal part of the vulva which is strongly curved upwards, ending with several short ‘teeth’, and externally *M. mycalesoides* might be more related to *M. magnifica* than to *M. nevadensis* because both have large ventral ocelli on both fore and hindwing and wavy, dark, prominent postdiscal line on both VFW and VHW. Mahecha-J. et al. (in press) reported that *M. nevadensis* is associated with *M. mycalesoides*, but they didn’t include *M. magnifica* in the analysis. Additionally, unpublished data from Pyrcz et al. (in prep.), using the advancement of next-generation sequencing (NGS) technologies, have been found that *M. nevadensis* forming a clade with *M. mycalesoides* and is the sister group of the rest of the members of the ‘cyclopina’. In our results, *M. mycalesoides* is within the same clade with *M. nevadensis*, but it is not forming a separate clade from the other members of the ‘cyclopina’ clade.

On the other hand, *M. cyclopina*, *M. cyclopella*, *M. cyclops*, *M. typhlops* have a posterior portion of the aedeagus, upturned tip to the valva and short subuncus, similar to certain southern Andean such as *M. mycalesoides*, *M. magnifica*, and *M. nevadensis* (Pyrcz et al. 2006), hence it is possible to include *M. cyclopella* within the ‘cyclopina’ clade, and unpublished data from Pyrcz et al. (in prep.), using NGS technologies, have been reported to the relationship between *M. cyclopella* with *M. cyclopina*. However, it’s necessary to include the other possible members of the ‘cyclopina’ clade (e.g. *M. cyclopella*, and *M. thyphlopsella*) in the phylogenetic analyses to know the systematic position of these species.

'pauperata' clade

Pyrcz et al. (2006) described as subspecies *M. satura lamasi* and *M. satura pauperata* based on the phenotypic similarity due to the absence of the pale VHW postdiscal band, and in females, the band is well-developed and chalky white, and both
with smaller ocelli on the VHW and a small ocellus in cell M1-M2 on the VFW. However, Mahecha-J. et al. (in prep.) found, employing molecular and morphology characters, that these subspecies were two different species and were not relating, and these subspecies were transferred by them to species-level, and in effect, *M. lamasi* is related with *M. granatus, M. reducta, and M. placida* (‘navarre’ clade), and *M. pauperata* is the sister group of the *M. ronda*, a new species proposed by Mahecha-J. et al. (in prep.). Besides, Mahecha-J. et al. (in prep.) observed that the status of subspecies of *Manerebia inderena mirena* from Ecuador was uncertain, and it was proposed by them, based on delimitation-species tools, as a new putative species called *Manerebia* n. sp.3 in our study, and it is the phylogenetic sister of the *M. ronda* and *M. pauperata*, supporting our results.

‘satura’ clade

Pyrcz (2004) commented to *M. benigni* shares with *M. satura* and *M. diffusa* a large FWD androconial patch situated in the median part of the wing. HWD is brown except for brick red suffusion at tornus, somewhat reminiscent of *M. haywardi*. Additionally, *M. benigni* is superficially very similar to *M. zoippus* which occurs in southern Peru and Bolivia is smaller, and it has no red suffusion on the HWD anal area, a smaller FWD androconial patch and different male genitalia. Besides, *M. diffusa* resembles at first sight the sympatric *M. benigni*, and both have a faint HWV milky white median band, which is a unique feature among all the congeners in this part of the Andes. *M. benigni* differs immediately form *M. diffusa* by the rusty anal suffusion on the HWD and a large brick over cast of the HWV, but the affinities of *M. diffusa* are currently difficult to assess due to its male genitalia are most similar to *M. haywardi* but are not unlike *M. benigni* (Pyrcz 2004).

Subsequently, Pyrcz et al. (2006) suggested that *M. satura* is closely related to the allopatric *M. franciscae, M. mammuthus*, and *M. navarrae*, in where the male genitalia are distinctive, with the distal, narrower portion of the valva being relatively short, and often only bearing a few large spines, rather than numerous smaller spines. However, according to our phylogenetic results, *M. benigni* is more related to *M. satura* and *Manerebia* n. sp.4, but we couldn’t include to *M. diffusa*. For his part, *M. rubescens* has an external similarity to *M. trirufa* by the wide, orange patch, which spreads over distal 1/3 of the HWD (Pyrcz 2004), but according to our results, *M. trirufa* is part of the
‘rufanalis’ clade. *M. haywardi* superficially resembles *M. inderena* and several other Ecuadorian species characterised by the wide HWV yellow median band, however all of them having markedly different male genitalia (Pyrcz 2004), but we found to *M. haywardi* is the sister species of *Manerebia* n. sp.6, although we couldn´t include to nominal species of *M. inderena*.

Mahecha-J. *et al.* (in press.), using COI gene, reported the association between *M. benigni* and *M. satura*, but they didn't include other species associated with the ‘satura’ clade. For that reason, it’s necessary to include the other possible members of the ‘satura’ clade (e.g. *M. diffusa*, and *M. reducta*) in our phylogenetic analyses to know the systematic position of these species. On the other hand, unpublished data from Pyrcz *et al.* (in prep.), using NGS technologies, is supporting the ‘satura’ clade which is proposed by us, but in their analysis appears *M. ronda* (‘pauperata’ clade) related to *M. benigni* and *M. diffusa*.

The phylogenetic relationships between ‘pauperata’ clade and ‘satura’ clade are supported by a good branch value, but it’s not enough for taking on that both they both belong to the same clade. Moreover, Mahecha-J. *et al.* (in press.) reported that members of the ‘pauperata’ group were related to members of the ‘rufanalis’ group, and ‘satura’ group with ‘leaena’ group + *M. punku* + ‘cyclopina’ group.

‘leaena’ clade

Pyrcz *et al.* (2006) suggested to *M. apiculata*, *M. leaena*, *M. germaniae*, and *M. pluviosa* might be associated for presenting in the male genitalia a toothed projection near the base of the ampulla and a strongly arched uncus but these characters are similar to other species such as *M. navarrae*, *M. satura*, *M. franciscae*, and *M. mammuthus*, and additionally are externally similar to them, being probably these morphological characters homoplasies between them. Although, they have other characters distinctive male genitalia and molecular analyses to allow cataloged them as different species (Pyrcz 2004; Pyrcz *et al*. 2006; Mahecha-J. *et al.* in. press). According to our molecular results, *M. apiculata*, *M. leaena*, and *M. germaniae* are conforming to the clade called by us as ‘leaena’, and this phylogenetic relations was found too by Mahecha-J. *et al.* (in. press) using the COI gene, but they didn’t include *M. pluviosa*. To may include *M. pluviosa* within the ‘leaena’ clade it’s necessary to obtain the molecular sequence and to
make the corresponding phylogenetic analyses but according to Pyrcz et al. (2006) is possible this species would be part of this clade.

‘levana’ clade

Pyrcz et al. (2006) commented that the relationships of *M. levana* to other congeners were uncertain, and it might be associated with *M. pervaga* based on morphological characters. Mahecha-J. et al. (2021) proposed the ‘levana’ group when described the *M. clarita* and *M. bernito* from Colombia, and did remarks about the relationship between *M. pervaga* and *M. levana*, being more related *M. levana* to *M. bernito*, and *M. pervaga* with *M. clarita*. Based on our results, *M. pervaga* might be more related to *M. bernito*, but we have to include *M. levana* and *M. clarita* in our phylogenetic analyses to know the phylogenetic relationships of these species within ‘levana’ clade. Moreover, Pyrcz et al. (in .prep.), using NGS technologies, have found that *M. bernito* is associated with *M. clarita*, but they haven’t included *M. levana* and *M. pervaga* yet, although the existence of the ‘levana’ clade is valid.

‘rufanalis’ clade

*M. rufanalis* and *M. fernandina* were considered by Pyrcz et al. (2006) as subspecies (*M. r. rufanalis* and *M. r. fernandina*) due to morphological simmilarity. However, Mahecha-J. et al. (in press.) observed that *M. r. rufanalis* and *M. r. fernandina* was a differsents species supported by the genetic distances (> 2%), but they didn’t make the correspondent taxonomic arrangement. Then, Mahecha-J. et al. (in. prep.) transferred to *M. r. rufanalis* and *M. r. fernandina* to species-level, supported by the genetic distances too (> 2%), and they found that *M. rufanalis*is the phylogenetic sister of the *M. fernandina*, as evidenced by our results as well.

On the other hand, *M. trirufa* can be immediately recognised from other species within the genus, except *M. rubescens* (‘satura’ clade), by the wide, orange patch, which spreads overdistal 1/3 of the HWD, and *M. rubescens* has a wide HWV median, yellow band, whereas *M. trirufa* has no trace of any lighter median band (Pyrcz 2004). Besides, Mahecha-J. et al. (in press.) found that *M. trirufa* is related to *M. rufanalis* and *M. fernandina* supporting the ‘rufanalis’ clade.
’prattorum’ clade

*M. prattorum* is easily distinguished from its congeners in the northern Andes by the postdiscal orange band on the DHW only. The only species with somewhat similar pattern is *Manerebia lisa* (Weymer) which occurring in central Peru, but in that taxon the band is darker, with blurred edges, narrowing gradually from anal to costal margin. Moreover, the male genitalia of *M. lisa* show that the two species are not closely related, although, being similar to *M. golondrina* base on the male genitalia. Additionally, the size and wing shape of *M. prattorum* are similar to *M. mirena* and *M. milaena*, but *M. prattorum* lacks the ventral magenta or greyish sheen in the distal marginal areas that characterises *M. undulata*, and does not have the DHW submarginal ocelli that occur in *M. mirena* (Pyrcz et al. 2006).

On the other hand, Pyrcz et al. (2006) described *M. inderena antioquiana* and *M. inderena clara* as subspecies based on morphological characters. Moreover, *M. i. clara* is associated with *M. inderena fina* Pyrcz & Willmott and *M. inderena leaeniva* Pyrcz & Willmott, both from Ecuador, and *M. i. antioquiana* with the nominate subspecies (Pyrcz et al. 2006). Nevertheless, Mahecha-J. et al. (in press.), according to the delimitation-species tools and genetic distances which they used, found that *M. i. antioquiana and M. i. clara* are valid species, and Mahecha-J. et al. (in prep.) made the taxonomic changes correspondingly and demonstrated that *M. antioquiana* is related to *Manerebia* n. sp.8 from Colombia and might be allopatric species, and *M. clara* is the phylogenetic sister of the *Manerebia* n. sp.9 and might be allopatric species too.

’trimaculata’ clade

This clade is the most speciose within the genus, being almost exclusive from Ecuador (Pyrcz et al. 2006; Mahecha-J. et al. in prep.). According to Pyrcz et al. (2006), *M. trimaculata* is more related to *M. undulata* and *M. interrupta*, all characterized by a light greyish or magenta marginal sheen along the distal quarter of the ventral surface of both wings, and a thin, dark brown, undulate line passing through the center of the VHW discal cell. At the same time, Pyrcz et al. (2006) reported that *M. golondrina* was most closely related to *M. inderena fina* (now *M. fina*) due to the phenotypic similarity. However, based on our results, *M. trimaculata* is more related to *M. milaena*, and it’s within the group conformed by (*M. interrupta + M. fina) + M. golondrina*, supporting the results found by Mahecha-J. et al. (in prep.).
On the other hand, Pyrcz et al. (2006) proposed that *M. inderena similis* (now *M. similis*) is closely related to the neighboring *M. i. fina* (now *M. fina*) to the north by the broader, pale VHW postdiscal band which is more strongly yellow especially towards the anal margin, but based on our molecular results, *M. similis* is associate with *M. undulata* and *Manerebia* n. sp.2, supporting the observed by Mahecha-J. et al. (in prep.). Ultimately, *M. mirena* appears as the sister of the rest of the species that conformed to the ‘trimaculata’ clade, a result supported by Mahecha-J. et al. (in prep.) based on molecular data.

Finally, the genetic marker used here is of course still rather restricted but proved to be helpful in establishing intrageneric relationships. Nevertheless, much broader sampling across the genome, and including more species of the *Manerebia* will be needed to confidently place some systematic positions within the phylogeny of the genus *Manerebia*.

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CHAPTER IV

An exploration of the complex biogeographical history of the Neotropical butterfly *Manerebia* Staudinger (Nymphalidae: Satyrinae: Pronophilina)

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Abstract

The genus *Manerebia* Staudinger is an Andean butterflies group (Satyrinae, Pronophilina) distributed from northern Argentina to Venezuela. The *Manerebia* butterflies present a high phenotypic variation (polymorphisms), and several new species have been described during the last few decades, and others still await description. The genus is of interest from ecological, evolutionary, biogeographic, and conservation perspectives, but the historical biogeography patterns do not well known, and a biogeographic study is needed to help to be able to understand and infer patterns about the genus *Manerebia* evolutionary history in the Neotropic. For that reason, the aim of this study was to explore the biogeographical history of the genus *Manerebia* estimating times and rates of diversification for its lineages and employing a biogeographical analysis in order to reconstruct its evolutionary history. Our results allowed us to infer that the divergence time of *Manerebia* was between the late Miocene and Pliocene, and most extant lineages had already appeared in the Pleistocene. The genus had an overall early burst in the late Miocene / early Pliocene boundary followed by deceleration due to a decrease in speciation along to Pleistocene, and this pattern is
reflected for all clades in *Manerebia*. Dispersal and colonization events are possibly the most common processes within the genus, and our results confirm the role of the Andean geomorphological in the evolution of Neotropical biodiversity.

**Key words:** Divergence times, diversification rates, dispersal events, Andean geomorphological.

**Introduction**

The Andes is one of the great mountain systems of the world and it extends from northern Venezuela to southern Chile (Clark 2001; Hoorn et al. 2010). The Andes has been proposed as the main driver of diversification in the Neotropical region (Hoorn et al. 2010; Chazot et al. 2016) due to the complex geology and history in conjunction with the tropical location of its Central and Northern Andean regions have provided a unique evolutionary opportunity (Clark 2001; De-Silva et al. 2016). An improved understanding of the role of the Andes in Neotropical diversification should result from examining a large range of taxa and assessing the extent to which groups have been similarly affected or not by the Andes (De-Silva et al. 2016). In particular, insects represent the bulk of terrestrial diversity but remain under-represented in biogeographic research, despite a number of recent studies of Neotropical butterflies (e.g. Hall 2005; Casner & Pyrcz 2010; De-Silva et al. 2016; Chazot et al. 2016; Mahecha et al. 2019; Matos-Mavarí et al. 2019; 2021).

Butterflies of the subtribe Pronophilina (Nymphalidae, Satyrinae) are an excellent group to study historical biogeography patterns (Casner & Pyrcz 2010; Mahecha et al. 2019). The Pronophilina is a diverse subtribe of the Satyrini that occurs mainly at middle to high elevations in most montane areas of the Neotropical región (Padrón et al. 2021). The Pronophilina has been recognized as one of the most diversified groups of butterflies in mountain environments, distributed within narrow altitude ranges and presenting high levels of endemism (Adams 1985; Pyrcz et al. 2006; Pyrcz & Viloria 2007; Mahecha-Jiménez et al. 2011; Marín et al. 2014; Marin et al. 2017; Pyrcz et al. 2016; Álvarez-Hincapié et al. 2017; Mahecha et al. 2019; Padrón et al. 2021). Almost 90% of all recognized species of Pronophilina occur in the tropical Andes, from Venezuela to northern Argentina, and they are the best-represented group of butterflies in terms of diversity in mountain habitats, such as cloud forests and paramo, being
reported the highest diversity levels of Pronophilina in cloud forests between 2500 and 2900m (Adams 1985; 1986; Pyrcz 2004; Pyrcz & Garlacz 2012; Mahecha et al. 2019; Mahecha-J. et al. 2021; Padrón et al. 2021). It is in these habitats that the montane bamboo genus *Chusquea* (Poaceae) are particularly abundant, and known or presumed to be their larval host plants (Viloria 2007; Mahecha-Jiménez et al. 2011; Mahecha et al. 2019; Padrón et al. 2021). Besides, some species of Pronophilina apparently also use other Poaceae, such as *Stipa* and *Calamagrostis* grasses, woody cane (DeVries 1997), and *Guadua* bamboo (DeVries 1987; Pyrcz 2004; Padrón et al. 2021). Furthermore, the pronophilines have a strong relationship with altitude, and most species are distributed in well-defined, and sometimes very narrow, altitudinal bands (Adams 1985; 1986; Pyrcz & Wojtusiak 1999; 2002; Pyrcz 2004; Pyrcz & Garlacz 2012; Mahecha et al. 2019; Padrón et al. 2021), favoring that the pronophilines butterflies present a high percentage of endemic taxa along the Andean mountain ranges (Pyrcz 2004; Mahecha et al. 2019; Mahecha-J et al. 2021; Padrón et al. 2021). These two traits of narrow altitudinal range and endemism are features that are observed in the genus *Manerebia* Staudinger, 1897.

A remarkable feature of the genus *Manerebia* is the external similarity of several species which can only be identified with certainty through genitalia dissection and sometimes only with molecular data, which has led to considerable confusion in the literature and resulted in underestimating their true taxonomic diversity (Pyrcz et al. 2006; Mahecha-J. et al. 2021). Additionally, some species of *Manerebia* can be polymorphic and depending on the morphotype can have a band shortened; discontinuous or even completely absent (Pyrcz 2004; Pyrcz et al. 2006). The genus *Manerebia* is distributed between Venezuela and northern Argentina (Fig.1), and it is restricted to the cloud forests, paramo, and adjacent grasslands along to the Andes, and because of the inaccessibility of these areas in some places, and their small size too, *Manerebia* are both difficult to collect and often overlooked, and are thus rather poorly represented in most collections. Some species apparently have very restricted geographic ranges and little is known about their natural history (Pyrcz et al. 2006; Mahecha-J. et al. 2021). The most recent contributions to the taxonomy of *Manerebia* were done by Pyrcz (2004), Pyrcz et al. (2006), and Mahecha-J. et al. (2021).
The genus is of interest from ecological, evolutionary, biogeographic, and conservation perspectives, but the historical biogeography patterns do not well known, and the origins, evolutionary history and diversification of the *Manerebia* are poorly known and uncertain (Mahecha-J. *et al.* 2021), and a biogeographic study is needed to help to be able to understand and infer patterns about the genus *Manerebia* evolutionary history in the Neotropics (Pyrcz *et al.* 2006; Mahecha *et al.* 2019; Mahecha-J. *et al.* 2021). Therefore, the aims of our study were: (1) estimating the divergence times for the genus; (2) investigating the diversification pattern of the group by fitting likelihood models of clade speciation in order to test the hypothesis of rapid radiation during the diversification of *Manerebia*, and (3) propose a biogeographic scenario for the evolution of the genus.

**Material and methods**

*Taxon sampling*

The material examined in this study was obtained by TWP and collaborators during field-work in Colombia, Peru, Argentina, Bolivia, Ecuador (2010–2019), by Pierre Boyer and TWP (2000–2018), Carlos Prieto and OMJ during field-work in
Colombia (2013-2020), and by Keith Willmot and collaborators (2000–2019) during field work in southern Ecuador. Comparative material has been studied in the collections of CEPUJ (Nature Education Centre, formerly Zoological Museum, Jagiellonian University, Kraków, Poland), including the collection of Pierre Boyer, the FLMNH (McGuire Centre for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, USA), INABIO (Instituto Nacional de Biodiversidad, Quito, Ecuador), MUSA (Museo de Historia Natural, Universidad Nacional de San Agustín, Arequipa, Perú) and MUSM (Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Perú), Instituto de Ciencias Naturales of the Universidad Nacional de Colombia (ICN-MHN-L), Museo de Historia Natural of the Universidad de Caldas (MHN-UC), Museo Pontificia Universidad Javeriana (MPUJ), Colección entomológica of the Universidad Nacional de Colombia, Sede Medellín (UNAL-MED), E. Schmidt Mumm’s personal collection at Instituto Alexander von Humboldt, Jean F. LeCrom’s personal collection (C-JFL), and Carlos Prieto’s personal collection (P-CP). In total, 140 specimens of Manerebia, representing 48 of the 58 nominal species previously placed in the genus were used (Mahecha-J. et al. in prep) (Supplementary material 1).

**DNA extraction, amplification, and sequencing**

At the CEPUJ, DNA was isolated from a pair of legs, dry or ethanol-preserved, using a NucleoSpin® Tissue extraction kit (Macherey-Nagel Düren, Germany) and following the manufacturer's protocol. Amplification of part of the mitochondrial gene Cytochrome Oxidase I (COI) was performed using HybLCO and HybHCO primers with universal primer tails, respectively T7 Promoter(F) and T3(R) (Folmer et al. 1994, Wahlberg & Wheat 2008), with standard PCR protocol. PCR reactions were performed in a 20 µl reaction volume in Eppendorf Mastercycler® nexus thermocycler. The PCR cycling profile comprised an initial denaturation at 95°C for 5 min followed by 40 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 1 min, and a final extension period of 72°C for 10 min. Amplification products were checked by electrophoresis on 1% agarose gels stained with Midori Green (NIPPON Genetics). Finally, samples were sent to Macrogen Europe (Amsterdam, Netherlands) for purification and sequencing. DNA extraction, PCR and sequencing at the FLMNH followed methods described in Willmott et al. (2018).
Species-level phylogeny

The COI mitochondrial dataset consisted of 140 sequences with a length between 500-680 bp for 47 nominal species of *Manerebia*. Some available COI sequences were extracted from the Barcode of Life Data System (BOLD) (Ratnasingham & Hebert 2007) and NCBI (https://www.ncbi.nlm.nih.gov/nuccore), and used to reconstruct the phylogenetic tree (Supplementary material 1). The Chromatograms, and sequences from NCBI Genbank and BOLD obtained were edited and base calls checked using Geneious v. 4.8.5 (Drummond *et al* 2009). We searched for reading frame errors and unexpected stop codons by translating the nucleotide sequences to peptides using Geneious v. 4.8.5. COI sequences were aligned using MAFFT v. 7.245 (Katoh & Standley 2014) and then refined with Geneious v. 4.8.5.

The best-fit DNA sequence evolution model of our dataset was estimated to ModelFinder (Kalyaanamoorthy *et al*. 2017) which is included in IQ-TREE version 2.1.3 (Minh *et al*. 2020) and using the Bayesian Information Criterion (BIC) to choose the most suitable model (Pan *et al*. 2019). The GTR+I+G evolution model was selected to implement in the phylogenetic analyses. For all analyses, *Lymanopoda nevada* Krüger, *Idioneurula erebioides* (C. Felder & R. Felder, 1867), *Ianussiusa maso* (Godman, 1905), and *Diaphanos* were used as outgroup taxa according to results obtained by Mahecha-J. *et al*. (in prep.). Geneious v. 4.8.5 (Drummond *et al*. 2009) was used to format the resulting trees of each analysis, and then were refined in Inkscape 0.92.3 (2405546, 2021-06-09) (https://www.inkscape.org).

A phylogenetic analysis was inferred using the Maximum Likelihood (ML) in IQ-TREE v. 2.1.3 (Minh *et al*. 2020) with performed 10000 ultrafast bootstrap approximation algorithm replicates to explore branches support across the topology (Minh *et al*. 2013; Hoang *et al*. 2017), with the ‘bnni’ approach to reduce the risk of overestimating support values (Hoang *et al*. 2018) and an increased value of maximum number of iterations to stop (−nm 10 000) (Kamiński *et al*. 2021). In discussing support for obtained relationships, the following abbreviations are used: UFB, ultrafast bootstrap. Node support is defined as low/weak (UFB < 50), moderate (UFB = 50 – 84), high (UFB = 85 – 94) and strong (UFB ≥ 95) (Supplementary material 2).
Inasmuch as there are no described fossils of *Manerebia* or Pronophilina, we relied on secondary calibrations to time-calibrate the species tree. The selected calibration points were chosen from well-supported monophyletic groups: the crown age of the tribe Satyrini to 38 – 55 Mya (Matos-Maraví et al. 2021), the crown age of the subtribe Pronophilina to 28 – 33 Mya (Kumar et al. 2017), and the crown age of ‘Lymanopoda’ clade (*Lymanopoda* + *Ianussiusa* + *Idioneurula* + *Diaphanos*) to 19.5 – 25.7 Mya (Kumar et al. 2017). We also constrained the root with host-plant proposed for Pronophilina with a maximum age corresponding to the crown age of Poaceae to 112 Mya (Foster et al. 2017; Chazot et al. 2019a).

Bayesian estimation of divergence times was run using the STARBEAST2 v.0.15.5 package (Ogilvie et al. 2017) available in BEAST2 suite v. 2.5.2 (Bouckaert et al. 2019). An uncorrelated relaxed-clock was used (Matos-Maraví et al. 2019), and mitochondrial COI locus a gene ploidy of 0.5 was assigned (BEAST Developers 2017; Matos-Maraví et al. 2019; Núñez et al. 2020). In order to have a fixed topology, we turned off the topology operators in BEAUTi, and we specified the topology obtained with IQTREE made ultrametric with the ape package (Paradis et al. 2004; Chazot et al. 2019). The Birth-Death model (Gernhard 2008; Maurin 2020) and the Calibrated Yule speciation processes were used as the tree prior in separate analyses to investigate the impact of this parameter on the final age estimates (Matos-Maraví et al. 2014), with GrowthRate prior as log-normal (M=5, S =2) and relative DeathRate as uniform in [0, 1], while the popMean prior was set to log-normal (M=−7, S =2) (Jones et al. 2015; Matos-Maraví et al. 2019). In addition, the calibration points were modelled as uniform ranges (hard bounds).

The MCMC chains were run for 50 million generations and sampled once every 5000; convergence was checked using Tracer v.1.6 (Rambaut et al. 2014) to confirm sufficient effective sampling size (ESS > 200) (BEAST Developers 2017; Maurin 2020). Four independents runs were done and combined using LogCombiner v. 2.5.2 (Bouckaert et al. 2019). The dated ultrametric tree was obtained using TreeAnnotator v. 2.5.2 (Bouckaert et al. 2019) with a selected burn-in of 25%, to build it into a single
maximum clade credibility tree (Maurin 2020; Pola et al. 2021), and visualized using Figtree v1.4 (http://tree.bio.ed.ac.uk/software/figtree/), and the R package ggtree (Yu et al. 2017). To plot the ultrametric tree against stratigraphy, the R package Strap (Bell & Lloy 2015) was used, and then it was refined in Inkscape 0.92.3 (2405546, 2021-06-09) (https://www.inkscape.org).

**Diversification rates estimation**

We analyzed diversification patterns in the genus *Manerebia* by generating standard LTT plot and based on 1000 sampled Bayesian in the R package ape v. 5.5 (Paradis et al. 2004), and to test whether diversification rates have changed over time the gamma ($\gamma$) statistic (Pybus & Harvey 2000) was calculated using thr R package diversitree v. 09-16 (FitzJohn 2012; FitzJohn et al. 2021). Positive and negative values of ($\gamma$) indicate an increasing and decreasing diversification rate towards the present respectively (De-Silva et al. 2016). The gamma statistic ($\gamma$) was used to compare the rate-constant models (pure-birth and birth-death) using the R package ape v. 5.5 (Paradis et al. 2004). In addition, Log-likelihood (AIC) and the difference in AIC with the best model (AICc) were examined for each model using the R package GEIGER (Pennell et al. 2014).

To investigate possible diversification rate heterogeneity, we used the Bayesian Analysis of Macroevolutionary Mixtures (BAMM. 2.2.0) approach (Rabosky et al. 2014). This method uses reversible jump Markov Chain Monte Carlo and assumes that changes in diversification regimes along the branches of a tree follow a compound Poisson process (Rabosky et al. 2014). We also tested whether the diversification pattern of *Manerebia* is consistent with a diversity-dependent birth-death process by using the R package DDD (Etienne et al. 2011; Peña et al. 2015) and calculated speciation, extinction rates and carrying capacity for our tree. DDD uses hidden Markov models to test likelihood of speciation and extinctions rates under several models of evolution (constant-rate and diversity-dependent models) (Etienne et al. 2011). We ran BAMM for 1 million generations and discarded 10% of the data as burn-in. We inspected convergence and calculated the ESS values for the parameters before further analyses using in the R package Coda v. 0.19-4 (Plummer et al. 2021). BAMM fits several models of diversification to the data and calculates Bayes factors for evaluation of model fit. We identified the best model of diversification for our *Manerebia*
phylogeny. The result obtained in BAMM was checked, and output was analyzed in the R package BAMMtools 2.1.8 (Rabosky et al. 2014).

**Biogeographical reconstructions**

The ancestral range estimation was performed with the following software: R package BioGeoBEARS v.0.2.1 (Matzke 2013) and RASP (Reconstruct Ancestral State in Phylogenies) (Yu et al. 2015). Both software packages allowed us to customize dispersal rates matrices and time stratification events, as well as infer areas among the following historical biogeography frameworks: Dispersal-Vicariance Analysis (DIVA) (Ronquist 1997), Statistical Dispersal-Vicariance (S-DIVA) (Yu et al. 2010), Dispersal-Extinction Cladogenesis (DEC) (Ree & Smith 2008), and Bayesian inference of historical biogeography for discrete areas (BayArea) (Landis et al. 2013). In addition, Matzke (2014) included a new parameter in all the models accounting for what he calls the founder-event speciation. These are described as “jumping dispersal events (J)”, which are rare events that occur when a new population colonizes a new area (Matzke 2014). In particular, BioGeoBEARS has implemented model selection among six different historical biogeographic scenarios (DEC, DEC + J, DIVALIKE, DIVALIKE + J, BayAreaLike, BayAreaLike + J) (Matzke 2013; 2014). Although, recently highlighted there are conceptual and statistical issues with the DEC + J model implemented in BioGeoBEARS, which can sometimes favor unparsimonious numbers for “jumping dispersal events”; and as a result it will not reflect a more close approximation of the “true” model of range evolution (Ree & Sanmartín 2018; Sánchez-Herrera et al. 2020). Ancestral area probability was inferred using the divergence time tree obtained in BEAST2. Likelihood values of these models were compared using the likelihood ratio test, and the best-fit model based on the corrected Akaike Information Criterion (AICc_wt) weights was used to compare the different models to select the most likely biogeographical scenario.

For our analyses, we designated fourteen distinct geological areas based on Hazzi et al. (2018), which include; (A) Apurimac, (B) Central Cordillera of Peru, (C) Cordillera del Norte, (D) Cordillera of Merida, (E) Eastern Cordillera of Colombia, (F) Eastern side of the Central Andes, (G) Northern Central Cordillera, (H) Serrania del Pérja, (I) SNSM, (J) Southern Yungas, (K) Tumbensian mountains, (L) Western Cordillera of Colombia, (M) Western Cordillera of Peru, and (N) Yungas (Fig. 2).
Results

Times of divergence

There was a congruence between age estimations between the Birth-Death model (BDM) and the Calibrated Yule speciation processes (CYS) (BDM: \( \sim 5.40 \) Mya (95% HPD: 4.40 – 6.39 Mya; PP=1), and CYS: \( \sim 5.44 \) Mya (95% HPD: 4.45 – 6.43 Mya; PP=1). Therefore, our average in the time divergence reconstruction suggests that the
MRCA (Most Recent Common Ancestor) of the genus *Manerebia* diverged ~5.42 Mya (Fig.3). This finding suggests that the lineages leading to the extant species of *Manerebia* started divergence around the later Miocene / early Pliocene boundary, and most extant lineages had already appeared by ~3.53 – 4.7 Mya during the middle Pliocene / early Pleistocene, and according to our results, many divergences at the species level occurred in the last few Mya (~2.7 Mya) during middle Pleistocene (Fig.3). The estimated mean age for crown *Manerebia* is ~23.9 Mya (95% HPD: 19.6–28.1 Mya) diverging from the clade (*Lymanopoda + Ianussiusa + Idioneurula + Diaphanos*) around the middle Miocene.
FIGURE 3. Estimated dates of times of divergence for clades in *Manerebia*. Node support: low/weak (UFB <50, PP <0.50), moderate (PP = 0.50–0.84), high (PP = 0.85–0.94) or strong (PP ≥0.95) – Photo by Kertell Ken ©.

The ‘navarre’ clade began to diversify during the early Pleistocene ~2.7 Mya (95% HPD 1.83–3.49 Mya, PP=0.96). The ‘cyclopina’ clade began to diversify around the later Pliocene / early Pleistocene boundary ~3.53 Mya (95% HPD 2.51–4.58 Mya, PP=0.97). The ‘pauperata’ clade began to diversify during the last Pleistocene ~0.84 Mya (95% HPD 1.83–2.49 Mya, PP=0.98). The ‘satura’ clade appears around the later Pleistocene ~1.98 Mya (95% HPD 1.16–2.78 Mya, PP=0.97). The ‘leaena’ clade began to diversify during the later Pleistocene ~1.86 Mya (95% HPD 1.25–2.51 Mya,
The ‘levana’ clade began to diversify around the later Pleistocene ~1.29 Mya (95% HPD 1.07–2.15 Mya, PP=0.97). The ‘rufanalis’ clade appears during the early Pleistocene ~2.05 Mya (95% HPD 1.33–2.78 Mya, PP=0.98). The ‘prattorum’ clade began to diverge around the later Pleistocene ~1.22 Mya (95% HPD 0.66–1.51 Mya, PP=0.97), and the ‘trimaculata’ clade began to diversify during the later Pleistocene ~1.74 Mya (95% HPD 1.04–1.99 Mya, PP=0.96) (Fig.3).

Diversification analyses

Lineage-through-time and based on 1000 sampled Bayesian plots (Fig.4A, B), and the gamma statistic ($\gamma = -1.9881; p\text{-value} = 0.0468$) indicate an overall decreasing diversification rate for the genus *Manerebia*. The decreasing in the diversification began since the late Pliocene and early Pleistocene boundary, and during the Pleistocene until present the diversification rate is low in the genus. Moreover, based on gamma statistic, the best model for our *Manerebia* phylogeny was the Yule Pure-Birth ($x^2 = -114.49; p\text{-value} = 4.5276e-11; \ln\text{Lik} = -.78.55; AICc= 159.16$). These results are supporting the diversification analysis by LTT.
FIGURE 4. A. Diversification patterns in the genus *Manerebia* by generating standard LTT plot. B. Lineage-through-time (LTT) plot based on 1000 sampled Bayesian.
On the other hand, our BAMM analysis converged after a few generations and the ESS values were high (> 861). This analysis shows that the speed of diversification slows down gradually towards the present (Fig. 5A), consistent with a density-dependent scenario. BAMM estimated that there is one probable pattern of diversification for *Manerebia* with a constant-rate model without any significant shift in net diversification rates, and, however, this model is not significantly better than a model without significant increases in net diversification (Bayes factor < 1). This model shows that there is a relative faster diversification rate at the origin of *Manerebia* which gradually decreases towards the present (Fig. 5A). The speciation and extinction rates estimated by BAMM was about $\lambda = 1.3$ species/my and $\mu = 0.1$, being the speciation rate initially high and declined gradually towards the present, while the extinction rate has been stable during the evolution of *Manerebia* (Fig. 5B). We could observe that the speciation rate was high around the later Miocene and early Pliocene boundary, and the speciation rate decreased in order until the early Pleistocene (~3.8 Mya), and the extinction rate showed no increase until the early Pleistocene and then showed a little increase until the present ($\mu < 0.2$).

We used the speciation and extinction parameters estimated by BAMM for the phylogeny of *Manerebia* to maximize the loglikelihood and parameters under a diversity-dependent model of diversification in the R package DDD, and it was able to maximize the loglikelihood of speciation and extinction for *Manerebia* under a density-dependent model of evolution and taking into account the number of missing species in our sampling. We found that the carrying capacity for *Manerebia* might be 74 species, $\lambda = 0.8$ species/my, $\mu = 0.033$ species/my and loglikelihood = $-206.4$. 
FIGURE 5. A. BAMM phylorate plot showing the best model that fit the data, the density-dependent model of diversification. Colours of the branches indicate speed of diversification rates (cold colours = slow, warm colours = fast). B. Speciation/Extinction rate v.s time before present plot for the genus *Manerebia*.

**Biogeographical reconstructions**

In the ancestral area reconstruction analysis, the most likely biogeographical scenario model was DEC + J based on the AICc_wt (Table 1). In addition, the DEC + J model estimated 25 dispersal, 14 vicariance, and 1 extinction event. Ancestral area reconstruction analysis indicated the origin of the genus *Manerebia* is not clear yet because the analysis showed three possible ancestral areas: CEGI, DEGI, or CDGI (Node 95) (Fig.6), and the three areas have the same low
probability of occurrence (P=0.09). However, it’s possible to see the biogeography and evolution patterns in the genus *Manerebia* were affected by dispersal events (Node 95; P=0.9). The model predicted speciation events within the areas: A:2 (Apurimac), B:7 (Central Cordillera of Peru), E:2 (Eastern Cordillera of Colombia), F:2 (Eastern side of the Central Andes), G:21 (Northern Central Cordillera), L:2 (Western Cordillera of Colombia), and N:2 (Yungas).

**Table 1.** Results of Model Test by BioGeoBEARS.

<table>
<thead>
<tr>
<th>Model</th>
<th>LnL</th>
<th>numparams</th>
<th>d</th>
<th>e</th>
<th>j</th>
<th>AICc</th>
<th>AICc_wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEC</td>
<td>-180.8</td>
<td>2</td>
<td>0.023</td>
<td>0.0055</td>
<td>0</td>
<td>365.8</td>
<td>0.0027</td>
</tr>
<tr>
<td>DEC+J</td>
<td>-173.7</td>
<td>3</td>
<td>0.018</td>
<td>1.00E-12</td>
<td>0.019</td>
<td>354</td>
<td><strong>0.96</strong></td>
</tr>
<tr>
<td>DIVALIKE</td>
<td>-181.2</td>
<td>2</td>
<td>0.028</td>
<td>3.50E-09</td>
<td>0</td>
<td>366.6</td>
<td>0.0018</td>
</tr>
<tr>
<td>DIVALIKE+J</td>
<td>-177.1</td>
<td>3</td>
<td>0.022</td>
<td>1.00E-12</td>
<td>0.016</td>
<td>360.8</td>
<td>0.033</td>
</tr>
<tr>
<td>BAYAREALIKE</td>
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<td>0.021</td>
<td>0.4</td>
<td>0</td>
<td>397</td>
<td>4.40E-10</td>
</tr>
<tr>
<td>BAYAREALIKE+J</td>
<td>-189.4</td>
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<td>0.017</td>
<td>0.3</td>
<td>0.012</td>
<td>385.3</td>
<td>1.60E-07</td>
</tr>
</tbody>
</table>

A dispersal event originated the ‘cyclopina’ clade (Node 94; P=0.92), and another the rest clades of the genus (Node 89; P=0.9). In addition, a possible one extinction, vicariance and dispersal event occurred in the ‘cyclopina’ group (Node 94; P=0.05). Node 93 apparently had as the ancestral area to I (SNSM) presenting one dispersal and vicariance event (P=0.8). The possible route was from the I to B area and from B to N (Node 92; P=0.13), but it’s a few probabilities that have been that. Node 90 had one vicariance event originating to *M. n.*sp. and *M. cyclops* species, and three dispersal events from B to ANJ (P=0.2) resulting the rest of the species of the ‘cyclopina’ clade (Fig.6).

On the other hand, it’s possible to observe that the rest clades of *Manerebia* had an ancestral area in G (Northern Central Cordillera) (Node 89; P= 0.97) (Fig.6). Then was a one dispersal event from G to ABFHKM areas originating the clades ‘pauperata’, ‘rufanalis’, and ‘navarrae’ (Node 87; P= 0.87). Node 82 presented five dispersal events from the G area to FKM (Node 81; P=0.8) and BA (Node 79; P=0.7) originating the clades ‘pauperata’ and ‘rufanalis’ respectively. In the ‘pauperata’ clade were two vicariance events arising to *M. n.*sp3 and *M. pauperata – M. ronda*, and in the ‘rufanalis’ clade was one vicariance event originating the *M. reducta – M. trirufa*, and *M. n.*sp11. For another part, the ‘navarrae’ clade presented three dispersal events (Node 85; P=0.82 ; Node 84; P=0.89; and Node 83; P=0.87), and one vicariance (Node 86; P=0.85) that originated the species that conform to the clade (Fig.6).
FIGURE 6. Reconstruction of the ancestral areas according to the RASP and BioGeoBEARS analyses by the genus *Manerebia*. The model select was DEC + J. Blue circles shows dispersal events; green circles shows vicariance events; and yellow circles an extinction event. The colors for each ancestral area indicate the Biogeographical regions of Fig.2.
The clades ‘satura’, ‘leaena’, ‘levana’, ‘prattorum’, and ‘trimaculata’ presented two dispersal events from the G ancestral area (Node 75; P=0.93). The first dispersal event originated the ‘satura’ clade (Node 54; P=0.92), and the second event arose the clades ‘leaena’, ‘levana’, ‘prattorum’, and ‘trimaculata’ (Node 74; P=0.95). Into the ‘satura’ clade was observed two dispersal events: the first event arise the M. altafina, M. satura, and M. benigni subgroup (Node 50; P=0.9), and in addition, one vicariance event originated the M. altafina and M. satura species (Node 49; P=0.95), and the second dispersal event occurred in Node 53 resulting in the M. rubescens, M. weymeri, M. santiludovici, and M. haywardi subclade (P=0.92). Moreover, one vicariance event originated the M. santiludovici and M. haywardi species (Node 51; P=0.93) (Fig. 6).

Additionally, from Node 74 was two dispersal events resulting in one the ‘leaena’ clade and the other in the clades ‘levana’, ‘prattorum’, and ‘trimaculata’ (P=0.95). Possibly one dispersal event arose the ‘leaena’ clade (Node 72; P=0.94), and one vicariance event originated the M. germaniae and M. leaena species (Node 71; P=0.94). At the same time, one vicariance event occurred that induced the separating the ‘levana’ clade from the clades ‘prattorum’ and ‘trimaculata’ (Node 70; P=0.95). One dispersal event originated the ‘prattorum’ clade (Node 58; P=0.93), and others vicariance events were seen in Node 56 (P=0.94) and 57 (P=0.9) into the ‘prattorum’ clade originating the M. prattorum and M. prietoi – M. antioquiana, and M. clara – M. romeliana species respectively. Finally, three dispersal events occurred into the ‘trimaculata’ clade, one event originated the M. milaena and M. trimaculata species (Node 61; P=0.92); the second event was observed in Node 64 resulting the M. n.sp2, M. undulata – M. similis (P=0.84), and the third event arise the M. undulata and M. similis (Node 63; P=0.8) (Fig. 6).

Discussion

Manerebia diverged from the clade ((Lymanopoda + Ianussiusia) + (Idioneurula + Diaphanos)) during the early Miocene (~23.9 Mya), a divergence time similar found by Chazot et al. (2018) (~23 Mya), being a divergence more recent than Lymanopoda - Ianussiusia (27 Mya) (Casner & Pyrcz 2010), and Idioneurula - Diaphanos (25.7 Mya) (Chazot et al. 2018), both clades appeared in the late Oligocene and early Miocene boundary; in these epochs South America was approaching the western subduction zone in the Pacific Ocean, causing both the rise of the Andes and a southward extension of the Meso-American peninsula (Torsvik et al. 2017), and the coast of northern Brazil, Colombia, south-central Peru, central Chile, and large swathes of inland Patagonia were subject to a marine transgression (Rossetti et al. 2013). Timing of the split of these clades, between 20 to 30 Mya, corresponds with very early Andean orogeny, and
subsequent diversification of these genera (including *Manerebia*) likely occurred when uplift resumed 10–15 million years later (Casner & Pyrcz 2010).

Likewise, according to our results, *Manerebia* diverged in the late Miocene and early Pliocene boundary, similar to *Oleria* Hübner, 1816 (Danainae, Ithomiini) that diversified mainly during the late Miocene and early Pliocene boundary (95% HPD: 8.0–3.0 Ma) (De-Silva et al. 2016). Other Andean butterflies, *Morpho sulkowskyi* group (Satyrinae, Morphini), diverged around 10 and 18 Mya during the middle-late Miocene (Nattier et al. 2017). The divergence of these genera is possible that the uplift of the Andes and its acceleration from the Miocene–Pliocene may have triggered diversification by isolating populations on either side (Gregory-Wodzicki 2000; Garzione et al. 2008; Elias et al. 2009). Besides, during the Miocene, the tropical Andes began to rise initially in the south, with high elevations being attained progressively later to the north, but the Andes likely exceeded 1000m in elevation in Bolivia and southern Peru by ~20 Mya, similar elevations were achieved in the Ecuadorian and Colombian Andes only in the last ~10 mya (Gregory-Wodzicki 2000; Chazot et al. 2018). However, some Andean butterflies have diverged prior to these orogenic events, for example, Ithomiine calibrations imply that several entirely montane genera in Ithomiini diverged from their sister taxa before (see: Chazot et al. 2018).

Most of the clades of *Manerebia* diverged during the early-middle Pleistocene boundary, a similar pattern was found in the genus *Lymanopoda* (Satyrinae, Pronophilina), a species-rich montane group distributed along the Andes (Casner & Pyrcz 2010; Pyrcz et al. 2018; Mahecha et al. 2019). *Lymanopoda* first diversified between ~15 and ~10 Mya, the formation of major clades occurred by ~10 and ~8 Mya, and most of the species-level diversification occurred since the end of the Miocene, ~6 Mya until the Pleistocene even post-Pleistocene (Casner & Pyrcz 2010). Adams (1985), Pyrcz & Wojtusiak (2002), Casner & Pyrcz (2010), Pyrcz et al. (2016), and Mahecha et al. (2019) suggested that the distribution pattern (stair-step) of pronophiline butterflies is the consequence of vicariance facilitated through Pleistocene glaciation cycles, wherein warm periods caused species to move up-slope, become isolated and diversify, and glacial periods pushed faunas to lower elevations where reinforcement and dispersal into adjacent mountain ranges ensued, and they argued that repetitions of this cycle might produce vertical stacking of species’ ranges through completely allopatric speciation processes. While it appears that the Pleistocene certainly contributed to the species-level diversity of *Manerebia*, is possible that the diversification has taken place
through peripatric speciation, by colonizing some isolated - ‘island’ mountains from a larger – ‘continental’ block (Adams 1985; Sanmartín et al. 2010; Mahecha et al. 2019; Mahecha-J. et al. 2021). On the other hand, the Pleistocene was also important in the diversification in others Andean butetrflies as the Oleria amalda Andean group (De-Silva et al. 2016), Morpho sulkowskyi group (Nattier et al. 2017).

Overall, the Manerebia show a rapid early burst in diversification followed by a marked decreasing diversification rate during their history, which is also reflected in the Andean genus Oleria (De-Silva et al. 2016), and in a genus from the Holarctic región, Erebia Dalman, 1816, that inhabit cold environments (arctic-alpine) (Peña et al. 2015). Decreasing diversification rates, particularly density-dependent rates, have been interpreted as a signature of adaptive radiation (e.g. Rabosky & Lovette 2008; Etienne et al. 2012; De-Silva et al. 2016), and possibly due to filling of niche space (Peña et al. 2015). There is mounting evidence that ecological limits for speciation (and thus clade richness) are related to geographic area (Losos & Schluter 2000; Kisel & Barraclough 2010) probably due to its relationship with resource availability (Peña et al. 2015). Moreover, when the Andes arose they created new biotic and abiotic conditions along their slopes, modified the climate in the Neotropic (Hoorn et al. 2010; Chazot et al. 2019), and there is evidence that the Andes influenced the diversification of Neotropical lineages, primarily by driving increased speciation rates, perhaps most spectacularly in the high altitude páramo habitat (Madriñán et al. 2013; Chazot et al. 2019a; 2019b; Vieu et al. 2021) as might have been occurred in the genus Manerebia.

Important clues to identify adaptive radiations involve finding cases of taxa that took advantage of ecological opportunities, such as access to a novel area of distribution or usage of novel host plants (Peña et al. 2015; Stewart et al. 2015). Pronophilina butterflies have been as host plants the bamboo Poaceae, especially the genus Chusquea Kunth (Poaceae) (Pyrcz 2004; Pyrcz et al. 2006; Mahecha-Jiménez et al. 2011; Montero & Ortiz 2013; 2014a; 2014b; Mahecha et al. 2019; Padrón et al. 2021). The Poaceae family diverged about ~65 and ~112 Mya after the K–Pg (Cretaceous–Paleogene) boundary (Prasad et al. 2011; Magallón et al. 2015; Foster et al. 2017), and many genera have diversified around the Miocene and Pliocene (Fisher 2011; Magallón et al. 2015; Spalink et al. 2016; Foster et al. 2017). The genus Chusquea diverged between ~14.5 and ~6.5 Mya around the middle-last Miocene and early Pliocene boundary (Fisher 2011; Nattier et al. 2017) consistent with the divergence of the genus Manerebia, even with the genus Lymanopoda, which includes a majority of cloud forest
species (Casner & Pyrcz 2010), and with the expansion and diversification of the *M. sulkowskyi* group, which occurred before the Quaternary, and their larvae eat *Chusquea* bamboo too (Casner & Pyrcz 2010; Nattier *et al.* 2017). Other Pronophilina species that have as host plant *Chusquea* are *Corades chelonis* Hewitson, *Corades medeba* Hewitson, *Corades dymanisis* Thiem, *Corades chirone* Hewitson, *Pedaliodes pallantis* Hewitson, and *Lymanopoda schmidtii* Adams (Pyrcz 2004; Greeney *et al.* 2010; Montero & Ortiz 2012a; 2012b; 2014a; 2014b). The diversification of the host plant, *Chusquea*, was likely a key event in the diversification of the subtribe Pronophilina as a whole, coupled with further ecological specialization in most genera (Pyrcz 2004; Casner & Pyrcz 2010; Mahecha-Jiménez *et al.* 2011; Marín *et al.* 2017; Mahecha *et al.* 2019; Padrón *et al.* 2021). In addition to this, ecological adaptation, such as diversification on new host plants, may have been key to the formation of some sympatric sister species (e.g. *M. milaena – M. trimaculata*), and expansion in larval diet breadth could have provided opportunities for colonization of new habitats, a process also observed in other butterfly species such as *Oleria* (De-Silva *et al.* 2016).

Although, there are some Pronophilina species that have other host plants, such as *Panyapedaliodes dryamaea* Hewitson whose host plant is *Poa annua* Linnaeus (Poaceae) (Pyrcz 2004; Montero & Ortiz 2014c), and *Neopedaliodes zipa* Adams, whose larvae eat *Carex jamesonii* Boott (Cyperaceae) (Montero & Ortiz 2013). In the case of the genus *Poa*, the estimated age for the crown node is about ~18–10 Mya, in the Middle Miocene, and the radiation of species into the Americas is estimated at almost the same age, in the late Miocene–early Pliocene (~8–4 Mya) (Giussani *et al.* 2016), and the genus *Carex* diverged about ~20 Mya around the early Miocene (Spalink *et al.* 2016). Unfortunately, there aren't diversification studies for these genera yet that allow us to infer the possible relationship between the divergence of these genera de butterflies and plants. Maybe a switch in the host plant in stem Pronophilina was likely a key event in its diversification, coupled with ecological specialization due to climate variations that have occurred during the Miocene–Pliocene–Pleistocene, have had affected their diversification patterns (Adams 1985; Casner & Pyrcz 2010; Mahecha *et al.* 2019).

On the other hand, the Andes Mountains were constructed during Mesozoic–Cenozoic subduction of the oceanic lithosphere beneath the South American Plate. Ocean–continent convergence has resulted in a continuous, generally north trending orogenic belt that closely follows the western edge of South America (see: Horton
2018). The Central Andes started to uplift in the Eocene–Oligocene boundary, and in the north the mountains first reached high elevations, progressively extending northwards (Gregory-Wodzicki 2000; Picard et al. 2008; Mahecha et al. 2019), and the uplift continued in the Miocene and accentuated in the Pliocene, ~2 Mya (Kellog 1984; Mahecha et al. 2019) when the range reached elevations even higher than today. Therefore, based on our analyses, we hypothesized that the *Manerebia* most likely diverged in Middle Central Andes and it extending from southeast Yungas spread out to Cordillera Central of Peru (including Apurimac region), passed by the eastern side of the Central Andes, and arrived the Northern Central Cordillera and since there it expanded to the three Colombian cordilleras. We supported our hypothesis due to the ‘cyclopina’ clade apparently being the first group that diverged within *Manerebia* (~3.53 Mya, HPD 95% = 2.51 – 4.5 Mya), and their current distribution is from the Middle Central Andes until the Northern Andes in Venezuela, a similar pattern also observed in other butterfly species such as *Veladyris* Fox, 1945, *Velamysta* Haensch, 1909, and *Godryris* Boisduval, 1870 (Nymphalidae: Ithomiini: Godyridina) (Chazot et al. 2016). Additionally, the Northern Central Cordillera was likely the diversification area for *Manerebia* due to the majority of clades of the genus diverging within this bioregion, presenting a higher speciation rate according to our results, and likely the *Manerebia* species dispersed from Northern Central Cordillera to Northern Andes. Besides, Northern Central Cordillera was likely to have been important in *Manerebia* diversification and several distinct distribution patterns emerge (Mahecha et al. 2019; Mahecha-J. et al. 2021).

Furthermore, apparently, the Northeastern of the Central Andes and Northern Central Cordillera were diversification points to *Manerebia* according to our results, because the ‘trimaculata’ clade is the most speciose and is distributed mainly in this zone, so that is possible that the diversification of the *Manerebia'* clades have been affected mostly by dispersal events during the Pleistocene, generating peripatric speciation by founder effect, and not so much for vicariance (Mahecha et al. 2019; Mahecha-J. et al. 2021). Also, from middle to late Miocene the Northern Andes had about half their present average elevation, for example, in Colombia the Western and Central Cordilleras already had rather high elevations at the Oligocene–Miocene boundary (~24–21 Mya) (Nattier et al. 2017; Mahecha et al. 2019), and the Eastern Cordillera present height occurred between ~6–3 Mya (Graham 2009), and they probably only attained cloud forest altitudes during the Pleistocene (Nattier et al. 2017). These uplifts and climate change history may have generated the formation of a
complex topography expressed by depressions, valleys, rivers, etc., acting as biogeographical barriers for the dispersal of Pronophilina butterflies through different geological periods, such as the Pliocene–Pleistocene (Mahecha et al. 2019), and the radiation of current Manerebia species was around the middle Pleistocene. Hence our hypothesis about the colonization by isolated –‘island’ continental areas by dispersal is supported (Casner & Pyrcz 2010; Mahecha et al. 2019; Mahecha-J. et al. 2021). For instance, in the genus Ithomiola Felder, C y R Felder, 1865 (Riodinidae: Mesosemiini), Hall (2005) showed that diversification within the group consisted of recurrent speciation events across different altitudes, including numerous colonization events of the Andes from other areas. However, there are some examples of geographically allopatric sister species, for example, M. pauperata – M. ronda separated by the Marañon river valley in Peru; M. romeliana – M. clara by Patia valley in Colombia; and M. germaniae – M. leaena distributed at Colombian Central Cordillera and East Cordillera respectively (see Pyrcz et al. 2006; Mahecha et al. 2019; Mahecha-J. et al. 2021).

Finally, further paleoecological studies show that paramos underwent robust elevational shifts in vegetation zones during the Pleistocene (~2.7–0.02 Ma), resulting in extensive changes in surface area and connectivity, led to rapidly changing distributions (an expansion and contraction of the range) of montane species of the fauna and flora, which originated new linages (Torres et al. 2013; Hazzi et al. 2018; Flantua et al. 2019; Mahecha et al. 2019), for example, the recent divergence between M. ronda and M. pauperata (~0.11 Ma, HPD 95% = 0.06 – 1.03). We hypothesize that changes in altitudinal distribution of ecological belts associated to Pleistocene climatic variations (after 2.6 Ma) may have favoured dispersal of Pronophilina butterfly species (e.g. between the northernmost Colombian and Venezuelan) by creating temporarily existing ecological corridors, and consequently, the dispersal of cloud forest species from older ranges might have been controlled by climate–driven altitudinal shifts of habitats, like those that caused by the glacial cycles (Adams 1985; Casner & Pyrcz 2010; Nattier et al. 2017; Pyrcz et al. 2017; Pyrcz et al. 2018; Mahecha et al. 2019).

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and diversification of the predominantly Andean butterfly subtribe Pronophilina (Nymphalidae, Satyrinae) based on phylogenetic data generated using modern molecular methods”. Molecular analysis was partly carried out in the laboratory of the Nature Education Centre, Jagiellonian University, Kraków, Poland (CEPUJ). The research trip of O. Mahecha-J. to Poland in 2019 was supported by PROM Programme of international scholarship exchange of PhD students and academics of the Jagiellonian University and Polish National Agency for Academic Exchange, agreement number: PPI/PRO/2018/1/00001/U/001.

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CONCLUSIONS

Chapter I.

The use of the molecular phylogenetic approaches and a large taxonomic sampling for several species of Satyrini allowed demonstrated the monophyly of the genus *Manerebia*, and determine its taxonomic position within the subtribe Pronophilina and clarify its phylogenetic relationships within the subtribe. Besides, provided a better understanding of the subtribe level phylogenetic relationships within the Satyrini, and allowed us to make some taxonomical arrangements in the tribe. Besides, we found that using larger taxonomic sampling may help to improve the problems when using individual genes and it allows to build systematic relationships more robust.

Chapter II.

The phylogenetic analyses, together with the species delimitation methods and the morphological characters, allowed us to evaluate the high cryptic diversity within the genus *Manerebia*. Hence, our study highlights the importance of employe the integrative taxonomy framework for the detection of cryptic diversity in regions such as the Neotropics. Therefore, the genus *Manerebia* comprises 58 nominal species, but for the moment some remain undescribed yet, and the number of the species could increase.

Chapter III.

Based on sequence data from mitochondrial (COI) and using phylogenetic tools the first phylogenetic hypothesis for the genus *Manerebia* was proposed. The monophyly of the genus is corroborated supporting previous morphological and molecular hypotheses. Nine clades are proposed for the *Manerebia* along the Central and Northern Andes being the Northern Andes the zone with the most richness. Finally, our results permitted us to clarify some of the phylogenetic relationships within the genus to species-level, but some internal nodes near to root were unresolved in the phylogenetic trees, indicating the need to use larger taxonomic sampling or include other molecular and morphological characters (e.g. genomic data) that may help to solve these problems and allow to build systematic relationships more robust within the genus.
Chapter IV.

The historical biogeography analyses allow us to conclude that the divergence time of *Manerebia* was recent, between the late Miocene and early Pliocene boundary, and the current lineages appeared during the Pleistocene. The genus had an overall early burst in the late Miocene / early Pliocene boundary followed by deceleration due to a decrease in speciation along to Pleistocene, and this pattern is reflected for all clades in *Manerebia*. Dispersal and colonization events are likely the most common processes within the genus, being the peripatric speciation mode (in some cases vicariance) the most frequencies within the genus *Manerebia*. Finally, our results confirm the role of the Andean geomorphological in the evolution of Neotropical biodiversity.
RESEARCH PERSPECTIVES

This thesis approaches several topics on systematics, phylogenies, taxonomy and historical biogeography, in an attempt to integrate them as a way to explore the evolutionary history of the genus *Manerebia* in the Neotropical realm. Thus, I can pinpoint the main research perspectives according to each chapter:

**Chapter I.** I hope I am able to include more molecular markers for the species which we couldn't use in the thesis and increase the taxonomic sampling to improve the phylogenetic relationships in our study. I would like to make genomics analyses (e.g. NGS) to improve and clarify the Satyrini phylogenetic relationships and be able to compare it with the molecular classic tools. There is a need to increase the number of studies about the Ecological and Historical Biogeography in Satyrini and apply new phylogenetic tools for contributing to knowledge about the evolutionary history in the tribe Satyrini and especially, subtribe Pronophilina.

**Chapter II.** I would like to include the species of *Manerebia* that I was not able to examine here, and to be able to include morphological characters within the species delimitation analyses, and be able to corroborate our results. I would like to use other molecular tools, such as SNPs, to study the genetic variation within populations of *Manerebia* and be able to know better the genetic structure that allows us to will understand better the divergence and diversification process of the *Manerebia* species. Besides, it is necessary to sequence more molecular markers and use them within new phylogenetic analyses to improve our results obtained. We are going to describe the new *Manerebia* species, which are not have described yet, and further taxonomic studies are necessary to discover the cryptic diversity within the genus *Manerebia*.

**Chapter III.** The phylogeny of the genus *Manerebia* continues to be under reconstruction including more species and more sequences of different genetic markers. In addition, it is necessary to include the species which were not examined in the frame of our phylogenetic analyses in order to improve and clarify the phylogenetic relationships in within *Manerebia*. Also, I am going to include the morphological characters within the analyses to do a taxonomy integral, and we are able to obtain a phylogenetic hypothesis most robust. I would like to work with the whole genome of *Manerebia* species using next-generation sequencing (NGS), delivering a base-by-base view of all genomic alterations, including single nucleotide variants (SNV), insertions and deletions, copy number changes, and structural variations, and be able to compare it.
with traditional molecular technics (Sanger), and be able to use it in phylogenetic analyses, and populations studies.

**Chapter IV.** There is a need to increase the number of studies about the ecological and historical biogeography in the *Manerebia* butterflies along the Neotropical region. I would like to approach the questions about, as for example, the mechanisms which enabled *Manerebia* butterflies to conserve their extraordinary diversity in the Andes mountains ranges, and try to explain its current biogeographical patterns. The answers could be provided by the integration of species distribution and niche-modeling into the phylogenetic reconstruction. Finally, a meta-analysis of already published studies could provide an insight into the role of ecological and neutral speciation and its frequency in Andean tropical mountain systems of similar age and altitudes.
LIST OF PUBLICATIONS

Chapter II - III.


Chapter IV.

LIST OF ATTENDANCE


SUPPLEMENTARY MATERIAL

Chaper I.

Access link to Drive:

https://drive.google.com/drive/folders/1cBCUG1WJsTaAcRcfQaWM_HgLkAN3P_o4?usp=sharing

Chaper II.

Access link to Drive:

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Chaper III.

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Chaper IV.

Access link to Drive:

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