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Sandra Rose Schachat

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The evolution of wing pattern in Micropterigidae (Insecta: Lepidoptera)

By

Sandra R. Schachat

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Agriculture and Life Sciences
in the Department of Biochemistry, Molecular Biology, Entomology, and Plant
Pathology

Mississippi State, Mississippi

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Despite the biological importance of lepidopteran wing patterns, homologies between pattern elements in different lineages are still not understood. Though plesiomorphic wing veins influence color patterning even when not expressed in the adult wing, most studies of wing pattern evolution have focused on derived taxa with reduced venation. Here I address this gap with an examination of Micropterigidae, a very early-diverged family in which all known plesiomorphic lepidopteran veins are expressed in the adult wing. Differences between the coloration of transverse bands in *Micropterix* and *Sabatinca* suggest that homologies exist between the contrast boundaries that divide wing pattern elements. Because the wing pattern of *Sabatinca doroxena* very closely resembles the nymphalid groundplan when plotted onto a hypothetical nymphalid wing following the relationship between pattern and venation discussed here, it appears that the nymphalid groundplan may have originated from a *Sabatinca*-like wing pattern subjected to changes in wing shape.

DEDICATION

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CHAPTER I
INTRODUCTION

“[T]he questions as to whether longitudinal or transverse markings are the most primitive, the number of the possible longitudinal stripes, their use, and the kindred questions seem to be decided so much by the caprice of the individual investigator, that a non-specialist may be permitted to retire from the field until the parties concerned have been able to patch up some sort of a truce.” (Newbigin 1898)

Introduction

The evolution of striped patterns on insect wings predates the radiation of Lepidoptera by many millions of years (Lemche 1935, Beckemeyer and Byers 2001). However, among extant insects, moths and butterflies have the most famously diverse wing patterns, and interest in the evolution of these wing patterns grew dramatically during the early twentieth century. Some of the greatest figures in nineteenth-century biology, such as Darwin (1871), Wallace (1865), Bates (1862), and Müller (1878), studied the ecological and behavioral significance of wing patterning in macrolepidoptera, particularly butterflies. With the publication of *Le dessin des ailes des lépidoptères* in 1902, the Countess Maria von Linden brought attention to the morphological evolution of wing patterning in microlepidoptera, macrolepidoptera, and

insects from a number of other orders (von Linden 1902). During the following fifteen years, other entomologists researched wing pattern morphology in the early-diverging families Gracillariidae (Braun 1914) and Hepialidae (van Bemmelen 1916). Attention then shifted back to macrolepidoptera, especially butterflies, as the development and evolution of symmetry systems became a popular topic of study (Schwanwitsch 1924, Koch and Nijhout 2002).

Moth and butterfly wing patterns seem to have lost their appeal by the 1950s, but Nymphalid wing pattern development came back to the fore during the 1990s (Nijhout 1991) and continues to be an exciting area of study (Nijhout 2010, Otaki 2012). Recent phylogenetic advances, facilitated by molecular techniques, have allowed biologists in the field of Evolutionary Developmental Biology to combine development with systematics (Kodandaramaiah 2009, Oliver et al. 2014, Monteiro 2015). The expanding scope and resolution of molecular phylogenies will allow comparative studies of wing pattern evolution in the different major lineages of Lepidoptera, and a few such studies have already been conducted (Monteiro et al. 2006, Martin and Reed 2010). However, this area of inquiry is hindered by the fact that wing pattern homologies are still not understood. Different families of macrolepidoptera have different numbers of symmetry systems on the wing, and the origin of these symmetry systems is still unknown (Nijhout 1994). Disagreement also exists as to whether the primitive lepidopteran wing pattern elements are spots (van Bemmelen 1916, Nijhout 2003) or transverse bands (Eimer and Fickert 1897, von Linden 1902, Braun 1914, Lemche 1935, Brown and Powell 1991). At present, the most complete summary of wing pattern evolution in basal moths is nearly a century old (Lemche 1937) and the most recent review of wing pattern evolution across

Lepidoptera focuses almost entirely on symmetry systems in macrolepidoptera (Nijhout 2003).

So far during the twenty-first century, the field of lepidopteran systematics has advanced by leaps and bounds (Mutanen et al. 2010, Regier et al. 2013, Kawahara and Breinholt 2014, Heikkilä et al. 2015). These advances have paved the way for studies of lepidopteran wing pattern in the context of phylogeny, but initial attempts in this area have highlighted the need for a wider evolutionary context (Reed and Gilbert 2004). Many questions remain: whether homologies exist among the wing pattern elements of Lepidoptera and other insect orders (Lemche 1935); whether developmental constraints determine the location of wing pattern elements (Braun 1914, Lemche 1935, Brown and Powell 1991); and through which mechanisms a primitive lepidopteran wing pattern gave rise to symmetry systems (Nijhout 1994).

Early Studies of Lepidopteran Wing Pattern Evolution

When studies of lepidopteran wings expanded from ecology and biogeography to development, anatomy, and morphology at the end of the nineteenth century, wing veins were examined first (Adolph 1879, Redtenbacher 1886). Research at the turn of the twentieth century, like earlier efforts, focused largely on butterflies. Certain workers began with comparative studies of pigmentation (Hopkins 1891, 1895) and scales (Kellogg 1894, Mayer 1896); others turned immediately to patterning.

After studying the evolution of color patterns in vertebrates and mollusks (Beddard 1892), G. H. Theodor Eimer proposed a wing pattern groundplan for Papilionidae comprised of eleven transverse “longitudinal bands” on the forewing, which he numbered with roman numerals (1889). He then used phylogenies to reconstruct the

evolution of his proposed groundplan, postulating that different wing patterns can evolve via the bands' tendency to fuse. In a later contribution with Fickert, Eimer examined both Papilionidae and Nymphalidae to identify common wing pattern elements which they then recognized in other superfamilies throughout the Lepidoptera (1897); beginning with the Papilionoidea, they worked their way down toward the root of the lepidopteran tree. Notably, Eimer did not consider wing venation to be an important determinant of the location of wing pattern elements.

Though Eimer paved the way for the subsequent research discussed below, all of the main tenets of his work were long ago demonstrated to be incorrect. Schwanwitsch (1924) and Süffert (Süffert 1927) showed that the wing patterns of butterflies, including Papilionidae, are comprised of symmetry systems, not individuated longitudinal bands. While the symmetry systems themselves appear to be comprised of multiple longitudinal bands, Eimer's bands do not consistently match symmetry system components (Schwanwitsch 1928a). His nomenclatural system was therefore shown to be invalid within a few decades. Furthermore, Eimer's phylogenies were not accepted, nor were his phylogenetic reconstructions of wing pattern evolution (Schwanwitsch 1949) and the extrapolation of his papilionid wing pattern groundplan to other, more basal lepidopteran families was rejected by workers who focused primarily on microlepidoptera (Lemche 1937). But of all Eimer's assertions, the one that was overturned most quickly was the purported lack of correlation between wing pattern and wing veins.

Von Linden accepted Eimer's model of eleven transverse bands, but emphasized the relationship between pattern elements and venation (von Linden 1902). Like Eimer, she set out to determine whether butterfly wing patterns contain phylogenetic signal – a

task that ultimately led her to compare papilionoid and ephemeropteran wing patterns, examining dipteran, neuropteran, homopteran, and orthopteran wing patterns along the way. Her approach was similar to Eimer's in that she began by examining butterflies (*Papilio* and *Vanessa*) and slowly expanded her scope to include other lineages, but she paid far more attention to "representatives which [had] hitherto been neglected in terms of their evolution," particularly Geometridae. In addition, Von Linden was interested in developmental aspects of butterfly wing patterns, such as the order in which colors develop and the different developmental rates on the fore- and hindwing.

In 1914, the American entomologist Annette Frances Braun undertook a similar study to Eimer's, tracing wing pattern evolution on the phylogeny of a single genus. But unlike Eimer, Fickert, and Von Linden, Braun chose a basal genus whose primary wing pattern elements really are transverse bands and not symmetry systems: the former genus *Lithocolletis* (now divided into *Phyllonorycter* and *Cameraria*) in the family Gracillariidae. Braun divided *Lithocolletis* into two groups that largely correspond to *Phyllonorycter* and *Cameraria*, and a reexamination of her study could very well uphold her conclusions. Braun ultimately concluded that the location of transverse bands was constrained by "neuration," or venation, at the costal margin of the wing: regardless of whether a transverse band runs vertically or diagonally down the wing, it always reaches the costal margin between the same veins. Braun's studies of microlepidopteran taxonomy (1948, 1963), evolutionary morphology (1921, 1928), and wing venation (1933) have had a lasting influence (e.g., Lemche 1937, Feir et al. 1990, de Prins and Kawahara 2012) and her work is still held in very high regard (Gilligan 2006), but her ideas on wing patterns have largely been forgotten.

In 1916, J.F. van Bemmelen published a study of the wing venation and wing pattern in an even more basal group of moths, the Hepialidae. Van Bemmelen was far more forthright than Braun in his insistence that wing pattern be studied in relation to wing venation, and that basal groups form the foundation for such studies:

“[T]he . . . regularity and completeness of the primitive pattern depend on its connection with the course of the wing-veins, the markings either following these veins, or being arranged in the interspaces between them, without transgressing their boundaries. . . . [T]he definite wing-skeleton arises from the modification of a provisional and more primitive one, which shows smaller differences between fore- and hindwings, and greater similarity to a general ground-plan, holding good for all different groups of Lepidoptera. In those families, which for different reasons are considered the most primitive, the imaginal system of wing-veins shows the least degree of deviation from this general plan, and for the same reason the greatest similarity to the distribution of wing-veins in other insect-orders nearly related to the Lepidoptera, such as the Trichoptera” (van Bemmelen 1916).

Hepialidae have complex wing patterns comprised of many pattern elements, including transverse bands of the sort studied by Eimer, von Linden, and Braun, as well as “spots and blotches” (van Bemmelen 1916). Van Bemmelen focused on the spots and blotches, and considered transverse bands to arise secondarily from the fusion of spots. Unlike Eimer, van Bemmelen did not propose a new nomenclatural system or predictive model, and unlike Braun, he did not identify a precise relationship between veins and pattern elements that could be tested by examining other moth families. Instead, his 1916

contribution is largely conceptual – “proof that these chains of [wing pattern] modifications are phylogenetically older than the genera themselves. We may even push their origin still further back, to beyond the branching point of the Lepidopterous order into its different families.”

However, during the time when Braun and van Bemmelen were probing the nature of primitive lepidopteran wing pattern elements, “the branching point of the Lepidopterous order into its different families” was still unknown. Modern phylogenetic studies clearly show that Micropterigidae, Agathiphagidae, and Heterobathmiidae are the three most basal crown lepidopteran families (Regier et al. 2013, 2015, Heikkilä et al. 2015), but Agathiphagidae and Heterobathmiidae were not known to science until the second half of the twentieth century (Dumbleton 1952, Kristensen and Nielsen 1979) and the position of Micropterigidae was subject to dispute during the early twentieth century: certain workers did assert that Micropterigidae are the most basal living Lepidoptera (Packard 1895, Meyrick 1912, Tillyard 1919), another elevated *Micropterix* to ordinal status (Chapman 1917), and Comstock argued that Micropterigidae are terrestrial trichopterans, writing that, should Micropterigidae ultimately remain within the Lepidoptera, the family must be “near the stem form from which the Trichoptera and the Lepidoptera have been evolved” (1918). This debate continued into the second half of the century as two great systematic entomologists, Hinton and Hennig, disagreed over whether Micropterigidae belong in the Lepidoptera or a separate order (Engel and Kristensen 2013).

Long before the debate over the systematic position of Micropterigidae was settled and Agathiphagidae and Heterobathmiidae were described, studies of wing pattern shifted focus toward macrolepidoptera.

During the 1920s, two independent researchers, Fritz Süffert and B.N. Schwanwitsch, converged on a nomenclatural system now known as the “nymphalid ground plan” (NGP) to describe wing pattern homologies in butterflies (Schwanwitsch 1924, Süffert 1927). Though Schwanwitsch’s publication precedes Süffert’s, H. Frederik Nijhout’s modern incarnation of the NGP relies more heavily on Süffert’s nomenclature (Nijhout 1991). The NGP is comprised of individual wing pattern elements that traverse the wing from the anterior to the posterior axis, much like fasciae; indeed, though the wing pattern element closest to the termen of the wing is comprised of ocelli, or eyespots, all other elements of the NGP are comprised of transverse bands that, considered individually, could be discussed as fasciae. However, a central tenet of the NGP is the developmental linkage between adjacent fasciae, such that fasciae form the distal elements of three “symmetry systems” (Süffert 1927, Nijhout 1991). Therefore, although fasciae are apparent on the wings of nymphalids and other butterflies, these color patterns must be described with more complex terminology. The origin of symmetry systems is far from settled, and some authors have argued that symmetry systems likely arose from spots with concentric rings of color, not from fasciae (Nijhout 1994). The NGP has had its history documented extensively (Nijhout 1991, 2003) and is subject to continuous revision (Otaki 2012).

While Süffert and Schwanwitsch studied butterflies, the experimental geneticist Alfred Kühn settled on the flour moth *Ephestia kühniella* as the system of choice for his

investigations into heredity and development. From 1907 to 1910 Kühn conducted early research on the effect of temperature on butterfly wing patterns, which he finally published during the decade when symmetry systems were first described (Kühn 1926). Also during the 1920s, R. Goldschmidt's work on *Lymantria* wing patterns (Goldschmidt 1927) inspired Kühn to begin working with moths (Harwood 1993). Kühn ultimately chose *Ephestia*, which had already been bred as a laboratory animal (Whiting 1921, cited in Rheinberger 2000) and whose wing pattern offered many advantages: simple composition, extensive phenotypic and genotypic variation, and rapid development (Harwood 1993).

Kühn's student Karl Henke began a dissertation about color patterning in the fire bug *Pyrrhocoris apterus* (Henke 1924, cited in Rheinberger 2000), later switched to *Ephestia*, and ended up breeding and examining over 100,000 moths (Rheinberger 2000). His contributions with Kühn, summarized by Harwood (1993), concerned the genetics and heritability of wing pattern modifications (Kühn and Henke 1930, 1936).

The comparative morphological work of Hennig Lemche represents a brief return to microlepidoptera during the 1930s. Initially, Lemche observed that spots occur at the points where veins bifurcate in both Pyralidae and Noctuidae. He then extrapolated from this observation, proposing a predictive model for the location of dark bands, or fasciae, on insect wings: the basal edge of each fascia lies along the points where wing veins bifurcate. Lemche had therefore generalized from spots to fasciae, and from derived, obtectomeran Lepidoptera to all insects. Lemche outlined this model in the first of two publications on this topic (1935). The second publication (1937) was dedicated mainly to descriptions of wing pattern in microlepidoptera, and also proposed a hypothesis for the

origin of symmetry systems: each symmetry system originated when one fascia underwent “hypertrophy” (Nijhout 2003) and a light band appeared in its center. In other words, each single, wide, dark band gave way to a light band bordered on each side by a thinner, dark band.

During the 1940s and 1950s, work on heredity focused intensely on the molecular basis of inheritance, and young students did not flock to studies that relied heavily on morphology, such as the work of Kühn, Henke, and Schwanwitsch. Schwanwitsch remained active throughout the 1940s (Schwanwitsch 1943, 1948, 1949) and went on to publish a summary of this work (Schwanwitsch 1956). Henke shifted focus from experiments on developmental genetics to conceptual work on the underlying mathematics of pattern formation. He had briefly considered the Liesegang-phenomenon, which concerns patterns formed by concentric bands, during the 1930s (Henke 1936), but during the late 1940s he presented a schematic of lepidopteran wing patterns that could be produced as the Liesegang-phenomenon progresses (Henke 1948). The progression of Liesegang-phenomenon patterns is uncorrelated with phylogeny, and the order in which Henke proposes that patterns would form according to the Liesegang-phenomenon does not match the order in which such patterns have appeared during lepidopteran evolution. However, Henke’s Liesegang taxonomy of lepidopteran wing patterns does offer a conceptual framework through which patterns can be categorized and compared.

Ten years after Watson and Crick published the mechanism through which genes are passed on by DNA (Watson and Crick 1953), K.C. Sondhi wrote a review of animal color patterns that showed how ideas about inheritance were shaped by the evolutionary morphology studies of Henke, Schwanwitsch, and others (1963). Sondhi writes that

“morphological observations . . . served as a starting point for detailed experimental analysis of wing patterns” that focused on “modifying genetic or environmental influences”: morphology is a means through which development and inheritance can be understood. Here, Sondhi begins by discussing wing pattern in various lepidopteran lineages but goes on to focus mainly on *Drosophila*, discussing characters that continue to be of interest to evolutionary developmental biologists, especially bristles. Regarding *Drosophila*, Sondhi mentions early potential applications of Turing’s reaction-diffusion patterns (1963).

A Modern Resurgence

Lepidopteran wing patterns were rarely studied in a macroevolutionary context for a number of decades. During the 1980s, H. Frederik Nijhout mentioned the NGP in publications on developmental physiology (1985) and only a few more years passed before he began to publish on morphology and evolution in light of the NGP (1990). While expanding on the NGP in a book (1991), Nijhout discussed the history of the NGP its applicability to other macrolepidoptera. The NGP has continued to be a rich area of interest to the present day and is continually updated (Otaki 2012).

Also during the year 1991, a model was published that predicts the relationship between wing venation and pattern in microlepidoptera (Brown and Powell 1991). Like the NGP, this predictive model for microlepidoptera has been updated in subsequent publications (Baixeras 2002). However, this model has received far less attention than Nijhout’s revival of the NGP – which is perhaps unsurprising in light of the fact that macrolepidopteran lineages whose wings patterns include symmetry systems, such as butterflies, have long been preferred for studies of wing pattern because these lineages

include various model organisms and tend to be relatively large-bodied and therefore easier to handle.

Very recently, an attempt was made to trace the evolution of wing pattern in all Lepidoptera by mapping elements of the NGP onto a preliminary, family-level phylogeny of the order (Martin and Reed 2010). Like early workers who published their results over a century ago (Eimer and Fickert 1897, von Linden 1902), Martin and Reed have examined all lepidopteran wing patterns in terms of the NGP, have ignored the earliest-diverging families, and have used a phylogeny that is poorly resolved and now out-of-date (Regier et al. 2013, Kawahara and Breinholt 2014, Heikkilä et al. 2015). Martin and Reed have demonstrated that, over a century after Eimer and von Linden began studying the evolutionary morphology of lepidopteran wing patterns, early research questions of wing pattern homology are still of interest. At present, with new ideas about evolutionary morphology (Brown and Powell 1991, Baixeras 2002, Otaki 2012) and major advances in phylogeny (Regier et al. 2013, 2015, Kawahara and Breinholt 2014, Heikkilä et al. 2015), these early research questions about the origins of lepidopteran wing patterns are more tractable than ever before. However, such questions can only be answered if previously ignored, early-diverging lineages of Lepidoptera are examined on their own terms.

CHAPTER II

COLOR PATTERN ON THE FOREWING OF *MICROPTERIX*: INSIGHTS INTO
WING VENATION AND WING PATTERN HOMOLOGIES
IN LEPIDOPTERA

Abstract

Wing patterns are key taxonomic characters that have long been used in descriptions of Lepidoptera; however, wing pattern homologies are not understood among different moth lineages. Here, we examine the relationship between wing venation and wing pattern in the genus *Micropterix*, among the most basal extant Lepidoptera, in order to evaluate the two existing predictive models that have the potential to establish wing pattern element homologies for the order. The location of wing pattern elements along the costal margin of the wing in *Micropterix* is consistent with the predictions of the model proposed for Tortricidae by Brown and Powell in 1991, later modified by Baixeras in 2002. The predictive power of this model for such distantly related taxa suggests that the model may hold across various superfamilies within Lepidoptera, and that fasciae, not spots, are the most likely primitive wing pattern elements for the order. In addition, the location of wing pattern elements suggests that the wing vein commonly termed Sc₁ may in fact be a different vein, which Comstock identified in Trichoptera and referred to as “a.”

Keywords

Developmental constraints; fasciae; groundplan; microlepidoptera; Micropterigidae.

Introduction

Many recent studies have examined the evolution of wing patterns in butterflies (Nijhout 2001, Monteiro 2015) and other macrolepidoptera (Monteiro et al. 2006, Martin and Reed 2010, Collins 2013, Suzuki 2013). The wing patterns of these taxa, and of other relatively derived moths such as Pyraloidea, are based on symmetry systems, which occur in different arrangements in various lineages (Nijhout 2003) and consist of parallel lines in two or more colors overlaid on a light ground color (Schwanwitsch 1924, Henke 1928, Nijhout 1991, Koch and Nijhout 2002). Early-diverged moths, often small and brown, lack symmetry systems; both spots (van Bemmelen 1916, Nijhout 1994) and transverse bands (Lemche 1935, Brown and Powell 1991) have been proposed as primitive wing pattern elements. Largely due to the fact that these basal lineages are poorly studied, homologous wing pattern elements have not yet been established for the order.

The current lack of knowledge regarding wing pattern homology is of great concern because wing patterning has been used to describe and differentiate species throughout the history of Lepidoptera systematics. In recent years, great progress has been made in the application of molecular data toward the lepidopteran tree of life (Mutanen et al. 2010, Kawahara et al. 2011, Regier et al. 2013). Because of the strong support for an integrated morphological and molecular approach to systematics (Giribet 2015, Pyron 2015), particularly for Lepidoptera (Scotland et al. 2003, Will and Rubinoff 2004, Wahlberg et al. 2005, Simonsen et al. 2012, Heikkilä et al. 2013), the use of wing

patterns as taxonomic characters would supplement other morphological characters, e.g., genitalia and venation, and improve the resolution of the lepidopteran tree of life. Because genitalia and venation are skeletal elements, their different components are relatively easy to isolate. Wing pattern, in contrast, is repetitive and can change drastically with few or no changes to skeletal characters such as wing venation. However, the fact that wing pattern homologies are not understood prevents the use of this character in large-scale phylogenetic studies. Also due to the poor understanding of homology in this area, inconsistent terminology is used to describe wing pattern elements, especially between different families.

Predictive Models

Wing venation has long been suspected to constrain lepidopteran wing patterns (Braun 1914, van Bemmelen 1916, Schwanwitsch 1928b). Two models predict primitive forewing patterning for Lepidoptera (Figure 2.1); both assume that fasciae, not spots, are the primitive wing pattern elements. “Fasciae” are generally regarded to be transverse bands suffused with dark pigment, interspersed between interfascial areas that are suffused only with the lighter pigment corresponding of the ground color. The first model (Lemche 1935, 1937), termed the “vein-fork” model here, posits that the basal edge of each fascia falls directly on the points where veins branch within the wing (Figure 2.1A). The second, termed the “wing-margin” model here, predicts the location of fasciae based on pairs of strigulae, or light markings, that occur between veins at the costal margin of the wing in Tortricidae (Brown and Powell 1991, Baixeras 2002). Fasciae are interspersed with interfascial areas between alternating pairs of strigulae and thus are constrained by the wing venation (Figure 2.1B). Therefore, the points where veins meet

the wing costa constrain the location of fasciae although some pairs of strigulae are not separated by veins on the tortricid wing, in which certain ancestral veins are known not to be expressed. Other authors have explored the wing pattern structure in Tortricidae (Falkovitsh 1966, Danilevsky and Kuznetsov 1968, Kuznetsov 1989), but we emphasize Brown and Baixeras' "wing-margin" model here because of its predictive potential. Both models are based on relatively derived moths: the "vein-fork" model was originally inspired by Pyralidae and Noctuidae and later was evaluated in taxa ranging from Lepidoptera to Paleodictyoptera (Lemche 1935, 1937), and the "wing-margin" model has only been proposed for Tortricidae (Brown and Powell 1991, Baixeras 2002), later adopted with further explanation by Gilligan et al. (2008).

Because Lepidoptera have an especially depauperate fossil record (Sohn et al. 2012, 2015), ancestral wing patterns cannot be reconstructed with paleontological data alone; extant analogues for early taxa must be used. However, many decades have passed since basal Lepidoptera (monotrysian moths) were examined in light of any predictive models. If either of the models discussed here holds for the entire order, then evidence should abound in many families of Lepidoptera, particularly in the basal groups. Furthermore, basal Lepidoptera are especially important regarding the relationship between color pattern and wing venation because basal moths have a more complete suite of venation than derived moths.

Family Micropterigidae

Micropterigidae has been considered to be at the very base of the lepidopteran phylogeny since Meyrick (1912), either by itself (Kristensen 1984, Mutanen et al. 2010) or with Agathiphagidae as a sister group to the remaining Lepidoptera (Regier et al. 2013,

2015). The relationship of the genus *Micropterix* to other Micropterigidae has been in flux. Certain workers have long suspected that *Micropterix* occurs at the base of the family-level phylogeny, distantly related to all other micropterigid genera (Skalski 1976a, Kristensen and Nielsen 1979). A more recent molecular study has confirmed the monophyly of *Micropterix*, but recovered the genus within a larger clade (Gibbs and Lees 2014). The oldest possible *Micropterix* fossil dates to the Early-Late Cretaceous boundary, approximately 100 million years before the present (Kühne et al. 1973, Kozlov 1988, Sohn et al. 2012). The oldest definitive *Micropterix* fossils, belonging to the species *M. immensipalpa*, date to the Lutetian Stage of the Middle Eocene, approximately 48 to 41 million years before the present (Kusnezov 1941, Skalski 1976b, Kozlov 1988, Kupryjanowicz 2001, Sohn et al. 2012).

Micropterigid wing venation resembles the groundplan reconstructed for the common ancestor of all Lepidoptera (Figure 2.2). The present study focuses on *Micropterix* in particular because its forewing patterns consist exclusively of dark fasciae and light interfascial areas (Figure 2.3), whereas both fasciate and non-fasciate patterns are present in other micropterigid genera such as *Sabatinca*. Because wing patterns in *Micropterix* include only two colors, a light tan and dark purplish-brown, the distinction between fasciae and interfascial areas is straightforward and unambiguous. Wing patterns of *Micropterix* can vary among individuals of the same species, as well as between species (Zeller-Lukashort et al. 2007), but there is little variation in wing venation (Figure 2.4). The varied forewing color patterns in this genus are therefore a suitable living analog for the primitive fasciate wing patterns in ancestral Lepidoptera assumed by both models discussed here.

Methods

All of the specimens examined for this study are held in the USNM Entomology collections in Washington, DC, USA. A total of 13 species of *Micropterix* were examined. For each species included in the study, all available specimens were reviewed to determine the number of differentiated fasciae and the number of confluent fasciae, or color fields. For species in which all individuals have wing patterns with the same number of differentiated fasciae, the forewing of one individual was illustrated. When forewing patterns with varying numbers of differentiated fasciae were observed among individuals of the same species, the venation-pattern relationship was recorded and illustrated for one representative of each variation. Likewise, variants with suffused interfascial areas and lack of expression in fasciae at the costa were illustrated.

Scaled wings, instead of cleared wings, were examined in order to observe the precise relationship between wing pattern and venation. Micropterigid wings are thinly scaled, and the venation becomes visible when specimens are lit from below using a microscope stage light. The observed wing venation was confirmed by examination of a wing slide of *M. anderschella* (USNM 91791) and the published literature (Heath 1976). To verify that the illustrations fully represent the species to which they correspond, up to 10 specimens per species – for a total of up to 20 forewings – were examined under a light microscope. (Results are discussed primarily in terms of wings instead of specimens because, in a few cases, only one forewing could be examined per specimen due to wear or due to the angle at which the specimen had been pinned. Furthermore, a number of specimens have pattern arrangements that varied between the two forewings.) To create illustrations, one forewing was photographed while backlit so that both the patterning and

venation were visible. This photograph was used as a template for the wing venation/wing patterning schematic. The location of the wing vein 1A+2A could not be observed in all pinned specimens because of the overlap between the forewing and hindwing, and therefore had to be inferred based on previously described venation (Heath 1976); however, this vein is of no relevance to either model because it does not bifurcate in the sense of Lemche's model, nor does it reach the costal margin. Similarly, the outline of the jugal lobe had to be inferred because this feature was often folded in the specimens examined; its outline was inferred based on previous descriptions (Heath 1976). Inferred features are illustrated with dashed lines.

Support for the "vein-fork" model was assessed based upon whether the basal edges of fasciae lie along the points where veins bifurcate (Figure 2.1A); the points where veins meet the costal margin (costa) and inner margin (dorsum) of the wing were not taken into consideration because they are not part of this model. Support for the "wing-margin" model was assessed based on whether fasciae reach the costa between the same veins as in Tortricidae (Figure 2.1B); the points where veins bifurcate and meet the inner margin of the wing were not taken into consideration because they are not part of this model.

Süffert identified five basic pattern elements on lepidopteran wings: ripple patterns, dependent patterns (encompassing all pattern elements that depend on wing venation), eyespots (ocelli), crossbands (fasciae), and color fields (1929). These terms are in continuous use (Brown and Powell 1991, Nijhout 1994). Because none of the *Micropterix* wing patterns studied were found to contain ripple patterns or eyespots and because the aim of the present investigation is to determine whether dependent patterns

exist in *Micropterix*, the main terms used here are “fasciae” and “color fields.”

Differentiated fasciae are transverse bands that are bordered by interfascial areas on each side. “Dark color” refers to the dark purple/brown color associated with fasciae. “Ground color” refers to the light beige color associated with interfascial areas. Because this term is conventionally used, it is employed here for the sake of continuity; however, we caution that “ground color” is not meant to imply any sort of priority or developmental sequence, nor should this imply lack of pigmentation. The apparent boundary between a fascia and an interfascial area can change in a number of ways. “Color fields” are wider patches that are formed when an interfascial area is subject to “complete suffusion” – the interfascial area is suffused entirely with dark color, so that the two adjacent fasciae appear “confluent.” A plus sign (+) is used here to denote the confluent fasciae embedded in a single color field. “Incomplete suffusion” refers to instances in which an interfascial area is partially suffused with dark color at the costal margin of the wing such that dark scales surround the vein that the interfascial area straddles; thus, the fascia appears to have expanded. “Incomplete lack of expression” refers to instances in which a fascia is not fully expressed at the costal margin of the wing so that the ground color surrounds the vein that the fascia normally straddles; the interfascial area therefore appears to have expanded.

Results

We examined a total of 172 forewings representing 13 species of *Micropterix* (Table 2.1). *M. aglaella*, *M. allionella*, *M. anderschella*, *M. aureoviridella*, *M. rothenbachii*, *M. schaefferi*, and *M. tunbergella* have wing patterns with six differentiated fasciae, all separated by visible interfascial areas (Figure 2.5). Very few forks in the

venation lie along or immediately adjacent to the basal edge of any fasciae. The basal fascia extends from the costa to the dorsum in all species except *M. aglaella*, in which this fascia extends from costa to midwing. The subbasal fascia extends from Sc₁ on the costa to the dorsum. The median fascia extends from Sc₂ on the costa down to the dorsum. The postmedian fascia originates from R₁ on the costa but becomes confluent with the median fascia at midwing, with varying degrees of ground color between the two fasciae. The preterminal fascia originates from Rs₂ and extends to M₂ on the dorsum, usually becoming confluent with the median + postmedian fasciae near the dorsum. The terminal fascia is a spot of varying size at Rs₄. The interfascial area that separates the terminal and preterminal fasciae is difficult to see under some types of lighting, and may be imperceptible in specimens that are old or rubbed. All fasciae are separated by interfascial areas, with the two most apical interfascial areas straddling or abutting Rs₁ and Rs₃, respectively, at the costa. The positions of these fasciae on the costa relative to venation are the same as those in wing pattern model proposed for Tortricidae (Brown and Powell 1991, Baixeras 2002) except that Tortricidae have only one Sc vein, and Rs₄ intercepts the wing margin at the termen (rather than the costa in Micropterigidae) resulting in absence of a distinct terminal fascia. When present in tortricids, the remnant of the terminal fascia is sometimes termed an “apical spot.”

Three of the *M. rothenbachii* wings examined have four differentiated fasciae, plus one color field produced by confluence of the preterminal + terminal fasciae (Figure 2.6). The majority of species examined (9) include individuals whose wings have two color fields. Consequently, the forewing has four dark areas, only two of which are comprised of differentiated fasciae. *M. sicanella* has a unique wing pattern due to the

interfascial areas that have become suffused: its two color fields are formed by confluent basal + subbasal and preterminal + terminal fasciae (Figure 2.7). All examined specimens of *M. aruncella*, *M. aureatella*, *M. corcyrella*, *M. erectella*, and *M. rablensis* and 19 of 20 *M. aglaella* wings have color fields formed by suffusion of the interfascial areas between the median + postmedian and preterminal + terminal fasciae (Figure 2.8). The same pattern of suffusion of these interfascial areas can be seen in certain individuals belonging to *M. aureoviridella* and *M. rothenbachii*, two species that also include other specimens with six differentiated fasciae at the costa (Figure 2.5D,E and Figure 2.8D,H). In these species with both differentiated fasciae and color fields, as in the other species discussed above, very few forks in the venation lie along the basal edge of a fascia.

We found eight of the species examined (*M. aglaella*, *M. allionella*, *M. aruncella*, *M. aureatella*, *M. rablensis*, *M. rothenbachii*, *M. schaefferi*, *M. sicanella*) to contain individuals displaying incomplete suffusion of interfascial areas and/or lack of expression of fasciae at the wing costa (0). In most cases, this involves the interfascial area between the postmedian + preterminal fasciae. One type of incomplete suffusion appears in *M. allionella*, *M. aglaella*, *M. aruncella*, *M. aureatella*, *M. rablensis*, *M. rothenbachii*, and *M. sicanella*: the interfascial area is suffused with dark color along the edge of the preterminal fascia, forming a larger dark pattern element that also straddles the Rs_1 vein at the costa; this leaves a smaller patch of ground color that does not straddle or abut any vein at the wing costa (Figure 2.9A). On the wings of some *M. aureatella* specimens, the postmedian + preterminal fasciae are entirely confluent along the costa (Figure 2.9B); this may represent a further step in the suffusion process than that seen in Figure 2.9A. Suffusion of the basal-subbasal interfascial area is incomplete in *M. schaefferi* (Figure

2.10), and this is interpreted as an intermediate step between separate and confluent basal + subbasal fasciae.

Some specimens have one wing that follows the basic groundplan and one that shows incomplete suffusion or lack of expression at the costa. One *M. aglaella* specimen has a lack of expression in the postmedian fascia such that the adjacent interfascial area appears to straddle not only Rs_1 but also R_1 at the costa (Figure 2.9C); this type of lack of expression is also present on one wing of a *M. rablensis* specimen whose other wing shows no modifications of the basic groundplan. Due to lack of expression of the preterminal fascia at the costa, this same interfascial area appears to have expanded in the opposite direction on the wings of two other *M. rablensis* specimens, and one wing of a *M. sicanella* specimen: a patch of ground color straddles Rs_1 and also Rs_2 at the costa (Figure 2.9D).

A few other modifications of the groundplan due to incomplete suffusion/lack of expression occur on other areas of the costa. In *M. schaefferi*, the basal + subbasal fasciae are confluent along the costa, but not at midwing (Figure 2.10); this may be an intermediate step in the evolution of the wing pattern seen in *M. sicanella*, in which these fasciae are completely confluent (Figure 2.7). Lastly, *M. rothenbachii* shows two modifications of the groundplan involving the interfascial area that precedes the terminal fascia. In the first, there is incomplete suffusion of this interfascial area along the boundary with the preterminal fascia at the costa (Figure 2.11A); this may represent an intermediate step in the evolution of the wing pattern with complete suffusion that was observed in other *M. rothenbachii* specimens (Figure 2.6). Another *M. rothenbachii* specimen shows an opposite modification of the groundplan, in that there is lack of

expression of the terminal fascia at the costa, making this interfascial area appear larger (Figure 2.11B).

Overall, *Micropterix rothenbachii* is the most variable of the 13 species examined. Of the 20 wings examined, 15 have six differentiated fasciae and no color fields (Figure 2.5E, Figure 2.11A). One wing is unique in having no apparent terminal fascia due to lack of expression (Figure 2.11B). Two wings have color fields formed by suffusion of the interfascial area between the preterminal + terminal fasciae (Figure 2.6). Two wings have only two differentiated fasciae, basal and subbasal, and two color fields formed by confluence of the median + postmedian and preterminal + terminal fasciae (Figure 2.8).

Discussion

Because very few forks in the wing veins align with the edges of fasciae, *Micropterix* shows little support for Lemche's "vein-fork" model. Forewing patterns in *Micropterix* fit Brown and Baixeras' "wing-margin" model. The five fasciae known from tortricids occur along the wing costa exactly as predicted by the model. On the wing costa in both Tortricidae and *Micropterix*, the basal and subbasal fasciae occur basal to the Sc vein (Sc₂ in *Micropterix*), the median fascia straddles the Sc vein, the postmedian fascia straddles R₁ (R_{1b} in *Micropterix*), and the preterminal fascia straddles Rs₂. A terminal fascia abuts or straddles Rs₄ in *Micropterix*, but this fascia was not defined for Tortricidae in the "wing margin" model because this vein terminates on the outer margin (termen), not the costa, of tortricid wings (Figure 2.1B). Rather, the terminal fascia in *Micropterix* may correspond with what is known in some species of Tortricidae as an "apical spot." Regardless, when present in *Micropterix*, the terminal fascia follows the

pattern that was first recognized in the five tortricid fasciae: among the Rs veins, each fascia straddles/abuts one vein at the costa, and all fasciae are separated by an interfascial area that also straddles/abuts one vein at the costa. The *Micropterix* groundplan requires a slight alteration of the “wing-margin” model due to differences in wing shape between Micropterigidae and Tortricidae – in both families, the underlying concept is the same: at the costa, each fascia and each interfascial area straddles or abuts one vein; beyond R₁, all primitive veins are visible in both Micropterigidae and Tortricidae and the venation-fascia relationship can be readily observed.

None of the instances of incomplete suffusion or lack of expression at the costa violate the “wing-margin” model. When an interfascial area is incompletely suffused with dark color, causing an adjacent fascia to appear larger, this seemingly enlarged fascia continues to straddle the vein originally predicted by the model. When there is lack of expression of a fascia, causing an adjacent interfascial area to appear larger, the interfascial area continues to straddle the vein originally predicted by the model.

In the original “wing-margin” model proposed for Tortricidae (Figure 1B), fasciae straddle alternating veins on both the costal and inner margins of the wing. In *Micropterix*, the wing pattern groundplan is not nearly as clear on the inner margin (dorsum) as it is on the costal margin (costa) due to extensive suffusion of interfascial areas. The evidence available from *Micropterix* suggests no firm conclusions about the relationship between fasciae and the inner margin.

Developmentally, this groundplan requires a mechanism through which vein position could pattern elements even when veins are not expressed. Because this phenomenon has also been observed in other Lepidoptera – for example, the “Cu2” and

“Pc” eyespots on nymphalid wings are separated by a vein that is not maintained in the adult wing (Süffert 1927) – such a developmental mechanism must exist. Studies of wing vein development in microlepidoptera are lacking. In butterflies, the transcription factors Notch and Distal-less serve as markers during wing vein development (Reed and Serfas 2004) and the transcription factors Spalt, Cubitus interruptus, and Engrailed are sector- or compartment-specific (Keys et al. 1999, Monteiro 2015); transcription factors of this sort could produce the *Micropterix* groundplan. At present, most of the genes involved in insect wing vein development, such as *dpp*, are known only from *Drosophila* (Bier 2000, Sotillos and de Celis 2006, Araujo et al. 2011, Raftery and Umulis 2012), with their potential functions occasionally considered for other insect groups (McMillan et al. 2002, de Celis and Diaz-Benjumea 2003, Monteiro 2015).

In *Micropterix*, as in Tortricidae, not all boundaries between fasciae and interfascial areas (marked by strigulae on tortricid wings) are separated by veins – three additional veins would be needed in order for each fascia to straddle one vein and for all fasciae to be separated by one vein. This is likely due to ancestral veins that are missing in these taxa. Between veins R_{1b} and Rs₄, all of which are present in *Micropterix*, fasciae and interfascial areas all straddle or abut one vein. Between the base of the wing and the R_{1b} vein, veins are known to be missing; *Micropterix* has only two veins in this area of the wing, but other micropterigid genera, as well as extinct basal Lepidoptera, have four: h, Sc₁, Sc₂, and R_{1a} (Skalski 1979, Nielsen and Kristensen 1982, Zhang et al. 2013, Gibbs 2014). If the two additional, ancestral veins – h and Sc₁ – are included in the *Micropterix* wing pattern groundplan, only one more vein is needed in order for all fasciae to straddle one vein and to be separated by one vein. Such a vein – located basally to R₁ along the

costa, and missing in Lepidoptera – can be found between the h and Sc₁ veins on the forewings of some basal Trichoptera, e.g. *Rhyacophila fuscula* (Figure 2.12), as well as the basal, Permian mecopteroid genus *Agetopanorpa* (Comstock 1918, Minet et al. 2010). Comstock termed this vein “a” in *Rhyacophila* (1918). This vein occurs in few species and, perhaps for this reason, is not mentioned in recent treatments of the trichopteran wing groundplan (Schmid 1989), but its presence was confirmed by our own examinations of *Rhyacophila fuscula* in the Mississippi Entomological Museum. When plotted in order along the costa of the *M. anderschella* forewing, the aforementioned veins produce a groundplan in which each fascia-interfascial boundary is separated by one vein (Figure 2.13). This suggests a new groundplan for primitive wing patterning in ancestral moths (Figure 2.14).

This groundplan has implications for wing vein homology in Lepidoptera. Of the two visible veins that precede R_{1b} in *Micropterix*, one is straddled by the subbasal fascia at the costa and the other is straddled by the median fascia. According to the “wing-margin” model, these veins must be “a” and Sc₂. However, in basal moths such as Micropterigidae and ancestral Lepidoptera, these veins have long been referred to as Sc₁ and Sc₂ (Comstock 1918, Tillyard 1919, Scoble 1995, Mey 2011, Zhang et al. 2013). Our analysis of wing pattern suggests that, particularly in taxa that are closely related to *Micropterix*, the vein that is often termed Sc₁ may in fact be Comstock’s trichopteran “a.”

Conclusions

Along the costa, fasciae always occur between the same wing veins regardless of how many instances of suffusion or lack of expression have occurred. The fascia-venation relationship is the same in *Micropterix* as in Tortricidae despite the many

millions of years of evolutionary history that separate these two lineages. The similar wing pattern groundplans in Micropterigidae and Tortricidae suggest that fasciae, not spots, are the primitive wing pattern elements for Lepidoptera. The results reported here also suggest that these fasciae are homologous between the families Micropterigidae and Tortricidae, which would strongly imply that these wing pattern elements are primitive in Lepidoptera and homologous in all taxa in which they are present. Future research should focus on other genera within the Micropterigidae, and on the many superfamilies of Lepidoptera that bridge the phylogenetic gap between Micropterigidae and Tortricidae, in order to determine the prevalence of fasciate wing patterns that fit the “wing-margin” model.

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Tables

Table 2.1 Numbers of differentiated fasciae and color fields on the forewings examined for this study.

Species	6 differentiated fasciae; 0 color fields	4 differentiated fasciae; 1 color field	2 differentiated fasciae; 2 color fields	Total
<i>M. aglaella</i>	1		19	20
<i>M. allionella</i>	6			6
<i>M. anderschella</i>	20			20
<i>M. aruncella</i>			20	20
<i>M. aureatella</i>			20	20
<i>M. aureoviridella</i>	2		2	4
<i>M. corcyrella</i>			10	10
<i>M. erctella</i>			2	2
<i>M. rablensis</i>			20	20
<i>M. rothenbachii</i>	15	3	2	20
<i>M. schaefferi</i>	6			6
<i>M. sicanella</i>			4	4
<i>M. tunbergella</i>	20			20
TOTAL	70	3	99	172

For an explanation of terms used, see the last paragraph of the Methods.

Table 2.2 Total number of wings examined, and number of wings displaying incomplete suffusion or incomplete lack of expression at the wing costa, per species.

Species	Total	Incomplete . .. at costa
<i>M. aglaella</i>	20	9
<i>M. allionella</i>	6	2
<i>M. anderschella</i>	20	
<i>M. aruncella</i>	20	6
<i>M. aureatella</i>	20	15
<i>M. aureoviridella</i>	4	
<i>M. corcyrella</i>	10	
<i>M. ertella</i>	2	
<i>M. rablensis</i>	20	6
<i>M. rothenbachii</i>	20	14
<i>M. schaefferi</i>	6	3
<i>M. sicanella</i>	4	2
<i>M. tunbergella</i>	20	
TOTAL	172	57

For an explanation of terms used, see the last paragraph of the Methods.

Figures

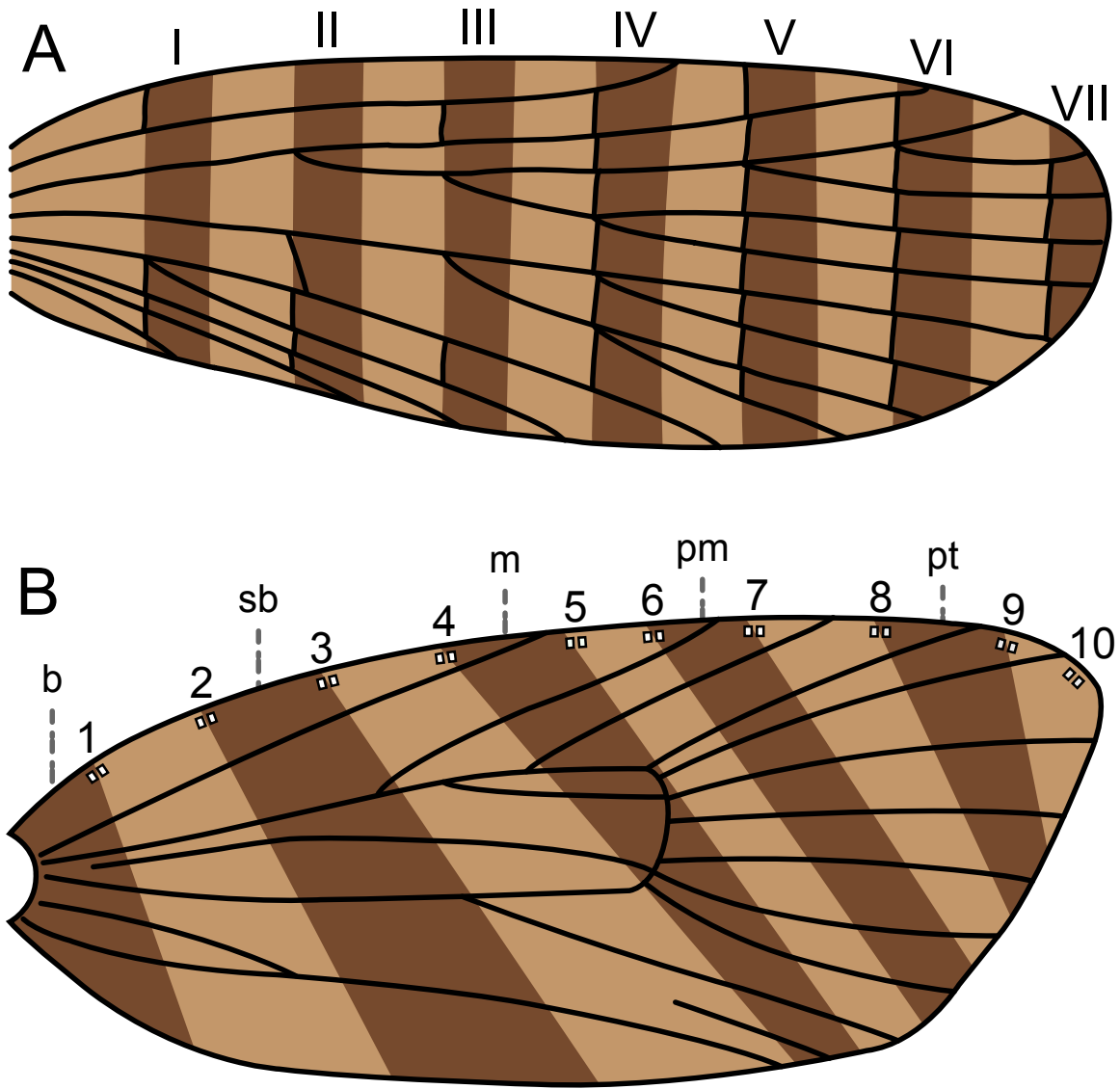


Figure 2.1 The two predictive models that relate wing pattern to wing venation.

The beige areas of the wing represent the lighter, interfascial areas; the dark brown areas represent the fasciae. (A) Lemche's "vein-fork" model, shown on a panorpoid wing (Lemche 1935). (B) Brown and Baixeras' "wing-margin" model (Baixeras 2002).

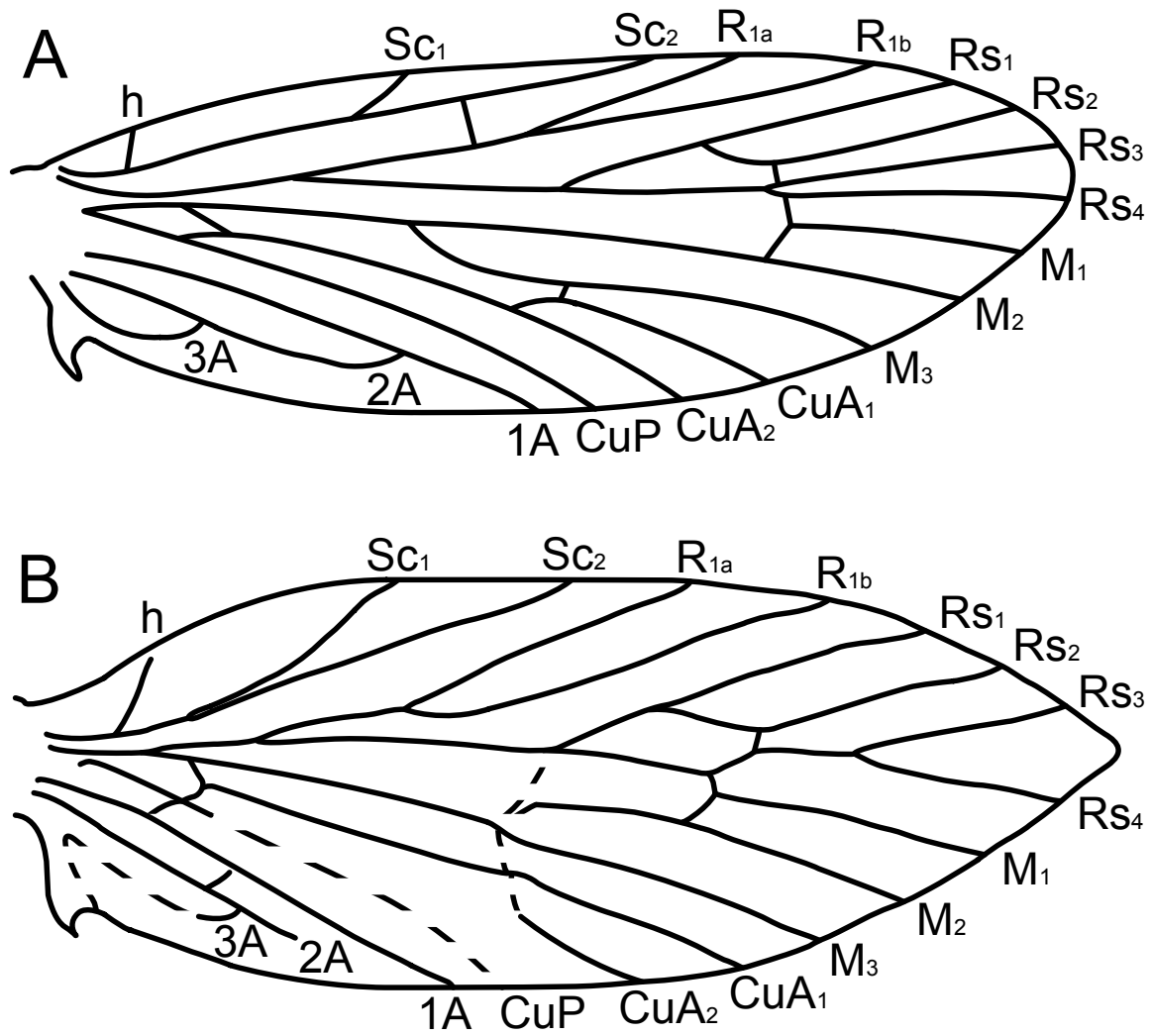


Figure 2.2 Primitive Lepidopteran wing venation.

(A) The wing venation groundplan for ancestral Lepidoptera (Zhang et al. 2013). (B) A micropterigid wing venation groundplan, based on the genus *Sabatinca* (Scoble 1995).

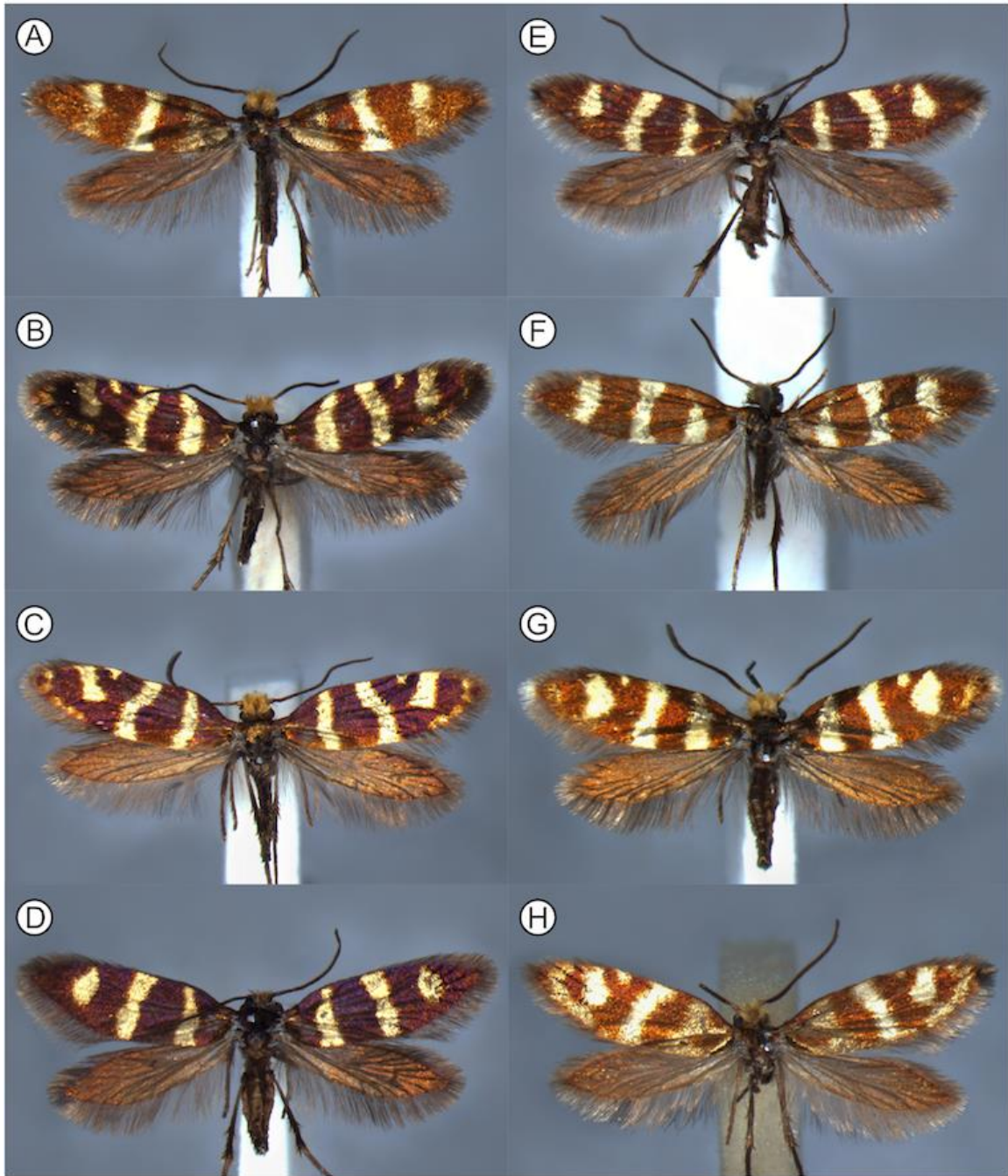


Figure 2.3 Photographs of some of the species examined in the present study, showing a sampling of the variety of *Micropterix* wing patterns.

(A) *M. aglaella*. (B) *M. allionella*. (C) *M. anderschella*. (D) *M. aureatella*. (E) *M. aureatella*. (F) *M. rablensis*. (G) *M. rothenbachii*. (H) *M. schaefferi*.

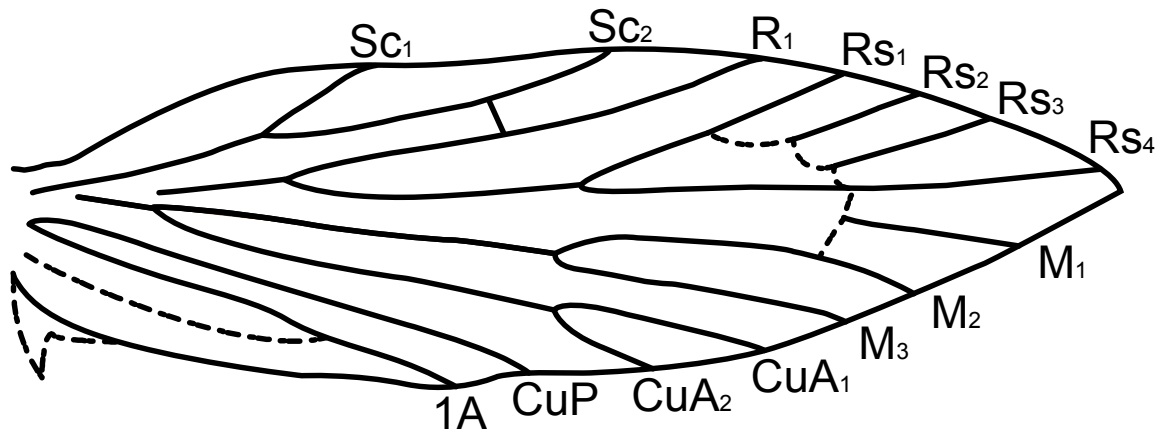


Figure 2.4 Wing venation of *Micropterix anderschella* labeled according to currently recognized nomenclature as currently recognized.

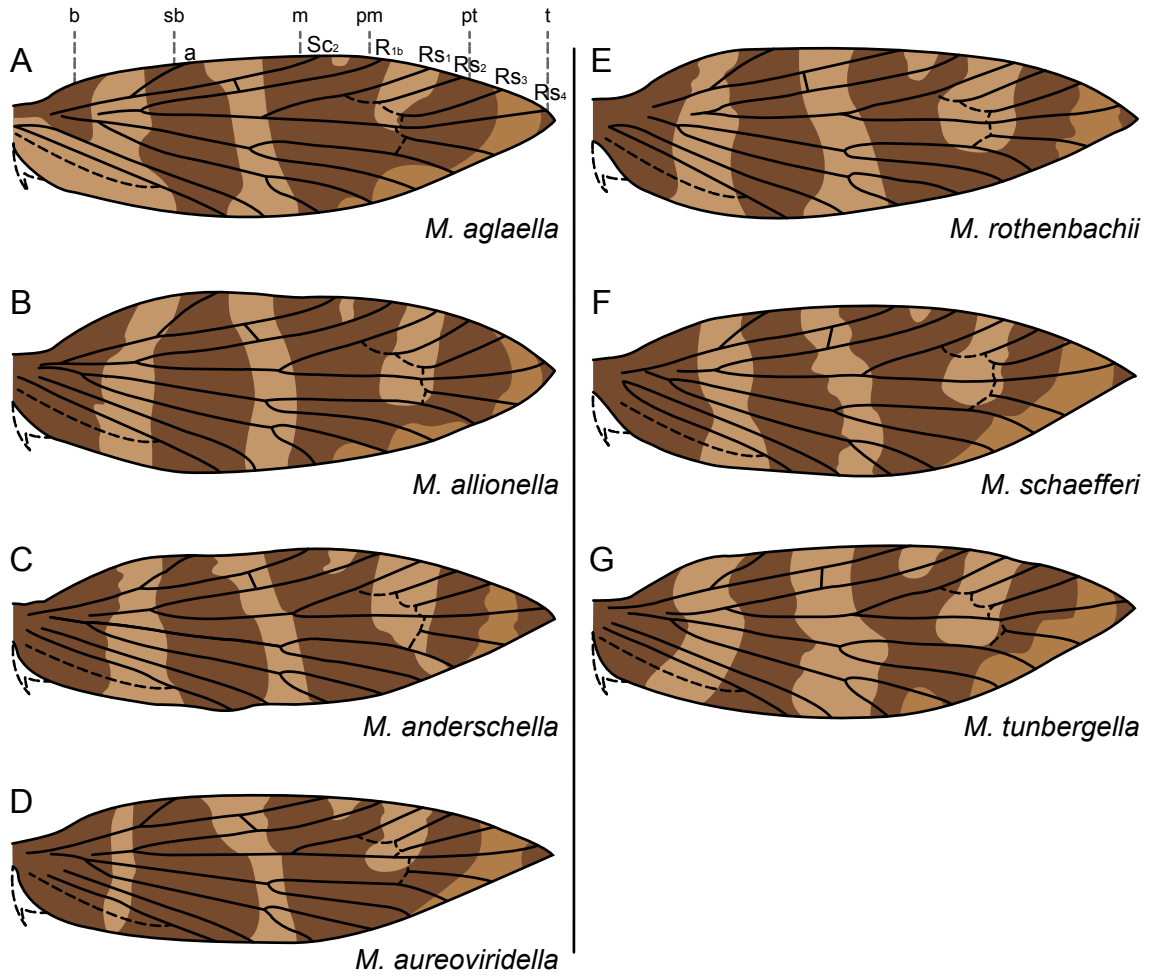


Figure 2.5 *Micropterix* wing patterns with no color fields and no incomplete suffusion or lack of expression at the costa.

For an explanation of the nomenclature used for veins on the wing costa, see Discussion. Legend: **b**: basal; **sb**: subbasal; **m**: median; **pm**: postmedian; **pt**: preterminal; **t**: terminal.

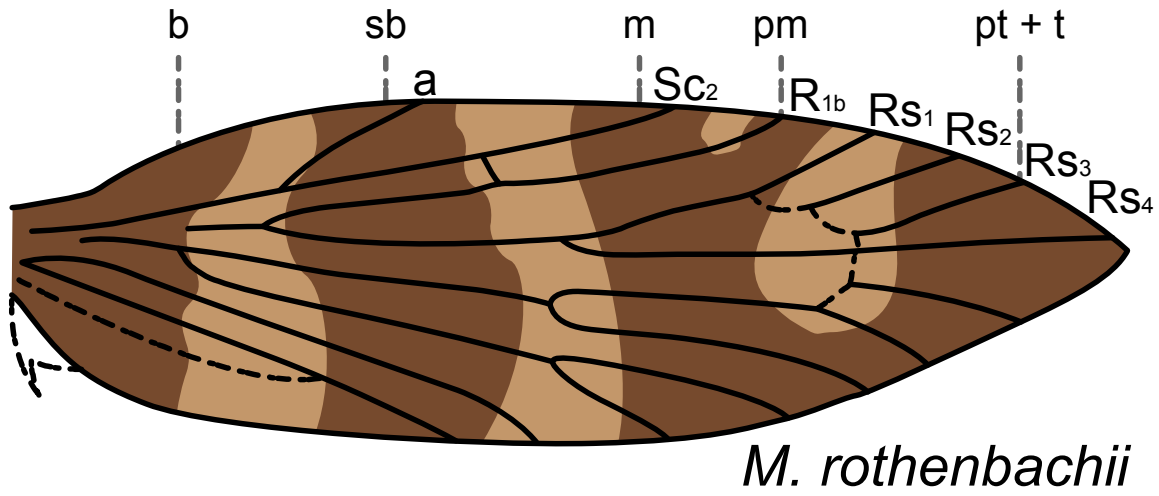


Figure 2.6 A *Micropterix* wing pattern with one color field and no incomplete suffusion or lack of expression at the costa.

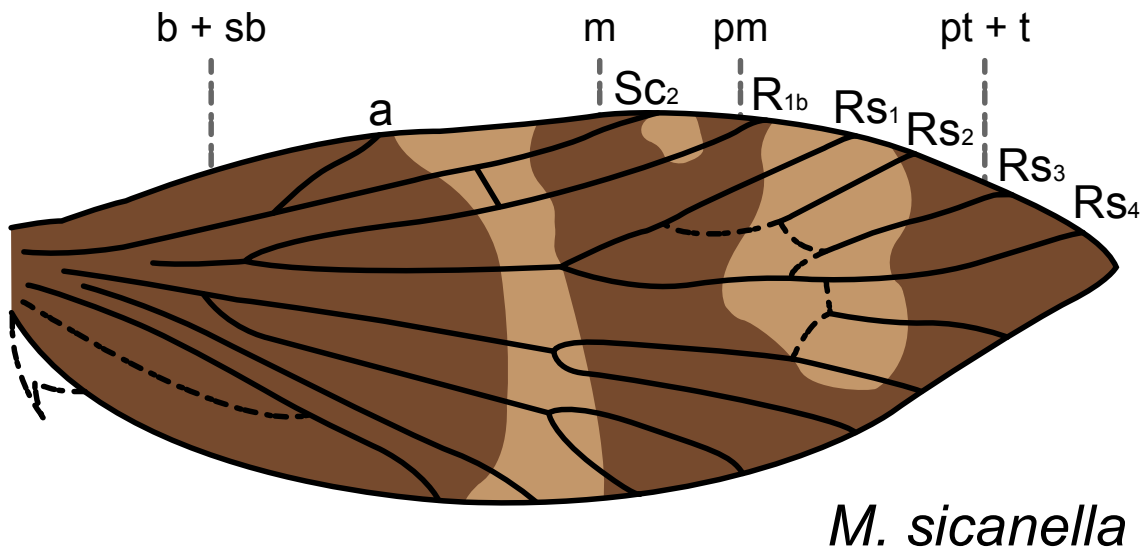


Figure 2.7 The wing pattern of *M. sicanella*, with two color fields and no incomplete suffusion or lack of expression at the costa.

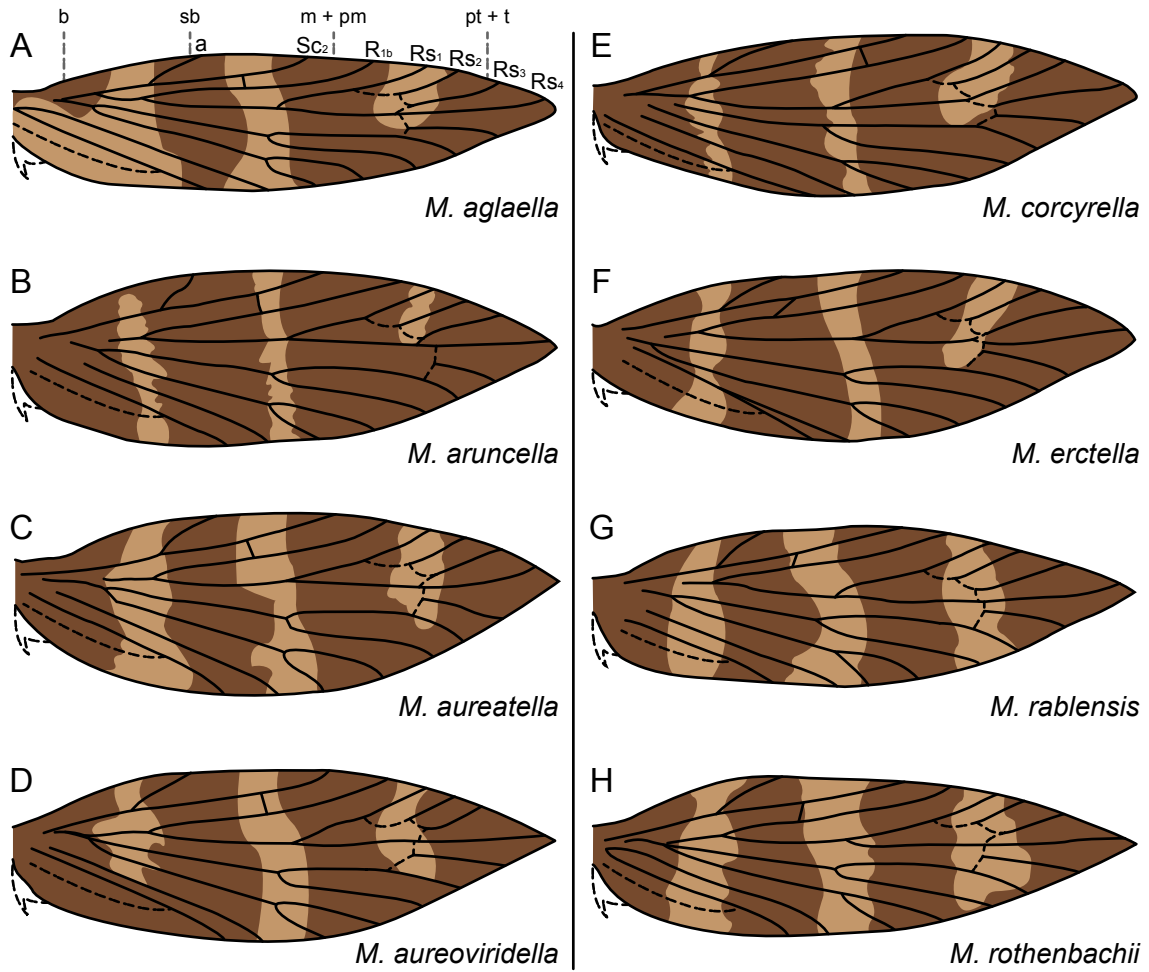


Figure 2.8 *Micropterix* wing patterns with two color fields and no incomplete suffusion or lack of expression at the costa.

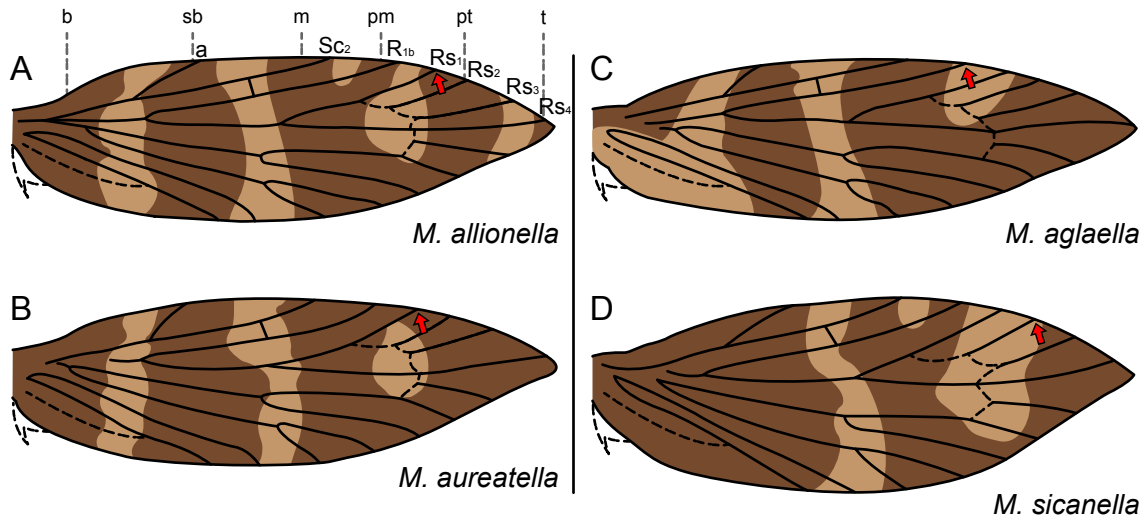


Figure 2.9 *Micropterix* wing patterns with variations of the groundplan at the costal margin of the wing: incomplete suffusion of the interfascial area between the postmedian + preterminal fasciae (A, B) and incomplete lack of expression surrounding this same interfascial area (C, D).

The red arrows point to areas of incomplete suffusion/incomplete lack of expression at the wing costa.

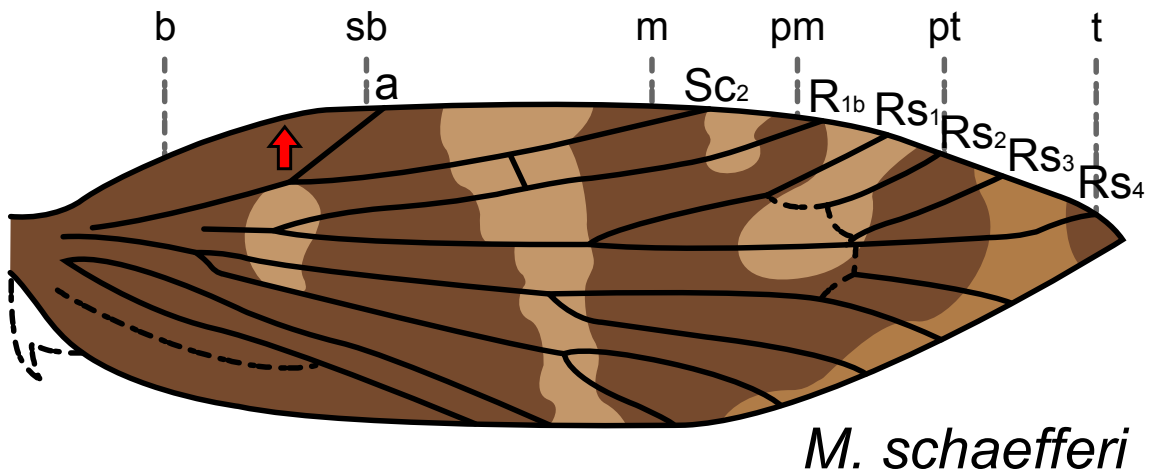


Figure 2.10 A wing pattern of *M. schaefferi* with incomplete suffusion of the interfascial area between the basal + subbasal fasciae at the costa.

The red arrows points to the area of incomplete suffusion at the wing costa.

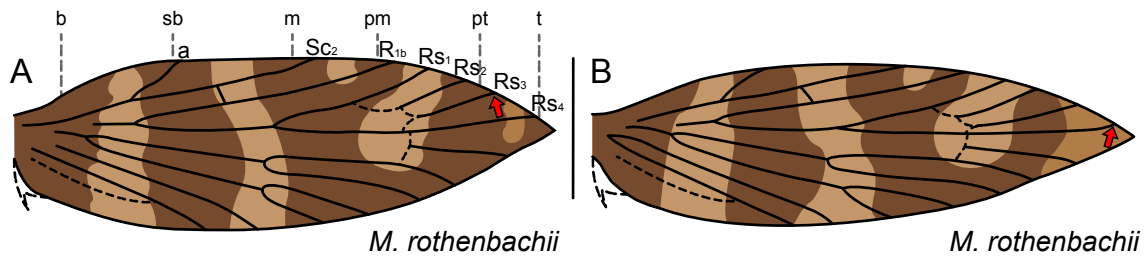


Figure 2.11 *M. rothenbachii* wing patterns with instances of incomplete suffusion of the interfascial area between the preterminal + terminal fasciae at the costa (A) and incomplete lack of expression surrounding the costal margin of this same interfascial area (B).

The red arrows point to areas of incomplete suffusion/incomplete lack of expression at the wing costa.

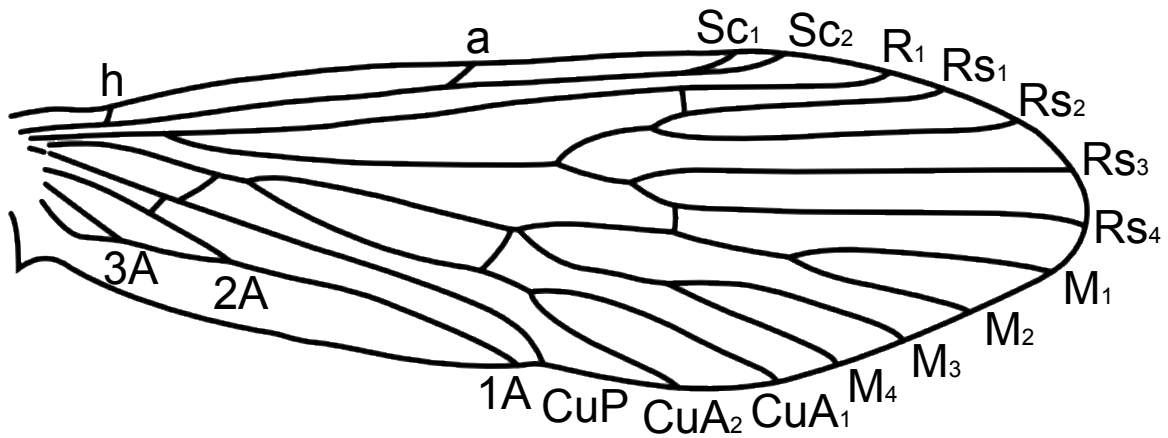


Figure 2.12 The wing venation of *Rhyacophila fuscula* (Trichoptera).

From *The Wings of Insects* (Comstock 1918).

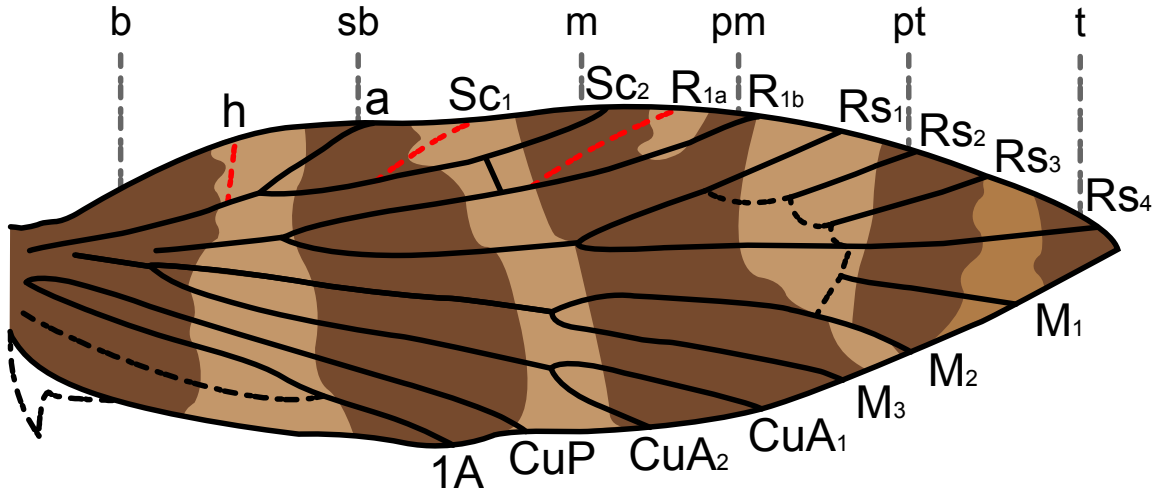


Figure 2.13 The wing pattern groundplan of *M. anderschella*, showing the possible distribution of primitive veins that are not visible in *Micropterix*.

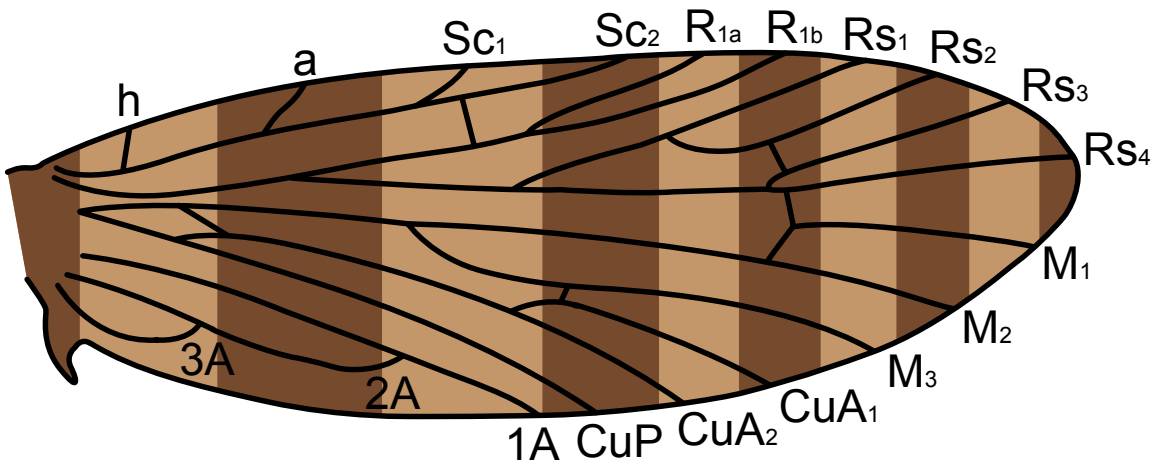


Figure 2.14 A hypothesized primitive wing pattern groundplan for Lepidoptera. Based on the most recent hypothesis for primitive wing venation (Zhang et al. 2013).

CHAPTER III

FOREWING COLOR PATTERN IN MICROPTERIGIDAE (LEPIDOPTERA):
HOMOLOGIES BETWEEN CONTRAST BORDERS, AND A REVISED
HYPOTHESIS FOR THE ORIGIN OF SYMMETRY SYSTEMS

Abstract

Despite the great importance of lepidopteran wing patterns to various biological disciplines, homologies between wing pattern elements in different moth and butterfly lineages are still not understood. Among other reasons, this may be due to an incomplete understanding of the relationship between color pattern and wing venation; many specific wing pattern elements have a known relationship with venation, but a framework to unite all wing pattern elements with venation is lacking. Though plesiomorphic wing veins are known to influence color patterning even when not expressed in the adult wing, most studies of wing pattern evolution have focused on derived taxa with a reduced suite of wing veins. The present study aims to address this gap through an examination of Micropterigidae, a very early-diverged moth family in which all known plesiomorphic lepidopteran veins are expressed in the adult wing. The relationship between wing pattern and venation was observed in 66 species belonging to 9 genera. Differences between the coloration of transverse bands in *Micropterix* and *Sabatinca* suggest that homologies exist between the contrast boundaries that divide wing pattern elements, and that color is not a reliable indicator of homology. Because the wing pattern of *Sabatinca doroxena*

very closely resembles the nymphalid groundplan when plotted onto a hypothetical nymphalid wing following the relationship between pattern and venation discussed here, it appears that the nymphalid groundplan may have originated from a *Sabatinca*-like wing pattern subjected to changes in wing shape.

Keywords

Developmental constraints; microlepidoptera; nymphalid groundplan; *Sabatinca*; symmetry systems.

Introduction

Color pattern in the animal kingdom has been an area of intense study for well over a century (Newbigin 1898). Insects in the order Lepidoptera were the subject of groundbreaking research during the early years of evolutionary biology (Bates 1862, Wallace 1865, Darwin 1871, Müller 1878) and remain a tremendously popular system for the study of color pattern in a variety of disciplines, ranging from theoretical biology to taxonomy, developmental biology, and ecology (Sekimural et al. 2000, Finkbeiner et al. 2014, Wilts et al. 2014, Monteiro et al. 2015). However, a disproportionate number of studies of Lepidoptera – such as those cited thus far – have focused on butterflies.

Butterfly wing patterns follow the “nymphalid groundplan,” which consists mainly of symmetry systems: pairs of transverse bands that run from the costal to the dorsal wing margin, symmetrical not in shape but in color (Nijhout 1991). Symmetry systems have been known for nearly a century (Schwanwitsch 1924, Süffert 1927), were subject to a revival during recent decades (Nijhout 1991), and remain an active area of inquiry (Otaki 2012). Although symmetry systems provide the foundation for numerous

publications on evolution and development, their evolutionary history remains obscure. Two hypotheses have been put forth to explain the origin of symmetry systems, both largely speculative. First, Lemche (1937) hypothesized that symmetry systems originated when primitive transverse bands of a single color became bisected with another color, so that one band appears to split into two (Figure 3.1A). This is called the “split-band” hypothesis here. Decades later, Nijhout (1994) hypothesized that symmetry systems originated when primitive irregular spots became concentric (with a circular outline surrounding the central spot), became aligned into parallel rows running from the costal margin to the dorsal margin, and then fused into three symmetry systems (Figure 3.1B). This is called the “fused-spot” hypothesis here. On the wings of moths in the families Hepialidae and Zygaenidae, concentric spots have been noted to show varying degrees of fusion (Nijhout 1994). However, bands that are formed by fused spots do not bear a particularly strong resemblance to nymphalid symmetry systems, and the directionality of change between spots and bands cannot yet be inferred because the necessary phylogenetic topology is still lacking.

These two hypotheses are founded on different assumptions. Lemche, after closely studying wing pattern in many families of microlepidoptera, arrived at the assumption that transverse bands are the primitive wing pattern element for Lepidoptera. Nijhout arrived at the assumption that spots, not transverse bands, are the primitive wing pattern element for Lepidoptera, because “[t]he vast majority of Panorpid and Trichopteroid wing patterns (like those of many primitive Lepidoptera) are in fact made up of irregular spots, and insofar as these groups are sister groups of the Lepidoptera, spotted patterns are most likely to represent the p[r]imitive (plesiomorphic) condition for

the Lepidoptera” (Nijhout 2003). However, the connection between trichopteran spotted wing patterns and the plesiomorphic character state for Lepidoptera is not entirely straightforward. In Trichoptera as in all other Panorpoidea aside from Lepidoptera, color pattern is associated with the wing membrane, but in Lepidoptera, color pattern is associated with wing scales. Because color pattern elements on the wings of Trichoptera and Lepidoptera occur in very different anatomical structures – membrane versus scales – proposed homology of color pattern elements between these two orders is dubious.

The two mutually exclusive hypotheses for the origin of symmetry systems rely on conflicting assumptions; a test of these assumptions would be a first step toward rejecting one, or perhaps ultimately both, of these hypotheses. In addition to the primitive state for lepidopteran wing pattern elements, another question should be resolved in order to reconcile microlepidopteran wing pattern with the origin of symmetry systems: the influence of wing venation on wing pattern development. In Lepidoptera with symmetry systems, such as butterflies, certain types of wing pattern elements – such as venous stripes – are unquestionably dependent on venation (Nijhout 1991). Other pattern elements, such as melanic band pattern elements, are not so obviously dependent on venation and may develop even if a species’ typical suite of venation is not expressed in the adult wing (Reed and Gilbert 2004). However, observations over many decades have confirmed that plesiomorphic veins can continue to influence the development of butterfly wing pattern elements, such as eyespots, even if the veins are not expressed in the adult wing (Süffert 1927, Oliver et al. 2009), so the presence of a wing pattern element in the absence of expressed corresponding venation does not necessarily indicate that wing venation is irrelevant to that pattern element. Because many plesiomorphic

lepidopteran wing veins are not distinguishable in the adult wings of butterflies and other higher Lepidoptera, either due to fusion or lack of expression, the relationship between venation and color patterning is especially difficult to deduce in these taxa and has been granted little consideration.

Though we do know with absolute certainty that plesiomorphic wing veins can influence color pattern elements (such as eyespots) even when not expressed, the relevant groundplan of primitive lepidopteran wing venation remains less certain. Because color patterns that arise from scales are unique to Lepidoptera, a reasonable working hypothesis is that the veins with potential to influence the development of color pattern are those that were present in the ancestral lineage in which scales first originated, regardless of whether these primitive scales expressed color. However, identification of this lineage, and the wing veins that it possessed, is hindered by the nature of the lepidopteran fossil record. The timing of the split between Trichoptera and Lepidoptera is unknown; the earliest definitive Trichoptera and Lepidoptera both date to the Mesozoic, but the recent finding of putative caddisfly cases from the Early Permian would move this split much farther into the past (Mouro et al. 2016). Moths have a remarkably poor fossil record (Sohn et al. 2015) and putative stem-group fossils are plagued by taxonomic uncertainty (Sohn et al. 2012). In the earliest known fossil of a true moth, *Archaeolepis mane*, only one branch of the Sc vein is visible (Whalley 1986, Grimaldi and Engel 2005); because early-diverging moths such as Micropterigidae overwhelmingly possess a two-branched Sc vein, and because a multi-branched Sc vein is the plesiomorphic character state for ancestral Amphiesmenoptera (Kukalova-Peck and Willmann 1990, Minet et al. 2010, Sukatsheva and Vassilenko 2013), *A. mane* is highly unlikely to

represent the ancestral state for lepidopteran wing venation. Other Jurassic fossil moths offer limited additional information about ancestral wing venation. New discoveries are very rare (Zhang et al. 2013), and fossils previously assigned to the extinct trichopteran family Necrotauliidae have been shifted to Lepidoptera on the basis of wing venation – more specifically, a 3-branched medial vein (Ansorge 2002). Unsurprisingly in light of the fact that assignment of Jurassic amphiesmenopteran fossils to Lepidoptera depends largely on similarities with venation in extant moths, the most recent hypothesis for primitive lepidopteran venation (Zhang et al. 2013) bears a striking resemblance to wing venation in Micropterigidae such as *Sabatinca*. There is reason to doubt that this hypothesis is complete: it contains a three-branched M vein, as is found in *Sabatinca* and other Micropterigidae, but the presence of a four-branched M vein in Permotrichoptera (Kukalova-Peck and Willmann 1990, Minet et al. 2010), Mesozoic caddisflies (Liu et al. 2014), extant caddisflies (Comstock 1918), and the extant lepidopteran family Agathiphagidae (Common 1973) – recently shown to belong to the earliest-diverging branch of Lepidoptera, alongside Micropterigidae (Regier et al. 2013, 2015) – suggests that more veins may need to be added to the reconstruction of primitive moth venation. Given the paucity of data available to inform hypotheses of primitive lepidopteran wing venation, the possibility certainly exists that additional wing veins known from other Amphiesmenoptera may have also been present in early moths, and may therefore continue to influence the development of color patterns in extant Lepidoptera.

Micropterigidae: Systematics and wing pattern morphology

Modern molecular studies have confirmed that Micropterigidae, along with Agathiphagidae, are the most basal extant moths (Regier et al. 2013, 2015). Relationships

within the Micropterigidae were recently explored in a molecular “preliminary phylogeny” based on the COI gene, which included 47 species of *Sabatinca*, 12 other micropterigid genera, and trichopteran outgroups (Gibbs and Lees 2014). Results of the molecular analysis were consistent with previous hypotheses based on morphology alone. The deepest split within the Micropterigidae has resulted in two biogeographically distinct clades: one in the Southern Hemisphere (Gondwanan) and one in the Northern Hemisphere (Laurasian). This Gondwanan-Laurasian split was also found in another recent study that included fewer micropterigid taxa but more comprehensive gene sampling (Regier et al. 2015). The Laurasian clade consists of the early-diverging genus *Micropterix*, whose wing pattern has already been examined (Schachat and Brown 2015); *Epimartyria*, a genus with three species, two of which have wing patterns comprised of large, light brown spots against a dark brown ground color (Davis and Landry 2012); and various genera whose wings are covered entirely is dark brown, purplish, or reddish scales (Hashimoto 2006). The Gondwanan clade contains many genera whose wing patterns include two or more colors, and is the focus of the present study. This clade contains two lineages: *Sabatinca* plus the “southern sabatinoid” lineage, which is dispersed throughout the Southern Hemisphere and includes *Austromartyria*, *Hypomartyria*, and *Agrionympha*; and the second, far less diverse “Australian group” which is restricted to Australia, New Zealand, and New Caledonia and is dominated by *Tasmantrix* and also includes *Aureopterix*, *Zealandopterix*, and *Nannopterix*; the terms “southern sabatinoid” and “Australian group” are used here in accordance with previous contributions (Gibbs and Lees 2014).

A recent examination of wing pattern in *Micropterix* (Micropterigidae) found a consistent relationship between wing venation and color pattern (Schachat and Brown 2015). In *Micropterix*, alternating light and dark brown transverse bands straddle one vein each along the costal margin of the wing (Figure 3.2). This interpretation relies on three veins that are not present in the adult wing of *Micropterix*, two of which – h and R1a – are known from other Micropterigidae such as *Sabatinca*, and have been included in the most recent reconstruction of ancestral wing venation for Lepidoptera (Zhang et al. 2013), and one other – a third branch of Sc, occurring here between the branches referred to as Sc₁ and Sc₂ – that is widely known from the amphiesmenopteran fossil record (Kukalova-Peck and Willmann 1990, Minet et al. 2010, Sukatsheva and Vassilenko 2013) and from the basal trichopteran genus *Rhyacophila* (Comstock 1918). This plesiomorphic three-branched Sc vein has also been put forth as tentative explanation for the hindwing venation of the micropterigid *Paramartyria semifasciella* (Hashimoto 2006). Genera such as *Sabatinca*, which have a more complete suite of plesiomorphic wing veins than *Micropterix*, are excellent candidates for further testing of the wing pattern groundplan proposed based on *Micropterix*.

The wing pattern groundplan of *Micropterix* as discussed above follows the predictions of a model based on the far more derived microlepidopteran family Tortricidae (Brown and Powell 1991, Baixeras 2002), referred to as the “wing-margin” model. A competing model, proposed decades earlier by Hennig Lemche (1935, 1937) and referred to as the “vein-fork” model, predicts that the basal edge of each fascia, or dark transverse band, will fall along the points where veins bifurcate. Examinations of the wing pattern of *Micropterix* did not support the “vein-fork” model.

Studies of micropterigid wing scales have found that they are always internally “fused” and therefore lack a cavity to hold pigment sacs (Simonsen 2001). Photos and written descriptions show that micropterigid wing scales are often iridescent (Lees et al. 2010, Gibbs 2014, Gibbs and Lees 2014).

Methods

The specimens examined for this study are held in the Australian National Insect Collection in Canberra, Australia; Victoria University in Wellington, New Zealand; and the Smithsonian Institution in Washington DC, USA. Only forewings were examined, because hindwings have very light scales of only one color. A total of 918 wings were examined, which may have included one or both forewings from a given specimen – the only wings that were excluded are those in which the relationship between venation and patterning cannot be deduced because the scales are worn off or the wing is broken. These 918 wings represent 66 species and 9 genera of Micropterigidae. Taxa were selected to match those sampled in the existing preliminary micropterigid phylogeny (Gibbs and Lees 2014). Sampling differences between the preliminary phylogeny and the present study are as follows: *Micropterix* was not included here because this genus has already been examined (Schachat and Brown 2015); *Sabatinca* spp. 5b, 49, and 50 were not included here because these species are only known from specimens preserved in ethanol; and *Epimartyria auricrinella* and the genera *Paramartyria*, *Palaeomicroides*, *Issikiomartyria*, *Kurokopteryx*, and *Neomicropteryx* were not included here because these species’ wings are of only a single color, a dark brown similar to the color of dark bands in *Micropterix* (Hashimoto 2006, Davis and Landry 2012). Additional species belonging to the genera *Epimartyria* (*E. bimaculella*) and *Tasmantrix* (*T. calliplaca*, *T. lunaris*, *T.*

nigrocornis, *T. phalaros*, and *T. tasmaniensis*), and species representing the additional genera *Agrionympha* (*A. capensis*, *A. fuscoapicella*, and *A. sagitella*) and *Nannopterix* (*N. choreutes*) were included here, despite being absent from the preliminary phylogeny, because specimens were available and because the affinities of the additional genera have already been discussed in the published literature (Gibbs and Kristensen 2011, Gibbs 2014).

The methods used here to examine wing pattern morphology parallel those developed by Schachat and Brown (2015) and are as follows: For each species, one forewing from one specimen was selected to form the basis of the illustration of that species' wing pattern. The wings selected were those that had intact color pattern, minimal overlap between the forewing and hindwing, and minimal overlap between the wing and the small block holding the minuten pin. This allowed maximum light to shine through the backlit wing. Scaled wings, instead of cleared wings, were examined in order to observe the precise relationship between color pattern and venation. Micropterigid wings are thinly scaled, and the venation becomes visible when specimens are lit from below using a microscope stage light. The observed wing venation was confirmed by examination of published illustrations of wing venation (Gibbs 2010, Gibbs and Kristensen 2011, Davis and Landry 2012) and by examination of a wing slide prepared by Don Davis and Jean-Francois Landry and held at the USNM for *Epimartyria bimaculella*, and by examination of wing slides prepared by George Gibbs and held at Victoria University for all other species; for the 7 species for which wing slides are not available (*Sabatinca viettei*, *S. sp. 36*, *S. sp. 39*, *S. sp. 43*; *Agrionympha capensis*, *A.*

sagitella, *A. fuscoapicella*), the wing slide of a sister species was examined for *Sabatinca* and published illustrations were consulted for *Agrionympha* (Gibbs and Kristensen 2011).

To verify that the illustrations fully represent the species to which they correspond, a total of up to 20 forewings were examined under a light stereomicroscope. (Results are discussed primarily in terms of wings instead of specimens because, in a few cases, only one forewing could be examined per specimen due to wear, due to the angle at which the specimen had been pinned, or because one wing had been removed to make a wing slide. Furthermore, a number of specimens have color patterns that varied between the two forewings.) Variations were noted at all locations along the costa where veins terminate, with the frequent exception of the humeral vein, which often cannot be detected on scaled specimens. Variations were also noted in between the two visible branches of the Sc vein, because an ancestral vein in this location has been hypothesized to constrain wing pattern (Schachat and Brown 2015), and variations were noted at the location where the Rs₄ vein terminates because, although this vein does not terminate along the costa in any of the species examined for the present study, it does terminate along the costa in *Micropterix* (Lees et al. 2010) and occasionally in fossil Micropterigidae (Kurz 2015). To create illustrations, a forewing was photographed while backlit so that both the patterning and venation were visible. This photograph was used as a template for a wing venation/wing patterning illustration created in the vector graphics application Affinity Designer. All intraspecific variation was incorporated into one single illustration per species. For each species, the illustrated wing pattern is that which is most prevalent; each variation is noted by a number and illustrated with a line comprised of red dashes alternating with the color that is present in the variation. Furthermore,

supplemental material includes a written description of each pattern variation as well as prevalence data (APPENDIX A).

The location of the wing vein 1A+2A could not be observed in all pinned specimens because of the overlap between the forewing and hindwing, and therefore had to be inferred based on wing slides and previously described venation (Gibbs 2010, Gibbs and Kristensen 2011, Davis and Landry 2012); however, this vein is of no relevance to the model because it does not reach the costal margin. Similarly, the jugal lobe was often folded in the specimens examined; its outline was inferred based on wing slides and previous descriptions. These and other inferred features are illustrated with dashed lines. In the descriptions that follow, the humeral vein is often excluded from statements regarding wing veins that terminate along the costa because this vein is so often difficult to observe.

Terminology is employed as follows. A “wing pattern element” formed by two or more adjacent wing scales of the same color, and can take the form of a spot, band, patch, etc., and the term “band” is used as shorthand for “transverse band,” which is a band that runs more or less between the costal and dorsal margins of the wing. The term “fascia” is rarely used here; this term has recently been subject to varying interpretations regarding wing pattern in Micropterigidae, having been applied to both light bands (Zeller-Lukashort et al. 2007, Lees et al. 2010) and to dark bands (Schachat and Brown 2015). The term “band,” as used here, encompasses both interpretations of “fascia.” Similarly, the term “ground color” is avoided here because this term usually signifies the color that covers the greatest amount of wing surface area, but this can vary between very light and very dark brown even among closely related species within the same genus. For the sake

of simplicity, the term “color” is applied broadly, to encompass all distinguishable colors, shades, tones, and tints. Therefore, white, silver, blue, brown, and black are discussed as “colors,” and different tints, tones, or shades – for example, light and dark shades of brown – are considered to be separate colors. The use of the term “color” here does not discriminate between structural colors and those derived from pigments. Nomenclature for wing venation (Figure 3.2) mainly follows Wootton (1979), with the exception of the humeral vein (“h”). The wing veins referred to with conventional nomenclature are those that are visible in the adult wing of *Sabatinca*. Schachat and Brown hypothesized that a third branch of Sc, known to be plesiomorphic for Amphiesmenoptera, plays a key role in micropterigid wing pattern; this hypothesized vein is referred to here as “pSc,” for plesiomorphic Sc (Figure 3.2).

Results

Forewing pattern in New Zealand *Sabatinca*

In the *Sabatinca* clade shown to be the most basal in the genus, called the “*calliarcha* group” (Gibbs and Lees 2014), wing patterning consists of either three or four different colors in each species (Figure 3.4). *S. lucilia* has the simplest wing pattern in this group, with a small, dark pattern element straddling the humeral vein; a very lightly-colored, uninterrupted band straddling Sc₁ at the costa, surrounded on either side by dark bands; another very light band straddling R_{1b} at the costa, also surrounded by dark bands on both sides but interrupted by a dark patch connecting the two dark bands; and one last very light band, straddling Rs₄ and M₁ along the dorsum and sometimes Rs₃ along the costa, also bordered by a dark band along its basal edge (Figure 3.4C). *S. heighwayi* has a somewhat similar pattern in that it includes very light bands surrounded

by dark bands, but the bands on *S. heighwayi* are more numerous and not as wide (Figure 3.4A). The wing pattern of *S. calliarcha* is more complex still, with four colors and a number of variations (Figure 3.4B). Some transverse bands are connected by patches of the same color. Concentric spots occur near the apex of the wing: on the costal margin these are comprised of small, dark intravenular patches surrounded by a rim of very light scales, with the opposite arrangement on the dorsal margin.

In the “*chrysargrya* group,” the clade that includes the majority of New Zealand *Sabatinca* species sampled, the fasciate wing pattern of *S. aurella* is the most straightforward (Figure 3.5E). Dark and medium brown wing pattern elements alternate along the wing; the four distal-most dark pattern elements contain areas of light scales in the center, but these light scales never interrupt the contiguous dark border. At and beyond the terminal Sc branch along the costal margin, the dark and medium wing pattern elements each straddle one vein. The wing pattern of *S. doroxena* is very similar to that of *S. aurella*, except that the location of certain pattern elements varies slightly between individuals, and the pattern includes four colors instead of three (Figure 3.5D). The wing pattern of *S. aenea* includes many small spots and therefore is not strictly “fasciate,” particularly along the apical half of the wing; nevertheless, it is similar to that of *S. aurella* in that, with a single exception, the largest of the dark pattern elements straddle/about alternating wing veins along the costal margin (Figure 3.5G). However, wing pattern in this species is quite variable, and in certain specimens, pattern elements – some extremely small – straddle every single wing vein beyond Sc instead of occurring in an alternating pattern.

The wing pattern of *S. aemula* is similar to that of *S. aurella* in that the lightest scales form transverse markings that are bordered by the darkest scales on the wing (Figure 3.5H). Two major differences between *S. aemula* and *S. aurella* are apparent: firstly, the wing pattern of *S. aemula* is not entirely fasciate, as the darkest scales often form spots, and secondly, medium-colored scales straddle alternating veins along the costa of *S. aurella* but straddle only one vein, h, along the costa of *S. aemula*. The wing pattern of *S. chrysargyra* is broadly similar to that of *S. aemula* in terms of the positioning of pattern elements relative to veins along the costa, but contains spots of varying sizes instead of any discernible fasciae (Figure 3.5I). In *S. chrysargyra*, unlike *S. aurella* and *S. aemula*, the darkest pattern elements are spots and do not occur adjacent to the lightest pattern elements.

The wing patterns of other species in the “*chrysargyra* group” are not as easily understood in terms of the wing pattern of *S. aurella*, and are discussed in order of complexity as follows: In *S. ianthina*, the predominance of dark wing pattern elements is such that every single vein is straddled by dark scales along the costa (Figure 3.5F). *S. quadrijuga* also has a wing pattern that consists overwhelmingly of dark scales; certain lighter wing pattern elements do straddle veins at the costa, but this occurs only at the h and Sc veins (Figure 3.5A). *S. caustica* and *S. chalcophanes* share a banding pattern in which fasciae converge toward the middle of the dorsum (Figure 3.5B,C). In both species, wing pattern is quite variable at the costal margin of the wing and all veins that reach the costa, including the humeral vein, are surrounded by dark scales in at least some specimens.

The wing patterns of *S. incongruella* and *S. demissa*, the only two New Zealand species that belong to the “*incongruella* group,” cannot be said to consist exclusively of fasciae or spots. In *S. incongruella*, for which the relationship between patterning and venation could only be observed for one wing, the pattern consists of four colors (Figure 3.6A). Fasciae have very jagged edges and spots occur toward the dorsum. In *S. demissa*, large, dark spots occur at the points where veins meet the costa and where veins bifurcate; all veins along the costa (except for the humeral vein) are surrounded by dark scales in at least some individuals (Figure 3.6B). Smaller spots occur elsewhere on the wing and are usually much lighter in color.

Forewing pattern in New Caledonia *Sabatinca*

The two New Caledonian species shown to be most basal, sp. 33 and sp. 4, have somewhat fasciate wing patterns consisting of three colors. In sp. 33, only two the lightest and darkest colors reach the costa (Figure 3.6C). The darkest brown straddles the humeral vein, and then alternating veins: Sc₁, R_{1a}, R_{S1}, and sometimes R₃. In sp. 4, all three colors reach the costa (Figure 3.6D). The main transverse bands alternate between light and medium brown, with small dark brown spots and bands appearing at the basal edge of the light bands.

Sabatinca sp. 31 has two light bands, outlined by very dark scales, straddling veins R_{1a} and R_{S1} at the costa (Figure 3.7A). *Sabatinca* sp. 36 has a wing pattern that consists primarily of dark scales; lighter wing pattern elements are few, and do not straddle any veins along the costa (Figure 3.7B). In *Sabatinca kristenseni* and sp. 17, veins are often abutted by two wing pattern elements at the costa; the only veins that are straddled by a single wing pattern element are R_{S1}, straddled by dark scales, and R_{S2},

straddled by light scales (Figure 3.7C,D). In *Sabatinca* sp. 6, dark pattern elements always straddle veins Sc₁, Sc₂, Rs₁, and Rs₃, and sometimes straddle R_{1a} and R_{1b}, along the costa (Figure 3.7E). Five very light bands also reach the costa but these never straddle, and rarely abut, any veins. In *Sabatinca delobeli* and sp. 28, dark pattern elements straddle all veins except for Rs₃ at the costa (Figure 3.7F,G).

Whereas the vast majority of Micropterigidae have wings patterns comprised only of different shades of brown, the wing pattern of *Sabatinca* sp. 48 includes four colors: beige, pale blue, and two shades of dark brown (Figure 3.8A). The Sc₁ vein abuts a dark brown pattern element, and all other veins are straddled by dark brown pattern elements along the costa. *S.* spp. 43, 20, 47, 29, 46, and 11 all have similar wing patterns, somewhat reminiscent of those of *S.* sp. 4, *aemula*, and *heighwayi*, with light brown bands against a medium background bordered, most often on the basal edge, by small dark stripes and spots (Figure 3.8B-G). In these six species, light bands surround/about Sc₁ and Sc₂. In *S.* sp. 29, R_{1a}, Rs₁, and Rs₃ are most often surrounded by medium colored scales, and R_{1b} and Rs₂ are surrounded by light scales (Figure 3.8E). In the other five species in this clade, dark scales nearly always surround/about R_{1a}, R_{1b}, and Rs₁ at the costa, and medium scales usually surround/about Rs₂ and Rs₃.

In *Sabatinca* sp. 12, all veins that terminate along the costa are surrounded by dark scales in some or all of the specimens examined (Figure 3.9A). As in *S.* sp. 6 (Figure 3.9E), various light bands occur along the costa, but never straddle or abut any veins. In *S.* sp. 18, Sc₁ usually abuts a dark wing pattern element, and dark scales surround all other veins at the costa (Figure 3.9B). *S.* sp. 10 has a broadly similar wing pattern to that of *S.* sp. 18, but Sc₁, Rs₃, and sometimes R_{1b} are surrounded by bluish scales (Figure

3.9C). *Sabatinca* spp. 15 and 37 have wing patterns unlike those of any other *Sabatinca* species; these two species' color patterns are quite different from each other, with the exception of small, bluish wing pattern elements toward the wing apex. In *S.* sp. 15, dark scales surround Sc₂, Rs₁, and Rs₂ at the costa; all other veins are surrounded by scales in one of two shades of lighter brown (Figure 3.9D). In *S.* sp. 37, Sc₁ is abutted basally by dark scales and apically by light scales, and beyond this vein, dark and light pattern elements occur in an alternating fashion from Sc₂ (surrounded by light scales) through Rs₂ (Figure 3.9E). At the wing apex, Rs₃ violates this pattern of alternation, as it is surrounded by the same light pattern element as Rs₂.

Figure 3.10 is comprised of two well-supported clades: the sp. 22 clade, consisting of *Sabatinca* spp. 22, 21, 5 and 7; and the *viettei* clade, consisting of *Sabatinca* spp. 44, 39, 32, 45, and *viettei* (Gibbs and Lees 2014). Species in the sp. 22 clade have wing patterns comprised of light bands, with each light band bordered with small dark bands and spots, mostly along the basal edge (Figure 3.10A-D), very reminiscent of the wing patterns found in *Sabatinca* spp. 43, 20, 47, 46, and 11 (Figure 3.8). In all species of the sp. 22 clade, light bands straddle/about Sc₁ and Sc₂, dark scales straddle/about R_{1a} and Rs₁, and medium scales straddle/about R_{1b} and Rs₂. In spp. 21 and 7, dark spots sometimes straddle Rs₃. The *viettei* clade also contains wing patterns with dark bands occurring basally to light bands (Figure 3.10E-I), but whereas the sp. 22 clade contains many wing patterns in which light bands are surrounded by dark pattern elements on both sides, the opposite is true for the *viettei* clade: light spots sometimes appear basally to the dark bands. However, the relationship between color pattern and venation along the costa is very similar in the sp. 22 and *viettei* clades. In the *viettei* clade as in the sp. 22 clade, light

bands straddle/about Sc₁ and Sc₂, dark bands straddle/about R_{1a} and Rs₁, and medium scales straddle/about R_{1b}. In the *viettei* clade, unlike the sp. 22 clade, a dark band always straddles Rs₃. In sp. 39, medium scales straddle Rs₂; in all other species in the *viettei* clade, a single band straddles Rs₁ and Rs₃ as well as Rs₂.

Forewing pattern in other Micropterigidae

In genera other than *Sabatinca*, forewing pattern is simpler, consisting of only two or three shades of brown, usually with fewer wing pattern elements due to the absence of numerous dark spots. Along the costa of *Austromartyria porphyrodes*, light bands sometimes straddle Sc₁ and Sc₂; dark scales straddle all other veins (Figure 3.11A). In *Hypomartyria micropteroides*, one light band nearly reaches the costa between Sc₁ and Sc₂, another straddles R_{1b} and another abuts Rs₂ and straddles Rs₃ (Figure 3.11B). In the three *Agrionympha* species examined (*A. capensis*, *A. fuscoapicella*, and *A. sagitella*), light bands are surrounded on either side by thinner, very dark bands (Figure 3.11C-E). Similarly to *Hypomartyria micropteroides*, one light band reaches the costa between Sc₁ and Sc₂. Another light band straddles R_{1a} in *A. sagitella*, and R_{1b} in *A. capensis* and *A. fuscoapicella*. In *A. capensis* and *A. sagitella*, a third light band straddles Rs₃; this band is absent in *A. fuscoapicella*.

In *Nannopterix choreutes*, a dark band abuts the basal edge of Sc₁ at the costa and a medium band straddles Rs₂ (Figure 3.11F). In *Aureopterix micans*, dark bands straddle Sc₁, Sc₂, and Rs₁ at the costa. Sometimes the band that straddles Rs₁ also straddles Rs₂ and Rs₃, and, less often, R_{1b} (Figure 3.11G). In *Aureopterix sterops*, dark bands consistently straddle Sc₂ and Rs₂ at the costa; the band that straddles Rs₂ sometimes extends to Rs₁ and R_{1b} (Figure 3.11H). In *Zealandopterix zonodoxa*, the only light wing

pattern element that consistently reaches the costal margin of the wing is a small spot that occurs at the apex and does not straddle any veins; in some specimens, one light band occurs in the “pSc” are of the costa between Sc₁ and Sc₂, and another straddles Rs₁ (Figure 3.11I).

Wing pattern in *Tasmantrix* consists of two shades of brown (Figure 3.12). In all species examined here except for *T. lunaris*, a light band reaches the costa between Sc₁ and Sc₂; in *T. phalaros*, *T. tasmaniensis*, and *T. thula*, this band sometimes straddles Sc₁. Another light band straddles R_{1b} in *T. tasmaniensis* and almost reaches this vein in *T. lunaris*, sometimes straddling R_{1a} in both species. In all other species (in which this band does not appear), small spots occur along the costa but never straddle any veins. A light pattern element straddles Rs₄ in *T. calliplaca*, *T. tasmaniensis*, and *T. thula*, nearly abutting this vein in *T. fragilis*.

Lastly, in *Epimartyria* – the only genus examined here that belongs to the Laurasian, Northern Hemisphere clade (Gibbs and Lees 2014) – both species, *E. bimaculella* and *E. pardella*, have the same wing pattern along the costa: a single light pattern element usually straddles R₁ and occasionally straddles Sc₂. (Figure 3.13).

Discussion

Both models put forth to explain wing pattern homology in microlepidoptera, the “wing-margin” model and the “vein-fork” model, assume a wing pattern that, at a first approximation, is comprised of one relatively light color and one relatively dark color. Though both models are based on taxa whose wing patterns include more than two colors, evaluation of these models is most straightforward for taxa whose wing patterns include only two colors. Six of the genera examined here – *Austromartyria*,

Hypomartyria, *Aureopterix*, *Zealandopterix*, *Tasmantrix*, and *Epimartyria* – have wing patterns with only one light and one dark shade of brown, and all of these wing patterns are consistent with the “wing-margin” model. However, none of these genera provide as robust a test of the model as *Micropterix* because none have more than four pairs of alternating light and dark bands.

Other than *Micropterix*, *Epimartyria* is the only genus in the Laurasian, Northern Hemisphere clade that has a wing pattern comprised of two colors (Figure 3.13). The wing patterns of both *E. bimaculella* and *E. pardella* could be said to be consistent with the “wing-margin” model, in that the single light pattern element at the costa straddles only one vein. However, because so few differentiated pattern elements occur on the wing of *Epimartyria*, this genus does not provide any additional insight into the applicability of the “wing-margin” model to Micropterigidae.

The wing patterns of all taxa in the “Australian group” are consistent with the “wing-margin” model as observed in *Micropterix*. The only light band that reaches the costa in most *Tasmantrix* species corresponds to the interfascial area that separates the subbasal and median fasciae in *Micropterix*, and the light band that reaches the costa in *T. lunaris* and *tasmaniensis* corresponds to the interfascial area between the median and postmedian fasciae in *Micropterix*, with additional lack of expression of the postmedian fascia at R_{1b} in *T. tasmaniensis*. The wing pattern of *Zealandopterix* is dominated by dark pattern elements and consists of a small spot at the base of the wing that does not reach the costa, a smaller spot at the wing apex that does not straddle any veins, a light band that reaches the “pSc” area of the costa that separates the subbasal and median fasciae (Figure 3.2) as in *Tasmantrix* and *Micropterix*, and a light band that straddles Rs_1 ,

corresponding to the interfascial area that separates the postmedian and preterminal fasciae in *Micropterix*. Wing pattern in *Tasmantrix* and *Zealandopterix* is therefore consistent with the “wing-margin” model with confluence of fasciae due to suffusion of interfascial areas, except with the addition of small light spots between fasciae. In contrast to the other genera in the “Australian group,” *Aureopterix* has a wing pattern dominated by light pattern elements. Nevertheless, the wing pattern of *Aureopterix micans* is broadly consistent with the *Micropterix* groundplan – dark bands straddle Sc₁ and Sc₂ exactly as predicted by the “wing-margin” model, corresponding to the subbasal and median fasciae; R_{1b} is sometimes straddled by a dark band, corresponding to the postmedian fascia; and Rs₂ is straddled by a dark band corresponding to the preterminal fascia, which has become confluent with the postmedian fascia. The wing pattern of *Aureopterix micans* can therefore be derived from the “wing-margin” model through lack of expression of the basal fascia and confluence of the postmedian and preterminal fasciae. The wing pattern of *Aureopterix sterops* is very similar, except that the basal fascia is partially expressed, the subbasal fascia does not straddle Sc₁ at the costa due to incomplete lack of expression, and the postmedian and preterminal fasciae are not confluent as frequently. Between *A. micans* and *A. sterops*, all fasciae and interfascial areas predicted by the “wing-margin” model are present, with the sole exception of the terminal fascia which is absent by necessity because Rs₄ does not terminate along the costa in *Aureopterix*.

In the two “southern sabatinoid” genera whose wing patterns contain two colors, *Austromartyria* and *Hypomartyria*, the only veins ever straddled by light bands are Sc₁, Sc₂, and R_{1b}. These three veins do form an alternating series, as they are interspersed

between h, “pSc,” R_{1a}, and R_{s1}, and so at first glance the southern sabatinoid wing patterns appear consistent with the “wing-margin” model. However, the model predicts that these veins should be straddled by dark bands. The contrast boundaries between wing pattern elements in *Austromartyria* and *Hypomartyria* are consistent with those predicted by the “wing-margin” model, but the colors of these pattern elements are not.

Only three of the *Sabatinca* species examined have wing patterns of just two colors: *S. quadrijuga*, *S. ianthina*, and *S. sp. 36*. The only light wing pattern elements that reach the costa in *S. quadrijuga* straddle Sc₁ and Sc₂ as in *Austromartyria*; no light wing patterns straddle any veins along the costa of *S. ianthina* or *S. sp. 36*. The latter two species, though phylogenetically and geographically distant from each other (Figure 3.15), have very similar wing patterns: an overwhelmingly dark wing with very light pattern elements occurring basal to the humeral vein, in the “pSc” area, between R_{1a} and R_{1b}, straddling or very close to R_{s4}, and between M₃ and CuA₁; *S. ianthina* also has a light band between R_{s2} and R_{s3} and small light spots between M₁ and M₂. This wing pattern could be derived from the *Micropterix* groundplan through complete suffusion of various interfascial areas, and incomplete suffusion of those that remain; both complete and incomplete suffusion of interfascial areas have been observed in various *Micropterix* species (Schachat and Brown 2015). The small light bands along the costa in these two species correspond to the *Micropterix* interfascial area that straddles “pSc” and the *Micropterix* interfascial area that straddles R_{1a}, with incomplete suffusion adjacent to the median fascia. The additional light band in *S. ianthina* corresponds to the *Micropterix* interfascial area that straddles R_{s3}, with incomplete suffusion adjacent to the terminal fascia. The light band that straddles R_{s4} in many *S. sp. 36* specimens could be attributed

to lack of expression of the terminal fascia along the dorsum and incomplete suffusion of the adjacent interfascial area along the costa. Both of these groundplan modifications have been observed in *M. rothenbachii*, though not in the same specimen (Schachat and Brown 2015). Because few *Sabatinca* species have wing patterns comprised of only two colors, and because these wing patterns are characterized by extensive suffusion of interfascial areas, this genus adds little to our understanding of micropterigid wing patterns that are comprised strictly of one light shade and one dark shade of brown.

Wing patterns with three or more colors

Wing patterns include three or more colors in *Nannopterix*, *Agrionympha*, and the vast majority of *Sabatinca* species. In *Nannopterix choreutes*, all veins except for Rs_2 are straddled by light scales at the costa and so no firm conclusions can be drawn regarding the “wing-margin” model or homology in any other sense. In *Agrionympha*, light bands are bordered by very dark, thin bands. Among various other possible mechanisms, these very dark bands could have arisen in the manner predicted by Lemche’s “split-band” hypothesis, with each pair of dark bands originating from a single, ancestral dark band that was bisected by a very light band. In the three *Agrionympha* species examined here, a light band and the two very dark bands that border it all fall within the “pSc” region, occasionally abutting Sc_1 but never straddling either of the Sc veins expressed in the adult wing; because all two-colored micropterigid wing patterns are consistent with the “wing-margin” model, which predicts a single wing pattern element in the “pSc” are between Sc_1 and Sc_2 , this suggests that each light band, plus the two very dark bands that border it, function together as a single wing pattern element. The three *Agrionympha* species examined have a another “split-band”-type wing pattern element at R_1 , but because this

pattern element straddles different veins in different taxa – R_{1b} in *A. capensis* and *A. fuscoapicella* and R_{1a} in *A. sagitella* – it is difficult to determine how this pattern element, and therefore *Agrionympha* wing patterns as a whole, might relate to the “wing-margin” model.

In *Sabatinca*, the relationships between wing pattern elements of different colors seem to vary greatly among species. For example, in the *chrysargyra* group – a small, well-supported clade – *S. aurella* and *S. doroxena* have fasciate wing patterns in which the one or two most basal dark bands are of a single color, but all others are bisected by a very light color. These wing patterns essentially provide an illustration of the “split-band” hypothesis, because the basal bands conform exactly to Lemche’s hypothesized ancestral state for microlepidopteran wing pattern and the others conform exactly to Lemche’s hypothesized incipient symmetry systems. A few other *Sabatinca* species, such as *S. lucilia*, have wing pattern elements that somewhat resemble the “split-band,” but not as unambiguously so. In *S. caustica* and *S. chalcophanes*, the darkest wing pattern elements occur only at the costal and dorsal wing margins and are connected by medium-brown bands. In *S. chrysargyra*, the darkest pattern elements are small spots that straddle veins at the wing margin and the lightest pattern elements are much larger bands that do not straddle veins.

In the *incongruella* group, three-color wing patterns are even more varied. *S. demissa* has large, dark spots at the points where veins reach the costa or bifurcate, and small, light spots elsewhere. In *S. sp. 33*, only the lightest and darkest wing pattern elements reach the costa, with the exception of R_{s3} in some specimens. *S. sp. 6* and the distantly related *S. sp. 12* have color patterns very similar to that of *S. chrysargyra*. Many

Sabatinca species from New Caledonia have wing patterns somewhat similar to the “split-band”-type patterns of *S. doroxena* and *S. aurella* from New Zealand, but the thin, dark bands of *S. doroxena* and *S. aurella* often do not appear as bands at all in the New Caledonian species and instead are either broken up into small spots or are absent altogether, particularly at the apical, or distal, margin of each light band. However, the relationship between patterning and venation differs markedly between the “split-band”-type *Sabatinca* species of New Zealand and New Caledonia: whereas the “split-band”-type pattern elements straddle alternating veins along the costa in the New Zealand species and in *Sabatinca* sp. 31 from New Caledonia, it is common for every single vein along the costa to be surrounded by a “split-band”-type pattern element in New Caledonian species.

Sabatinca sp. 37 has a wing pattern of only two colors except at the apical area. Its wing pattern is not exactly fasciate – if this wing pattern is indeed derived from an ancestral, fasciate pattern, the edges of the fasciae have become rather sinusoidal, creating a reticulate pattern comprised of elements that simultaneously resemble both fasciae and spots. However, these sinusoidal fasciae do straddle alternating veins along the costa: the area basal to Sc₁, the “pSc” area, R_{1a}, and Rs₁. (Rs₃ is straddled by a light band that also straddles both Rs₂ and Rs₄.) Because the various colors of the *Sabatinca* sp. 37 wing pattern are limited to restricted areas of the wing, potential relevance to the “wing-margin” model may be easier to deduce.

Many *Sabatinca* species from New Caledonia have wing patterns with spots and transverse bands in light brown, dark brown, and iridescent blue; these wing patterns have been hypothesized to mimic the jumping spiders that appear on the island (Gibbs

and Lees 2014). Though the putative spider mimics have many pattern elements, it is still most common for dark bands to straddle all veins along the costa in these species. Dark scales surround (or in one case, abut) every single vein at the costa in *Sabatinca* spp. 18 and 48, and surround or abut nearly all veins in *S. kristenseni* and spp. 10 and 17. The putative spider mimic wing patterns rely on a large number of light bands and spots, and, given the wing patterns of other *Sabatinca* species discussed so far, a straightforward way to derive a wing pattern with many separate light markings would be for each vein to be surrounded by dark scales, and for one light wing pattern element to occur between each pair of adjacent veins along the costa. However, this does not appear to be the case with the New Caledonian spider mimics. Instead, based on their positions along the costa, the separate blue and light brown bands appear to have originated from a single light band that split into two.

In summary, third and fourth colors in *Sabatinca* wing patterns seem to have originated independently multiple times and through a variety of mechanisms, often obscuring homologies with more straightforward pattern elements seen on the wings of other micropterigid genera with wing patterns comprised of only two colors, such as *Tasmantrix*, *Austromartyria*, and *Micropterix*. In *Sabatinca* sp. 37 the four colors in the wing pattern are largely confined to specific areas along the proximo-distal axis and in *S.* sp. 33 colors are confined to specific areas along the anterior-posterior axis, but in all other species, different colors are dispersed throughout the wing. In *S. demissa*, the color of a spot corresponds with its proximity to the points where veins bifurcate and terminate. In *S.* sp. 6, the darkest spots along the costal margin always straddle veins and the lightest spots never do. In *S. doroxena* and *S. aurella*, the lightest color on a wing, either beige or

white, seems to have originated within the central areas of the dark brown bands. In various putative spider mimics, blue and light brown bands are adjacent to each other and may have originated when one band split into two, losing its self-symmetry. Many species from New Caledonia have transverse light bands surrounded by dark bands or spots on both sides; there is a consistent relationship between venation and both of the band colors – light bands always straddle Sc_1 and Sc_2 , and a dark band always straddles R_{1a} – suggesting that both of these colors, whether they arose from the hypertrophy seen in *S. doroxena* and *S. aurella* or by some other mechanism, are developmentally individuated.

Implications for Lemche’s “vein-fork” model

Lemche’s “vein-fork” model for homology between wing pattern elements was originally based on observations of the location of spots on the wings of Pyralidae and Noctuidae (Lemche 1935). Lemche found that spots often occurred at the points where veins bifurcate, and from this observation he hypothesized that these bifurcations lie along the basal edges of fasciae. This model therefore implies that fasciae and spots are homologous; because Lemche hypothesized that fasciae are the primitive wing pattern elements for Lepidoptera (Lemche 1937), one would therefore expect the spots in Pyralidae, Noctuidae, and many other moths to have arisen via incomplete expression of bands.

The “vein-fork” model initially appeared to be irrelevant to Micropterigidae, and therefore quite possibly of limited relevance to the evolution of wing pattern in Lepidoptera, because the model does not predict the location of fasciae in *Micropterix* (Schachat and Brown 2015). The data presented here show that the model is of similarly

limited utility for predicting the location of fasciae on the wings of other micropterigid taxa. However, in two *Sabatinca* species that appear to be distantly related – *S. demissa* and *S. sp. 6* – prominent large, dark spots occur at many of the points where veins reach the wing margin and at the point where the M vein bifurcates (Figure 3.15). In *S. demissa*, additional dark spots occur where Rs and CuA bifurcate; many more spots occur elsewhere on the wing, but are either much smaller or much lighter in color than those that occur where veins meet the wing margin and where M bifurcates. It is striking that the largest dark spots appear in the same locations relative to venation in both *S. demissa* and *S. sp. 6*, because these two species' color patterns are otherwise dissimilar: *S. sp. 6* has large spots and bands that are very light in color whereas *S. demissa* has small, medium-brown spots against a very light ground color. Because prominent spots at the bifurcation of M are rare in Micropterigidae and appear to have originated independently twice in *Sabatinca* alone (Figure 3.15), it appears that Lemche erred in assuming that the spots in Pyralidae and Noctuidae are homologous and ancestral within and beyond the Lepidoptera (1935). The origination of such spots at vein bifurcations may well be a real phenomenon, but appears to have occurred convergently in various lepidopteran lineages and would therefore be homoplastic.

Ancestral states and the nature of wing pattern homology

Because the basal *Sabatinca* species with the most obviously fasciate wing patterns – *S. aurella* and *S. doroxena* – have alternating light and dark bands straddling or abutting one vein each along the costa, just as in *Micropterix*, it is likely that the wing pattern elements of *Sabatinca* and *Micropterix* are homologous. Even the shapes of individual wing pattern elements are similar between *Sabatinca* and *Micropterix*: for

example, in *S. aurella* and *S. doroxena*, the band that straddles R_{1a} is quite small and does not even extend halfway toward the dorsum from the costa (Figure 3.5D,E); the same is true for the wing pattern element that corresponds with R_{1a} in *Micropterix* (Schachat and Brown 2015). However, this pattern element is light in *Micropterix*, and would therefore be called an “interfascial area” according to the “wing-margin” model (Brown and Powell 1991, Baixeras 2002), but is outlined by dark scales in *Sabatinca* and would therefore be called a “fascia” according to the same model. This, along with a similar observation from the “southern sabatinoid” genera discussed above, suggests that microlepidopteran homologies between fasciae and interfascial areas, should they exist, occur among contrast boundaries as opposed to wing pattern element color. One contrast boundary occurs between each pair of adjacent plesiomorphic veins, and a series of alternating light and dark transverse bands will develop such that one band occurs within each pair of adjacent contrast boundaries. Either series of alternating bands – the series that straddles [h, “pSc”, R_{1a}, RS₁, RS₃] or the series that straddles [Sc₁, Sc₂, R_{1b}, RS₂, RS₄] – could develop a darker scale color. In other words, the color of wing pattern elements is likely a misleading signifier of homology, with the contrast boundaries between pattern elements being far more reliable. Terms such as “ground color” and “background color” may be convenient for use in taxonomic descriptions but appear not to be meaningful in the context of evolutionary history.

Reconstruction of an ancestral wing pattern state for all Micropterigidae is problematic at present because the rate of discovery of Gondwanan taxa remains high, and so various genera are not represented in the current, preliminary phylogeny. However, present knowledge supports some tentative conclusions. Firstly, bands appear

to be a far more likely primitive wing pattern element for Micropterigidae than spots. The most basal Laurasian genus, *Micropterix*, has a wing pattern comprised entirely of bands. In the Gondwanan clade, bands predominate over spots in *Sabatinca* and are even more overwhelmingly dominant in all other genera: spots only appear in *Tasmantrix*, but occur far less consistently between species than bands do. The predominance of bands in the most basal Laurasian genus and in all Gondwanan genera that could possibly be described as “basal” strongly indicates that ancestral Micropterigidae would have had banded wing patterns. Secondly, because both the Laurasian and Gondwanan clades include taxa with alternating dark and light bands straddling veins along the costal margin, the common ancestor of Micropterigidae most likely had the potential to develop a wing pattern similar to that of *Micropterix* that could later become confluent in many areas, leading to a wing pattern with fewer apparent wing pattern elements. Third, because the basal genera *Micropterix* and *Tasmantrix* both have a light band along the “pSc” region of the costa, surrounded on either side by dark bands that straddle Sc₁ and Sc₂, it appears most likely that ancestral Micropterigidae had dark bands straddling the veins [Sc₁, Sc₂, R_{1b}, R_{S2}, R_{S4}], with the sabatinoid groundplan of dark bands straddling [h, “pSc”, R_{1a}, R_{S1}, R_{S3}] originating later (Figure 3.14).

The clade comprised of *Sabatinca doroxena*, *aurella*, *ianthina*, *aenea*, *aemula*, and *chrysargrya* contains spectrum of wing patterning with fasciate patterns on one extreme (represented by *S. doroxena* and *aurella*); an intermediate state in which the dark bands are not contiguous, and additional dark pattern elements surround almost most veins along the costal margin of the wing (represented by *S. aemula*); a distinct intermediate state in which wing pattern consists mainly of only two colors and the

largest pattern elements continue to straddle veins along the costa as in *S. doroxena* and *aurella*, but as above, smaller dark pattern elements accumulate around most veins at the costal margin (represented by *S. aenea*); and another extreme with no bands, only light and dark spots of various sizes including small dark spots straddling all veins at the costa with the possible exception of the humeral vein (represented by *S. chrysargyra*) or with dark wing pattern elements so predominant that light wing pattern elements are small and few, and do not straddle any veins at the costa (represented by *S. ianthina*).

The phylogenetic structure needed to infer the direction in which evolutionary change occurred is tentative at present. Two analyses were presented when the preliminary phylogeny was originally published: one in which sampling included many micropterigid genera but only eleven species of *Sabatinca* from New Zealand, and another in which sampling included forty-seven species of *Sabatinca*, fourteen of which are from New Zealand. These two phylogenies contain conflicting topologies for the clade in question, with the former resolving *S. chrysargrya* and *aemula* as the most basal species in the clade, and the latter resolving *S. doroxena* as the most basal species in the clade followed by *S. aurella* (Gibbs and Lees 2014). An additional analysis in a separate publication, which includes eighteen species of *Sabatinca* from New Zealand, found *S. aurella* and *doroxena* to be sister to the rest of the clade (Gibbs 2014). Because the two phylogenies with the densest sampling of New Zealand *Sabatinca* show *S. aurella* and *doroxena* to have diverged earlier than *S. aenea*, *aemula*, and *chrysargrya*, it is most likely that fasciate wing patterns are the ancestral condition for this group and that the bands slowly broke up into spots, and new spots formed along the costa, after the divergence of *S. aurella* and *doroxena*.

The number of primitive fasciae in Lepidoptera

Though the “wing-margin” and “vein-fork” models share the assumption that transverse bands are the primitive wing pattern element for Lepidoptera, the two models differ in that the “vein-fork” model proposed a primitive groundplan with seven dark bands whereas the “wing-margin” proposes a primitive groundplan with either five or six dark bands, depending on whether Rs_4 terminates along the costa. *Sabatinca doroxena* has a wing pattern of five dark bands that is entirely consistent with the “wing-margin” model’s prediction about the location of contrast boundaries. The band that straddles the humeral vein is part of a V-shaped pattern element that could have originated from a fasciate wing pattern in one of two ways: either two dark bands became confluent at the dorsal margin, or one dark band was split by a light pattern element that runs from the costa nearly to the dorsum. In *Sabatinca aurella*, this putative split is complete: the two apparent dark bands appear basal to Sc_1 along the costa, with one apparent dark band straddling the humeral vein and one that does not straddle any vein. There are two simple explanations for this apparent split from one dark band into two. The first is that only one dark band occurred basal to Sc_1 in ancestral *Sabatinca*, this band is nearly split in *S. doroxena*, and appears to have split into two bands in *S. aurella* though both are derived from a single primitive band. The second explanation is that two dark bands occurred basal to Sc_1 in ancestral *Sabatinca*, which would require an additional two plesiomorphic branches of Sc – completely unknown from Trichoptera as well as Lepidoptera – to influence the development of wing pattern in extant Lepidoptera. The first explanation, of one primitive band that appears to split into two, is far more conservative in that it does not require the presence of plesiomorphic veins unknown from crown Amphiesmenoptera

(the clade that includes all living moths and caddisflies), and is arguably also the more plausible of the two explanations given that multiple bands preceding Sc₁ are not known from any micropterigid genus besides *Sabatinca*.

Even in the unlikely event that additional contrast boundaries between h and Sc₁ turn out to be a reality, a groundplan with these additional contrast boundaries could only include the seven dark bands proposed by Lemche on wings where Rs₄ terminates along the costa, which rarely occurs. One possible groundplan that could underlie wing patterns with seven dark transverse bands would include a single dark band straddling every vein at the costa, instead of straddling alternating veins. Dark spots such as those that have accumulated where all veins reach the costa in many *Sabatinca* species (*S. caustica*, *chalcophanes*, *chrysargyra*, *demissa*, sp. 6, sp. 12) could extend down toward the dorsal margin of the wing in order to form these bands. This hypothesized groundplan could be tested in future studies by examining the relationship between wing venation and color pattern in additional microlepidopteran lineages.

The continued importance of morphology for lepidopteran wing pattern evolution

The earliest studies of wing pattern evolution in Lepidoptera were based primarily on morphology, with preliminary phylogenetic context (Eimer 1889, Eimer and Fickert 1897, von Linden 1902). The first rigorous examination of wing pattern morphology in the context of phylogeny is over a century old (Braun 1914) and is roughly contemporaneous with influential studies of other aspects of lepidopteran morphology (Eyer 1924). In the wake of the publication of the nymphalid groundplan (Schwanwitsch 1924, Süffert 1927), comparative morphology was a popular area of study that overlapped heavily with early experimental work on heredity (Kühn and Henke 1930,

1936, Lemche 1935, 1937, Ford 1937). During the current era, morphological insights continue to inform our understanding of the systematics of Lepidoptera (Kristensen 2003, Heikkilä et al. 2015) and of the nymphalid groundplan (Otaki 2012); wing pattern homologies are designated through a combination of developmental, phylogenetic, and morphological data (Oliver et al. 2012); and morphological data continue to shed light on the developmental genetics of color pattern, particularly when combined with phylogeny (Schachat et al. 2015). At present, developmental studies of wing pattern in Micropterigidae are not possible; no lab colony has been established, despite years of effort. However, the similarities between wing pattern of the micropterigid type genus and distantly related microlepidoptera (Schachat and Brown 2015) suggest that the morphology of micropterigid wing patterning may hold many valuable insights despite the lack of corresponding developmental data.

A revised hypothesis for the origin of symmetry systems

Because micropterigid wing pattern is consistent with Lemche's assumption that transverse bands, not spots, are the primitive wing pattern element for Lepidoptera, Nijhout's "fused-spot" hypothesis is an unlikely explanation for the origin of symmetry systems. In contrast, the wing patterns of *Sabatinca doroxena* and *aurella* are entirely consistent with Lemche's "split-band" hypothesis, which requires a band that was originally of a single color to take on another color while remaining self-symmetrical. In both species, the dark band that straddles the humeral vein is comprised of a single color, but the bands that straddle "pSc", R_{1a}, R_{S1}, and R_{S3} are all bisected by a much lighter color, exactly as predicted by Lemche. A projection of the wing pattern of *Sabatinca*

doroxena onto a typical nymphalid wing further supports the “split-band” hypothesis, given that the projection is in accordance with the “wing-margin” model (Figure 3.16).

Because micropterigid and nymphalid wings are so different in size, shape, and venation, the wing pattern of *Sabatinca doroxena* could be projected onto a nymphalid wing in any number of ways. However, the exacting predictions of the “wing-margin” model allow this to occur in only one way. In *S. doroxena*, the basal half of the nearly-split band that straddles the humeral vein is comprised of a single color, the apical half is bisected by a lighter color, and one last band – again, bisected by a lighter color – reaches the margin of the wing basal to the terminal branch of the subcostal vein. According to the nymphalid groundplan, three such bands – a one-color band at the base of the wing, followed by two concentric, two-color bands – reach the costa before Sc terminates. On the wing of *Sabatinca doroxena*, three additional concentric, two-color bands occur between the terminal branch of Sc and the terminal branch of Rs. However, because Sc terminates so close to the apex in nymphalids, the nymphalid wing simply does not contain sufficient space for three two-color bands, or symmetry systems, beyond Sc – much in the same way that the pattern element straddling Rs_4 in *Micropterix* originates along the costa and develops into a band, and is called the “terminal fascia,” but the pattern element straddling this same vein in Tortricidae does not occur along the costa of the wing and can only exist as a spot (Schachat and Brown 2015). And so in Nymphalidae, there is only space for one two-color band, or symmetry system, beyond Sc; two very thin one-color bands appear between this symmetry system and the terminus of the wing, such that the total number of wing pattern elements beyond Sc is the same in Nymphalidae as it is in *Sabatinca doroxena*; the fact that the two terminal wing pattern

elements in Nymphalidae do not resemble those in *Sabatinca doroxena* is a necessity according to the “wing-margin” model due to differences in wing shape between these two taxa (Figure 3.16).

Most elements of the nymphalid groundplan have a corresponding pattern element in *Sabatinca doroxena* and *S.aurella*. The only nymphalid groundplan elements not accounted for in the wing patterns of *S. doroxena* and *S. aurella* are the discal spot (DS) and the distal portion of the distal band of the central symmetry system (dBC). The discal spot could have arisen if the central symmetry system, corresponding to the pattern element that located in the “pSc” area of the wing in *Sabatinca*, is originated from a band that hypertrophied not once but twice. The distal portion of the distal band of the central symmetry system, marked with an asterisk (Figure 3.16C), could have originated if a very thin band, akin to the silvery striae in Tortricidae, appeared alongside the central symmetry system but later became decoupled from it and moved toward the apex of the wing.

The resemblance of certain *Sabatinca* wing patterns to the nymphalid groundplan suggests a revised version of Lemche’s “split-band” hypothesis for the origin of symmetry systems, in which symmetry systems originate from one-color bands that are bisected by another color and become concentric but the location of these bands along the wing is constrained by veins at the costa, as postulated by the “wing-margin” model, instead of the points where veins bifurcate. This novel combination of two compatible concepts that had previously been discussed in isolation, the “wing-margin” model for band location and the “split-band” hypothesis for the origin of symmetry systems, fits the nymphalid groundplan very closely and is, at present, better supported by empirical data

than either of the two previous proposed explanations because the wing patterns of *S. doroxena* and *S. aurella* so closely match the nymphalid groundplan.

Conclusions

From an examination micropterigid of wing patterns that are comprised of two colors, it appears that the ancestral state for this family – and therefore quite possibly for the order Lepidoptera – is a wing pattern of alternating light and dark bands, with each band straddling one vein along the costa. This ancestral state conforms to the predictions of the “wing-margin” model, originally based on Tortricidae (Brown and Powell 1991, Baixeras 2002). However, a comparison of the wing patterns of *Micropterix* with *Sabatinca doroxena* and *S. aurella* shows that the “wing-margin” correctly predicts the location of transverse bands and the contrast boundaries between them, but cannot predict which series of bands will be light brown and which will be dark brown. The wing pattern elements of *S. doroxena* and *S. aurella* – simple bands of a single dark color, and two-color bands in which dark scales surround a central light area – illustrate both stages of “split-band” symmetry system formation hypothesized by Lemche (1937) and therefore strongly support his hypothesis that symmetry systems originated when dark bands were bisected, or hypertrophied, by light bands. When the wing pattern of *Sabatinca doroxena* is plotted on to a nymphalid wing following the constraints proposed by the “wing-margin” model, the resulting hypothetical wing pattern very strongly resembles the nymphalid groundplan. Because the “wing-margin” model correctly predicts the location of wing pattern elements in distantly related lepidopteran lineages (Micropterigidae, Tortricidae), and, in combination with the “split-band” hypothesis, can predict the nymphalid groundplan based on wing pattern in *Sabatinca*, the “wing-margin”

model and the “split-band” hypothesis appear to have great potential to explain wing pattern diversity in the order Lepidoptera.

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Figures

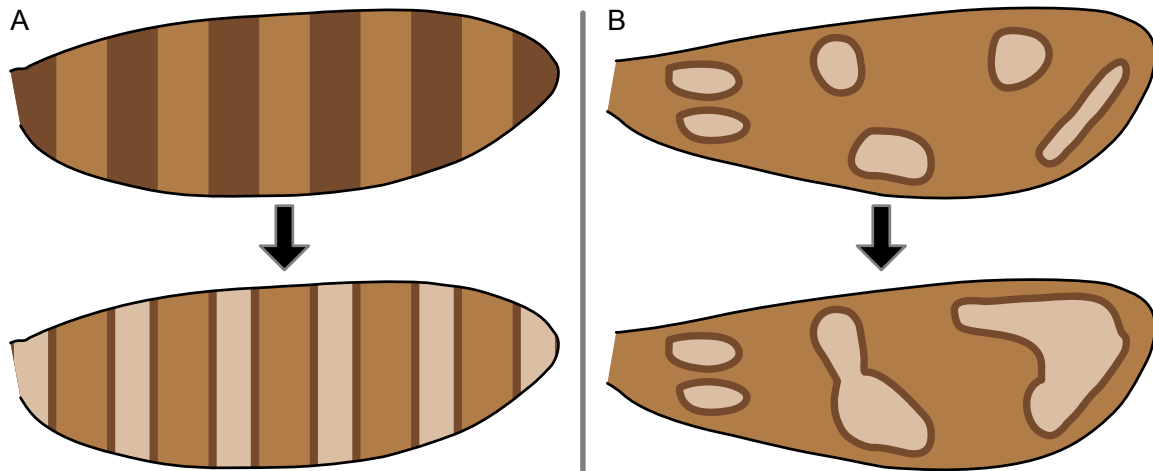


Figure 3.1 The two existing hypotheses for the origin of symmetry systems from primitive microlepidopteran wing pattern elements.

(A) Lemche's "split-band" hypothesis. (B) Nijhout's "fused-spot" hypothesis.

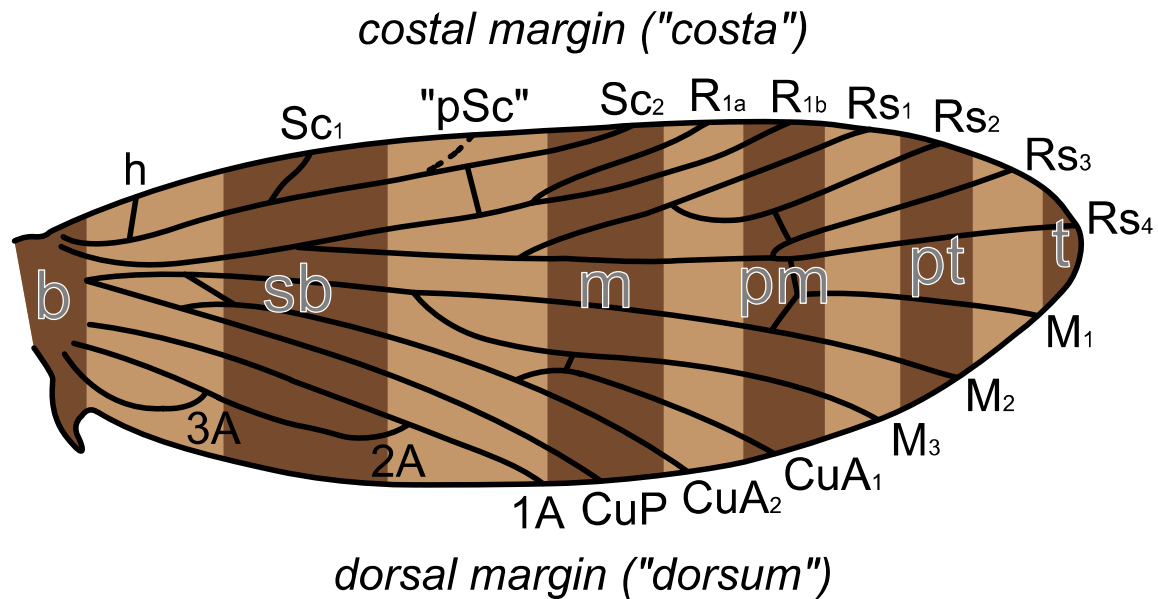


Figure 3.2 The *Micropterix* wing pattern groundplan.

The forewing color pattern groundplan of *Micropterix* (Schachat and Brown 2015), plotted onto a hypothesized ancestral wing for Lepidoptera (Zhang et al. 2013). The vein labeled "pSc" is a known plesiomorphic wing vein for Amphiesmenoptera hypothesized by Schachat and Brown to influence color pattern. Abbreviations for other veins are as follows: **h**: humeral; **Sc**: subcosta; **R**: radius; **Rs**: radial sector; **M**: media; **CuA**: anterior cubitus; **CuP**: posterior cubitus; **A**: anal.

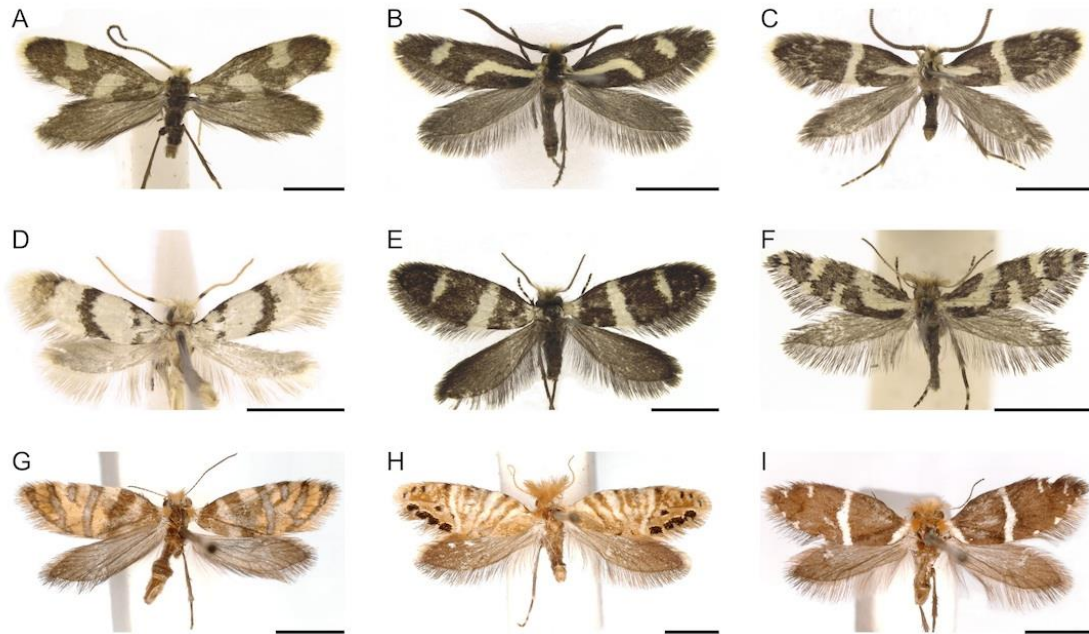


Figure 3.3 Photographs of various Micropterigidae examined for the present study.

(A) *Epimartyria pardella*. (B) *Tasmantrix lunaris*. (C) *Tasmantrix nigricornis*. (D) *Aureopterix sterops*. (E) *Austromartyria porphyrodes*. (F) *Agrionympha capensis*. (G) *Sabatinca aurella*. (H) *Sabatinca calliarcha*. (I) *Sabatinca ianthina*.

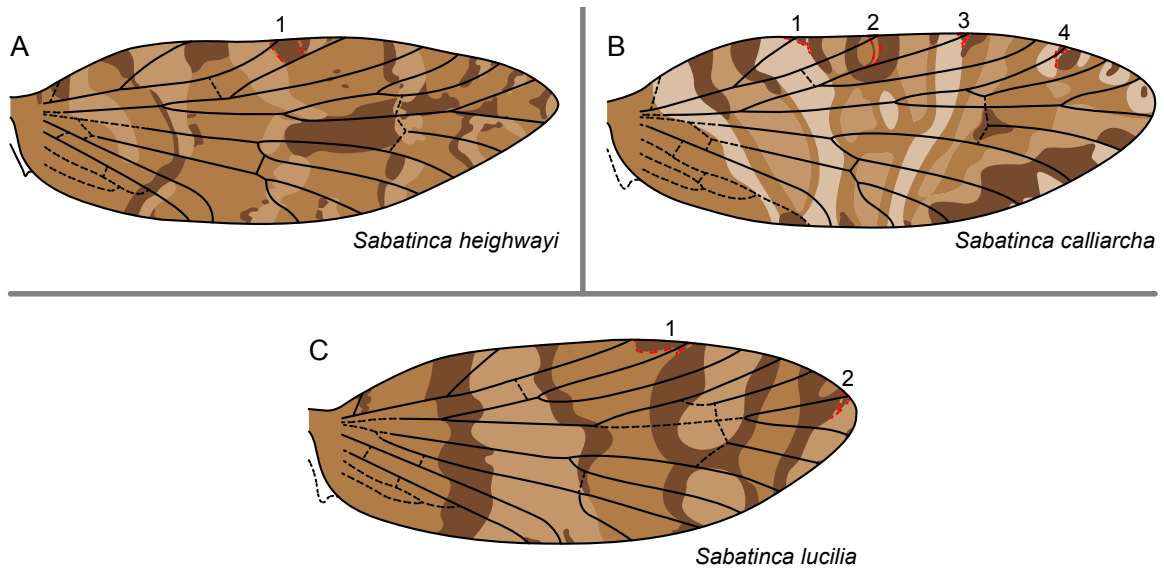


Figure 3.4 Wing pattern of the most basal *Sabatinca* species, which occur in New Zealand and belong to the *calliartha* group.

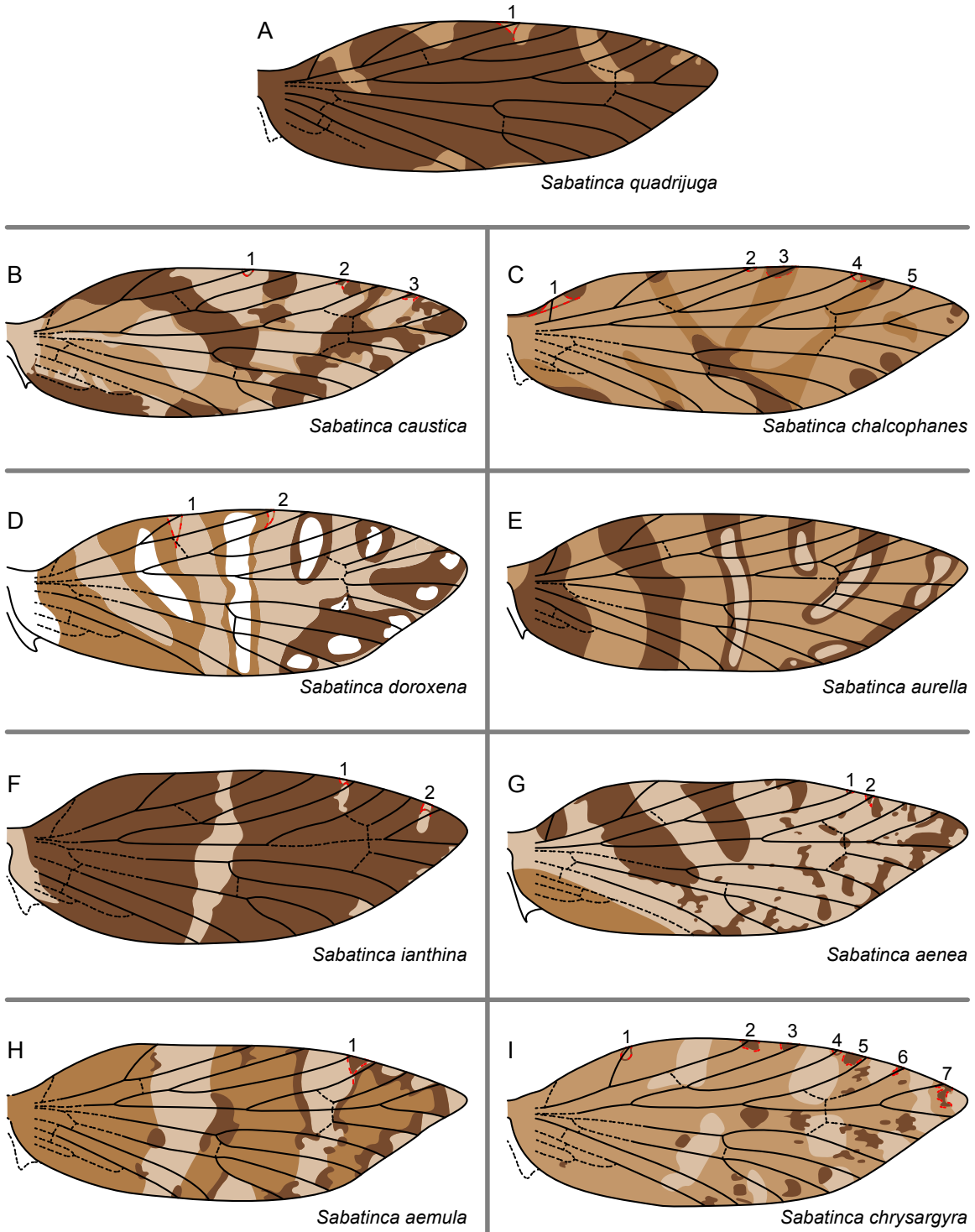


Figure 3.5 Wing pattern of the majority of New Zealand *Sabatinca* species sampled, which belong to the *chrysargyra* group.

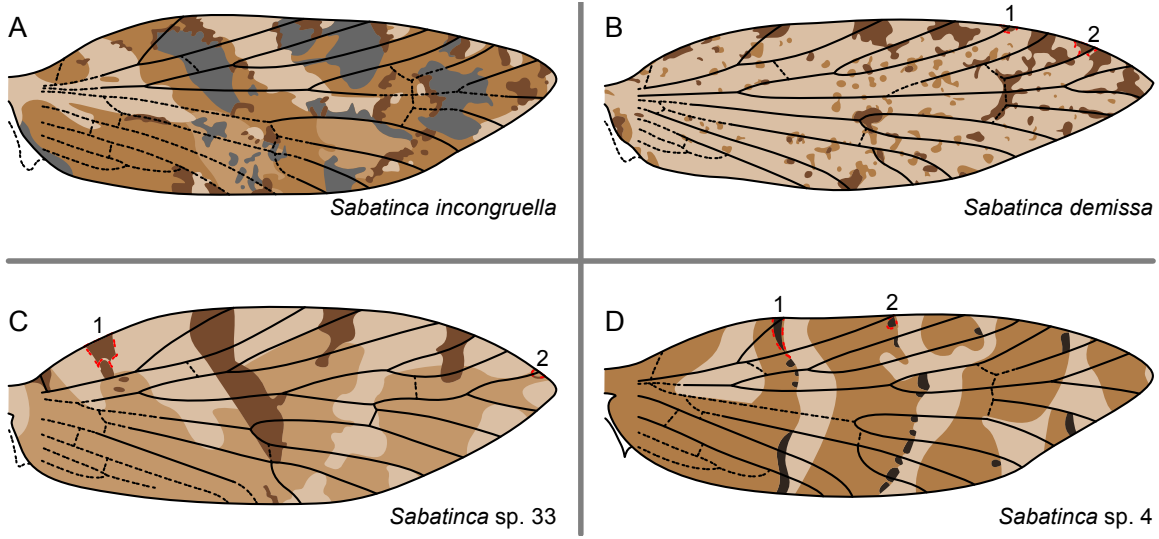


Figure 3.6 Wing pattern of the earliest-diverging species of the *incongruella* group, from New Zealand and New Caledonia.

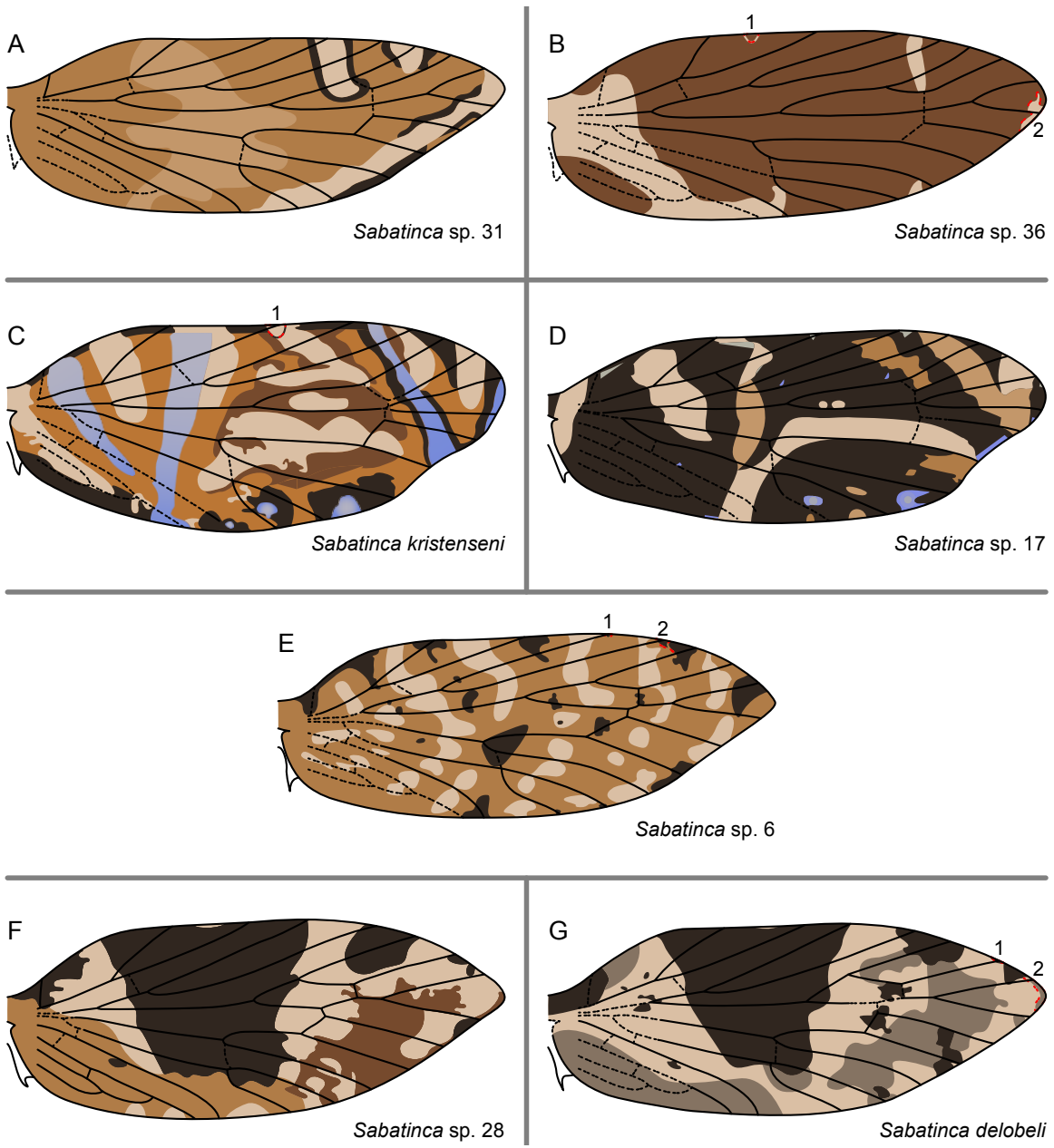


Figure 3.7 Wing pattern in the *incongruella* group from New Caledonia, continued.

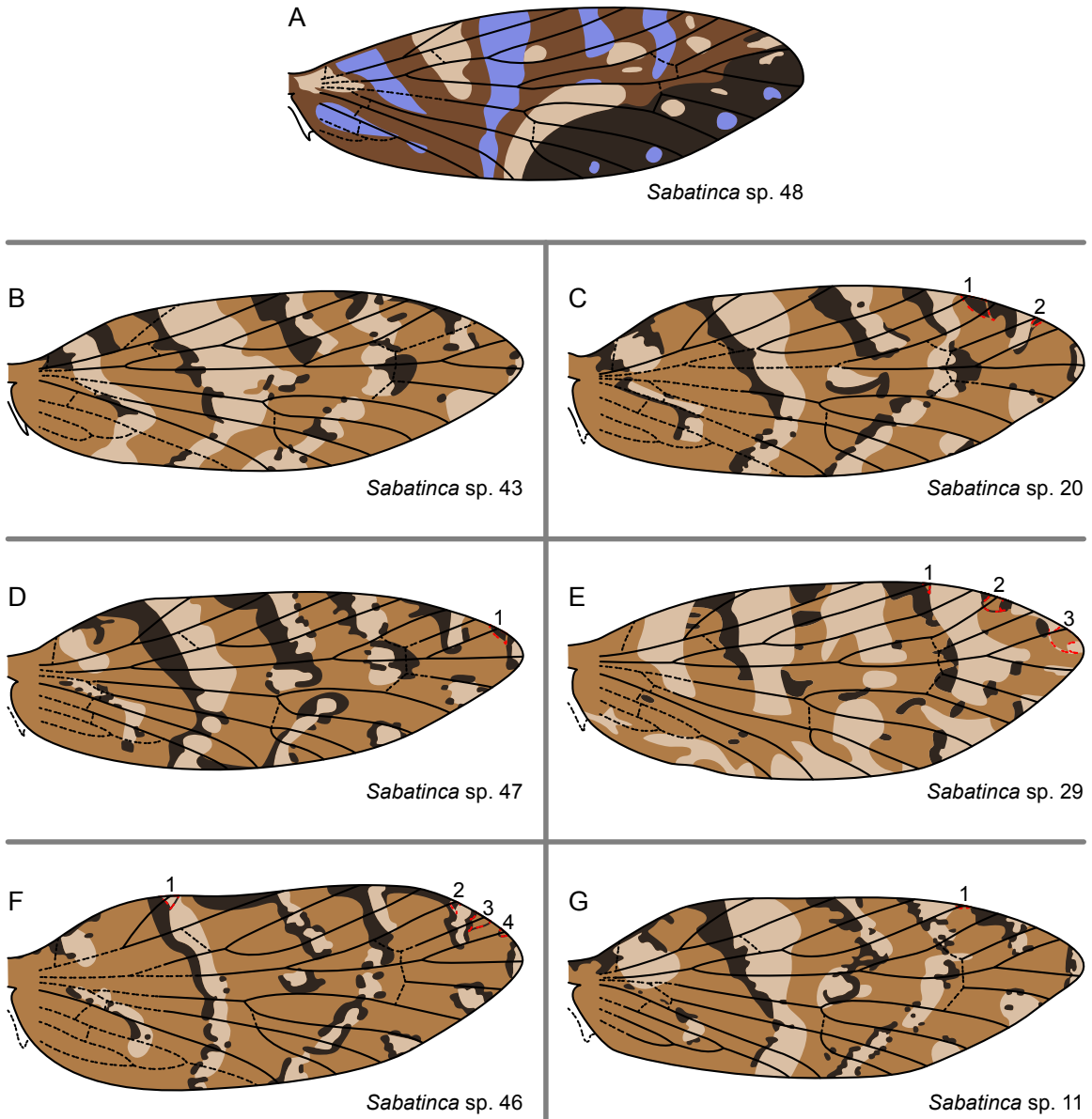


Figure 3.8 Wing pattern in the *incongruella* group from New Caledonia, continued.

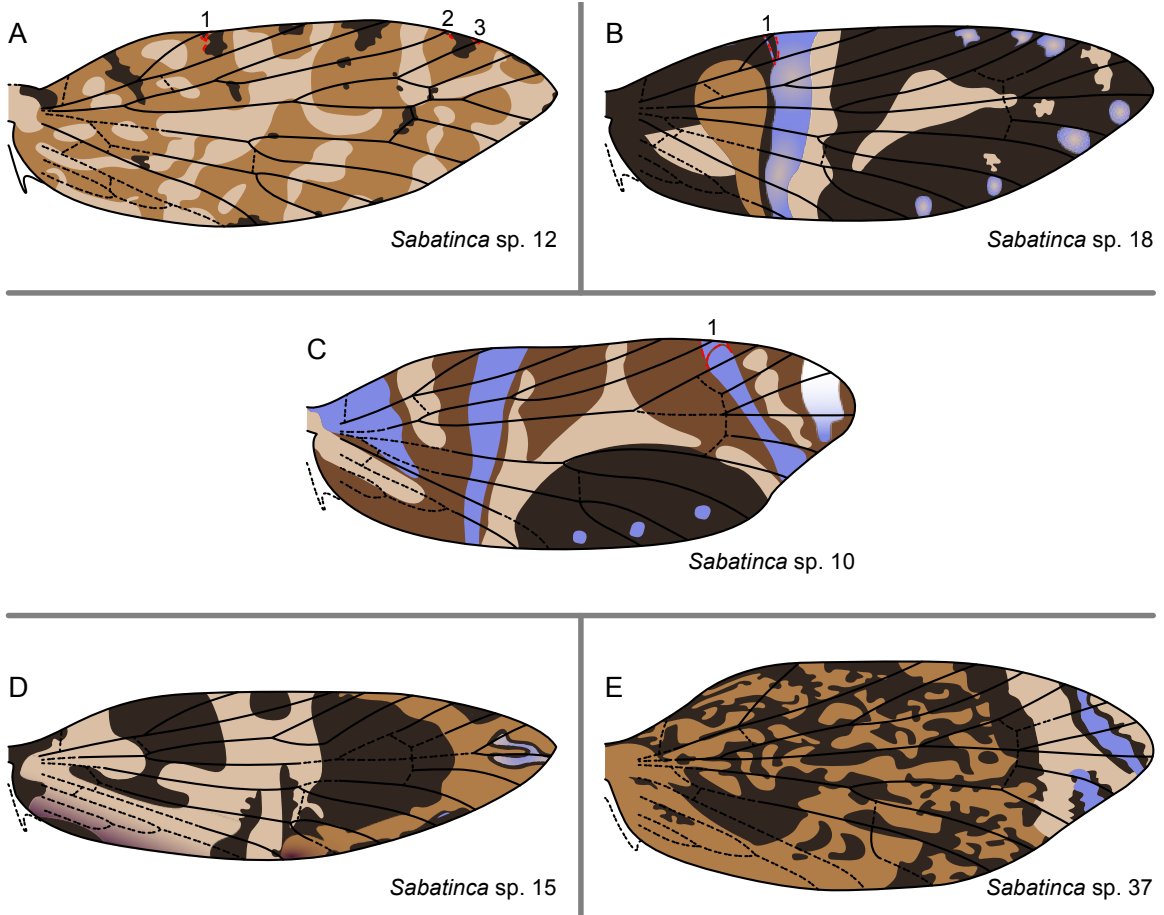


Figure 3.9 Wing pattern in the *incongruella* group from New Caledonia, continued.

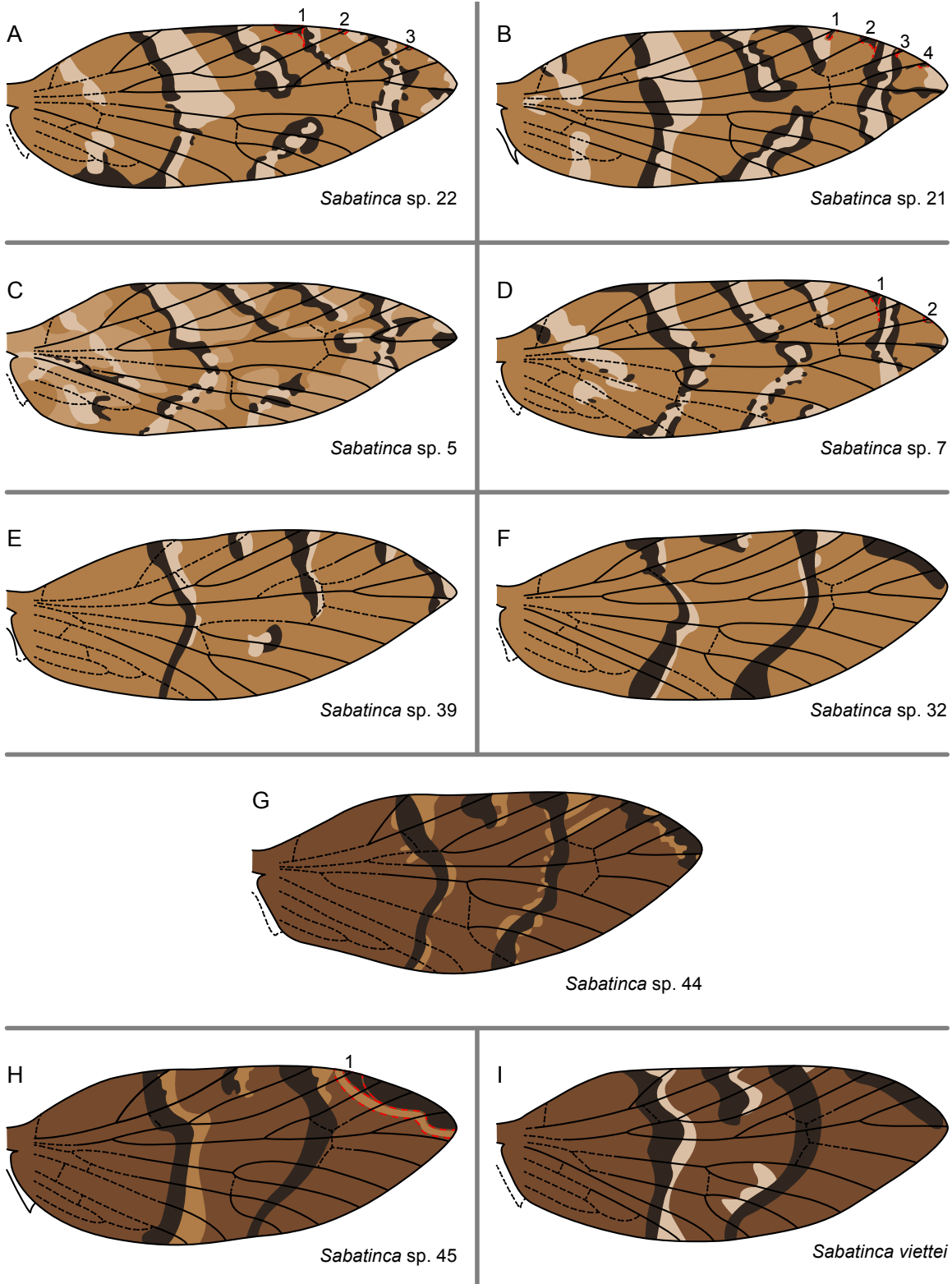


Figure 3.10 Wing pattern in the *incongruella* group from New Caledonia, continued.

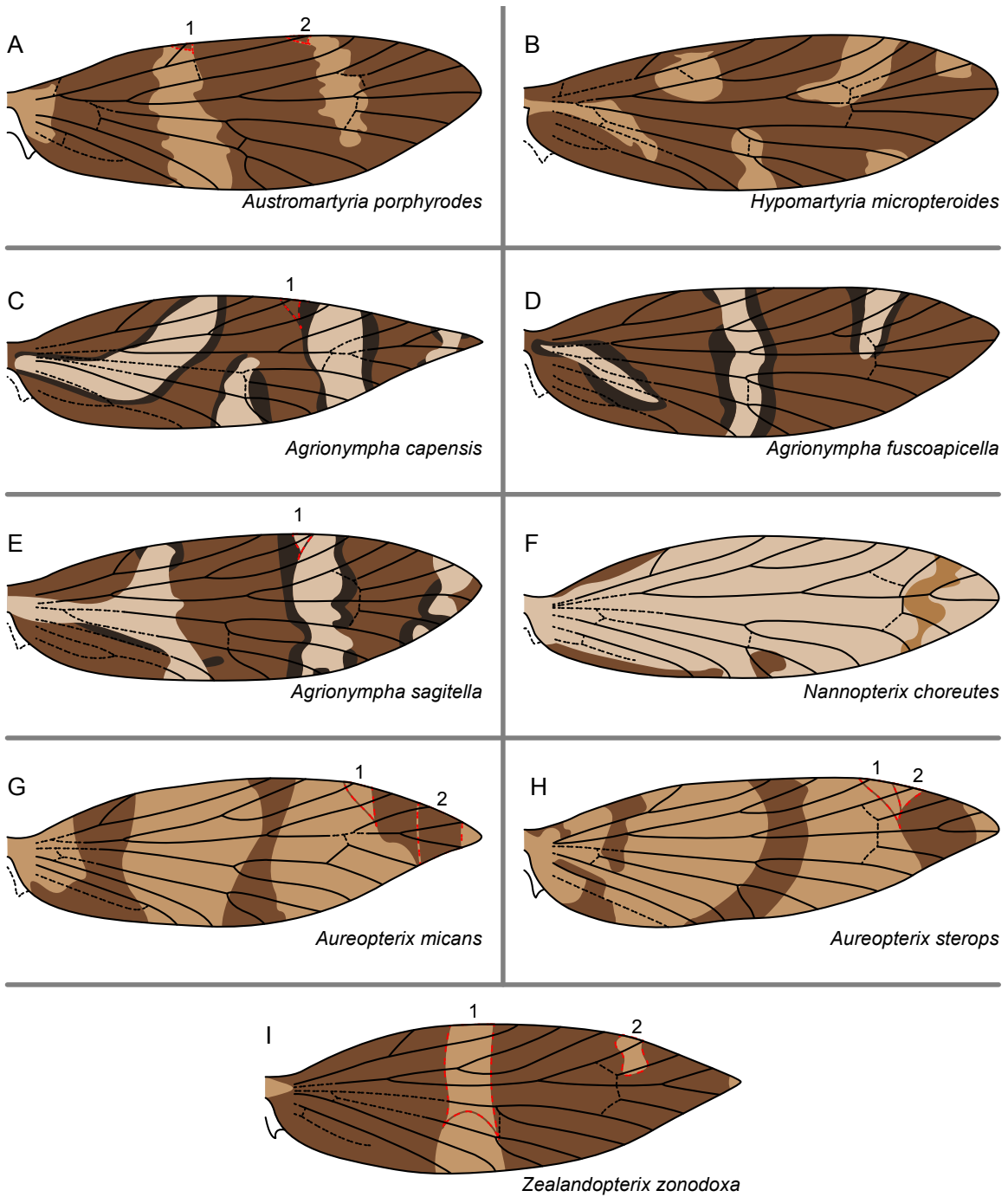


Figure 3.11 Wing pattern in Micropterigidae other than *Sabatinca*, *Tasmantrix*, *Micropterix*, and *Epimartyria*.

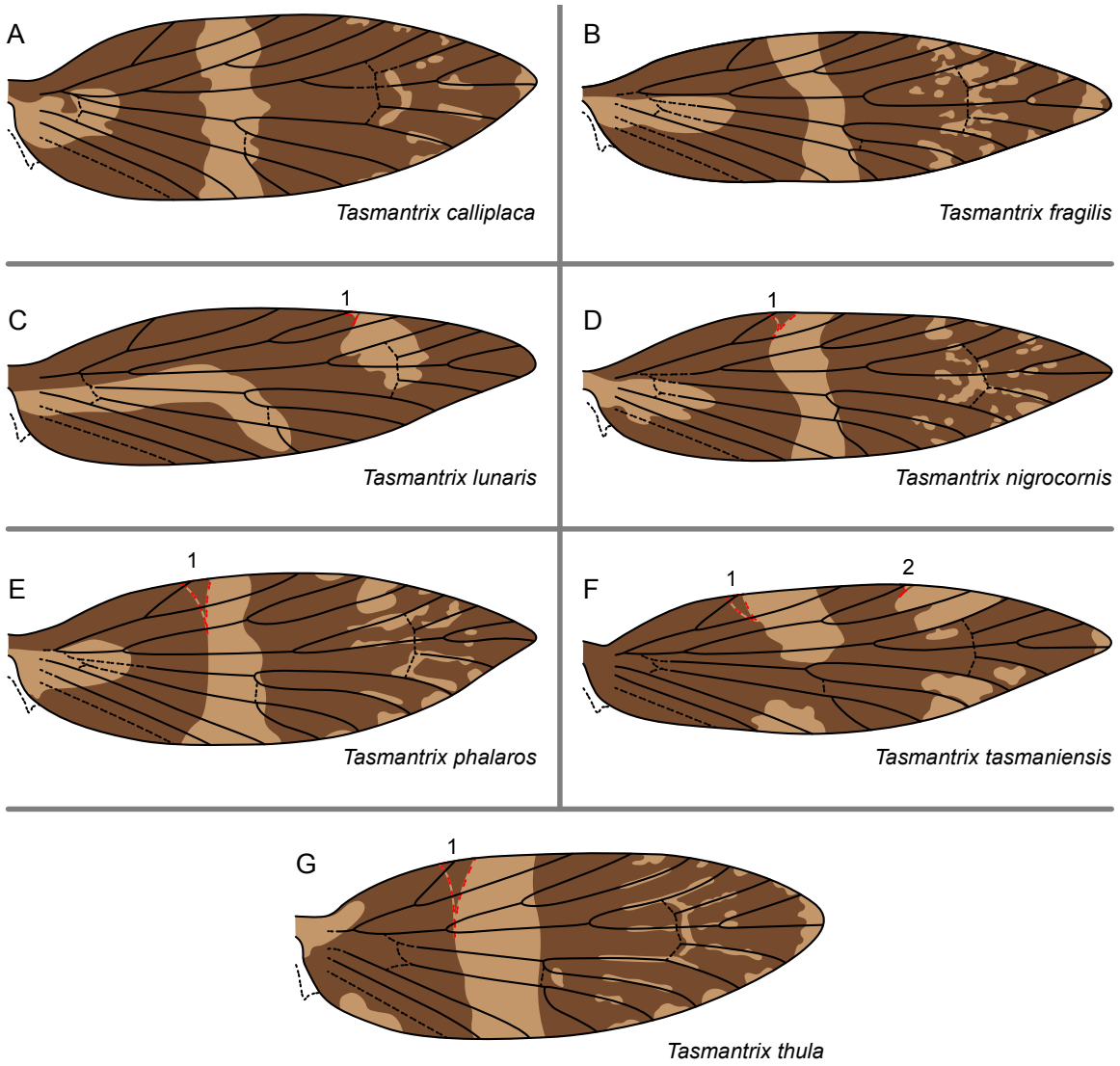


Figure 3.12 Wing pattern in *Tasmantrix*.

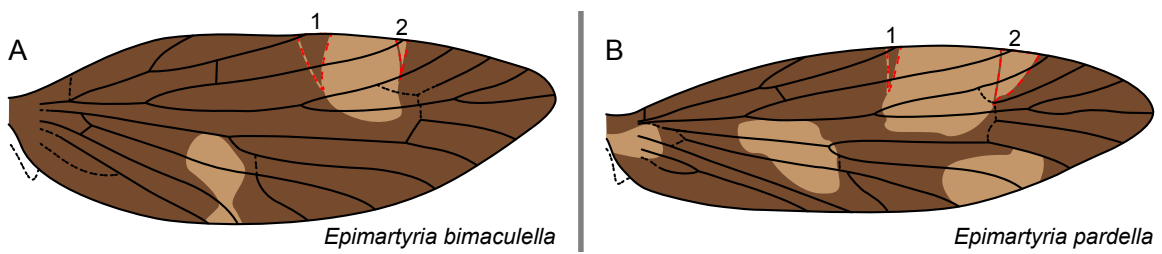


Figure 3.13 Wing pattern in *Epimartyria*.

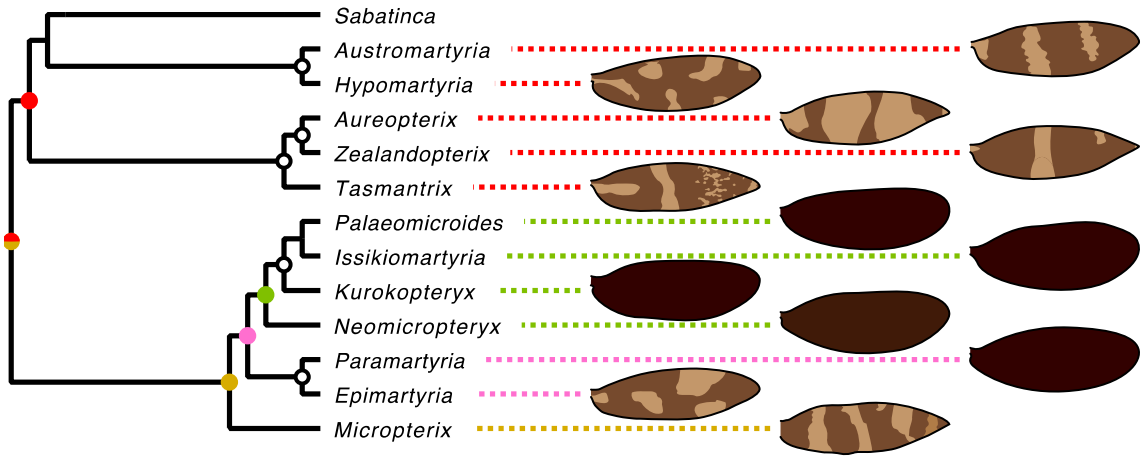


Figure 3.14 Wing pattern of Micropterigidae other than *Sabatinca* in the context of the preliminary phylogeny published by Gibbs & Lees (2014).

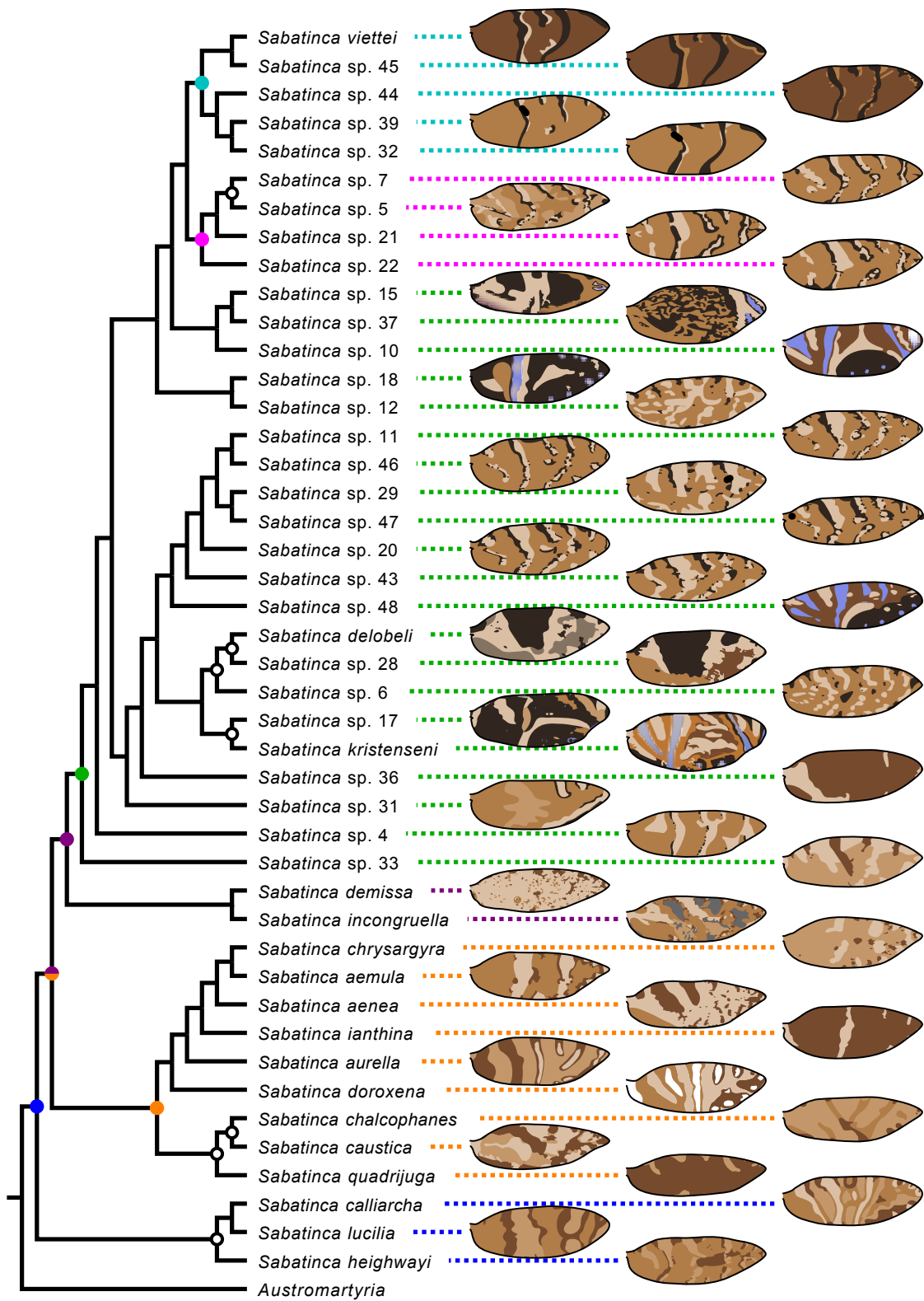


Figure 3.15 Wing pattern of *Sabatinca* in the context of the preliminary phylogeny published by Gibbs & Lees (2014).

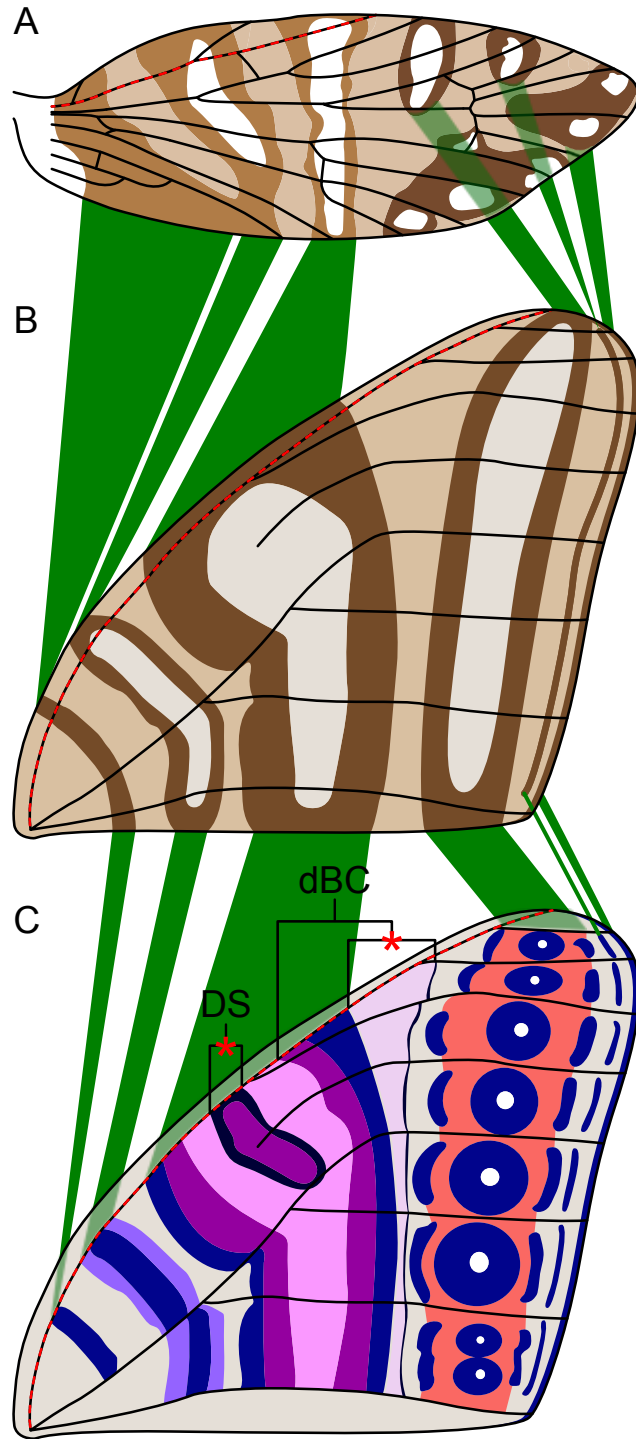


Figure 3.16 Comparison of the wing pattern of *Sabatinca doroxena* with the nymphalid groundplan.

(A) The observed wing pattern of *Sabatinca doroxena*. (B) The wing pattern of *Sabatinca doroxena* plotted onto a typical wing venation groundplan for Nymphalidae, preserving the relationship between venation and pattern outlined by the “wing-margin” model. (C) The Nymphalid groundplan (Otaki 2012), with the discal spot (DS) and the distal band of the central symmetry system (dBC) labeled; the red asterisk indicates the portion of dBC that has no counterpart in *Sabatinca*.

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APPENDIX A

VARIATION IN FOREWING COLOR PATTERN IN MICROPTERIGIDAE

Table A.1 Non-*Sabatinca* wing pattern variation

Collection	Genus	Species	GP	V1	V2	V1&2
ANIC	<i>Austromartyria</i>	<i>porphyrodes</i>	14	2	4	
VUW	<i>Hypomartyria</i>	<i>micropteroides</i>	1			
VUW	<i>Agrionympha</i>	<i>capensis</i>	19	1		
VUW	<i>Agrionympha</i>	<i>sagitella</i>	19	1		
VUW	<i>Agrionympha</i>	<i>fuscoapicella</i>	6			
VUW	<i>Aureopterix</i>	<i>micans</i>	16	2	2	
ANIC	<i>Aureopterix</i>	<i>sterops</i>	9		8	3
VUW	<i>Nannopterix</i>	<i>choreutes</i>	12			
VUW	<i>Zealandopterix</i>	<i>zonodoxa</i>	8		8	4
ANIC	<i>Tasmantrix</i>	<i>calliplaca</i>	20			
ANIC	<i>Tasmantrix</i>	<i>fragilis</i>	12			
ANIC	<i>Tasmantrix</i>	<i>lunaris</i>	8	2		
ANIC	<i>Tasmantrix</i>	<i>nigrocornis</i>	18	2		
ANIC	<i>Tasmantrix</i>	<i>phalaros</i>	16	4		
ANIC	<i>Tasmantrix</i>	<i>tasmaniensis</i>	10	1	3	
ANIC	<i>Tasmantrix</i>	<i>thula</i>	18	2		
USNM	<i>Epimartyria</i>	<i>bimaculella</i>	8	2	7	3
ANIC, USNM	<i>Epimartyria</i>	<i>pardella</i>	10	7	1	

Legend: ANIC: Australian National Insect Collection, VUW: Victoria University Wellington, USNM: United States National Museum, GP: Groundplan (as illustrated), V1 = Variation 1, etc.

Explanation of variations:

- *Austromartyria porphyrodes*
 1. Sc₁ straddled by light scales at costa
 2. Sc₂ surrounded by light scales at costa
- *Agrionympha capensis*
 1. R_{1a} surrounded by darkest scales at costa
- *Agrionympha sagitella*
 1. R_{1a} surrounded by darkest scales at costa
- *Aureopterix micans*
 1. R_{1b} surrounded by dark scales at costa
 2. Rs₂ surrounded by light scales at costa
- *Aureopterix sterops*
 1. R_{1b} surrounded by dark scales at costa
 2. Rs₁ surrounded by dark scales at costa
- *Zealandopterix zonodoxa*
 1. Light area between Sc₁ and Sc₂ does not reach the costa
 2. Rs₁ surrounded by dark scales at costa
- *Tasmantrix lunaris*
 1. R_{1a} surrounded by light scales at costa
- *Tasmantrix nigrocornis*
 1. Sc₁ surrounded by light scales at costa
- *Tasmantrix phalaros*
 1. Sc₁ surrounded by light scales at costa

- *Tasmantrix tasmaniensis*
 1. Sc₁ surrounded by light scales at costa
 2. R_{1a} surrounded by dark scales at costa
- *Tasmantrix thula*
 3. Sc₁ surrounded by light scales at costa
- *Epimartyria bimaculella*
 1. Sc₂ surrounded by light scales at costa
 2. R₁ surrounded by dark scales at costa
- *Epimartyria pardella*
 1. Sc₂ surrounded by light scales at costa
 2. R₁ surrounded by dark scales at costa

Table A.2 *Sabatinca* wing pattern variation from New Zealand

Species	GP	V1	V2	V3	V4	V 1,2	V 2,3	V 2,4	V 2,5	V 3,4	V 4,5	V1, 2,5	V5, 6,7	V2, 3,5, 6	V2, 3,5, 6,7
<i>aemula</i>	10	2													
<i>aenea</i>	7	6	3												
<i>aurella</i>	20														
<i>calliarcha</i>	6	3	4	2	1										
<i>caustica</i>	7		5			6	2								
<i>chalcophanes</i>	7		4				1	2	2		2	2			
<i>chrysargyra</i>	8	2			1		4			1			1	2	1
<i>demissa</i>	11	6	2			1									
<i>doroxena</i>	8	2	2												
<i>heighwayi</i>	5	1													
<i>ianthina</i>	15	2	1			2									
<i>incongruella</i>	1														
<i>lucilia</i>	7	1	2												
<i>quadrijuga</i>	12	2													

All *Sabatinca* specimens examined are held in the collection of Victoria University, Wellington. **Legend:** GP: Groundplan (as illustrated), V1 = Variation 1, etc.

Explanation of variations:

- *Sabatinca aemula*
 1. R_{1b} surrounded by light area at costa
- *Sabatinca aenea*
 1. R_{1b} surrounded by dark area at costa
 2. R_{s1} surrounded by light area at costa
- *Sabatinca calliarcha*

1. Sc₁ surrounded by light scales at costa
 2. Sc₂ surrounded by dark scales at costa
 3. R_{1a} surrounded by light scales at costa
 4. Rs₁ surrounded by light scales at costa
- *Sabatinca caustica*
 1. Sc₂ surrounded by darkest scales at costa
 2. R_{1b} surrounded by lightest scales
 3. Rs₂ surrounded by light scales at costa
 - *Sabatinca chalcophanes*
 1. h surrounded by dark scales at costa
 2. Sc₂ surrounded by dark scales at costa
 3. R_{1a} surrounded by medium scales at costa
 4. R_{1b} surrounded by dark scales at costa
 5. Rs₂ surrounded by dark scales at costa
 - *Sabatinca chrysargyra*
 1. Sc₁ surrounded by darkest scales at costa
 2. Sc₂ surrounded by lightest scales at costa
 3. R_{1a} surrounded by lightest scales at costa
 4. R_{1b} surrounded by darkest scales at costa
 5. Rs₁ surrounded by lightest scales at costa
 6. Rs₂ surrounded by lightest scales at costa
 7. Rs₃ surrounded by lightest scales at costa

- *Sabatinca demissa*
 1. R_{1b} surrounded by light scales at costa
 2. R_{s2} surrounded by light scales at costa
- *Sabatinca doroxena*
 1. Sc₁ surrounded by medium scales at costa
 2. Sc₂ surrounded by medium scales at costa
- *Sabatinca heighwayi*
 1. Sc₂ surrounded by medium brown scales at costa
- *Sabatinca ianthina*
 1. Light area between R_{1a} and R_{1b} does not reach costa
 2. Light area between R_{s2} and R_{s3} does not reach costa
- *Sabatinca lucilia*
 1. R_{1a} surrounded by medium scales at costa
 2. R_{s3} surrounded by lightest scales at costa
- *Sabatinca quadrijuga*
 1. Sc₂ surrounded by dark scales at costa

Table A.3 *Sabatinca* wing pattern variation from New Caledonia

Species	GP	V1	V2	V3	V4	V1,2	V2,3	V2,4	V3,4	V1,3,4	V2,3,4
4	10	2	1			7					
5	6										
6	11	2	7								
7	10	2	8								
10	18	2									
11	18	2									
12	9	6	4				1				
15	8										
17	12										
18	10	2									
20	9	5	4			2					
21	6	4			2				2	1	1
22	17		1			1	1				
28	11										
29	6			6		4					
31	2										
32	12										
33	4	1	2								
36	6	4	2								
37	2										
39	1										
43	2										
44	13										
45	5	4									
46	7	1	1	1	4			2	2		

Table A.3 (Continued)

47	7	1									
48	12										
<i>delobeli</i>	10	8	2								
<i>kristenseni</i>	14	6									
<i>viettei</i>	6										

All *Sabatinca* specimens examined are held in the collection of Victoria University, Wellington. **Legend:** GP: Groundplan (as illustrated), V1 = Variation 1, etc.

Explanation of variations:

- *Sabatinca* sp. 4
 1. Sc₁ surrounded by dark scales at costa
 2. Sc₂ surrounded by dark scales at costa
- *Sabatinca* sp. 6
 1. R_{1a} surrounded by medium scales at costa
 2. R_{1b} surrounded by medium scales at costa
- *Sabatinca* sp. 7
 1. Rs₁ surrounded by medium scales at costa
 2. Rs₃ surrounded by darkest scales at costa
- *Sabatinca* sp. 10
 1. R_{1b} surrounded by brown scales at costa
- *Sabatinca* sp. 11
 1. R_{1b} surrounded by medium scales at costa
- *Sabatinca* sp. 12

1. Sc₁ surrounded by medium scales at costa
 2. R_{1b} surrounded by medium scales at costa
 3. Rs₁ surrounded by medium scales at costa
- *Sabatinca* sp. 18
 1. Sc₁ surrounded by bluish scales at costa
 - *Sabatinca* sp. 20
 1. R_{1b} surrounded by medium scales at costa
 2. Rs₂ surrounded by medium scales at costa
 - *Sabatinca* sp. 21
 1. The distal edge of R_{1b} is abutted by medium scales at the costa
 2. Rs₁ surrounded by medium scales at costa
 3. Rs₂ surrounded by medium scales at costa
 4. Rs₃ surrounded by medium scales at costa
 - *Sabatinca* sp. 22
 1. R_{1a} surrounded by medium scales at costa
 2. R_{1b} surrounded by darkest scales at costa
 3. Rs₂ surrounded by darkest scales at costa
 - *Sabatinca* sp. 29
 1. R_{1a} surrounded by darkest scales at costa
 2. Rs₁ surrounded by darkest scales at costa
 3. Rs₃ surrounded by lightest scales at costa
 - *Sabatinca* sp. 33
 1. Darkest scales don't reach costa at area between h and Sc₁

2. Darkest scales surround costa at Rs_3
- *Sabatinca* sp. 36
 1. Light scales meet costa between Sc_1 and Sc_2
 2. Dark scales straddle/abut Rs_4 at termen
 - *Sabatinca* sp. 45
 1. R_{1b} surrounded by medium scales at costa; the dark band that straddles $Rs_{1,2,3}$ at costa is not bordered by a contiguous band of light scales
 - *Sabatinca* sp. 46
 1. Sc_2 surrounded by darkest scales at costa
 2. Rs_1 surrounded/abuted by lightest scales at costa
 3. Rs_2 surrounded by darkest scales at costa
 4. Rs_3 surrounded by dark scales at costa
 - *Sabatinca* sp. 47
 1. Rs_3 surrounded by medium scales at costa
 - *Sabatinca delobeli*
 1. Rs_2 surrounded by darkest scales at costa
 - *Sabatinca kristenseni*
 1. Sc_2 surrounded by darkest scales at costa