

# Population Genetics and Phylogeny of the Bush Brown Butterfly in Genus Mycalesis Hübner, 1818 (Lepidoptera: Nymphalidae) in Peninsular Thailand 

Sakiyah Morlor

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Zoology Prince of Songkla University 2017


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| ชื่อวิทยานิพนธ์ | พันธุศาสตรประชากรและวงศ์วานวิวัฒนาการของผีเสื้อตาลพุ่มในสกุล |
| :--- | :--- |
|  | Mycalesis Hübner, 1818 (Lepidoptera: Nymphalidae) ใน |
|  | คาบสมุทรไทย |
| ผู้เขียน | นางสาวซากียะ มอลอ |
| สาขาวิชา | สัตววิทยา |
| ปีการศึกษา | 2560 |

บทคัดย่อ
ประเทศไทยตั้งอยู่ในเขตร้อนชื้นซึ่งมีความหลากหลายทางชีวภาพสูงโดยเฉพาะ บริเวณคาบสมุทรไทย เป็นบริเวณรอยต่อการแพร่กระจายของพันธุ์พืชและสัตว์บางชนิดระหว่าง ภูมิภาคอินโดจีนและภูมิภาคซุนดา ผีเสื้อตาลพุ่มในสกุล Mycalesis เป็นกลุ่มที่มีการแพร่กระจายใน เขตภูมิภาคเอเชียตะวันออกเฉียงใต้และออสเตรเลีย ลักษณะเด่นของผีเสื้อกลุ่มนี้คือ มีรูปแบบของ ปีกที่แตกต่างกัน 2 รูปแบบได้แก่ รูปแบบในฤดูฝน และรูปแบบในฤดูแล้ง โดยรูปแบบปีกในฤดู แล้งมีการลดรูปของจุดตาของปีกด้านล่าง ซึ่งทำให้ยากต่อการจำแนกชนิด วัตถุประสงค์ของ การศึกษาครั้งนี้เพื่อสำรวจและจำแนกชนิดผีเสื้อตาลพุ่ม ตรวจสอบความผันแปรทางพันธุกรรม และศึกษาความสัมพันธ์เชิงวิวัฒนาการของผีเสื้อตาลพุ่มในคาบสมุทรไทย โดยได้ทำการเก็บ ตัวอย่างผีเสื้อตาลพุ่มใน 6 จังหวัดตามแนวเทือกเขาในภาคใต้ ได้แก่ จังหวัดชุมพร จังหวัด สุราษฏร์ธานี จังหวัดนครศรีธรรมราช จังหวัดพังงา จังหวัดตรัง และจังหวัดนราธิวาส ตั้งแต่เดือน กุมภาพันธ์ 2558 ถึง เดือนกรกฎาคม 2559 โดยใช้สวิงจับแมลงและกับดักผลไม้ จากนั้นนำมาจัด รูปร่าง ถ่ายรูปตัวอย่างและจำแนกชนิดโดยใช้ลักษณะของปีกและอวัยวะสืบพันธุ์ นอกจากนี้ได้นำ ขาคู่กลางมาใช้ในการสกัดดีเอ็นเอเพื่อตรวจสอบความผันแปรทางพันธุกรรม และศึกษา ความสัมพันธ์เชิงวิวัฒนาการระดับโมเลกุล โดยใช้ยีนในไมโทคอนเดรีย ( COI ) และยีนในนิวเคลียส (EF-1 $\alpha$ และ wingless)

จากการศึกษาพบผีเสื้อตาลพุ่มทั้งหมด 214 ตัว สามารถจำแนกได้ 7 ชนิด ได้แก่ Mycalesis intermedia, M. perseus, M. perseoides, M. visala, M. orseis, M. janardana และ M. mineus ซึ่งพบรูปแบบปีกของรูปแบบในฤดูฝนและรูปแบบในฤดูแล้ง โดยการศึกษานี้ได้บรรยาย ลักษณะของอวัยวะสืบพันธุ์เพศผู้ของผีเสื้อตาลพุ่มทั้ง 7 ชนิด จากการศึกษาความผันแปรทาง พันธุกรรมของผีเสื้อตาลพุ่มแต่ละชนิด พบว่ามีความแตกต่างกันในแต่ละยีน โดย $M$. mineus มี ความผันแปรทางพันธุกรรมภายในชนิดต่ำทั้งสามยีน จากผลการศึกษาความสัมพันธ์เชิงวิวัฒนาการ

โดยการรวมยีนพบว่า ผีเสื้อตาลพุ่มในคาบสมุทรไทยมีบรรพบุรุษร่วมกันโดยสมาชิกใน Mineus group สามารถแบ่งได้สองเคลด จากผลการศึกษาครั้งนี้ยังแสดงให้เห็นว่า ผีเสื้อตาลพุ่มใน คาบสมุทรไทยมีจุดกำเนิดในช่วงเวลา 32.47 ล้านปีในยุคโอลิโกซีน

Thesis Title Population Genetics and Phylogeny of the Bush Brown Butterfly in Genus Mycalesis Hübner, 1818 (Lepidoptera: Nymphalidae) in Peninsular Thailand<br>Author<br>Miss Sakiyah Morlor<br>Major Program Zoology<br>Academic Year 2016


#### Abstract

Thailand is located in tropical rainforest with rich in biodiversity, especially peninsular Thailand, which is an important transition zone of fauna between the Indo-Chinese and Sundaic sub-regions. Mycalesis radiated spectacular in Southeast Asia and Australia, there are two distinct seasonal morphs (wet and dry season form). In dry season form, the submarginal ocelli pattern on the underside of wing is reduced that make difficult for identification. The aims of this study were observed species and identification, investigated genetic variation and phylogenetic relationships of Mycalesis in peninsular Thailand. The survey of Mycalesis in peninsular Thailand was conducted in 6 provinces, comprising of Chumphon, Surat Thani, Nakhon Si Thammarat, Phangnga, Trang and Narathiwat during February 2015 to July 2016. The adult specimens were collected by using insect nets and fruitbaited traps, then wing setting, photographs and genitalia dissections were carried out. Mitochondrial gene (COI) and two nuclear gene ( $E F-1 \alpha$ and wingless) were investigated to study the genetic variation and constructed phylogenetic relationships in Mycalesis populations.

Totally 214 Mycalesis specimens were collected, that could be identified to seven species; M. intermedia, M. perseus, M. perseoides, M. visala, M. orseis, M. janardana and M. mineus. In this study, two distinct seasonal morphs were found as wet and dry season forms, male genitalia also were described. Difference of genetic variation was found in each gene of Mycalesis species, but present as low in all genes of M. mineus. Phylogenetic relationships based on combined gene sequences


indicated that the Mycalesis was monophyletic group, which Mineus group can be divided into two major clades. Estimating divergence time of Mycalesis in peninsular Thailand was 32.47 mya in Oligocene period.

## ACKNOWLEDGEMENT

This thesis could not accomplished without the help and support of many people. First, I would like to express my deep gratitude to my advisor, Dr. Singtoe Boonrotpong who provided me support, patient guidance and enthusiastic encouragement. He invested a lot of energy to improve my thesis. Special thanks should be given to my Co-advisor, Assoc. Prof. Dr. Nantasak Pinkaew. Who gave me very useful advice and supported in the taxonomic work and useful critiques of the thesis.

I am thankful to my committees of both proposal and thesis defense, Assoc. Prof. Dr. Surakrai Permkam from Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University who was my proposal defense committee chairperson and Asst. Prof. Dr. Taeng On Prommi from Department of Science, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus who was my thesis defense committee chairperson and also Asst. Prof. Dr. Theerakamol Pengsakul.

I am also grateful to Dr. Patamarerk Engsontia, who helped and gave me a useful suggestion about Genbank submission. In addition, many thanks to Dr. Danjuma Solomon and Dr. Krittika Kaewchumnong, who corrected my English language and provided constructive comment for manuscript.

I would like to thank Ms. Sopita Muadsub and Mr. Paradorn Dokchan from Department of Entomology Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, who helped and suggested about genitalia dissection technique and took photos of male genitalia of Mycalesis specimens.

My sincere thanks also goes to Dr. Abdullah Samoh, Mrs. Natrada Mitpuangchon, Ms. Areeruk Nilsai and all members of the Entomology Research Unit for helped and support to collect the samples in the field.

Financial support for this study was provided through a scholarship of the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission (NRU).

Finally, I could not have succeeded this study without my warm family's support. My father, mother, brother, sister, my husband and my daughter. They gave me inspiration, encouragement and love.

Sakiyah Morlor

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## LIST OF ABBREVIATIONS AND SYMBOLS

| AFLP | $=$ | amplified fragment length polymorphism |
| :---: | :---: | :---: |
| pb | $=$ | base pair (s) |
| ${ }^{\circ} \mathrm{C}$ | $=$ | degree celcius |
| $\mathrm{CH}_{3} \mathrm{CO}_{2} \mathrm{~K}$ | $=$ | potassium acetate |
| CHP | $=$ | Chumphon province |
| COI | $=$ | cytochrome oxidase subunit I |
| DNA | $=$ | deoxyribonucleic acid |
| EDTA | $=$ | ethylenediamine tetraacetic acid |
| EF-1 $\alpha$ | $=$ | elongation factor 1 alpha |
| et al. | $=$ | and others |
| F | $=$ | forward |
| hr | $=$ | hour |
| i.e. | $=$ | id est (that is) |
| km | $=$ | kilometer |
| KOH | $=$ | potassium hydroxide |
| mM | $=$ | millimolar |
| M | $=$ | molar |
| min | $=$ | minute |

## LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

| mtDNA | $=$ | mitochondrial deoxyribonucleic acid |
| :---: | :---: | :---: |
| NaCl | = | sodium chloride |
| NST | = | Nakhon Si Thammarat province |
| NTW | = | Narathiwat province |
| PNG | = | Phang-Nga province |
| R | = | reward |
| RAPD | $=$ | random amplified polymorphic DNA |
| rpm | $=$ | revolution per minute |
| SDS | $=$ | sodium dodecyl sulfate |
| SSCP | $=$ | single strand conformation polymorphism |
| STN | $=$ | Surat Thani province |
| TRG | $=$ | Trang province |
| Tris-HCL | $=$ | Tris-(hydroxymethyl)-aminomethane hydrochloric acid |
| $\mu \mathrm{l}$ | $=$ | microliter |
| UnH | $=$ | underside of hindwing |
| UpF | $=$ | upperside of forewing |

## CHAPTER 1

## GENERAL INTRODUCTION

## Background and Rationale

Lepidoptera is a large order that the most economically important pests in the class Insecta (Ozden, 2003). Many schemes of classification exist for the Lepidoptera, some informal method divided the order into Microlepidoptera and Macrolepidoptera according to average body size of the included species. Another informal scheme divided the Lepidoptera into moths, butterflies, and skippers (Pedigo, 1996). Butterflies have been widely used as a bioindicator and model organisms to study the impact of habitat fragmentation, climate change and evolution (Benedick et al., 2007; Brakefield, 2012; Ghazanfar \& Raza, 2015; Hill et al., 2006; Williams et al., 2003).

Butterflies of the subtribe Mycalesina (Nymphalidae; Satyrinae) are an emerging model in evo-devo, much has been studied about the development, genetic and evolution especially, African butterfly Bicyclus anynana. All species in this subtribe have a wing pattern element that appear to be eyespots (ocelli), they are very extensively in number, pattern, color, size and position (Brakefield, 2012). This group has distribute in Old World tropics, they have radiated successfully in almost all habitat types in Africa, Madagascar and Indo-Australian region. The most recent study of these group focused on revised classification and phylogenetic relationships among genus (Aduse-Poku et al., 2015).

Genus Mycalesis is one of the member in subtribe Mycalesina, comprises more than 100 taxa in the Oriental and the Australian regions, this group is in need of revision (Parsons, 1998; Tennent, 2000). This genus shows striking seasonal polymorphism in wing pattern, as a mechanism to survive the wet and dry seasonal changes in their habitats (Braby, 1994). Polyphenism is accompanied with behavioral, reproductive and habitat changes (Braby, 1994; Brakefield, 2012; Islam et al., 2010), these phenomena is characterized by the reduction of the eyespot in dry
seasonal morph. This makes Mycalesis species identification difficult (Kondandaramaiah et al., 2010).

Thailand is situated in Southeast Asia, which lies between the northern Indochinese and Southern Sundaic biogeographical regions (Cobert \& Hill, 1996). The Thai peninsular is the junction between two of the planet's biodiversity hotspots. Various part of peninsular Thailand have been submerged or isolated by water as sea levels rose and fell over the past, these change isolated many animals, plant species and peninsular populations of many butterfly species are recognized as separate from population elsewhere in mainland Southeast Asia (Ek-Amnuay, 2012).

The study is aimed to: investigate the species composition and distribution of Mycalesis population in peninsular Thailand and investigate the genetic variations and phylogenetic relationship of Mycalesis species in peninsular Thailand, inferred from mitochondrial and nuclear genes.

## Research Questions

What is the phylogenetic relationships of butterfly in genus Mycalesis in peninsular Thailand?

1. Are there major genetic differences in the populations of Mycalesis in peninsular Thailand?
2. Are there Mycalesis in peninsular Thailand monophyly?

## Review of Literatures

Mycalesis Hübner is a large tropical genus represented by many species in the Oriental and Australian regions (Braby, 1994; Common \& Waterhouse, 1981; Corbet \& Pendlebury, 1978; D'Abrera 1971, 1985).

Taxonomy and generalization
Order Lepidoptera
Superfamily Papilionoidea
Family Nymphalidae
Subfamily Satyrinae
Tribe Mycalesini
Subtribe Mycalesina
Genus Mycalesis (Hübner, 1818)

The butterflies of the Mycalesis group is an enormous genus of medium size (around $35-70 \mathrm{~mm}$ ), species are characterized by the evenly rounded wings, the upperside ( Up ) of wings are mostly brown. There is a pale postdiscal band on the brighter underside of wings (Un) and a series of submarginal ocelli (eyespot) on both wings (Figure 1). On the forewing the base of vein 12 and the radius combined in strong swelling, and the bases of the cubitus and vein 1 b are less strongly swollen. Sexes are usually more or less similar (Figure 2) (Pinratana, 1988; Monastyrskii, 2005). Males have secondary sexual character that comprises of a costal nacreous on the upperside of the hindwing $(\mathrm{UpH})$, which has a patch of specialized scales covered by hair tuft and appear to be used in conjunction with a polished area nearly always containing a brand above the dorsum on the underside of the forewing (UnF) (Figure 1) (Monastyrskii, 2005).


Figure 1 Character of eyespot (ocelli) and postdiscal band on the underside of wing (Un) and male sexual character on hindwing of genus Mycalesis (Up).


Figure 2 Male (left) and female (right) of genus Mycalesis.

Seasonal forms are strongly developed. The Mycalesis, show striking seasonal polyphenism in the Un wing pattern: the wet seasonal forms are characterized by conspicuous submarginal ocelli (eyespot) pattern on the underside of wing, in the dry seasonal forms are rather drab, being paler with greatly reduced ocelli (Braby, 1994; Brakefield \& Reitsma, 1991; Corbet \& Pendlebury 1978; Kirk, 1982). The phenotypic plasticity associated with marked changes in behavior and breeding
status and associated changes in habitat favorability (Brakefield, 1987). The dry seasonal forms are usually reproductively dormant, less active, long-lived, aggregated in moist refugia and tent to rest amongst dead brown leaves, their wing pattern are thus well camouflaged against the resting background during the dormancy period (Braby, 1994; Brakefield, 1987; Brakefield \& Reitsma, 1991). By contrast, the wet seasonal form tent to reproduce more directly, is more active, generally do not aestivate in refugia (Braby, 1994; Brakefield and Larsen, 1984) and the prominent ocelli possibly function as anti-predator devise, either to startle or to deflect attacks of predator from the vulnerable body (Braby, 1994; Brakefield \& Larsen, 1984; Young, 1979). The majority of this genus prefers lowland habitats, low-flying species and preferring understory shade (Monastyrskii, 2005).

## Life cycles

Mycalesis has a life cycle with four distinct phases: Eggs are generally spherical, whitish usually smooth and size $1.2-1.3 \mathrm{~mm}$ (Figure 3A). Egg are laid singly or in egg-masses. The majority of satyrid ovipositing activity occurs in the evening. Larvae (Figure 3B) of this genus feed on monocotyledon plant species belonging to the Poaceae and Cyperaceae (Monastyrskii, 2005). In the $5^{\text {th }}$ stage of larva, body is pale cream-yellow at eclosion changing to light green after feeding, scattered primary setae; terminal abdominal segment with short bifid tail. Head capsule is shiny black, sometimes dark brown, with pair of prominent dorsal knobs and several long tapering setae, larva is long 2.0-3.0 mm (at eclosion) to 4.3 mm . Pupa (Figure 3C), with dull green or chocolate brown color, pupa is long 15-16 mm and suspended head downwards, they take along ten days to hatch to adult butterfly (Figure 3D).


Figure 3 General life cycle of Mycalesis in each stage, A) egg B) larva C) pupa D) adult

## The study of species distribution and ecology of genus Mycalesis

Mycalesis species are widely dispersed across Indo-Australian regions, including Sri Lanka, India, Southeast Asia, Australia, New Guinea and the East of Solomon Islands (Kodandaramaiah et al., 2010). There are many studies on Mycalesis as: Braby (1994) has studied seasonal polyphenism of several species of Mycalesis in northern Australia, Islam et al. (2010) studied on wet and dry season polymorphism of Mycalesis mineus in Bangladesh. Mycalesis orseis has been used to examine of habitat fragmentation on genetic diversity in Borneo. The genetic variation in Mycalesis based on mtDNA gene in Sri Lanka was studied by Goonesekera and Ranawaka (2013).

The study of butterflies in Thailand began in 1787, when Fabricius first described four species of butterflies from the country. Taxonomic research on Thai
butterflies was continued by Druce in 1874 (Ek-Amnuay, 2012; Lekakul et al., 1977). Bro. Amnuay Pinratana published a set of six volumes entitled "Butterflies in Thailand" from 1981-1996. In addition, much work has been done by Japanese scientist, including a team of Osaka City University expedition during 1957-1958, Yata (1989-1995) published a revision of the pierid genus Eurema, Aoki \& Uemura (1984) published "Studies on the genus Ypthima (Satyridae) in Thailand", and Aoki \& Yamagushi published "Notes on Butterflies of the genus Mycalesis (Satyridae) collected in Thailand, together with description of a new species". A year later they described another new species of Mycalesis from northern Thailand (Ek-Amnuay, 2012).

Following to Ek-Amnuay (2012) reported that there are 27 species spreading throughout Thailand (Table 1). Among these, M. fusca, M. janardana, M. perseus, M. mineus, M. visala, M. orseis were restricted to the peninsular Thailand. Boonvano (1998) reported four species of Mycalesis at Ton Nga-Chang Wildlife Sanctuary, Songkla province, includings M. fuscum, M. mineus, M. oroatis and M. janardana. Wangthaveesup (2008) reported 12 species of Mycalesis in Hala - Bala forest, Yala and Narathiwat Provinces, consisted of M. fusca fusca, M. perseus cepheus, M. mineus macromalayana, M. intermedia, M. anapita anapita, M. janardana sagittigera, M. anaxiodes, M. oroatis ustulata, M. orseis nautilus, M. mnasicles perna, M. maianeas maianeas and M. persoides.

## Population genetics

Population genetics is the study of genetic variation within and among populations and the evolutionary factors that explain this variation. Its foundation is the Hardy-Weinberg law, which is maintained as long as population size is large, mating is at random, mutation, selection and migration. Allele frequencies and genotype frequencies may change from one generation to the next. Variation in allele frequencies is found throughout the genome, and can be examined by genetic diversity and evolutionary patterns (Keats \& Sherman, 2013).

## Phylogenetic and Molecular technique

Phylogenetic is the study of the evolutionary history and relationships among individuals or groups of organisms (species or populations), these relationships are discovered through phylogenetic inference methods that observed heritable traits, such as DNA sequences or morphology under a model of evolution of these traits. The result of these analyses is a phylogeny or Phylogenetic tree (Yang \& Rannala, 2012). Phylogenetic studies have been conducted on several insects such as, butterflies (Aduse-Poku et al., 2015; Kodandaramaiah et al., 2010; Pena et al., 2006; Torres et al., 2001), black flies (Sriphirom et al., 2014) and Mosquito (Fang et al., 2017; Sum et al., 2014).

There are many molecular technique for getting genetic information for example, DNA hybridization, Random amplified polymorphic DNA (RAPD), Amplified fragment length polymorphism (AFLP), single-strand conformational polymorphism (SSCP), microsatellite and DNA sequencing. Those technique also provide different results and use depends on time, cost and previous background information. For instance, microsatellite, RAPD and SSCP are powerful to resolve the genetic differences within study of species (Avise, 2004). Furthermore, DNA sequencing is widely used in many aspects because base alignments can compare explicit on intraspecific and interspecific variation in the population. Thus, nucleotide sequence with specific markers has been used in resolving taxonomic status and the genetic differences of populations (Wichachucherd, 2014).

Mitochondrial DNA (mtDNA) has been the most popular marker used to investigate the molecular diversity in animals (Galtier et al., 2009). Following Avise et al. (1987) and Moritz et al. (1987), population geneticists and molecular biologists have adopted this tool with little reserve. Virtually every molecular study of animals species in the field involves mtDNA haplotyping at some stage. Not surprisingly, a mitochondrial fragment, COI, was recently elected as the standardized tool for molecular taxonomy and identification (Galtier et al., 2009; Ratnasingham \& Hebert 2007).

The reasons for the adoption of mtDNA as marker of choice are wellknown. Experimentally, mtDNA is relatively easy to amplify because it appears in
multiple copies in the cell. Mitochondrial gene content is strongly conserved across animals, with very few duplications, no intron, and very short intergenic regions (Gissi et al. 2008). Mitochondrial DNA is highly variable in natural populations because of its elevated mutation rate, which can generate some signal about population history over short time frames. Thus, COI was useful in fully resolving topologies within species of some butterfly species such as the Chrysoritis in Lycaenidae (Rand et al., 2000), as well as within groups of the Papilionidae (Caterino \& Sperling, 1999). Included, the systematics and biogeography of Mycalesina (Aduse-Poku et al., 2015; Kodandaramaiah et al., 2010; Monteiro \& Pierce, 2001).

However, nuclear gene also widely used for molecular study, which has shown utility in reconstructing species level to subfamily level relationships in some insects especially in Lepidoptera (Brower and DeSalle, 1998; Campbell et al., 2000). Previous studies (Campbell et al., 2000; Pena et al., 2006; Torres et al., 2001) indicated that $E F-1 \alpha$ and wingless genes further resolved relationships among species groups within the same genus, also useful in resolving clades at subfamily level in Nymphalidae.

Therefore, analysis of sequence variations of mtDNA and nuclear gene is an outstanding approach to study the population genetics and the phylogenetic relationships within population of the butterfly genus Mycalesis.

## Research objectives

1. To investigate the species composition and distribution of Mycalesis in peninsular Thailand
2. To examine the genetic variation and phylogenetic relationships of Mycalesis in peninsular Thailand

Table 1 Species list of Mycalesis in Thailand

| Species | Distribution in Thailand | Distribution in the world |
| :---: | :---: | :---: |
| 1. M. anapita anapita Moore, 1858 | TT | Thailand, India, Burma, Malay peninsula, Sumatra. A further subsp. in Borneo |
| 2. M. fusca fusca (C. \& R. Felder, 1860) | PT | Peninsular Thailand, Malay peninsula, Sumatra. Other subsp.in Nais, Borneo, Java |
| 3. M. mnasicles Hewitson, 1864 |  |  |
| 3a. M. mnasicles perna Fruhstorfer, 1906 | TT | Thailand, Burma, Vietnam, Malay peninsula. Nominate subsp. in Sumatra and Borneo |
| 4. M. janardaana Moore, 1858 |  |  |
| 4a. M. janardaana sagittigera Fruhstorfer, 1908 | PT | Peninsular Thailand, Malay peninsula, Sumatra. other subsp. throughout Sundaland Philippines and Sulawesi |
| 5. M. sangaica Butler, 1877 |  |  |
| 5a. M. sangaica tunicula Fruhstorfer, 1911 | TT, except PT | Thailand, Burma, Laos, Vietnam. other subsp. in China and Taiwan |
| 6. M. perseus (Fabricius, 1775) |  |  |
| 6a. M. perseus blasius (Fabricius, 1798) | N | Thailand, Assam (India), Burma |
| 6b. M. perseus cepheus Butler, 1867 | PT | Peninsular Thailand, Malay peninsula, Sumatra, Borneo, Java. Throughout Oriental region, extending to Australia |
| 7. M. mineus (Linnaeus, 1758) |  |  |
| 7a. M. mineus mineus (Linnaeus, 1758) | TT, except PT | Thailand, north India, Burma, Vietnam, South China |
| 7b. M. mineus macromalayana Fruhstorfer, 1911 | PT | Peninsular Thailand, Malay Peninsula, Sumatra. other subsp. in Sri Lanka, Peninsular India, Nicobars and Taiwan |
| 8. M. intermedia (Moore, 1892) | TT | Thailand, Burma, Vietnam, Malay peninsula |
| 9. M. thailandica Aoki \& Yamaguchi, 1984 | TT, except PT | Thailand |
| 10. M. distanti Moore, 1892 | TT | Thailand, Vietnam, Burma, north Malay peninsula |
| 11. M. perseoides (Moore, 1892) | TT | Thailand, Assam (India), Burma, Vietnam, Malay peninsula |
| 12. M. visala Moore, 1858 |  |  |

Table 1 Continued.

| Species | Distribution in Thailand | Distribution in the world |
| :---: | :---: | :---: |
| 12a. M. visala visala (Moore, 1858) | TT, except PT | Thailand, Vietnam, Burma, north India |
| 12b. M. visala phamis Talbot \& Corbet, 1939 | PT | Peninsular Thailand, Malay peninsula, other subsp. in peninsular India and Andaman Is |
| 13. M. orseis Hewitson, 186 |  |  |
| 13a. M. orseis nautilus Butler, 1867 | PT | Peninsular Thailand, Malay peninsula, other subsp. in Sumatra, Nias, Borneo, Palawan |
| 14. M. francisca Stoll, 1780 |  |  |
| 14a. M. francisca sanatana Moore, $1858$ | TT | Thailand, north India, Burma, Vietnam. other subsp. in China, Taiwan, Japan |
| 15. M. inayoshii Aoki \& Yamaguchi, 1995 | N | Thailand, Laos |
| 16. M. gotama Moore, 1858 |  |  |
| 16a. M. gotama charaka Moore, 1875 | N | Thailand, Assam (India), Burma, Vietnam. other subsp. in China, Taiwan, Japan |
| 17. M. anaxias Hewitson, 1862 |  |  |
| 17a. M. anaxias aemate Fruhstorfer, $1911$ | TT, except PT | Thailand, Burma, Laos, Vietnam. Other subsp. in India, Malay peninsula, Sumatra |
| 18. M. siamica Riley \& Godfrey, 1921 | E | Thailand, Laos, Nominate subsp. from north Vietnam |
| 19. M. adamsoni Watson, 1897 | TT, except PT | Thailand, Assam (India), Burma |
| 20. M. anaxioides Marshall, 1883 | TT | Thailand, Burma, Malay peninsula |
| 21. M. maianeas maianeas Hewitson, 1864 | TT | Peninsular Thailand, Malay peninsula, Borneo. Further subsp. in Sumatra |
| 22. M. suaveolens Wood - Mason \& de Niceville, 1883 |  |  |
| 22a. M. suaveolens duguidi Tytler, $1926$ | N | Thailand, Burma, Laos, Vietnam. Other subsp. in India, Taiwan |
| 23. M. nicotia Westwood, 1850 | N | Thailand, Burma, Laos, Vietnam, India |
| 24. M malsara Moore, 1858 | TT, except PT | Thailand, Burma, Vietnam, north India |
| 25. M. annamitica annamitica Fruhstorfer, 1906 | TT, except PT | Thailand, Vietnam. Further minor subsp. in Assam (India), Burma |

Table 1 Continued.

| Species | Distribution <br> in Thailand | Distribution in the world |
| :---: | :---: | :--- |
| 26. M. lepcha Moore, 1880 |  |  |
| 26a. M. lepcha kohimensis Tytler, <br> 1914 | TT, except PT | Thailand, Burma, Laos. Further <br> subsp. in India |
| 27. M. oroatis Hewitson, 1864 |  |  |
| 27a. M. oroatis surkha Marshall, <br> 1882 | TT, except PT | Thailand, Burma |
| 27b. M. oroatis ustulata Distant, <br> 1885 | PT | Thailand, Burma, Australia |

# CHAPTER 2 <br> Species composition of Mycalesis (Nymphalidae: Satyrinae) in peninsular Thailand with male genitalia description 

## Introduction

Mycalesis Hübner is a large tropical genus with more than 100 species distributed in the Oriental and Australian regions (Braby, 1994; Common \& Waterhouse, 1981; Corbet \& Pendlebury, 1978; D'Abrera 1971, 1985). This genus is characterised by evenly rounded wings, which is almost brown, and a series of subterminal ocelli (eyespots) on the underside of wing. The difference of structure in wing venation, eyespot and the genitalia separate the group from closely allied genera such as Orsotriaena Wallengren and the African Bicyclus Kirby and Henotesia Butler (Corbet \& Pendlebury, 1978). Mycalesis are reported to display polyphenism with seasonal and geographical variation in morphology, they are two distinct seasonal morphs (wet and dry season form). In Thailand, according to Ek-Amnuay (2012) reported that there are twenty seven species, some species are restricted in some part of Thailand such as M. gotama are endemic in northern, M. siamica are restricted Eastern, M. orseis and M. fusca are only found in Peninsular Thailand.

Morphology and function of genitalia in subfamily Satyrinae have been actively carried out since 1930 (Klots \& Rochester, 1931). Structure of male genitalia is a principle characteristic of most families of Lepidoptera. There are many studies and description on male genitalia of Papilionoidae including Satyrinae (Coutis, 1983; Dubatolov et al., 1994; Klalicek \& Povolny, 1992; Lukhtonov, 1995; Parshall, 1989; Shirozu \& Shima, 1979). The Asian Satyrinae was studied, for example in Malaysia and Vietnam (Corbet, Pendlebury \& Eloit, 1978; Monastyrskii, 2005). However, there are a few studies in Thailand especially in genus Mycalesis. Therefore, this study aims to investigate the species composition and distribution of Mycalesis in peninsular Thailand with description of the male genitalia.

## Materials and Methods

## Selection of study site

Study sites were selected on the basis of the mountain ranges in peninsular Thailand consist of Chumphon province (CHP), Surat Thani province (STN), Nakhon Si Thammarat province (NST), Phang-Nga province (PNG), Trang province (TRG) and Narathiwat province (NTW) (Figure $1-2$ ) details of each sampling site is given in Table 1. Mycalesis were collected during February 2015-July 2016 by using insect nets (Figure 3) and fruit-baited traps (Figure 4), which 10 fruitbaited traps were set along 1 km by line transect in each six sampling sites. Each traps was randomly placed on the line transect at a distance 100 m . The fruit-baited trap was sampled for seven consecutive days at each sampling site. Collected butterflies were immediately pressed the thorax carefully for minimizing damages to their external organs (scales, legs and wing veins) (Islam et al., 2010). Mycalesis was preserved in triangular paper envelopes and brought to the laboratory.

## Species identification

Mycalesis specimens were identified to species level by using identification key, descriptions of Corbet, Pendlebury and Eloit (1978), Ek-Amnuay (2012), Monastyrskii (2005), Pinratana (1988) and Talbot (1947).

## Genitalia dissection

The abdomen were detached from the thorax and placed in individual glass vials that contain about $500 \mu \mathrm{l}$ of $10 \% \mathrm{KOH}$ solution for 24 hr . Abdomen were transferred to a petri dich with distilled water and cleaned under the stereomicroscope by using a pair of straight forceps. EosinY was used for dying around 10 min and washed in $20 \%$ alcohol. After that they were dyed in Chlorozal black for 5 seconds and finally washed in $20 \%$ alcohol. Genitalia were separated from the abdomen and placed in $70 \%$ ethanol solution for cleaning. To preserve the genitalia immediately after the dissection, genitalia were preserved in a glycerin-filled small tube. Images of the genitalia were taken by using a Leica Microscope DM750. Images were adjusted
with Adobe Photoshop CS6, only the neutralizing the background and balancing the contrast across samples. The structures were not edited (Muadsub, 2014).

Table 1 Details of each sampling sites

| Study site | Province | Coordination | Description of study site |
| :--- | :--- | :--- | :--- |
| Ban Ta yang, <br> Mueang | Chumphon | $10^{\circ} 45.798^{\prime} \mathrm{N}$ <br> $99^{\circ} 23.032^{\prime} \mathrm{E}$ | Open aria, oil palm <br> agriculture, grass land |
| Ban Phumriang, <br> Chaiya | Surat Thani | $09^{\circ} 23.340^{\prime} \mathrm{N}$ <br> $99^{\circ} 15.240^{\prime} \mathrm{E}$ | Open aria, oil palm <br> agriculture, bamboo, <br> grassland |
| Khlong Krabue, <br> Pak phanang | Nakhon Si <br> Thammarat | $08^{\circ} 20.020^{\prime} \mathrm{N}$ <br> $100^{\circ} 09.562^{\prime} \mathrm{E}$ | Open aria, oil palm <br> agriculture, rice field, <br> grassland |
| Ban Bang Yai, <br> Takuapa | Phang-Nga | $08^{\circ} 90.241^{\prime} \mathrm{N}$ <br> $98^{\circ} 40.505^{\prime} \mathrm{E}$ | Open aria, oil palm <br> agriculture, bamboo, <br> grassland |
| Khao Chong, <br> Na Yong | Trang | $07^{\circ} 33.360^{\prime} \mathrm{N}$ <br> $99^{\circ} 46.724^{\prime} \mathrm{E}$ | Closed canopy, open aria, <br> oil palm agriculture, <br> bamboo, grassland |
| Ban Kayu Khla, <br> Waeng | Narathiwat | $05^{\circ} 58.655^{\prime} \mathrm{N}$ <br> $101^{\circ} 54.392^{\prime} \mathrm{E}$ | Open aria, rubber <br> plantation, rice field, <br> bamboo, grassland |



Figure 1 Map of peninsular Thailand showing locations of six sampling sites.


Figure 2 Habitat of collecting sites. A) Ban Ta yang, Chumphon province B) Ban Phumriang, Surat Thani province C) Khlong Krabue, Nakhon Si Thammarat province D) Ban Bang Yai, Phang-Nga province E) Khao Chong, Trang province and F) Ban Kayu Khla, Narathiwat province.


Figure 3 Butterfly sampling by using insect nets.


Figure 4 Butterfly sampling by using fruit-baited trap

## Result

## Species composition and distribution of Mycalesis in peninsular Thailand

The butterflies of genus Mycalesis were sampled, with a total of 214 individuals, described to seven species comprising of five members of Mineus group belonged to M. intermedia, M. visala, M. perseoides, M. mineus and M. perseus. The rest of Mycalesis were M. orseis of Francisca group and M. janardana of Janardana group (Table 2).

The result of Table 3 and Figure 5 showed that the species diversity, Chumphon province there are two Mycalesis species consisted of M. perseoides and M. mineus, there are three species composed of M. perseoides, M. mineus and M. intermedia were found in Surat Thani province. Four species, M. perseus, M. intermedia, M. perseoides and M. visala were found in Nakhon Si Thammarat province. Four Mycalesis species, M. perseus M. mineus, M. perseoides, and M. visala were found in Phang-Nga province. In addition, six species of Mycalesis consisted of M. orseis, M. janardana, M. mineus, M. intermedia, M. perseoides and M. visala were found in Trang province. However, five species, M. mineus, M. intermedia, M. perseoides, M. visala and M. perseus, occurred in Narathiwat province.

The result showed that high number of species was found at Trang province with six species, lowest number of species was found in Chumphon province with two species. Furthermore, M. perseoides was dominant species which found in all sampling sites across peninsular Thailand (Figure 5). M. orseis and M. janardana were found only one site from Trang province. In this study we found two distinct seasonal form (wet and dry season forms) in all site except Trang province.

Table 2 Species composition of Mycalesis in six sampling sites

| Study site | species | Number of individuals |  |  | Seasonal forms |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Male | Female | Total | wsf. | dsf. |
| Chumphon February, 2015 | M. mineus | 3 | 1 | 4 | + | + |
|  | M. perseoides | 26 | 11 | 37 | + | + |
|  | Mycalesis sp. 1 | 0 | 2 | 2 | - | + |
|  | Mycalesis sp. 2 | 0 | 1 | 1 | - | + |
|  |  | 29 | 15 | 44 |  |  |
| Surat Thani <br> April, 2015 | M. mineus | 5 | 3 | 8 | + | + |
|  | M. intermedia | 1 | 0 | 1 | + | - |
|  | M. perseoides | 15 | 6 | 21 | + | + |
|  | Mycalesis sp. 3 | 0 | 1 | 1 | - | + |
|  | Mycalesis sp. 4 | 0 | 2 | 2 | - | + |
|  | Mycalesis sp. 5 | 0 | 1 | 1 | - | + |
|  | Mycalesis sp. 6 | 0 | 1 | 1 | - | + |
|  | Mycalesis sp. 7 | 1 | 1 | 2 | - | + |
|  |  | 22 | 15 | 37 |  |  |
| Nakhon Si Thammarat April, 2015 | M. intermedia | 2 | 0 | 2 | + | - |
|  | M. perseus | 10 | 7 | 17 | + | + |
|  | M. perseoides | 9 | 0 | 9 | + | + |
|  | M. visala | 0 | 1 | 1 | + | - |
|  | Mycalesis sp. 1 | 1 | 0 | 1 | - | + |
|  |  | 22 | 8 | 30 |  |  |
| Phang-Nga February, 2015 | M. mineus | 6 | 1 | 7 | + | + |
|  | M. perseus | 5 | 3 | 8 | + | + |
|  | M. perseoides | 0 | 2 | 2 | + | - |
|  | M. visala | 1 | 0 | 1 | - | + |
|  | Mycalesis sp. 1 | 1 | 1 | 2 | - | + |
|  | Mycalesis sp. 4 | 0 | 1 | 1 | - | + |
|  |  | 13 | 8 | 21 |  |  |

Table 2 (continued)

| Study site | Species | Number of individuals |  | Seasonal form |  |  |
| :--- | :--- | :---: | :---: | :---: | :---: | :---: |
|  |  | Male | Female | Total | wsf. | dsf. |
|  | M. janardana | 1 | 1 | 2 | + | - |
|  | M. orseis | 1 | 0 | 1 | + | - |
|  | M. intermedia | 11 | 18 | 29 | + | - |
|  | M. mineus | 0 | 2 | 2 | + | - |
|  | M. perseoides | 1 | 0 | 1 | + | - |
|  | M. visala | 2 | 1 | 3 | + | - |
|  |  | $\mathbf{1 6}$ | $\mathbf{2 2}$ | $\mathbf{3 0}$ |  |  |
| Narathiwat <br> May, 2015 | M. intermedia | 3 | 3 | 6 | + | - |
|  | M. mineus | 5 | 3 | 8 | + | - |
|  | M. perseus | 1 | 4 | 5 | + | - |
|  | M. perseoides | 17 | 6 | 23 | + | + |
|  | M. visala | 1 | 1 | 2 | + | - |
|  |  | $\mathbf{2 7}$ | $\mathbf{1 7}$ | $\mathbf{4 4}$ |  |  |
| Total individuals | $\mathbf{1 2 9}$ | $\mathbf{8 5}$ | $\mathbf{2 1 4}$ |  |  |  |

Table 3 Species diversity and distribution of Mycalesis in six sampling sites.

|  | Study site |  |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| Species | CHP | STN | PNG | NST | TRG | NTW |
| M. janardana | - | - | - | - | + | - |
| M. orseis | - | - | - | - | + | - |
| M. intermedia | - | + | + | + | + | + |
| M. mineus | + | + | + | - | + | + |
| M. perseus | - | - | + | + | - | + |
| M. perseoides | + | + | + | + | + | + |
| $M$. visala | - | - | + | + | + | + |



Figure 5 Species diversity and distribution of Mycalesis in each sampling sites.

## Male genitalia description

The morphology of seven Mycalesis species were described. In general, the tegumen in lateral view of M. orseis and M. janardana formed a shallow dome and the rest of Mycalesis was flattened. The shape of tegumen in dorsal view was pentagonal. The width of combined length of the end of saccus to the tip of uncus varied from 0.2-0.4. The fenestrular was hardly defined in all species. The uncus was usually bent downward. The length of the gnathos was usually longer than uncus except M. orseis and M. janardana. The vinculum bent backward in all species. The saccus was elongated and bent upward or parallel to tegumen. The valva had a unique feather to the species

## 1. Mycalesis orseis Hewitson, 1864

Description: Tegumen in lateral view forming shallow dome; tegumen in dorsal view pentagonal, maximum width about 0.2 of combined length of the end of saccus to the tip of uncus. Fenestrula hardly defined; uncus elongate, slightly parallel, with short hook at tip, with moderately elongated gnathos; gnathos in lateral view moderately upward curve medially; gnathos in dorsal view bent inward at distal end; vinculum strongly bent backward; saccus elongate parallel to tegumen. Valva in lateral view convex outward and slightly longer than vinculum, sacculus weakly developed; distal half slightly tapered with pointed tip, aedeagus bent upward distal one-third and longer than vinculum; basal portion tapering (Figure 6).


Figure 6 Male genitalia and adult of M. orseis; Genitalia, A) lateral view B) dorsal view $C$ ) aedeagus in lateral view. Adult, D) upperside E) underside (scale bar $=1 \mathrm{~cm}$ ).

## 2. Mycalesis janardana Moore, 1858

Description: Tegumen in lateral view forming dome; tegumen in dorsal view pentagonal, maximum width about 0.3 of combined length of the end of saccus to the tip of uncus. Fenestrula hardly defined; uncus elongate, basal tick, strongly tapering to bent downward apex, with rather slightly elongated gnathos; gnathos in lateral view bent downward; gnathos in dorsal view bent inward at distal end; vinculum strongly bent backward; saccus elongated and bent upward; valva in lateral view convex outward and bent inward at distal end, valva in lateral view slightly longer than vinculum; sacculus weakly developed; distal end of harpe slightly tapered and bent inward. Aedeagus bent upward distal end one-third and longer than vinculum, basal portion bent inward and tapering (Figure 7).


Figure 7 Male genitalia and adult of M. janardana; Genitalia, A) lateral view B) dorsal view C) aedeagus in lateral view. Adult, D) upperside E) underside (scale bar $=1 \mathrm{~cm}$ ).

## 3. Mycalesis mineus (Linnaeus, 1758)

Description: Tegumen in lateral view flat; tegumen in dorsal view pentagonal, maximum width about 0.4 of combined length of the end of saccus to the tip of uncus. Fenestrula hardly defined; uncus slightly elongate and bent downward apex, with elongated gnathos; gnathos in lateral view moderately curve upward at distal end, gnathos in dorsal view bent inward at distal end. Vinculum strongly bent backward; saccus elongated parallel to tegumen. Valva in lateral view with large sacculus subtriangular; neck distinct; cucullus with dorsal lobe subtriangular with posterior edge dentate, distal end long bifid, slightly dentate at tip; ventral margin round smooth with dense hair. Aedeagus bent upward distal end one-fourth with sub triangular at tip and longer than vinculum; basal portion with dorsal semi-circle (Figure 8).


Figure 8 Male genitalia and adult of M. mineus; Genitalia, A) lateral view B) dorsal view $C$ ) aedeagus in lateral view. Adult, D) upperside E) underside (scale bar $=1 \mathrm{~cm}$ ).

## 4. Mycalesis perseus (Fabricius, 1775)

Description: Tegumen in lateral view flat; tegumen in dorsal view pentagonal, maximum width about 0.3 of combined length of the end of saccus to the tip of uncus. Fenestrula hardly defined; uncus elongate strongly tapering bent downward apex, with elongate gnathos; gnathos in lateral view moderately curve, weakly upward at distal end, gnathos in dorsal view bent inward at distal end, vinculum strongly bent backward, saccus elongated parallel to tegumen. Valva in lateral view with large sacculus subtriangular; neck distinct; cucullus with dorsal lobe subsemi-circle with posterior edge dentate, distal end short bifid, slightly dentate at tip; ventral margin round smooth with dense hair. Aedeagus bent upward distal end one-third and longer than vinculum; basal portion with dorsal subsemi-circle (Figure 9)


Figure 9 Male genitalia and adult of M. perseus; Genitalia, A) lateral view B) dorsal view $C$ ) aedeagus in lateral view. Adult, $D$ ) upperside $E$ ) underside (scale bar $=1 \mathrm{~cm}$ ).

## 5. Mycalesis perseoides (Moore, 1892)

Description: Tegumen in lateral view flat; tegumen in dorsal view pentagonal, maximum width about 0.3 of combined length of the end of saccus to the tip of uncus. Fenestrula hardly defined; uncus elongate, parallel to tegumen, strongly tapering bent downward apex, with elongated gnathos; gnathos in lateral view moderately curve upward, gnathos in dorsal view bent inward at distal end. Vinculum strongly bent backward; saccus elongated parallel to tegumen. Valva in lateral view with large sacculus subtriangle; neck distinct; cucullus with dorsal lobe subsemi-circle, with posterior edge dentate, distal end bifid with long tip, some saw tooth-like, slightly dentate at tip; ventral margin round, smooth with dense hair. Aedeagus bent upward distal end one-third with subtriangular tip and longer than vinculum; basal portion with dorsal semi-circle (Figure 10)


Figure 10 Male genitalia and adult of M. perseoides; Genitalia, A) lateral view B) dorsal view C) aedeagus in lateral view. Adult, D) upperside E) underside (scale bar $=1 \mathrm{~cm}$ ).

## 6. Mycalesis visala Moore, 1858

Description: Tegumen in lateral view flat; tegumen in dorsal view pentagonal, maximum width about 0.3 of combined length of the end of saccus to the tip of uncus. Fenestrula hardly defined; uncus elongate, parallel to tegumen, tapering bent downward apex, with elongated gnathos; gnathos in lateral view strongly curve upward medially, gnathos in dorsal view bent inward at distal end. Vinculum strongly bent backward; saccus elongate parallel to tegumen. Valva in lateral view with large sacculus subtriangle; neck distinct; cucullus with dorsal lobe subsemi-circle, with posterior edge dentate, distal end bifid with long tip some saw tooth-like, slightly dentate at tip; ventral margin round smooth with dense hair. Aedeagus bent upward distal end one-third, longer than vinculum, basal portion with dorsal semi-circle (Figure 11).


Figure 11 Male genitalia and adult of $M$. visala; Genitalia, A) lateral view B) dorsal view $C$ ) aedeagus in lateral view. Adult, D) upperside E) underside (scale bar $=1 \mathrm{~cm}$ ).

## 7. Mycalesis intermedia (Moore, 1892)

Description: Tegumen in lateral view flat; tegumen in dorsal view pentagonal, maximum width about 0.3 of combined length of the end of saccus to the tip of uncus. Fenestrula hardly defined; uncus elongate parallel to tegumen, strongly tapering bent downward apex, with elongated gnathos; gnathos in lateral view moderately curve upward medially, gnathos in dorsal view bent inward at distal end. Vinculum strongly bent backward; saccus elongated, bent backward. Valva in lateral view with large sacculus subtriangle; neck distinct; cucullus with dorsal lobe subsemi-circle, with posterior edge dentate, distal end bifid with long tip some saw tooth-like, slightly with hook at tip, ventral margin round, and smooth with dense hair. Aedeagus bent upward distal end one-third with sub triangular at tip and longer than vinculum, basal portion with dorsal semi-circle (Figure 12)


Figure 12 Male genitalia and adult of M. intermedia; Genitalia, A) lateral view B) dorsal view C) aedeagus in lateral view. Adult, D) upperside E) underside (scale bar = 1 cm ).

## Discussion

According to previous report (Ek-Amnuay, 2012; Pinratana, 1988; Wangthaveesup, 2008) there are 15 species of Mycalesis distribute throughout peninsular Thailand, but in this study, a total of seven species were found. The result showed that $M$. perseoides is common species was found in all collecting sites. It was possible related with their host plant in each habitat type, this species can found several habitat such as open area, vegetation, grasslands, oil palm agriculture and bamboo forest (Lin, 2014). The food plant of Mycalesis is mainly in the family Poaceae and Cyperacae (Ackery, 1988). The study of Mycalesis host plant in Thailand were lack of knowledge, there are reported in some species such as, Cyperus revifolius has been recorded as larva host plant of M. mineus. Axonopus compressus has been recorded as larva host plant of M. intermedia. However, Ottochloa nodosa and Ischaemum ciliare, have been recorded as larva host plant of M. perseoides in Singapore (Tan, 2013).

The result in figure 5 showed that Khao Chong,Trang province have high species richness (number of species) with six species, associate with there are several microhabitat in collecting site (closed canopy, open aria, grass land, oil palm agriculture and bamboo forest) more than another collecting site. The finding was supported with Young et al. (2012) reported that the characteristic of vegetation cover led to different to microhabitat and might be a major factor the diversity of butterfly.

In this study, we collected the samples covered wet season and dry season. Mycalesis species were found into two seasonal form that wet and dry season forms. M. orseis and $M$. janardana were found only wet season form. The rest of, $M$. perseoides, M.visala, M. perseus, M. intermedia and M. mineus were found two distinct seasonal forms. According to Brakefield (1984) indicated that many species of Satyrinae and some others group of butterfly in tropical have a distinct seasonal forms. Braby, 1994 and Islam et al. (2010) reported that the development of seasonal morphs is determined by many physical factor such as temperature, humidity and photoperiod. In this study, those factor was not observed. However, previous study
was suggested that temperature play an importance role in regulating wing phenotype in several Lepidoptera (Janzen, 1984)

Brakefield and Reitsma (1991) and Windig (1992) showed that different temperature in final instar larva of Bicyclus safitza induced different seasonal forms. Rienks (1985) and Jones et al. (1985) also demonstrate that small changes in photoperiod in conjunction with temperature can affect wing phenotype in two tropical Pieridae. Islam et al. (2010) report consistent with Brakefiled (1987) and Brakefield and Reitsma (1991) indicated that the turnover from dry season form to wet season form coincided with rising temperature when increase in humidity and food plant occur. In contrast, the turnover of wet season form to dry season form was found at declining temperature, low humidity and less food plant.

Braby (1994) and Brakefield (1984) proposed that the prominent eyespot pattern in wet season form possibly function as anti-predator devices. In contrast, dry season form butterflies have reduced ocelli and lack of eyespots this maybe probably for their survival. These type of phenotypic plasticity is associated with changes in habitat favorably (Braby, 1994; Islam et al., 2010).

## CHARPTER 3

Genetic variation in Mineus group of Mycalesis (Nymphalidae: Satyrinae) in peninsular Thailand: inferred from mitochondrial and nuclear genes

## Original Article

# Genetic variation in Mineus group of Mycalesis (Nymphalidae: Satyrinae) in peninsular Thailand: inferred from mitochondrial and nuclear genes 

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#### Abstract

The aim of this study was to investigate genetic variation and phylogenetic relationships of the Mineus group of the genus Mycalesis in peninsular Thailand inferred by mitochondrial and nuclear gene markers (COI, EF-l $\alpha$ and wingless). The Mycalesis specimens were sampled from six provinces in peninsular Thailand. Seven species of the genus Mycalesis were identified. In this study, two distinct seasonal morphs were found as wet season and dry season forms. Phylogenetic relationships based on combined gene sequences indicated that the Mineus group of Mycalesis was monophyletic group. However, M. intermedia, M. visala and M. perseoides might be complex species. Five haplotype patterns were observed in M. perseoides indicating that Chumphon province was the origin of $M$. perseoides. Divergence time of the Mineus group was 23.03 mya during Oligocene in peninsular Thailand.


Keywords: Mycalesis, genetic differentiation, haplotype network, peninsular Thailand, phylogeny

## Introduction

Butterflies of the subtribe Mycalesina (Nymphalidae: Satyrinae) were described into 7 genera based on molecular and morphological evidences consisting of genus Lohora, Heteropsis, Mycalesis, Mydosama, Culapa, Bicyclus and Hallelesis (Aduse-Poku et al., 2015). This subtribe is restricted in the Old World tropics in which Mycalesis is a dominant genus with over 100 estimated described species. Moreover, this genus has been widely dispersed in Indo-Australian region such as in Sri Lanka, India, Southeast Asia, Australia, New Guinea and the east of Solomon Islands (Kodandaramaiah et al., 2010). The majority of genus Mycalesis prefers lowland habitats and can be found in many kinds of habitat such as forest edges, grasslands and savannas. The butterflies are low-flight and prefer understory shade (Monastyrskii, 2005). The food plants were found in the family of Poaceae and Cyperaceae (Ackery, 1988; Torres et al., 2001).

Butterflies in subtribe Mycalesina are an important model to understand the evolutionary study, ecology, development, genetics and phenotypic plasticity of tropical butterflies (Braby, 1994; Brakefield, 1984; 2012; Islam et al., 2010; Torres et al., 2001). For instance, the study of seasonal polymorphism of Mycalesis in Australia indicated the mechanism of survival between wet and dry seasonal change in their habitat (Braby, 1994). In addition, physical factors such as temperature, rainfall, humidity and photoperiod can determine the variation of wet and dry season forms (Islam et al., 2010). Interestingly, phenotypic variation of tropical butterfly in terms of morphological characters in the dry and wet seasonal forms are important. However, the phenotypic character in the dry seasonal form are not recognized, because the eyespots reduced. In a previous study, morphometric characterization of Mycalesis butterflies in Sri-Lanka showed that phenotypic characters are different, for examples, coloration of wings and dorsal forewing ocelli of adult Mycalesis species (Goonesekera, Poorten, \& Ranawaka, 2014). Furthermore, genetic variation in Mycalesis from Sri Lanka based on analysis of COI gene indicated that the variation of haplotype network in Mycalesis was not different and also was restricted on their location. Genetic distances were significantly different among local species (Goonesekera \& Rawanaka, 2013).

Peninsular Thailand is placed on the Sundaic shelf, which is an important transition zone between the Indo-Chinese and Sundaic sub-regions. Both sub-regions are separated by the Isthmus of Kra which was proposed a natural barrier for dispersal of fauna (Lohman et al., 2011). According to vegetation structure in the peninsular Thailand, two types of forest community consist of evergreen rain forest and mixed moist deciduous forest (the southern part of Thailand, Thai type) and evergreen rain forest (Kangar-Pattani line, Malayan type)(Santisook, 2012). However, the study on Mycalesis was not attended, especially in terms of diversity and abundance. Moreover, Ek-Amnuay (2012) and Pinratana (1988) reported that there are 27 species spreading throughout Thailand. Among these, M. fusca, M. janardana, M. perseus, M. mineus, M. visala, M. orseis were restricted to the peninsular Thailand. On the other hand, Evan (1932) divided Mycalesis species into five groups, i.e., Gotama group, Mineus group, Oroatis group, Nicotia group, and Patnia group. The members of Mineus group are M. perseus, M. mineus, M. igilia, M. visala, M. perseoides, M. subtida, M. mercea, M. khasia, M. rama, M. evansii, M. mystes, M. adolphei, M. intermedia and M. oculus (Evan, 1932; Talbot \& Corbet, 1939). Nowadays, the phylogenetic relationship of the subtribe Mycalesina based on molecular data is established as monophyly. Mycalesis species are clustered into two clades as Mycalesis I consists of the taxa from mainland Southeast Asia and Mycalesis II belongs the taxa from Indo-Australia. However, both clusters are not monophyletic group (Aduse-Poku et al., 2015; Kodandaramaiah et al., 2010). In addition, there is lack of knowledge on phylogenetic relationships and genetic variation of Mycalesis (Mineus group) in peninsular Thailand.

This study aims to investigate the phylogenetic relationship and genetic variation of Mycalesis (Mineus group) inferred from one mitochondrial and two nuclear genes. Within the hypothesis of phylogenetic study, divergence times of Mycalesis group have been estimated. The finding of this study can contribute and clarify the Mycalesis (Mineus group) in peninsular Thailand.

## Materials and Methods

## Taxon sampling

Butterflies of the genus Mycalesis were collected in six provinces along the mountainous ranges of peninsular Thailand. Six sampling sites were at Chumphon province (CHP), Surat Thani province (STN), Nakhorn Si Thammarat province (NST), Phang-Nga province (PNG), Trang province (TRG) and Narathiwat province (NTW) during June 2015-July 2016. Adult butterflies were collected using insect nets and baited traps with 10 fruit-baited traps set up along 1 km line transect at each sampling site. Each trap was randomly placed on the line transect at a distance 100 m . The baited-trap was sampled during seven consecutively days at each sampling site. Adult butterflies were preserved in trianglular paper. A pair of middle legs were removed from the specimens and placed in $95 \%$ ethanol for DNA extraction. The rest of the specimens were in a box.

## Species identification

Mycalesis species were identified to species level using identification keys descriptions and illustrations by Corbet, Pendlebury and Eloit (1978), EkAmnuay (2012), Monastyrskii (2005), Pinratana (1988) and Talbot (1947).

## DNA extraction, PCR amplification and sequencing

Total DNA was extracted using applied protocol from Collins et al. (1987). The sample was homogenized with a pestles in a 1.5 micro centrifuge tube with $50 \mu \mathrm{l}$ of lysis buffer including $0.8 \mathrm{M} \mathrm{NaCl}, 0.16 \mathrm{M}$ sucrose, 0.06 M EDTA, $0.5 \%$ SDS and 0.1 M Tris- HCl pH 8.6. Two $\mu \mathrm{l}$ of Proteinase K was added and the homogenate was incubated at $65^{\circ} \mathrm{C}$ overnight. Seven $\mu 1$ of $8 \mathrm{M} \mathrm{CH}_{3} \mathrm{CO}_{2} \mathrm{~K}$ was added and the tube was incubated for 30 min at $-20^{\circ} \mathrm{C}$. Then it was centrifuged at room temperature for 15 min at $13,400 \mathrm{rpm}$. Supernatant was removed to a sterile tube and $95 \%$ ethanol added and centrifuged again at $13,400 \mathrm{rpm}$ for 15 min . After discarding supernatant, the pellet was washed with $70 \%$ ethanol and centrifuge again. The pellet
was dried and suspended in $50 \mu \mathrm{l}$ of TE buffer ( 10 mM Tris, 1 mM EDTA pH 8.0) and maintained at $-20^{\circ} \mathrm{C}$.

DNA was amplified from a mitochondrial gene Cytochrome Oxidase subunit I (COI) and two nuclear genes, Elongation factor 1 alpha ( $E F-1 \alpha$ ) and wingless genes. The polymerase chain reaction was done using 2 X Blue/Red mix DNA polymerase master mix (RBC Bioscience, Taiwan) following manufacturer's protocol. The primers of three genes follow Kodandaramaiah et al. (2010) with COI using primer LCO - HCO (LCO ; 5' GGTCAACAAATCATAAAGATATTGG 3' F and HCO : 5' TAAACTTCAGGGTGACCA AAAAATCA 3 'R) with following protocol : $95^{\circ} \mathrm{C} 7 \mathrm{~min}$ for initial denature, 40 cycles for $95^{\circ} \mathrm{C} 30 \mathrm{~s}, 50^{\circ} \mathrm{C} 30 \mathrm{~s}$ and 72 ${ }^{\circ} \mathrm{C} 1 \mathrm{~min}$, final extension at $72{ }^{\circ} \mathrm{C}$ for 10 min . EF-1 $\alpha$ using primer Starsky-Luke (Starsky : 5’'CACATYAACATTGTCGTSATYGG 3' F and Luke : $5^{\prime}$ CATRTTGTCKCCGTGCCAKCC 3 ' R ) with following protocol : $95^{\circ} \mathrm{C} 7 \mathrm{~min}$ for initial denature, 40 cycles for $95^{\circ} \mathrm{C} 30 \mathrm{~s}, 55^{\circ} \mathrm{C} 30 \mathrm{~s}$ and $72{ }^{\circ} \mathrm{C} 1 \mathrm{~min}$, final extension at $72{ }^{\circ} \mathrm{C}$ for 10 min . wingless using primer Wingnut $1 \mathrm{~A}-$ Wingnut-3 (Wingnut 1A : 5’-GAAATGCGNCARGARTGYAA-3' F and Wingnut-3 : 5’-ACYTCRCARCACCARTGRAA-3' R ) with following protocol : $80{ }^{\circ} \mathrm{C} 1 \mathrm{~min}$ for initial denature, 40 cycling for $94{ }^{\circ} \mathrm{C} 1 \mathrm{~min}, 52{ }^{\circ} \mathrm{C} 2 \mathrm{~min}$ and $72{ }^{\circ} \mathrm{C} 1 \mathrm{~min}$, final extension at $72{ }^{\circ} \mathrm{C}$ for 10 min . PCR products were checked by $1.5 \%$ agarose gel electrophoresis and sent to sequencing with BIONEER (south Korea).

## Genetic variation and phylogenetic analysis

Sequences were viewed by Chromas Lite version 2.5.1 software and aligned using Bioedit version 7.1.3 software (Hall, 1999). The aligned sequences were arranged by MEGA version 6.0 to analyze nucleotide composition and pairwise genetic distance (Tamura, Stecher, Peterson, Filipski, \& Kumar, 2013). DnaSP version 5.10.01 (Rozas \& Rozas, 1999) was use to estimate number of haplotype ( $h$ ), haplotype diversity $(H d)$, nucleotide diversity $(\pi)$, invariable site, parsimony informative site, number of polymorphic sites $(S)$ and Tajima's $D$ test (Nei \& Kumar, 2000). Sequences were submitted to GenBank for accession numbers (Table 1).

Table 1 List of Mycalesis sequences were obtained for this study

| Species | Localities | Coordinate | Accession Number |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | COI | EF-1 $\alpha$ | wingless |
| M. perseoides | CHP | $\begin{aligned} & 10^{\circ} 45.798^{\prime} \mathrm{N} \\ & 99^{\circ} 23.032^{\prime} \mathrm{E} \end{aligned}$ | MG461862 | MG461892 | MG461922 |
|  |  |  | MG461863 | MG461893 | MG461923 |
|  |  |  | MG461864 | MG461894 | MG461924 |
|  |  |  | MG461865 | MG461895 | MG461925 |
| M. perseoides | STN | $09^{\circ} 23.340^{\prime} \mathrm{N}$ | MG461871 | MG461901 | MG461931 |
|  |  | $99^{\circ} 15.240^{\prime} \mathrm{E}$ | MG461872 | MG461902 | MG461932 |
| M. perseoides | NST | $08^{\circ} 20.020^{\prime} \mathrm{N}$ | MG461875 | MG461905 | MG461935 |
|  |  | $100^{\circ} 09.562^{\prime} \mathrm{E}$ | MG461880 | MG461910 | MG461940 |
| M. perseoides | NTW | $05^{\circ} 58.655^{\prime} \mathrm{N}$ | MG461887 | MG461917 | MG461947 |
|  |  | $101{ }^{\circ} 54.392$ ' ${ }^{\text {E }}$ | MG461891 | MG461921 | MG461951 |
| M. intermedia | STN | $09^{\circ} 23.340{ }^{\prime} \mathrm{N}$ | MG461873 | MG461903 | MG461933 |
|  |  | $99^{\circ} 15.240^{\prime} \mathrm{E}$ |  |  |  |
| M. intermedia | NST | $08^{\circ} 20.020^{\prime} \mathrm{N}$ | MG461874 | MG461904 | MG461934 |
|  |  | $100^{\circ} 09.562^{\prime} \mathrm{E}$ | MG461877 | MG461907 | MG461937 |
| M. intermedia | TRG | 07³3.360 ${ }^{\text { }} \mathrm{N}$ | MG461882 | MG461912 | MG461942 |
|  |  | $99^{\circ} 46.724^{\prime} \mathrm{E}$ | MG461885 | MG461915 | MG461945 |
|  |  |  | MG461886 | MG461916 | MG461946 |
| M. intermedia | NTW | $05^{\circ} 58.655^{\prime} \mathrm{N}$ | MG461888 | MG461918 | MG461948 |
|  |  | $101{ }^{\circ} 54.392{ }^{\prime} \mathrm{E}$ | MG461889 | MG461919 | MG461949 |
|  |  |  | MG461890 | MG461920 | MG461950 |
| M. mineus | STN | $09^{\circ} 23.340^{\prime} \mathrm{N}$ | MG461869 | MG461899 | MG461929 |
|  |  | $99^{\circ} 15.240^{\prime} \mathrm{E}$ | MG461870 | MG461900 | MG461930 |
| M. visala | NST | $\begin{aligned} & 08^{\circ} 20.020^{\prime} \mathrm{N} \\ & 100^{\circ} 09.562^{\prime} \mathrm{E} \end{aligned}$ | MG461876 | MG461906 | MG461936 |
| M. visala | TRG | $\begin{aligned} & 07^{\circ} 33.360^{\prime} \mathrm{N} \\ & 99^{\circ} 46.724^{\prime} \mathrm{E} \end{aligned}$ | MG461883 | MG461913 | MG461943 |
| M. perseus | NST | $08^{\circ} 20.020^{\prime} \mathrm{N}$ | MG461878 | MG461908 | MG461938 |
|  |  | $100^{\circ} 09.562^{\prime} \mathrm{E}$ | MG461879 | MG461909 | MG461939 |
| M. orseis | TRG | 07³3.360 ${ }^{\text { }} \mathrm{N}$ | MG461881 | MG461914 | MG461944 |
|  |  | $99^{\circ} 46.724^{\prime} \mathrm{E}$ |  |  |  |
| M. janardana | TRG | $07^{\circ} 33.360^{\prime} \mathrm{N}$ | MG461884 | MG461911 | MG461941 |
|  |  | $99^{\circ} 46.724^{\prime} \mathrm{E}$ |  |  |  |

Genetic differentiation and phylogenetic tree of the butterfly genus Mycalesis in peninsular Thailand using COI, EF-1 $\alpha$ and wingless genes were determined. Kimura 2-parameter model was used to calculate the genetic distance. Haplotype network of the M. perseoides was generated using TCS program version 1.7 (Clement, Posada, \& Crandall, 2000). Phylogenetic analysis of the butterfly genus Mycalesis was conducted for COI sequence and for the combined dataset (COI, EF-1 $\alpha$ and wingless). Bayesian analysis with MrBayes version 3.2.6 using General Time Reversible (GTR) model by GAMMA distribution was done for 10,000,000 generations with a sampling frequency of 100 generations (Huelsenbeck \& Ronquist, 2001). Phylogeny was viewed and edited by FigTree version 1.3.1 (Rambaut, 2009). Species divergence time was investigated by the molecular clock using fossil record of Lethe corbieri by Neighbor-joining method (NJ) in MEGA program version 6.0.

## Result

The butterflies of genus Mycalesis were sampled during February 2015 to July 2016 with a total of 214 individuals, Seven species were grouped into five members of Mineus group consisting of M. intermedia, M. visala, M. perseoides, M. mineus and M. perseus. The rest of Mycalesis were M. orseis and M. janardana. The Mycalesis species was found to be distributed in each province; Chumphon province (CHP) consisted of M. mineus and M. perseoides. Four Mycalesis species ( $M$. perseus, M. mineus, M. perseoides and M. visala) were found in Phang-Nga province (PHG), Phuket mountain range. Three species, M. mineus, M. intermedia and M. perseoides were found in Surat Thani province (STN). Furthermore, four species, ( $M$. perseus, M. intermedia, M. perseoides and M. visala), were found in Nakhon Si Thammarat province (NST). In addition, six species of Mycalesis, (M. orseis, M. janardana, M. mineus, M. intermedia, M. perseoides and M. visala), were found in Trang province (TRG), Nakhon Si Thammarat mountain range. However, five species, M. mineus, M. intermedia, M. perseoides, M. visala and M. perseus, occurred in Narathiwat province (NTW), San kala khiri mountain range. The result showed that M. perseoides was dominant species in all sampling sites across peninsular Thailand (Figure 1). Interestingly, Mycalesis species in peninsular Thailand showed two
distinct seasonal morphs; wet season form and dry season form, were M. mineus and M. perseoides. This shows relationship between genetic variation and phenotypic plasticity (Figure 2). The phenotypic character of M. mineus and M. perseoides in the dry season form and the wet season form are different, especially in dry season forms they are difficult to be identified because the characters such as less colorations of wings and eyespots are reduced. On the other hand, for the wet season form, the colorations of wings and eyespots are prominent characters.


Figure 1 Distribution of Mycalesis species in six study sites of peninsular Thailand. Pie chart segments are proportional to the number of individuals collected at each location.


Figure 2 Two species of Mycalesis in peninsular Thailand: M. mineus (left column) and M. perseoides (right column); A) representative examples of wet season form (top row) and dry season form (bottom row); B) male genitalia and aedeagus. All specimens are males. Adult butterfly ( scale bar $=1 \mathrm{~cm}$ ).

## Genetic variation

The aligned sequences were $632 \mathrm{bp}(C O I), 485 \mathrm{bp}(E F-1 \alpha)$ and 379 bp (wingless). The COI sequence showed higher invariant sites than $E F-1 \alpha$ and wingless. Among 632 bp of $C O I$ sequence, 73 sites were parsimony informative. The $E F-1 \alpha$ sequence contained 24 variable parsimony informative sites while wingless contained only 16 variable parsimony informative sites. Genetic diversity indices of the three genes showed COI gene had 0.04019 nucleotide diversity $(\pi), 25.32$ average number of nucleotide differences ( $K$ ) and 125 polymorphic sites $(S)$. Furthermore, the nucleotide diversity, average number of nucleotide differences and number of polymorphic sites of EF-1 $\alpha$ and wingless were $0.02293,88,10.93$ and $0.01591,52$, 6.03 respectively. However, the number of haplotype ( $h$ ) and haplotype diversity ( $H d$ ) were high in wingless gene, that belonging 23 and 0.980 . The result of Tajima's $D$ test revealed that all genes were not significant (Table 2).

Table 2 Statistic for individual gene segments.

| parameters | COI | EF-1a | wingless |
| :---: | :---: | :---: | :---: |
| Number of samples | 29 | 29 | 29 |
| Aligned positions | 632 | 485 | 379 |
| Polymorphic site |  |  |  |
| Invariable sites | 505 | 389 | 327 |
| - Singleton variable sites | 52 | 64 | 36 |
| - Parsimony informative sites | 73 | 24 | 16 |
| - Nucleotide diversity ( $\pi$ ) | 0.04019 | 0.02293 | 0.01591 |
| - Number of polymorphic sites ( $S$ ) | 125 | 88 | 52 |
| Average number of nucleotide differences ( $K$ ) | 25.32 | 10.93 | 6.03 |
| Haplotype diversity |  |  |  |
| - Number of haplotype (h) | 14 | 19 | 23 |
| - Haplotype diversity ( $H d$ ) | 0.771 | 0.887 | 0.980 |
| Tajima's Test |  |  |  |
| - Tajima's $D$ | -1.23492* | -2.08307* | -2.20456* |

Note: * not significant.
M. perseoides and M. perseus possessed the greatest intraspecific genetic differentiation in the nuclear gene ( $E F-1 \alpha$ values were 1.76 and 4.70 , and wingless values were 0.80 and 1.10 , respectively). However, intraspecific genetic divergence for $M$. intermedia and M.visala were high in COI gene (2.70 and 6.20, respectively) (Table 3).

Table 3 Intraspecific genetic divergence among the members of Mycalesis Mineus group in peninsular Thailand based on the Kimura 2-parameter model.

| Species | COI | EF-1 $\boldsymbol{\alpha}$ | Wingless |
| :--- | :--- | :--- | :--- |
| M. perseoides | $0.33(0.000-0.006)$ | $1.76(0.000-0.042)$ | $0.80(0.003-0.013)$ |
| M. intermedia | $2.70(0.000-0.066)$ | $1.00(0.000-0.019)$ | $0.67(0.000-0.013)$ |
| M. mineus | $0.25(0.002-0.003)$ | $0.40(0.000-0.004)$ | $0.65(0.005-0.008)$ |
| M. visala | $6.20(0.000-0.062)$ | $1.00(0.002-0.015)$ | $0.40(0.003-0.005)$ |
| M. perseus | $0.20(0.002)$ | $4.70(0.047)$ | $1.10(0.011)$ |

Note: The analyses involved 29 nucleotide sequences. All positions containing gaps and missing data were eliminated. There are a total of 360 positions for COI, 476 positions for $E F-1 \alpha$ and 379 positions for wingless. All values are mean (\%) (min.max.). For M. perseus had only one value.

According to haplotype network of Mycalesis species in peninsular Thailand, the haplotype network of $M$. perseoides based on $E F-1 \alpha$ gene in four provinces (CHP, STN, NST and NTW) was generated. The result showed that five haplotype patterns were found and the haplotype diversity was 0.667 . Furthermore, number of polymorphic sites, nucleotide diversity and Average number of nucleotide differences were 22, 0.00949 and 4.556 respectively (Table 4).

Table 4 Statistic of $M$. perseoides based on $E F-1 \alpha$ sequence.

| parameters | $\boldsymbol{E F - 1 \boldsymbol { \alpha }}$ |
| :--- | :---: |
| Number of samples | 10 |
| Aligned positions | 485 |
| Polymorphic site |  |
| - $\quad$ Invariable sites | 458 |
| - | Singleton variable sites |
| - | Parsimony informative sites |
| - | Nucleotide diversity $(\pi)$ |
| - | Number of polymorphic sites $(S)$ |
| - | Average number of nucleotide |
|  | differences $(K)$ |
| - | Number of haplotype $(h)$ |
| - | Haplotype diversity $(H d)$ |
| - | Tajima's D |

Note: * not significant.

The haplotype relationship of five haplotype is shown in Figure 3. Haplotype 1 of $M$. perseoides $(n=6)$ indicated that Chumphon province might be the origin of $M$. perseoides in peninsular Thailand. However, the haplotype of $M$. perseoides in Chumphon is different from the Surat Thani (haplotype 3, $\mathrm{n}=1$ ) in one mutation step of nucleotide substitution. Moreover, haplotype 4 (Nakhon Si Thammarat, $\mathrm{n}=1$ ) is 19 mutation steps of nucleotide substitution away from Chumphon haplotype pattern. However, the haplotype 5 (Narathiwat, $\mathrm{n}=1$ ) is different in two mutation steps of nucleotide substitution from Chumphon haplotype pattern.


Figure 3 Haplotype network for $E F-1 \alpha$ sequence of M. perseoides in peninsular Thailand. Solid lines on branches refer to mutation step.

## Phylogenetic relationships

Phylogenetic analyses based on the combined dataset included 1,496 bp from 29 samples of Mycalesis species and one outgroup $O$. medus sequence from GenBank, DQ338766 (COI), DQ338906 (EF-1 $\alpha$ ) and DQ338633 (wingless). The phylogenetic tree of Bayesian approach showed that the Mineus group of Mycalesis in peninsular Thailand was monophyletic with a strong support (posterior probability, BI $=100$ ). This tree was divided into two major clades, A and B (Figure 4).


Figure 4 Bayesian tree for the combined dataset of COI, EF-1 $\alpha$ and wingless sequences of Mycalesis species in peninsular Thailand. Posterior probabilities base on Likelihood ratio test for Maximum likelihood.

Clade A consisting of M. perseoides, M. intermedia, M. visala and M. mineus was strongly supported as monophyly (posterior probability, $\mathrm{BI}=100$ ). Clade A can be divided into two subclade: A1 and A2. In subclade A1, M. visala from Nakhon Si Thammarat province and Trang province were grouped together. However, the result indicated that $M$. perseoides from Chumphon, Surat Thani, Nakhon Si Thammarat and Narathiwat provinces and M. intermedia from Surat Thani, Nakhon Si Thammarat and Narathiwat provinces in subclade A1 were grouped together. The result indicated that this might be species complex of Mineus group. Sublcade A2 consisted of $M$. intermedia from Trang province and Narathiwat province and $M$. mineus from Surat Thani province and Phang-Nga province. The result showed that each species was clearly separated in different branch. Furthermore, clade B composed of M. perseus from Nakhon Si Thammarat province and it was strongly supported as monophyly ( $\mathrm{BI}=100$ ). The rest of Mycalesis (M. janardana of Janardana group and M. orseis of Francisca group from Trang province) were collected.

## Divergence time estimates

The divergence time in the genus Mycalesis in this study was estimated from age of fossil record of Lethe corbieri during the Oligocene in southeast France, approximately 28.4 to 23.03 million years ago (mya) (Nel, Nel, \& Balme, 1993). The analysis in Neighbor-joining (NJ) indicated that Mycalesis in peninsular Thailand was diverged between 32.47 mya during Oligocene period (Figure 5). Initial split the janardana group and Mineus group arised ca. 23.03 mya during Oligocene period. The molecular phylogram showed that Mineus group it was divided into two clades (A and B) ca. 11.76 mya during Miocene period. In clade A, Mycalesis species were separated approximately 10.66 mya into two subclades (A1 and A2). Subclade A1 arised ca. 9.72 mya and is composed of M. perseoides from Chumphon, Surat Thani, Nakhon Si Thammarat and Narathiwat province, M. intermedia from Surat Thani, Nakhon Si Thammarat and Narathiwat provinces and M. visala from Nakhon Si Thammarat and Trang provinces colonizing around 0.78 mya during Pleistocene period. Subclade A2 consisted of $M$. mineus from Surat Thani and Phang-Nga
provinces ( 0.65 mya) during Pleistocene. However, Clade B consisted of $M$. intermedia from Trang and Narathiwat provinces colonizing around 0.29 mya during Pleistocene period.


Figure 5 The neighbor-joining tree is obtained from COI gene of Mycalesis species and out group in peninsular Thailand. Values on branches refer to divergence times. Scale bar indicates million years ago (mya) for divergence time molecular clock.

## Discussion

Molecular technique provides a powerful tool to investigate the population dynamics of organism and also enable more detailed understanding on the relationship between populations (Sum et al. 2014). Various genetic markers have been used to illustrate the genetic differentiation that occurs in butterfly populations, especially mitochondrial and nuclear gene markers. In this study, a good candidate gene marker is revealed as the mitochondrial gene (COI) with high variability in polymorphic sites (invariable sites, singleton variable sites, parsimony informative sites, nucleotide diversity, number of polymorphic sites and Average number of nucleotide differences of interspecific genetic differentiation). However, the number of haplotype and haplotype diversity were high in nuclear genes (EF-1 $\alpha$ and wingless). Although, four Mycalesis (Mineus groups); M. visala, M. intermedia, M. perseus and M. perseoides were highly different in intraspecific genetic divergence in each gene. Intraspecific genetic variation of $M$. visala had high diversity in COI and M. perseus in EF-1 $\alpha$ and wingless. M. mineus had low intraspecific genetic diversity in all genes, indicating high degree of gene flow within population.

Phylogenetic relationship of Mycalesis (Mineus group) in peninsular Thailand was monophyletic with strong support based on Bayesian analysis. Mycalesis (Mineus group) can be divided into two major clades (clade A and B). At species level, M. mineus of Surat Thani province and Phang-Nga province were grouped together in clade A2, consistent with intraspecific genetic distance which was low in all genes. Therefore, their genetic relationship is closely related within population. However, M. Intermedia and M. perseoides were species complex which were weakly supported $(\mathrm{BI}=69)$. However, those species were not clearly related in this study. These three species were different in terms of morphological characters of male genitalia. On the other hand, the genetic divergence among species of $M$. intermedia, M. perseoides and M. visala was low. This result suggested that the gene introgression in mtDNA and nuclear gene likely happened during species expansion. According to species delimitation, their connecting populations have been influenced by gene flow. Intraspecific genetic differentiation (introgression) would affect species
integrity. Recent studies have proposed that the correlation between intraspecific genetic differentiation and gene flow was a negative correlation. Therefore, species delimitation might be more effective with markers experiencing high degree of gene flow (Petit \& Excoffier, 2009).

This finding of the monophyly of Mineus group in peninsular Thailand is consistent with the scenario of Kodandaramaiah et al. (2010) indicating that the Mycalesis species was clustered into two clades as Mycalesis I and Mycalesis II. Mycalesis I belonged to Mycalesis species from mainland Southeast Asia (China, Vietnam, Laos, Bangladesh and Thailand) and Mycalesis II belonged to the taxa from Indo-Australia (Indonesia, Australia, Papua New Guinea and Solomon Island). In this study, all members of Mycalesis in peninsular Thailand might be clustered in clade Mycalesis I. The finding of the monophyletic group of Mycalesis (Mineus group) in peninsular Thailand was supported by divergence time as well. The result showed that Mycalesis species in peninsular Thailand diverged ca. 32.47 mya during Oligocene period. Mycalesis Mineus group arised at approximately 23.03 mya in Oligocene period, consistent with the reported of Aduse-Poku et al. (2015) proposing that the butterfly subtribe Mycalesina and Lethina diverged from each other about 39.8 mya during Eocene period. Furthermore, divergence time of Mycalesis in Southeast Asia was observed to have occurred around the Oligocene and Eocene boundary, roughly at the same time as diverged the endemic African genera Bicyclus and Hallesis. Furthermore, the origin of the subtribe Mycalesina was in Asia and later distributed to other regions (Aduse-Poku et al., 2015). However, Mycalesis Mineus group was divided into two clades approximately 11.76 mya in mid Miocene period.

According to Pena and Wahlberg (2008), the fossil record of butterfly reconstructed a divergence time of subfamily Satyrinae and the result showed that subfamily Satyrinae might dispersed to other continental with their adaptive radiation of food plants approximately 25 mya during Oligocene epoch (Brakefield, 2012; Cerling et al., 1997; Sage, 2004). The larvae of these butterflies feed on grasses (Poaceae) that are dominant in shaded and open habitats, especially the tropical rain forest (Pena \& Wahlberg, 2008). Interestingly, M. perseoides is a good example to
answer the haplotype network and migration route of Mycalesis species. The haplotype network of the EF-1 $\alpha$ sequence of $M$. perseoides suggested that the haplotype pattern of Chumphun $(\mathrm{n}=6)$ is assumed to be the origin then it was divided into Surat Thani pattern $(\mathrm{n}=1)$, Narathiwat pattern $(\mathrm{n}=1)$ and Nakhon Si Thammarat pattern ( $\mathrm{n}=1$ ). In this study, the result indicated that M. perseoides have a genetic connectivity microhabitat between populations of $M$. perseoides in peninsular Thailand. Furthermore, this study has determined relationship between genetic variation and phenotypic plasticity of Mycalesis populations in Thailand. Mycalesis species in peninsular Thailand were found in two distinct seasonal morphs, wet and dry season forms such as in M. mineus and M. perseoides. The morphology of male genitalia was distinguishing character to identify species in this genus. The butterfly in subtribe Mycalesina is well known important model to understand development, genetics and evolutionary of phenotypic plasticity as a response to wet and dry seasonal environment in their habitat. This phenomena has been influenced on seasonal fluctuation and reproductive dormancy. Temperature and humidity play an important role to regulate diel activity (Islam et al., 2010). Furthermore, the dry season form was usually reproductively inactive, long-lived, and has less diel activity. By contrast, wet season form was reproductively active more diel activity (Braby, 1994; Islam et al., 2010).

## Acknowledgements

We would like to express our thanks to Ms. Sopita Muadsub and Mr. Paradorn Dokchan from Department of Entomology Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, who helped and suggested genitalia dissection technique and took a photos of male genitalia. We are also grateful to the members in Entomology Research Unit for help and support to collect the samples in the field. This work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission (SCI540531M), the Graduate school of Prince of Songkla University and Department of Biology, Faculty of Science, Prince of Songkla University, Thailand.

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## CHAPTER 5

## CONCLUSION

From the result of this thesis, a total of 214 individuals adult Mycalesis were collected by insect nets and fruit-baited trap during February 2015 to July 2016. It can be identified to seven species. In this study, M. perseoides was most dominant and distributed at all collecting site along peninsular Thailand. Some species such as M. orseis and M. janardana were only found in Trang province (Figure 5, Chapter 2). We found two seasonal forms of wing pattern (wet and dry season forms) in all collecting sites except Khao Chong at Trang province was found only wet season form. Morphological variation of wing pattern in two seasonal forms can occur in Mycalesis species because of phenotypic plasticity that associated with environment conditions such as temperature, humidity and photoperiod in their habitat. Morphology of male genitalia were also described here.

We used different markers to reveal genetic diversity and genetic variation of Mycalesis in peninsular Thailand, as different markers have different level of variations (Hind \& Saunders, 2013; Whichachucherd, 2014). The result of our study using the three molecular markers (COI, EF-1 $\alpha$ and wingless), revealed as mtDNA with high variability in polymorphic sites such as invariable sites, parsimony informative sites, nucleotide diversity, number of polymorphic sites and average number of nucleotide differences. Haplotype diversity and number of haplotype were high in nuclear markers, but Tajima's $D$ test were negative in all gene. Intraspecific genetic divergence of some Mycalesis species low, that could be have genetic connectivity (gene flow) among species, consistent with the scenario of Petit and Excoffier (2009) proposed that the correlation between intraspecific genetic differentiation and gene flow was negative correlation.

Aduse-Poku et al., (2015) proposed that Asia as the area of origin of Mycalesis and later distributed to Indo-Australian region, includes Brunei, Indonesia,

Malaysia, Papua New Guinea, Singapore and peninsular Thailand. The result of the haplotype network of $E F-1 \alpha$ sequence of $M$. perseoides showed that the haplotype pattern of Chumphon is assume to be the origin and it was divided into Surat Thani, Nakhon Si Thammarat and Narathiwat (Figure 3, Chapter 3) . This result suggested that the gene introgression likely happened during species radiation. M. perseoides, is a good candidate to answer the migration route of Mycalesis species in peninsular Thailand.

Phylogenetic results using the combined dataset clearly indicated that Mycalesis in peninsular Thailand as monophyletic group. For Mineus group it can be divided into two major clades, although the result showed monophyly of Mycalesis in Mineus group, M. perseoides and M. intermedia in clade A1 were still unclear (Figure 4, Chapter 3). According to previous reported of Kodandaramaiah et al. (2010) indicated that Mycalesis was clustered into two clades (Mycalesis I and Mycalesis II). Mycalesis I belonged to Mycalesis species from mainland Southeast Asia (China, Vietnam, Laos, Bangledesh and Thailand), consists M. gotama, M. francisca, M. orseis, M. anaxias, M. mynior, M. perseus, M. perseoides, M. intermedia, M. visala and M. mineus. Therefore, all members of Mycalesis in this study might be clustered in Mycalesis I. As well as the divergence time of Mycalesis was estimated and the result showed that Mycalesis in peninsular Thailand diverged around 32.47 mya during Oligocene period (Figure 5, Chapter 3). According to Pena and Wahlberg (2008) proposed that the butterflies might be dispersed with their adaptive radiation of food plant during Oligocene epoch. The larva of these butterflies feed on Poaceae (grasses) that are dominant in shaded and open habitats, especially the tropical rain forest.

However, further research are needed, with large sample sizes representing more locations covering the different elevation and climatic zone. More replicate samples collecting in each site are required to determine the species diversity and distribution in Southeast Asia. Moreover, to investigate the gene expression of wingless gene in wet and dry season forms of Mycalesis species in Thailand.

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APPENDIX A Intraspecific genetic diversity of Mycalesis perseoides among population in peninsular Thailand

| population | $\mathbf{N}$ | $\boldsymbol{S}$ | $\boldsymbol{K}$ | $\boldsymbol{\pi}$ |
| :--- | :---: | :---: | :---: | :---: |
| Chumphon | 4 | 1 | 0.500 | 0.00103 |
| Surat thani | 2 | 1 | 1.000 | 0.00206 |
| Nakhon Si Thammarat | 2 | 19 | 19.000 | 0.03958 |
| Narathiwat | 2 | 2 | 2.000 | 0.00414 |
| All | 10 | 23 | 4.556 | 0.00949 |

N : Number of sequences, S : Number of polymorphic sites, K: Average number of nucleotide differences, $\boldsymbol{\pi}$ : Nucleotide diversity

APPENDIX B Nucleotide composition of Mycalesis species for individual's gene segment.

| Species | COI |  |  |  |  | EF-1a |  |  |  |  | Wingless |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | T(U) | C | A | G | Total | T(U) | C | A | G | Total | T(U) | C | A | G | Total |
| M. janardana | 40.5 | 15.5 | 29.6 | 14.4 | 632 | 21.2 | 28.0 | 25.4 | 25.4 | 485 | 14.7 | 32.1 | 20.8 | 32.4 | 379 |
| M. orsies | 39.1 | 16.3 | 30.4 | 14.1 | 632 | 20.6 | 27.6 | 24.7 | 27.0 | 485 | 16.4 | 30.3 | 22.2 | 31.1 | 379 |
| M. intermedia | 39.6 | 15.6 | 31.0 | 13.8 | 632 | 20.0 | 28.2 | 25.3 | 26.6 | 485 | 14.5 | 31.9 | 20.0 | 33.6 | 379 |
| M. minues | 40.8 | 14.1 | 31.0 | 14.1 | 632 | 19.7 | 28.6 | 24.9 | 26.8 | 485 | 14.7 | 31.7 | 20.0 | 33.6 | 379 |
| M. persues | 39.6 | 15.2 | 31.0 | 14.3 | 632 | 21.2 | 27.5 | 25.6 | 25.6 | 485 | 14.8 | 31.7 | 20.3 | 33.3 | 379 |
| M. perseoides | 39.6 | 15.4 | 31.3 | 13.6 | 632 | 20.2 | 27.7 | 25.6 | 26.5 | 485 | 14.6 | 32.0 | 20.0 | 33.4 | 379 |
| M. visala | 39.6 | 15.5 | 31.2 | 13.7 | 632 | 20.1 | 28.2 | 25.3 | 26.4 | 485 | 14.4 | 32.0 | 20.2 | 33.4 | 379 |
| Avg. | 39.8 | 15.4 | 30.8 | 14.0 | 632 | 20.4 | 28.0 | 25.3 | 26.3 | 485 | 14.9 | 31.7 | 20.5 | 33.0 | 379 |

APPENDIX C Interspecific genetic divergence of Mycalesis among population for individual's gene segment

COI

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1. M. janardana |  |  |  |  |  |  |  |
| 2. M. orseis | 0.113 |  |  |  |  |  |  |
| 3. M. perseus | 0.095 | 0.116 |  |  |  |  |  |
| 4. M. mineus | 0.084 | 0.113 | 0.048 |  |  |  |  |
| 5. M. intermedia | 0.097 | 0.117 | 0.052 | 0.043 |  |  |  |
| 6. M. perseoides | 0.098 | 0.119 | 0.054 | 0.046 | 0.057 |  |  |
| 7. M. visala | 0.097 | 0.117 | 0.052 | 0.040 | 0.059 | 0.056 |  |

## EF-1 $\alpha$

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1. M. janardana |  |  |  |  |  |  |  |
| 2. M. orseis | 0.067 |  |  |  |  |  |  |
| 3. M. perseus | 0.074 | 0.071 |  |  |  |  |  |
| 4. M. mineus | 0.064 | 0.061 | 0.019 |  |  |  |  |
| 5. M. intermedia | 0.067 | 0.064 | 0.022 | 0.008 |  |  |  |
| 6. M. perseoides | 0.071 | 0.067 | 0.025 | 0.011 | 0.011 |  |  |
| 7. M. visala | 0.064 | 0.061 | 0.019 | 0.005 | 0.003 | 0.005 |  |

## Wingless

| Species | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ |
| :--- | :---: | :---: | :---: | :---: | :---: |
| 6 | $\mathbf{7}$ |  |  |  |  |
| 1. M. janardana <br> 2. M. orseis |  |  |  |  |  |
| 3. M. perseus | 0.065 |  |  |  |  |
| 4. M. mineus | 0.113 | 0.096 |  |  |  |
| 5. M. intermedia | 0.065 | 0.045 | 0.072 |  |  |
| 6. M. perseoides | 0.109 | 0.101 | 0.131 | 0.017 | 0.061 |
| 7. M. visala | 0.065 | 0.045 | 0.066 | 0.002 | 0.015 |

APPENDIX D List of Mycalesis species ware used in this study and submitted to
Genbank

| Seq. | Species | Localities | Specimen N . | Isolate |
| :---: | :---: | :---: | :---: | :---: |
| 1 | M. perseoides | CHP | CH5 | MpersdS011 |
| 2 | M. perseoides | CHP | CH22 | MpersdS012 |
| 3 | M. perseoides | CHP | CH30 | MpersdS013 |
| 4 | M. perseoides | CHP | CH33 | MpersdS014 |
| 5 | M. mineus | PNG | PH7 | MminS021 |
| 6 | M. mineus | PNG | PH10 | MminS022 |
| 7 | M. mineus | STN | ST4 | MminS031 |
| 8 | M. mineus | STN | ST5 | MminS032 |
| 9 | M. perseoides | STN | ST8 | MpersdS031 |
| 10 | M. perseoides | STN | ST19 | MpersdS032 |
| 11 | M. intermedia | STN | ST25 | MintS031 |
| 12 | M. intermedia | NST | NK4 | MintS041 |
| 13 | M. perseoides | NST | NK6 | MpersdS041 |
| 14 | M. visala | NST | NK9 | MvisS041 |
| 15 | M. intermedia | NST | NK12 | MintS042 |
| 16 | M. perseus | NST | NK22 | MpersS041 |
| 17 | M. perseus | NST | NK23 | MpersS042 |
| 18 | M. perseoides | NST | NK28 | MpersdS042 |
| 29 | M. janardana | TRG | TR1 | MjanS051 |
| 20 | M. intermedia | TRG | TR2 | MintS051 |
| 21 | M. visala | TRG | TR3 | MvisS051 |
| 22 | M. orseis | TRG | TR15 | MorsS051 |
| 23 | M. intermedia | TRG | TR19 | MintS052 |
| 24 | M. intermedia | TRG | TR21 | MintS053 |
| 25 | M. perseoides | NTW | NR13 | MpersdS061 |
| 26 | M. intermedia | NTW | NR21 | MintS061 |

## APPENDIX D (Cont.)

| Seq. | Species | Locatities | Specimen N. | Isolate |
| :--- | ---: | :---: | :---: | :--- |
| $\mathbf{2 7}$ | M. intermedia | NTW | NR25 | MintS062 |
| $\mathbf{2 8}$ | M. intermedia | NTW | NR30 | MintS063 |
| $\mathbf{2 9}$ | M. perseoides | NTW | NR39 | MpersdS062 |

APPENDIX E Nucleotide sequencing of Mycalesis individual's gene segment for this study

## COI gene

Seq1 [organism=Mycalesis perseoides] [isolate=MpersdCOIS011]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAACATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC CTCСTCTCTCATCAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGTAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCCTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

Seq2 [organism=Mycalesis perseoides] [isolate=MpersdCOIS012]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAATATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC СТССТСТСТСATCAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAACTTA

## AATACTTCTTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

seq3 [organism=Mycalesis perseoides] [isolate=MpersdCOIS013]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAACATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC СТССТСТСТСATCAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCCTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT
seq4 [organism=Mycalesis perseoides] [isolate=MpersdCOIS014]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAATATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC СТССТСТСТСATCAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAACTTA AATACTTCTTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT
seq5 [organism=Mycalesis mineus] [isolate=MminCOIS021]
ATAGTAGGTACTTCTTTAAGTCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATTGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTTCCTC GTATAAATAATATAAGATTTTGACTTTTACCTCCTTCTTTAGTACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATATC CСССССТTTCATCAAATATTGCCCATGGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATTAATTT TATTTCAACAATTATTAATATACGAATTAATAGTATAACTTATGATCAAAT ACCCCTTTTTGTATGAGCTGTTGGAATTACAGCTCTATTACTATTATTATCT

TTACCTGTTTTAGCTGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCTTTTTTTGATCCAGCTGGAGGAGGAGATCCTATCCTTTATCAA CATTTATTTTGATTTTT

Seq6 [organism=Mycalesis mineus] [isolate=MminCOIS022]
ATAGTAGGTACTTCTTTAAGTCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATTGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTTCCTC GTATAAATAATATAAGATTTTGACTTTTACCTCCTTCTTTAGTACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATATC CСССССТТТСАТСАAATATTGCCCATGGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATTAATTT TATTTCAACAATTATTAATATACGAATTAATAGTATAACTTATGATCAAAT ACCCCTTTTTGTATGAGCTGTTGGAATTACAGCTCTATTACTATTATTATCT TTACCTGTTTTAGCTGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCTTTTTTTGATCCAGCTGGAGGAGGAGATCCTATCCTTTATCAA CATTTATTTTGATTTTT

Seq7 [organism=Mycalesis mineus] [isolate=MminCOIS031]
ATAGTAGGTACTTCTTTAAGTCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATTGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTTCCTC GTATAAATAATATAAGATTTTGACTTTTACCTCCTTCTTTAGTACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATATC CСССССТТТСАТСАAATATTGCCCATGGCGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATTAATTT TATTTCAACAATTATTAATATACGAATTAATAGTATAACTTATGATCAAAT ACCCCTTTTTGTATGAGCTGTTGGAATTACAGCTCTATTACTATTATTATCT TTACCTGTTTTAGCTGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCTTTTTTTGATCCAGCTGGAGGAGGAGATCCTATCCTTTATCAA CATTTATTTTGATTTTT

Seq8 [organism=Mycalesis mineus] [isolate $=$ MminCOIS032]
ATAGTAGGTACTTCTTTAAGTCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATTGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTTCCTC GTATAAATAATATAAGATTTTGACTTTTACCTCCTTCTTTAGTACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATATC CCCCССТTTCATCAAATATTGCCCATGGCGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATTAATTT TATTTCAACAATTATTAATATACGAATTAATAGTATAACTTATGATCAAAT

ACCCCTTTTTGTATGAGCTGTTGGAATTACAGCTTTATTACTATTATTATCT TTACCTGTTTTAGCTGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCTTTTTTTGATCCAGCTGGAGGAGGAGATCCTATCCTTTATCAA CATTTATTTTGATTTTT

Seq9 [organism=Mycalesis perseoides] [isolate=MpersdCOIS031]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAACATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC СТССТСТСТСАTCAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCCTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

Seq10 [organism=Mycalesis perseoides] [isolate=MpersdCOIS032]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAACATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC CTCСTСTCTCATCAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCCTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

Seq11 [organism=Mycalesis intermedia] [isolate=MintCOIS031]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAACATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC СТССТСТСТСАТСAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT

TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCCTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

Seq12 [organism=Mycalesis intermedia] [isolate=MintCOIS041]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAACATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC СТССТСТСТСАТСAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCCTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

Seq13 [organism=Mycalesis perseoides] [isolate=MpersdCOIS041]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAACATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC СТССТСТСТСАТСAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCCTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

Seq14 [organism=Mycalesis visala] [isolate=MvisCOIS041]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAACATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC СТССТСТСТСATCAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA

TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCCTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

Seq15 [organism=Mycalesis intermedia] [isolate=MintCOIS042]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAACATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC СТССТСТСТСATCAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCCTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

Seq16 [organism=Mycalesis perseus] [isolate=MpersCOIS041]
ATAGTAGGAACCTCTCTAAGCCTTATTATTCGAACAGAATTAGGAAACCC AGGATTTTTAATTGGAGATGATCAAATTTATAACACTATTGTAACAGCACA TGCTTTTATTATAATTTTTTTTATAGTAATACCTATTATAATTGGAGGATTC GGAAATTGATTAGTACCTTTAATATTAGGAGCTCCTGATATAGCTTTCCCT CGTATAAATAATATAAGATTTTGACTTTTACCACCTTCTTTAATGCTTTTAA TTTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATAT CCCCCTCTTTCATCAAATATTGCTCATGGAGGATCTTCAGTAGATTTAGCA ATTTTTTCATTACATTTAGCGGGAATTTCTTCAATTTTAGGAGCTATTAATT TTATTTCAACAATTATTAACATACGAGTTAATAATATATCTTATGATCAAA TACCTCTTTTCGTATGAGCTGTTGGAATTACAGCTTTATTACTTTTACTATC TTTACCTGTTTTAGCTGGAGCTATTACTATATTATTAACAGATCGAAATTT AAATACTTCTTTTTTTGATCCAGCCGGAGGAGGAGACCCTATTCTTTATCA ACATTTATTTTGATTTTT

Seq17 [organism=Mycalesis perseus] [isolate=MpersCOIS042]
ATAGTAGGAACCTCTCTGAGCCTTATTATTCGAACAGAATTAGGAAACCC AGGATTTTTAATTGGAGATGATCAAATTTATAACACTATTGTAACAGCACA TGCTTTTATTATAATTTTTTTTATAGTAATACCTATTATAATTGGAGGATTC GGAAATTGATTAGTACCTTTAATATTAGGAGCTCCTGATATAGCTTTCCCT CGTATAAATAATATAAGATTTTGACTTTTACCACCTTCTTTAATGCTTTTAA TTTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATAT

CCCCCTCTTTCATCAAATATTGCTCATGGAGGATCTTCAGTAGATTTAGCA ATTTTTTCATTACATTTAGCGGGAATTTCTTCAATTTTAGGAGCTATTAATT TTATTTCAACAATTATTAACATACGAGTTAATAATATATCTTATGATCAAA TACCTCTTTTCGTATGAGCTGTTGGAATTACAGCTTTATTACTTTTACTATC TTTACCTGTTTTAGCTGGAGCTATTACTATATTATTAACAGATCGAAATTT AAATACTTCTTTTTTTGATCCAGCCGGAGGAGGAGACCCTATTCTTTATCA ACATTTATTTTGATTTTT

Seq 18 [organism=Mycalesis perseoides] [isolate=MpersdCOIS042]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAACATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC СТССТСТСТСАТСAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCCTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

Seq19 [organism=Mycalesis janardana] [isolate=MjanCOIS051]
ATAGTAGGAACTTCTTTAAGTTTAATTATTCGAACAGAATTAGGAAATCCT GGATTTTTAATTGGTGATGATCAAATTTATAATACTATTGTAACCGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAATTCCTTTAATATTAGGAGCTCCTGACATAGCATTTCCAC GAATAAATAATATAAGATTTTGACTATTACCCCCCTCATTAGTACTTTTAA TTTCAAGAAGTATCGTAGAAAATGGAGCCGGTACAGGATGAACAGTTTAT СССССТСТTTCATCTAATATTGCTCATGGAGGCTCCTCTGTTGATTTAGCA ATTTTCTCTTTACATTTAGCCGGTATTTCATCAATTTTAGGGGCTATTAATT TTATTTCCACAATTATTAATATACGAATTAATAGTATATCCTATGATCAAA TACCTCTTTTTGTTTGAGCTGTGGGAATCACAGCTTTATTACTTTTATTATC ATTGCCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTT AAATACCTCTTTTTTTGATCCTGCTGGAGGAGGAGACCCTATTTTATACCA ACATTTATTTTGATTTTT

Seq20 [organism=Mycalesis intermedia] [isolate=MintCOIS051]
ATAGTAGGTACTTCATTAAGTCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTACAATACTATTGTAACAGCCCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCACTAATACTAGGAGCTCCTGATATAGCTTTTCCCC GTATAAATAATATAAGATTTTGACTTTTACCTCCTTCCTTAATACTTTTAAT


#### Abstract

TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTTTATC CCCCTCTTTCATCAAATATTGCTCATGGAGGATCTTCAGTAGATTTAGCAA TTTTTTCTCTGCATTTAGCGGGAATTTCTTCAATTTTAGGAGCTATTAATTT TATTTCAACAATTATTAATATACGAATTAACAATATAACTTACGATCAAAT ACCTCTTTTCGTATGAGCTGTTGGAATTACAGCTCTATTACTTTTATTATCT TTACCTGTTTTAGCCGGAGCTATTACTATGTTATTAACAGATCGAAATTTA AATACTTCTTTTTTTGACCCAGCTGGAGGAGGAGATCCTATTCTTTATCAA CATCTATTTTGATTTTT


Seq21 [organism=Mycalesis visala] [isolate=MvisCOIS051]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAACATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC CTCСТСТСТСATCAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCCTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

Seq 22 [organism=Mycalesis orseis] [isolate=MorsCOIS051]
ATAAAAAGTACTTCTTTGGGGNAAATTTTTCGAACAGAATTAGGAAAACC AGGATTTTTAATTGGAGATGATCAAATTTATAATACTATTGTTACAGCTCA TGCTTTTATCATAATTTTTTTTATGGTTATACCTATTATAATTGGAGGATTT GGAAATTGATTAGTGCCTCTAATATTAGGAGCTCCAGATATAGCCTTCCCC CGAATAAATAATATAAGTTTTTGACTTTTACCCCCCTCATTAATTTTATTAA TTTCAAGTTCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTGTAT CCCCCCCTTTCATCTAATATTGCTCACAGAGGATCCTCTGTTGACTTAGCA ATTTTTTCTCTTCATTTAGCTGGAATTTCCTCAATTTTAGGAGCTATTAATT TTATTTCCACAATTATTAATATACGAATTAATAATATATCATATGATCAAA TACCTCTTTTTGTATGAGCAGTAGGTATTACAGCTTTATTACTTTTATTATC TCTACCTGTACTAGCAGGAGCTATTACTATACTTCTTACAGATCGAAATTT AAATACCTCTTTTTTTGACCCTGCAGGAGGAGGAGATCCAATTCTTTATCA ACATTTATTTTGATTTTT

Seq 23 [organism=Mycalesis intermedia] [isolate=MintCOIS052]
ATAGTAGGTACTTCATTAAGTCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGACGATCAAATTTACAATACTATTGTAACAGCCCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCACTAATACTAGGAGCTCCTGATATAGCTTTTCCCC

GTATAAATAATATAAGATTTTGACTTTTACCTCCTTCCTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTTTATC CСССТСТTTCATCAAATATTGCTCATGGAGGATCTTCAGTAGATTTAGCAA TTTTTTCTCTGCATTTAGCGGGAATTTCTTCAATTTTAGGAGCTATTAATTT TATTTCAACAATTATTAATATACGAATTAACAATATAACTTACGATCAAAT ACCTCTTTTCGTATGAGCTGTTGGAATTACAGCTCTATTACTTTTATTATCT TTACCTGTTTTAGCCGGAGCTATTACTATGTTATTAACAGATCGAAATTTA AATACTTCTTTTTTTGACCCAGCTGGAGGAGGAGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

Seq24 [organism=Mycalesis intermedia] [isolate=MintCOIS053]
ATAGTAGGTACTTCATTAAGTCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGACGATCAAATTTACAATACTATTGTAACAGCCCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCACTAATACTAGGAGCTCCTGATATAGCTTTTCCCC GTATAAATAATATAAGATTTTGACTTTTACCTCCTTCCTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTTTATC CСССТСТТTСATCAAATATTGCTCATGGAGGATCTTCAGTAGATTTAGCAA TTTTTTCTCTGCATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATTAATTT TATTTCAACAATTATTAATATACGAATTAACAATATAACTTACGATCAAAT ACCTCTTTTCGTATGAGCTGTTGGAATTACAGCTCTATTACTTTTATTATCT TTACCTGTTTTAGCCGGAGCTATTACTATGTTATTAACAGATCGAAATTTA AATACTTCTTTTTTTGACCCAGCTGGAGGAGGAGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

Seq 25 [organism=Mycalesis perseoides] [isolate=MpersdCOIS061]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAACATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC СТССТСТСТСАТСAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCCTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

Seq 26 [organism=Mycalesis intermedia] [isolate=MintCOIS061]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG

GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAACATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC СТССТСТСТСATCAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCCTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

Seq27 [organism=Mycalesis intermedia] [isolate=MintCOIS062]
ACAGAAGGTACTTCATTAGGTCTTATTATTCGAACAGAATTAGGAAATCC AGGATTTTTAATTGGAGACGATCAAATTTACAATACTATTGTAACACCCCA TGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTT GGAAATTGATTAGTACCACTAATACTAGGAGCTCCTGATATAGCTTTTCCC CGTATAAATAATATAAGATTTTGACTTTTACCTCCTTCCTTAATACTTTTAA TTTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTTTAT CССССТСТTTCATCAAATATTGCTCATGGAGGATCTTCAGTAGATTTAGCA ATTTTTTCTCTGCATTTAGCGGGAATTTCTTCAATTTTAGGAGCTATTAATT TTATTTCAACAATTATTAATATACGAATTAACAATATAACTTACGATCAAA TACCTCTTTTCGTATGAGCTGTTGGAATTACAGCTCTATTACTTTTATTATC TTTACCTGTTTTAGCCGGAGCTATTACTATGTTATTAACAGATCGAAATTT AAATACTTCTTTTTTTGACCCAGCTGGAGGAGGAGATCCTATTCTTTATCA ACATCTATTTTGATTTTT

Seq28 [organism=Mycalesis intermedia] [isolate=MintCOIS063]
ATAGTAGGTACCTCTTTANGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAACATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC СТССТСТСТСАТСАAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCCTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

Seq29 [organism=Mycalesis perseoides] [isolate=MpersdCOIS062]
ATAGTAGGTACCTCTTTAAGCCTTATTATTCGAACAGAATTAGGAAATCCA GGATTTTTAATTGGAGATGATCAAATTTATAATACTATCGTAACAGCTCAT

GCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTG GAAATTGATTAGTACCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTC GTATAAATAACATAAGATTTTGACTCCTACCCCCTTCTTTAATACTTTTAAT TTCAAGAAGTATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTATACC СТССТСТСТСАТСAAATATTGCTCATAGAGGATCTTCAGTAGATTTAGCAA TTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCTATCAATTT TATTTCAACAATTATTAATATACGAATTAATAATATAACTTATGATCAAAT ACCTCTTTTTGTATGAGCTGTTGGAATTACAGCCTTATTACTTTTATTATCC TTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTA AATACTTCCTTTTTTGATCCAGCTGGAGGAGGTGATCCTATTCTTTATCAA CATCTATTTTGATTTTT

## EF-1 $\alpha$ gene

Seq1 [organism=Mycalesis perseoides] [isolate=MpersdEF-1alphaS011]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTTACCATCATCGACGCTCCTGGACACAGAGATTTCATCAAAAACATG ATCACCGGAACATCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq2 [organism=Mycalesis perseoides] [isolate=MpersdEF-1alphaS012]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTTACCATCATCGACGCTCCTGGACACAGAGATTTCATCAAAAACATG ATCACCGGAACATCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG
seq3 [organism=Mycalesis perseoides] [isolate=MpersdEF-1alphaS013]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTTACCATCATCGACGCTCCTGGACACAGAGATTTCATTAAAAACATG

ATCACCGGAACATCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG
seq4 [organism=Mycalesis perseoides] [isolate=MpersdEF-1alphaS014]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTTACCATCATCGACGCTCCTGGACACAGAGATTTCATCAAAAACATG ATCACCGGAACATCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG
seq5 [organism=Mycalesis mineus] [isolate=MminEF-1alphaS021]
ACGTACCATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGCAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTCTGGAAGTTCGAGACGGCCAAATACT ACGTCACCATCATCGACGCTCCCGGACACAGAGATTTCATCAAGAACATG ATCACCGGAACCTCGCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq6 [organism=Mycalesis mineus] [isolate=MminEF-1alphaS022]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGCAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTCACCATCATCGACGCTCCCGGACACAGAGATTTCATCAAGAACATG ATCACTGGAACCTCGCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTTGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq7 [organism=Mycalesis mineus] [isolate=MminEF-1alphaS031]

ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGCAAAGGT
TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTCACCATCATCGACGCTCCCGGACACAGAGATTTCATCAAGAACATG ATCACCGGAACCTCGCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACTGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq8 [organism=Mycalesis mineus] [isolate=MminEF-1alphaS032]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGCAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTCACCATCATCGACGCTCCCGGACACAGAGATTTCATCAAGAACATG ATCACCGGAACCTCGCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq9 [organism=Mycalesis perseoides] [isolate=MpersdEF-1alphaS031]
GCGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTTACCATCATCGACGCTCCTGGACACAGAGATTTCATCAAAAACATG ATCACCGGAACATCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq10 [organism=Mycalesis perseoides] [isolate=MpersdEF-1alphaS032]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTTACCATCATCGACGCTCCTGGACACAGAGATTTCATCAAAAACATG ATCACCGGAACATCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA

AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq11 [organism=Mycalesis intermedia] [isolate=MintEF-1alphaS031]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTTACCATCATCGACGCTCCTGGACACAGAGATTTCATCAAAAACATG ATCACCGGAACATCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq12 [organism=Mycalesis intermedia] [isolate=MintEF-1alphaS041]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTTACCATCATCGACGCTCCTGGACACAGAGATTTCATCAAAAACATG ATCACCGGAACATCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq13 [organism=Mycalesis perseoides] [isolate=MpersdEF-1alphaS041]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGAATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTTACCATCATCGACGCTCCTGGACACAGAGATTTCATCAAAAACAGA ATCACCGAAACATCTCAGGCCAATTGTGCGGTGTTGATCGTGGCCGCCGG ACCGGGTAAGTTCAAGGCCGGTATCTCCAAGAACGCCCGGACCCGGGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCNATACAGTGAGTCCCGTTTCGAGGA ATCNNAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTNACAACCCAG CTGCCGTCGCTTTCGTACCCATATCTGGCTGGCACG

Seq14 [organism=Mycalesis visala] [isolate=MvisEF-1alphaS041]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGCAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTCACCATCATCGACGCTCCCGGACACAGAGATTTCATCAAAAACATG

ATCACCGGAACCTCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCTACTGAGCCCCCATACAGTGAGTCACGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAT CTGCTGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq15 [organism=Mycalesis intermedia] [isolate=MintEF-1alphaS042]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTTACCATCATCGACGCTCCTGGACACAGAGATTTCATCAAAAACATG ATCACCGGAACATCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq16 [organism=Mycalesis perseus] [isolate=MpersEF-1alphaS041]
ACGTACTATCGAGAAGGNCGAGAAGGAGGTTCAAGAAATGCGCAAAGGT TCCTTCAAATACATCTGGATGTTGGANAAACGAAAGGCTGAGCGCGAGCT CTCTATCACCATCGACCTTGTTCTGTCCAAGTTCGAGACTGCTAAATTGTA CGTCACCATCATCGACGCTCCCGGACACAGAGATTTCATCAAGAACATGA TCACTGGAACCTCGCAGGCCGATTGTGCCGTGCTGATCGTTGCCGCCGGT ACCGGTGAGTTCGAGGCAGGTATCTCCAAGAACGGCCAGACTCGTGAGCA CGCGCTACTCGCCTTCACCCTCGGTGTCAAGCAGCTGATCGTGGGCGTCA ACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGAA ATCAAAAAGGAAGTGTCCTCATACATCAAGAAGATTGGTTACAACCCAGC TGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq17 [organism=Mycalesis perseus] [isolate=MpersEF-1alphaS042]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCTCAGGAAATGGGCAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACTGCCAAATACT ACGTCACCATCATCGACGCTCCCGGACACAGAGATTTCATCAAGAACATG ATCACTGGAACCTCGCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCAGGTATCTCCAAGAACGGCCAGACTCGTGAGC ACGCGCTACTCGCCTTCACCCTCGGTGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCATACATCAAGAAGNTTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq18 [organism=Mycalesis perseoides] [isolate=MpersdEF-1alphaS042]

ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT
TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTTACCATCATCGACGCTCCTGGACACAGAGATTTCATCAAAAACATG ATCACCGGAACATCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGANGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq19 [organism=Mycalesis janardana] [isolate=MjanEF-1 alphaS051]
ACGTTCTATTGAGAAGTTCGAGAAGGAGTTCCAGGAAATGGGCAAAGGCT CCTTCAAATATGCCTGGGTATTGGACAAACTAAAGGCTGAGCGCGAGCGT GGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACTGCCAAATACTA CGTCACCATCATCGACGCTCCTGGGCACAGAGATTTCATCAAGAACATGA TCACCGGAACCTCACAGGCCGATTGCGCCGTGCTGATCGTCGCCGCCGGT ACTGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAAACCCGTGAGCA CGCTCTGCTCGCCTTCACCCTCGGTGTCAAACAGCTGATCGTGGGCGTTAA CAAAATGGACTCCACTGAGCCCCCATACAGCGAGTCTCGTTTCGAGGAAA TCAAGAAGGAAGTGTCCTCCTACATCAAGAAAATCGGTTACAACCCAGCT GCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq20 [organism=Mycalesis intermedia] [isolate=MintEF-1alphaS051]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGCAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTCACCATCATCGACGCTCCCGGACACAGAGATTTCATCAAGAACATG ATCACCGGAACCTCGCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCTACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq21 [organism=Mycalesis visala] [isolate=MvisEF-1alphaS051]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTCACCATCATCGACGCTCCCGGACACAGAGATTTCATCAAAAACATG ATCACCGGAACCTCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCTACTGAGCCCCCATACAGTGAGTCACGTTTCGAGGA

AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAT CTGCTGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq22 [organism=Mycalesis orseis] [isolate=MorsEF-1alphaS051]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGCAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTGAAGGCTGAGCGCGAGCG TGGTATCACCATCGATATCGCTCTGTGGAAGTTCGAGACTGCCAAATACT ACGTCACCATCATCGACGCTCCCGGACACAGAGATTTCATCAAGAACATG ATCACTGGAACCTCACAGGCCGATTGCGCCGTGCTGATCGTCGCCGCAGG TACCGGCGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGTTGTTGGCCTTCACCCTCGGTGTCAAGCAGCTGATCGTGGGCGTCA ACAAAATGGACTCCACTGAGCCCCCGTACAGTGAGCCTCGTTTCGAGGAA ATCAAGAAGGAAGTGTCCTCCTACATCAAGAAGATTGGTTACAACCCAGC TGCTGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq 23 [organism=Mycalesis intermedia] [isolate=MintEF-1alphaS052]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGCAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTCACCATCATCGACGCTCCCGGACACAGAGATTTCATCAAGAACATG ATCACCGGAACCTCGCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq24 [organism=Mycalesis intermedia] [isolate=MintEF-1alphaS053]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGCAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTCACCATCATCGACGCTCCCGGACACAGAGATTTCATCAAGAACATG ATCACCGGAACCTCGCAGGCAGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCTACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq25 [organism=Mycalesis perseoides] [isolate=MpersdEF-1alphaS061]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTTACCATCATCGACGCTCCTGGACACAGAGATTTCATCAAAAACATG

ATCACCGGAACATCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACGGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCTTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq26 [organism=Mycalesis intermedia] [isolate=MintEF-1alphaS061]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTTACCATCATCGACGCTCCTGGACACAGAGATTTCATCAAAAACATG ATCACCGGAACATCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCTTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq27 [organism=Mycalesis intermedia] [isolate=MintEF-1alphaS062]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGCAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTCACCATCATCGACGCTCCCGGACACAGAGATTTCATCAAGAACATG ATCACCGGAACCTCGCAGGCAGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq 28 [organism=Mycalesis intermedia] [isolate=MintEF-1alphaS063]
ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTTACCATCATCGACGCTCCTGGACACAGAGATTTCATCAAAAACATG ATCACCGGAACATCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

Seq29 [organism=Mycalesis perseoides] [isolate=MpersdEF-1alphaS062]

ACGTACTATCGAGAAGTTCGAGAAGGAGGCCCAGGAAATGGGAAAAGGT
TCCTTCAAATACGCCTGGGTGTTGGACAAACTAAAGGCTGAGCGCGAGCG TGGTATCACCATCGACATCGCTCTGTGGAAGTTCGAGACGGCCAAATACT ACGTTACCATCATCGACGCTCCTGGACACAGAGATTTCATCAAAAACATG ATCACCGGAACATCTCAGGCCGATTGTGCCGTGCTGATCGTCGCCGCCGG TACCGGTGAGTTCGAGGCCGGTATCTCCAAGAACGGCCAGACCCGTGAGC ACGCGCTGCTCGCCTTCACCCTCGGCGTCAAGCAGCTGATCGTGGGCGTC AACAAAATGGACTCCACTGAGCCCCCATACAGTGAGTCCCGTTTCGAGGA AATCAAAAAGGAAGTGTCCTCGTACATCAAGAAGATTGGTTACAACCCAG CTGCCGTCGCTTTCGTACCCATTTCTGGCTGGCACG

## Wingless gene

Seq1 [organism=Mycalesis perseoides] [isolate=MpersdwinglessS011]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TTGACGGGGCGTCGCGGGTCATGATGCCCAACACCGAGGTGGAGGCGCCG TCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGGT ACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGTC AAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACCC CAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACCAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq2 [organism=Mycalesis perseoides] [isolate=MpersdwinglessS012]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGATGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACCAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC
seq3 [organism=Mycalesis perseoides] [isolate=MpersdwinglessS013]
GATGAGGCTGCCGACTTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCCCGGGTCATGATGCCCAACACCGAGGTGGAGGCGCCG TCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGGT ACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGTC AAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACCC CAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACTAGCA TCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGACC GAGACCATGTTCGTGGTGGAGCGCTGCAAC
seq4 [organism=Mycalesis perseoides] [isolate=MpersdwinglessS014]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGATGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACTAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC
seq5 [organism=Mycalesis mineus] [isolate=MminwinglessS021]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGATGCCCAATACCGAGGTGGAGGCGCCG TCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGATCGGTA CAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGTCA AGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACCCC AGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACGAGCA TCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGACC GAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq6 [organism=Mycalesis mineus] [isolate=MminwinglessS022]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGATGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCATAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACGAG CATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGA CCGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq7 [organism=Mycalesis mineus] [isolate=MminwinglessS031]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGAGCGTCGCGGGTCATGATGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACGAG CATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGA CCGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq8 [organism=Mycalesis mineus] [isolate=MminwinglessS032]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGATGCCCAATACCGAGGTGGAGGCGCCG

TCACAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGGT ACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGTC AAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACCC CAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACGAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq9 [organism=Mycalesis perseoides] [isolate=MpersdwinglessS031]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGAAGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACCAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq10 [organism=Mycalesis perseoides] [isolate=MpersdwinglessS032]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TTGACGGGGCGTCGCGGGTCATGATGCCCAACACCGAGGTGGAGGCGCCG TCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGGT ACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGTC AAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACCC CAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACCAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq11 [organism=Mycalesis intermedia] [isolate=MintwinglessS031]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGAAGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACCAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq12 [organism=Mycalesis intermedia] [isolate=MintwinglessS041]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGAAGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACTAGC

ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq13 [organism=Mycalesis perseoides] [isolate=MpersdwinglessS041]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGAAGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACTAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq14 [organism=Mycalesis visala] [isolate=MviswinglessS041]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGAAGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACTAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq15 [organism=Mycalesis intermedia] [isolate=MintwinglessS042]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGAAGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACCAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq16 [organism=Mycalesis perseus] [isolate=MperswinglessS041]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGATAGCT TCGACGGGGCATCGCGGGTCATGATGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCACGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAACCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCATCGCCGGGTTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACGAG CATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGA CCGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq17 [organism=Mycalesis perseus] [isolate=MperswinglessS042]

GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGATAGCT TCGACGGGGCGTCGCGGGTCATGATGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAACCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCACCGGGTTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACGAG CATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGA CCGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq18 [organism=Mycalesis perseoides] [isolate=MpersdwinglessS042]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TTGACGGGGCGTCGCGGGTCATGAAGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACTAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq19 [organism=Mycalesis janardana] [isolate=MjanwinglessS051]
GATGAGGTTGCCGACGTTCCGATCTGTAGGCGACGCCCTCAAAGACAGCT TCGACGGGGCGTCGCGGGTCATGATGCCCAACACGGAGGTAGAGGCGCC GTCCCAGCGAAACGACGCCGCGCCCCACCGGGTGCCGCGACGGGACCGG TACAGATTCCAACTTCGGCCGCACAACCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTATACTTGGAATCGTCGCCGGGCTTCTGCGAGAAGAACC CCAGGCTGGGCATTCCCGGTACGCACGGGCGTGCCTGCAACGACACGAGC ATCGGCGTCGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACAGGAC TGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq20 [organism=Mycalesis intermedia] [isolate=MintwinglessS051]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGAAGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAATGACACGAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq 21 [organism=Mycalesis visala] [isolate=MviswinglessS051]
GATGAGGCTGCCGACGTTCCGATCTGTATGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGAAGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT

CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACTAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq22 [organism=Mycalesis orseis] [isolate=MorswinglessS051]
GATGAGGCTGCCGACGTTCAGATCTGTAGGCGATGCCCTAAAAGACAGCT TCGACGGGGCGTCGCGGGTCATGATGCCCAACACCGAGGTAGAAGCGCC GTCTCAACGGAACGACGTTGCACCTCATAGGGTCCCGCGACGAGACCGGT ACAGATTTCAACTTCGGCCGCACAACCCTGACCACAAAACACCCGGGGTC AAGGACCTAGTATACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACCC CAGGCTGGGCATTCCGGGTACGCACGGGCGTGCCTGCAACGACACGAGTA TCGGCGTCGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGACC GAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq23 [organism=Mycalesis intermedia] [isolate=MintwinglessS052]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGAAGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACGAG CATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGA CCGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq24 [organism=Mycalesis intermedia] [isolate=MintwinglessS053]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGATGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCATGGGCGCGCCTGCAACGACACGAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq25 [organism=Mycalesis perseoides] [isolate=MpersdwinglessS061]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGAAGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGACTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACCAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq26 [organism=Mycalesis intermedia] [isolate=MintwinglessS061]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGAAGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGTCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACCAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq27 [organism=Mycalesis intermedia] [isolate=MintwinglessS062]
GATGAGGCTGCCGACGTTCCGATCTGTTTGCGACGCCCTGAATGACAGCT TCGACGGGGCGTCGCGGGTCATGAAGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGGCTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACGAG CATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGA CCGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq28 [organism=Mycalesis intermedia] [isolate=MintwinglessS063]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGATGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGACTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACCAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

Seq29 [organism=Mycalesis perseoides] [isolate=MpersdwinglessS062]
GATGAGGCTGCCGACGTTCCGATCTGTAGGCGACGCCCTGAAGGACAGCT TCGACGGGGCGTCGCGGGTCATGAAGCCCAACACCGAGGTGGAGGCGCC GTCGCAGCGGAACGACGCCGCACCTCACCGGGTGCCGCGACGAGACCGG TACAGGTTTCAACTTCGGCCGCACAATCCTGACCACAAAACACCCGGGGT CAAGGACCTAGTGTACTTGGAATCGTCGCCGGGCTTCTGCGAAAAGAACC CCAGACTGGGCATTCCGGGCACGCACGGGCGCGCCTGCAACGACACCAGC ATCGGCGTGGACGGCTGCGACCTCATGTGCTGCGGCCGCGGGTACCGGAC CGAGACCATGTTCGTGGTGGAGCGCTGCAAC

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