Delimitation and identification of crescent butterflies (Nymphalidae: *Phyciodes*) in Alberta using molecular and morphological techniques

by

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Abstract

Species delimitation can be challenging, especially in taxonomic groups that exhibit little morphological divergence. Many techniques and concepts have been developed for detecting species boundaries, and molecular methods are becoming increasingly common. Next generation sequencing techniques make it feasible to obtain hundreds to thousands of genome-wide markers, even for non-model organisms. This can provide powerful insights into species boundaries and the integrity of those boundaries in the presence of gene flow. However, classical taxonomic information, such as morphology, is often excluded from molecular studies, creating a disconnect between delimitation and identification. Integrative and iterative approaches to taxonomy that include morphological data maintain a link between delimitation and identification while providing a more complete understanding of the organisms being studied.

The *Phyciodes tharos* species group of nymphalid butterflies is currently thought to comprise four species. However, interspecific overlap and intraspecific variability of the wing patterns have resulted in a complicated taxonomic history with uncertainty regarding the level of divergence between species. Discordance of mitochondrial COI with traditional taxonomic identifications has added to this uncertainty but has been attributed to incomplete lineage sorting and contemporary introgression. In this thesis, I used an iterative approach to examine the species limits of this group using genome-wide single nucleotide polymorphisms (SNPs) and the barcoding region of the mitochondrial COI gene. I then quantitatively examined the utility of eighteen morphological characters for identification based on the genomic species lineages. I focused on Alberta, the only region where all four species occur, and no other species of the genus are present.

Genomic SNPs resolved all four species boundaries with strong support for *P. tharos* (Drury, 1773), *P. cocyta* (Cramer, 1777), and *P. pulchella* (Boisduval, 1852). *Phyciodes batesii* (Reakirt, 1865) did not form a monophyletic clade but did form a distinct cluster in all genomic analyses. Evidence of occasional hybridization and low levels of introgression indicate that these lineages maintain their genomic integrity when in contact. The COI haplotypes were discordant with genomic SNPs but provided evidence of unidirectional mitochondrial gene flow likely due to brood timing and opportunistic mating between species. Morphological characters exhibited extensive intraspecific variation and broad interspecific overlap. None of the character states were strictly diagnostic, but the proportions of character states exhibited for each species are provided as an identification resource.

"In naming the species after Cocytus, the River of Lamentation in the Underworld, Cramer seems to anticipate the woe in store for future students of crescentspot taxonomy."

-Robert Michael Pyle, The Butterflies of Cascadia

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List of abbreviations

ba – Phyciodes batesii

bp – base pair(s)

Char. – character

co – Phyciodes cocyta

COI – cytochrome c oxidase I gene

Cont. – contribution

Coord. – coordinate

ddRAD/ddRADseq – double-digest restriction site-associated DNA sequencing

DFW – dorsal forewing

DHW – dorsal hindwing

DiCA – discriminant correspondence analysis

DNA – deoxyribonucleic acid

DSLR – digital single-lens reflex camera

FW – forewing

MBSU – Molecular Biology Service Unit

MCA – multiple correspondence analysis

MCMC – Markov chain Monte Carlo

ML – maximum likelihood

mtDNA – mitochondrial DNA

NGS – next generation sequencing

PCA – principal component analysis

pu – Phyciodes pulchella

SNP – single nucleotide polymorphism

th – Phyciodes tharos

UFBoot – Ultrafast bootstrapping

VFW – ventral forewing

VHW – ventral hindwing

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Chapter 1

General introduction

1.1 Species delimitation: Methods and concepts

Recognizing species boundaries is foundational to biology and is particularly important for monitoring regional biodiversity and informing conservation (Bickford et al. 2007; Proshek et al. 2015; Stanton et al. 2019; Smith et al. 2022). The challenges associated with delimiting species have led to the continuous development of new techniques, and with them, new ways to conceptualize species (Luo et al. 2018). Several species concepts and definitions have been proposed over the years and disagreement about which of these is most appropriate is called the "species problem" (de Queiroz 2005). This is overcome, however, by the general lineage species concept which unifies all species concepts based on the overall agreement that species are evolutionary lineages (de Queiroz 1998, 2007). Thus, species concepts can be used as criteria for delimiting species under this unified concept and certain criteria are applicable to certain groups based on the processes underlying their divergence and the resulting patterns.

Traditional taxonomic classification, which remains an important component of modern systematics, relies primarily on morphological differences between species (Balakrishnan 2005; Ahrens et al. 2021). Designated type specimens and the characters they exhibit are used as references for determining identifications (phenetic and typological species concepts; Ruse 1969; Sneath 1976; Mayr 1996). This is practical since species are primarily perceived and identified based on morphology by both citizens and scientists (Lee 2004; Balakrishnan 2005; Zamani et al. 2021). However, hybrid phenotypes and intraspecific morphological variation that is not captured in species descriptions can preclude the identification of some individuals and can cast doubt on species definitions (Neff & Smith 1979; Stech et al. 2013; Dwyer et al. 2015; Dupuis et al. 2017b; Fioravanti et al. 2022).

Other types of data are also routinely used for assessing species boundaries and can help separate morphologically similar or identical species. For instance, ecological

differences can indicate divergent lineages that are adapted to different niches (ecological species concept, Van Valen 1976); failed courtship and hybrid breakdown can each indicate reproductive incompatibility (isolation, recognition, and biological species concepts; Paterson 1985; Masters et al. 1987; Mayr 1942); and monophyletic groups can indicate shared ancestry (phylogenetic species concept, Cracraft 1983).

The last couple of decades have seen an increased focus on using molecular data for species delimitation (Wiens 2007). Until recently, most studies have been limited to one or a few markers due to high sequencing costs and the computational power required to process multilocus data (Fontaneto et al. 2015; Fujisawa et al. 2016; Roe et al. 2017). The proposal of standardized single-marker 'barcodes' as a rapid and relatively inexpensive means to delimit and identify species (consistent with the typological species concept) led to the proliferation of DNA taxonomy (Caterino et al. 2000; Hebert et al. 2003; Lee 2004). The potential for large scale species discovery without the need for time-consuming morphological study promised to accelerate the global cataloguing of biodiversity (Wiens 2007; Zamani et al. 2021). However, individual markers are often discordant with one another and typically have a low success rate for delimiting shallow species relationships (Dupuis et al. 2012; Meier et al. 2022). Higher delimitation success using single markers is generally due to limited sampling (Sperling & Roe 2009; Zhang et al. 2010).

Next Generation Sequencing (NGS) methods, such as double-digest restriction site-associated DNA sequencing (ddRADseq, Peterson et al. 2012), have made it feasible to obtain hundreds to thousands of genome-wide loci, without the need for a reference genome (Metzker 2010; Hohenlohe et al. 2013; Andrews et al. 2016; Roe et al. 2017). Single nucleotide polymorphisms (SNPs) from these loci can provide insight into genomic patterns of divergence and gene flow between species (e.g. Rittmeyer & Austin 2014; Campbell et al. 2017). The genomic integrity species definition recognizes species as populations that remain genomically distinct upon contact (Sperling 2003). Conceptually, this definition is useful for genomic assessments of species in that it explicitly allows for occasional hybridization and low levels of gene flow between species.

Ideally, molecular data, including genome-wide markers, should be considered in conjunction with other types of data. Integrative (Dayrat 2005; Will et al. 2005) or iterative (Yeates et al. 2011) approaches, which utilize multiple types of data, have aided confirmation and discovery of the limits between many species (Burns et al. 2008; Padial & De La Riva 2009; Lumley & Sperling 2010; Bourguignon et al. 2013; Sistrom et al. 2013; Gratton et al. 2016; Bakkes et al. 2020; Reis et al. 2020; Smith et al. 2022). The signals provided by genome-wide loci and other forms of data (including, but not limited to, morphological characters, reproductive isolation, gene sequences, and ecological information) may not be congruent, but they each contribute to a more complete understanding of the focal taxa. Considering morphological information in the context of molecular information also maintains a link between species delimitation and identification (Balakrishnan 2005; Ahrens et al. 2021). Morphological characters can vary according to sex, season, and locality, and an integrative molecular-morphological approach may confirm patterns of intraspecific variation or help improve diagnostic characters.

1.2 Overview of the *Phyciodes tharos* species group

The *Phyciodes tharos* species group of nymphalid butterflies provides an excellent model system for exploring species boundaries in an iterative framework that uses genomic and morphological information. Our current understanding of the group has developed iteratively based on decades of observation and research on morphological characters, voltinism, natural history, chemical analysis, hybridization, and mitochondrial DNA. Currently, this Nearctic group is thought to comprise four species: *P. tharos* (Drury, 1773), *P. cocyta* (Cramer, 1777), *P. batesii* (Reakirt, 1865), and *P. pulchella* (Boisduval, 1852) (Scott 1994; Wahlberg et al. 2003a). However, the wing patterns – which are the primary basis for species-level identification – can appear similar between species and demonstrate intraspecific variation based on sex, region, and in some cases time of year (Scott 1994, 1998, 2006). Other morphological characters, including antennal club colour and genitalic characters, can be helpful for identification but are also variable and require closer examination than is possible in the field. This makes some

individuals difficult or impossible to identify in areas of sympatry, and has led to uncertainty about the species boundaries, particularly among *P. tharos*, *P. cocyta*, and *P. batesii*.

Phyciodes batesii was initially differentiated based on the morphology of exemplar adult specimens by Reakirt (1865) (as cited in Scott 1994). Some authors previously considered it a seasonal variety of *P. tharos*, but chemical analysis of fluorescent pigments by Rawson (1968) along with ecological differences (Layberry et al. 1998) and larval web spinning behaviour (McDunnough 1920; Bird et al. 1995) support its status as a distinct species. Recognition of *P. tharos* and *P. cocyta* as separate species is evident in the literature at least since Wright (1905) who included descriptions of "*Phyciodes Tharos"* and "*Phyciodes Pascoensis*", the latter of which is now considered a synonym of *P. cocyta*. Despite early recognition of each of these taxa as full species, they have since been treated by many authors as subspecies (e.g., dos Passos 1969; Tilden 1970; Oliver 1972, 1979a; Hooper 1973; Bauer 1975; Ferris & Brown 1981; Garth & Tilden 1986; Scott 1986b, 1992; Guppy & Shepard 2001; Pyle 2002), and our modern understanding of them is quite recent. Hooper (1973) was one of the first authors to recognize them as ecologically distinct and referred to them as the Prairie Pearl Crescent and the Woodland Pearl Crescent, each a subspecies of *P. tharos*. Bauer (1975) treated *P. tharos* as having four subspecies, two of which (*P. t. pascoensis* & *P. t. arctica*) represented what we now call *P. cocyta*, and their geographic range descriptions closely resemble the species range we recognize today (Figure 2.1).

Uncertainty about the boundaries between *Phyciodes* species led to a series of hybridization studies by Oliver (1972, 1978, 1979a, 1980). Two of these were particularly important for the *P. tharos* species group. Oliver (1979a) examined the compatibility of *P. tharos* from Pennsylvania and New York with *P. batesii* from New York. He found reduced egg, embryonic, pupal, and female viability along with developmental abnormalities in some crosses. All backcrosses except for one were especially affected at the embryonic stage. Though not explored in this study, Oliver (1979a) presented *P. tharos* in the northeastern United States as having two "types" – a southern Type A and a northern Type B based on geographic, ecological, and phenotypic

differences. Oliver (1980) explored the compatibility of these types; in crosses of Type A (from Pennsylvania) and Type B (from Pennsylvania, Vermont, or New York), the most consistent symptom of hybrid breakdown was a shift in development time of hybrid individuals. Specifically, females had either a reduced or dramatically lengthened development time, depending on the cross. Additionally, egg and embryonic viability were reduced, and sex ratios were skewed in a few crosses and backcrosses (however, this was also true for some intraspecific population-level hybrids). The conclusion was that the two types represent separate species, and that Type B was an eastern variant of *P. cocyta* (then called *P. t. pascoensis*). These findings led Opler & Krizek (1984) to designate *P. pascoensis* Wright, 1905 as a full species.

In his study on *Phyciodes*, Scott (1994) described every species and subspecies in detail and formalized the *P. tharos*, *P. mylitta*, and *P. phaon* species groups. Despite his doubts about the reproductive boundaries found between *P. tharos* Types A and B by Oliver (1980) and his own finding that these taxa act as subspecies in Colorado (Scott 1986b; *P. cocyta* as *P. t. morpheus*), he treated them as full species, resurrected the name *P. cocyta* (Cramer, 1777), and treated *P. pascoensis* Wright, 1905 as its synonym. He suggested that *P. tharos* is the most primitive species in the *P. tharos* species group based on similarities to *P. mylitta* in genitalic characters, wingspan, and pupal cone size, and that the group forms a morphological transformation series in the order: *P. tharos*, *P. cocyta*, *P. batesii*, and *P. pulchella*. The taxonomy of the *P. tharos* species group has since remained relatively stable other than subspecific changes (Scott 1998, 2006). However, the designation of the name *P. cocyta* (Cramer, 1777) is disputed by Glassberg (2022) based on its type locality of Suriname, where what we call *P. cocyta* does not occur. Instead, Glassberg uses the name *P. selenis* (Kirby, 1837). One proposed taxon – '*diminutor*' – has been treated as either a subspecies of *P. cocyta*, or as a full species (Scott 1998, 2006, 2008, 2014). It occurs in southeastern Canada and the northeastern United States and so is not included in this study. A detailed synonymic list of all *Phyciodes* species and subspecies is provided by Scott (2006).

So far, two studies have explored species boundaries in the *P. tharos* species group using the mitochondrial cytochrome c oxidase I gene (COI) in a phylogenetic

framework. Wahlberg et al. (2003a) assessed the group across North America and found unclear boundaries between all species except for *P. tharos* and *P. pulchella*. The main clades corresponded with *P. tharos*, *P. cocyta*, and *P. pulchella.* Individuals of *P. batesii* grouped with each of these species, but most were interdigitated with *P. cocyta*. Proshek & Houghton (2012) also found that *P. batesii* from Michigan and Ohio appeared in the *P. cocyta* clades of Wahlberg; however, most of their *P. cocyta* individuals grouped with *P. tharos*. Each of these studies attributed the conflict between morphology and the COI gene to extensive interspecific introgression and incomplete lineage sorting.

1.3 Thesis objectives

Species delimitation and identification in the *Phyciodes tharos* species group have proved challenging due to intraspecific morphological variation and overlapping interspecific phenotypes (Scott 1994). Ecological traits, phenology, behaviours, and reproductive isolation have each provided evidence that there may be four distinct lineages (Hooper 1973; Oliver 1979a, 1980). However, discordance between traditional delimitation criteria and the mitochondrial COI gene suggests that either the traditional species boundaries are not accurate or there is widespread introgression between species (Wahlberg et al. 2003a; Proshek & Houghton 2012). In this thesis, I evaluate species boundaries in the *P. tharos* species group in Alberta using genome-wide SNPs and examine COI sequence data and morphological characters in the context of the resulting genomic lineages.

The limitations of individual genetic markers for species delimitation, especially mitochondrial genes, are now widely recognized (Dupuis et al. 2012; Meier et al. 2022). The development of next generation sequencing methods has allowed the use of many more independent markers from across the nuclear genome for more robust inferences about species boundaries (Peterson et al. 2012; Roe et al. 2017). In **Chapter 2**, I conduct phylogenetic and cluster-based analyses using genome-wide SNPs to examine species boundaries in the *P. tharos* species group in Alberta. I interpret my results in the context of the genomic integrity species definition which allows for occasional introgression (Sperling 2003). I then conduct a phylogenetic analysis based on COI haplotypes,

including those from Wahlberg et al. (2003a), and evaluate discordance between the two types of data.

The province of Alberta, where the Rocky Mountains, prairies, and boreal forest meet, is an ideal place to examine species relationships in the *Phyciodes tharos* species group. It is one of the few provinces or states that is home to all four species (Figure 2.1) and is the only such jurisdiction where no other species of the genus are present (Layberry et al. 1998; Brock & Kaufman 2006). Thus, there is potential for interaction within the species group without direct interaction with other closely related species. Although I include specimens from localities across North America to ground the study in a broader context, the focus of my work is Alberta.

Accurate determination of the species boundaries in this group has implications for conservation. If all four species are genomically distinct, analysis of SNPs can potentially be extended to other regions to help confirm the presence of each species. This could improve the accuracy of conservation status assessments in areas where species appear to be declining or threatened (NatureServe 2022). Within Alberta, it is important to clarify the status of *P. batesii*, which is currently considered secure but for which identifications are uncertain due to morphological similarity to *P. tharos* and *P. cocyta* (Layberry et al. 1998; Brock & Kaufman 2006; Scott 2006; Glassberg 2017).

Molecular species delimitation on its own is of little use in the field. Relating molecular species boundaries back to traditional morphological characters is important in order to determine which characters, if any, are reliable for practical identification. Chapter 2 serves as the foundation for **Chapter 3**, in which I examine discrete morphological characters in the context of their genomic species boundaries. I provide a detailed summary of the characters commonly used in the literature and the proportions of the character states exhibited by each species, according to sex. The potential utility of some of the top performing characters is discussed.

In summary, this work addresses longstanding issues of species delimitation and identification in the *P. tharos* species group. An iterative approach using genomic SNPs,

COI gene sequence, and morphology provides a comprehensive assessment of species boundaries, patterns of introgression, and effectiveness of diagnostic characters.

Chapter 2

Assessing species boundaries of crescent butterflies (Nymphalidae: *Phyciodes*) in Alberta using DNA

2.1 Introduction

Species are a fundamental unit in biology, but delimitation of species boundaries is often challenging (Hey et al. 2003; Sites & Marshall 2003; McKay et al. 2014; Dellicour & Flot 2018). In some cases, morphological traits are not reliable (Chapter 3) and other data sources may provide more robust species assessments. Molecular techniques such as DNA sequencing, for instance, can be used to characterize species boundaries based on patterns of genetic divergence (Dupuis et al. 2017a,b; Campbell et al. 2020).

Until recently, most studies that used DNA sequencing to identify species or infer species boundaries relied on one or a few markers (e.g., Bartlett & Davidson 1991; Hebert et al. 2004; Lumley & Sperling 2010) due to technological constraints, cost of available sequencing techniques, and computational requirements of multilocus methods (Fontaneto et al. 2015; Fujisawa et al. 2016; Roe et al. 2017). Some markers, for example the mitochondrial cytochrome c oxidase I (COI) gene, were proposed as universal 'barcodes' for delimiting and identifying species on a large scale and in a standardized way that allowed comparison between studies (Caterino et al. 2000; Hebert et al. 2003). However, it is well known that signals from individual genes are often discordant with one another (Pamilo & Nei 1988; Doyle 1992, 1997; Maddison 1997; Meier et al. 2006; Knowles & Carstens 2007; Wahlberg et al. 2009) and that species delimitation success is greatly improved with additional loci (Roe et al. 2010; Dupuis et al. 2012; Dellicour & Flot 2018). Further, mitochondrial markers may exhibit higher levels of interspecific introgression and non-monophyly than some nuclear markers (Chan & Levin 2005; Meier et al. 2022).

The development of next-generation sequencing (NGS) technology – in particular, reduced representation techniques such as double-digest restriction site-associated DNA sequencing (ddRADseq, Peterson et al. 2012) – has made it feasible to

quickly, and cost-effectively, obtain hundreds to thousands of single nucleotide polymorphisms (SNPs) across the genome, even for species for which a reference genome is lacking (Metzker 2010; Hohenlohe et al. 2013; Andrews et al. 2016; Roe et al. 2017). This technology has revolutionized the field of systematics and presents new opportunities for re-assessing species delimitations and phylogenetic relationships in groups in which morphology is highly variable and individual genetic markers show patterns of non-monophyly (e.g., Dupuis et al. 2017a; Lavretsky et al. 2019; Campbell et al. 2020).

The *Phyciodes tharos* species group (Lepidoptera: Nymphalidae) is currently considered to be comprised of four Nearctic butterfly species: *P. tharos* (Drury, 1773), *P. cocyta* (Cramer, 1777), *P. batesii* (Reakirt, 1865), and *P. pulchella* (Boisduval, 1852) (Scott 1994; Wahlberg et al. 2003a). Together, their ranges cover most of North America (Figure 2.1), occurring in open areas and clearings in association with *Aster* species, their primary larval hostplants (Brock & Kaufman 2006; Glassberg 2017). In areas of sympatry between species, some *Phyciodes* individuals, particularly females, cannot be consistently identified using morphology, due to intraspecific variation that makes it difficult to find reliable diagnostic characters (see Chapter 3; Layberry et al. 1998; Douglas & Douglas 2005; Scott 2006; Hall et al. 2014; Schweitzer et al. 2018).

The confluence of all four species' ranges occurs in only a few regions, including Alberta, Canada, where no other *Phyciodes* species are present (Figure 2.1; Acorn 1993; Bird et al. 1995). Within Alberta, *P. tharos* is generally treated as being restricted to the southeastern prairie region, *P. cocyta* and *P. batesii* are sympatric across most of the province, and *P. pulchella* is restricted to the Rocky Mountains and foothills in the west. Although all four species are considered secure in the province, *P. batesii* is rarely encountered and tends to have a more localized distribution across its range (Opler & Krizek 1984; Glassberg 2017).

Phyciodes tharos and *P. cocyta* were considered conspecific until a few decades ago and have only recently been treated as separate species based on ecological differences, hybrid breakdown, differing numbers and phenological sequences of annual broods, and detailed morphological differences in all life stages (Hooper 1973; Oliver

1980; Opler & Krizek 1984; Scott 1994). Although this split is generally accepted, some authors maintain that they should be considered one species (Guppy & Shepard 2001; Pyle 2002; Glassberg 2017). *Phyciodes tharos* and *P. cocyta* adults can also be confused with *P. batesii*, and records of *P. batesii* are often questionable due to the likelihood of misidentification of these more common species (Brock & Kaufman 2006; Douglas & Douglas 2005; Scott 2006; Glassberg 2017; Schweitzer et al. 2018). This makes it difficult to ascertain the presence and monitor the status of *P. batesii* based on morphology. This species has apparently declined precipitously since the 1960s, having likely been extirpated in most of its eastern range in the United States, and is uncommon in its eastern Canadian range (Glassberg 1993, 2017; Brock & Kaufman 2006; Cech & Tudor 2005; Hall et al. 2014; NatureServe 2022). It is also considered threatened in the Peace River and Liard River areas of British Columbia (Guppy et al. 1994; Acorn & Sheldon 2006). Thus, it is important to confirm this species' presence and status in Alberta.

Molecular studies of the *P. tharos* species group to date have used one or a few genes for phylogenetic reconstruction, but these have not sufficiently clarified species boundaries. A phylogenetic assessment of *Phyciodes* using the COI gene demonstrated probable incomplete lineage sorting, contemporary genetic exchange, or both, between all possible species pairs in the *P. tharos* species group, except *P. tharos* and *P. pulchella* (Wahlberg et al. 2003a). This suggests overall close relationships in this species group marked by ongoing or only recently ceased contact between taxa. Notably, *P. cocyta* and *P. batesii* were almost entirely interdigitated with each other. Another study using COI found that *P. cocyta* individuals from Michigan and Ohio tended to have haplotypes consistent with either *P. tharos* or *P. batesii*, depending on which species had populations in closer proximity (Proshek & Houghton 2012). This suggests that interactions between species may differ geographically. So far, nuclear genes have not been utilized for the *P. tharos* species group, except for higher-level phylogenetic studies that used, at most, two nuclear genes, in combination with COI, and one or few individuals per species (Wahlberg & Zimmermann 2000; Wahlberg et al. 2003b, 2005; Wahlberg 2006; Wahlberg & Freitas 2007; Long et al. 2014). Thus, there is a significant lack of genomic resources for this group, which impedes comprehensive species assessments.

The main aim of this chapter is to examine species boundaries of the *P. tharos* species group in a setting that allows natural interaction between all four species. I focus my sampling on Alberta, which acts as a natural laboratory for exploring the distinctiveness of these taxa when they are not in contact with other *Phyciodes*. The sampling emphasis on this region will help confirm whether *P. batesii* – a species that is threatened, vulnerable, or extinct in many other regions (NatureServe 2022) – is present in the province and whether all four species are distinct from one another. I use phylogenetic and cluster-based methods to assess patterns in genomic SNPs, including possible patterns of introgression, and compare genome-wide SNPs to mitochondrial COI sequences to test for discordance between these data types. Since gene flow can be common between recently diverged species, I consider the results within the genomic integrity species definition (Sperling 2003).

2.2 Materials and methods

2.2.1 *Specimen collection and DNA extraction*

I collected *Phyciodes* specimens by aerial net in 2016 and 2017 across Alberta and in British Columbia (Appendix 1). Collaborators also collected specimens in Alberta, British Columbia, Ontario, New Mexico, and Texas (see acknowledgements) from 2016-2018 to compare the relationships among the Albertan populations to those among populations sampled elsewhere. I also retrieved previously collected specimens from the Sperling lab freezer. These were collected from 1989-2015 and were primarily from Alberta, with some material from Manitoba, British Columbia, Montana, Colorado, Oregon, California, New York, and North Carolina. One dried specimen from Alberta, which was part of a donated museum collection, was also included. Specimens were initially identified to species by eye and the identities of ambiguous individuals were subsequently informed by SNP analyses. In total, 152 ingroup specimens (46 *P. tharos*, 74 *P. cocyta*, 8 *P. batesii*, and 24 *P. pulchella*) and four outgroup *P. mylitta* specimens were used in this chapter.

Specimens were either stored alive in glassine envelopes or preserved in 95% ethanol, and then transferred to a -20 °C freezer until use. Legs and entire thoraces were

used for DNA extraction using a DNeasy Blood and Tissue Kit (Qiagen), following the manufacturer's protocols with an optional RNase A treatment (Sigma-Aldrich Canada Co.). The isolates were purified by ethanol precipitation and then suspended in 50-100ul Millipore water. DNA samples were assessed for quality and quantity using a Nanodrop ND-1000 Spectrophotometer (Thermo Scientific) and a Qubit Fluorometer (1.0 dsDNA BR assay kit; Invitrogen) and concentrations were adjusted to 20 ng/ul prior to sequencing.

Detached wings were kept in glassine envelopes for morphological identification and analysis (Chapter 3). Voucher wings were stored in glassine envelopes and have been deposited in the E. H. Strickland Entomological Museum with unique DNA extraction numbers and UASM museum collection numbers (Appendix 1). Remaining DNA samples and other voucher tissues such as heads, antennae, and abdomens are stored in vials in a -20 °C freezer in the Sperling lab and are labelled with DNA and UASM numbers. DNA data will be deposited to GenBank (Clark et al. 2016).

2.2.2 *NGS sequencing, alignment, and filtering*

The Molecular Biology Service Unit (MBSU) at the University of Alberta completed the NGS library preparation following the double digest restriction-site associated DNA sequencing approach (ddRAD) of Peterson et al. (2012) using 200 ng of input DNA and the restriction enzymes *PstI* and *MspI*. Samples were indexed using 8 base pair (bp) individual-specific indexes, and single-end sequencing was performed on an Illumina NextSeq 500 to produce 75 bp reads.

Sequence reads were demultiplexed using the *process_radtags* program in the *Stacks v2.0b* pipeline (Catchen et al. 2011, 2013). To avoid observed, occasional sequencing error in the *PstI* site, *cutadapt v1.18* (Martin 2011) was used to trim an additional 5 bp from the 5' end of each sequence read. In addition, because Stacks requires uniform sequence reads for *de novo* locus construction, I removed any reads containing remnant Illumina sequencing adapters. Final cleaned sequence reads were all 62 bp long. Sequence reads were assembled into putative *de novo* loci using the *Stacks*

v2.0b pipeline on the Graham cluster hosted by Compute Canada. I required a minimum of three raw reads to form putative alleles within individuals and two mismatches were allowed between reads within individuals to form loci. I allowed three mismatches between putatively homologous loci between individuals.

Individuals were assigned to one of five species (*P. tharos*, *P. cocyta*, *P. batesii*, *P. pulchella*, *P. mylitta*) based on putative morphological identifications, and both the *populations* program (part of the *Stacks v2.0b* pipeline) and VCFtools v0.1.14 (Danecek et al. 2011) were used to filter loci using a minimum genotype quality phred score of 30 and a minor allele frequency of 5%. A single SNP was output from each locus to reduce linkage disequilibrium, and all filtered loci were required to be present in at least 80% of individuals for each putative species. Lastly, I allowed global missing data rates to be a maximum of 20% for cluster-based analyses, which are sensitive to higher levels of missing data (Puechmaille 2016) and 50% for phylogenetic analyses, which are more tolerant of missing data (Wiens 2006; Huang & Knowles 2016). The final number of informative SNP loci used was 1477 for cluster-based analyses and 2360 for phylogenetic analysis.

2.2.3 *COI sequencing and alignment*

The 658 bp barcoding region of COI was amplified by polymerase chain reaction (PCR) using the primers LepF (5'-ATTCAACCAATCATAAAGATATTGG-3') and LepR (5'-TAAACTTCTGGATGTCCAAAAAATCA-3'). PCR began with a two-minute denaturation period at 94 °C followed by 35 cycles of 94 °C for 30 seconds, 45 °C for 30 seconds, and 72 °C for 2 minutes and then a final elongation at 72 °C for 5 minutes. Excess primers and nucleotides were removed from the product using exonuclease I and shrimp alkaline phosphatase (New England Biolabs). Sequencing was performed in both directions on a 3730 DNA Analyzer (Applied Biosystems) at the MBSU.

Forward and reverse sequences were merged in Geneious 10.2.6 (www.geneious.com) and base calls were manually checked. Consensus sequences were then aligned using MAFFT online (Katoh et al. 2017; Kuraku et al. 2013) under default

parameters. The multiple sequence alignment was also manually checked. Fifty-six additional sequences were downloaded from GenBank (Clark et al. 2016; Appendix 2) and aligned with the study sequences. All sequences were trimmed to 633 bp to match the length of the barcoding region that was consistently present in all sequences.

2.2.4 *Phylogenetic analyses*

Maximum likelihood phylogenetic analyses of 2360 SNPs (152 ingroup and 4 outgroup specimens) and 633 bp of COI (196 ingroup and 13 outgroup specimens) were performed in IQ-TREE versions 1.5.5 and 2.0.7, respectively (Nguyen et al. 2015) on Compute Canada. The perturbation strength for the tree search was 0.5 for both analyses. Ultrafast bootstrapping (UFBoot; Hoang et al. 2018) was performed for the SNP (2000 iterations) and COI (1000 iterations) analyses.

Models were selected based on the Bayesian Information Criterion in IQ-TREE's built-in ModelFinder (Kalyaanamoorthy et al. 2017). The TVM+R5 nucleotide substitution model was used for SNP analysis and the TN+R2 nucleotide substitution model was used for COI analysis. Ascertainment bias correction was not used for SNP analysis because there were constant or partially constant sites in the dataset resulting from lineage-specific polymorphisms in restriction sites. For both SNP and COI analyses, nodes were collapsed if they had lower than 50% UFBoot support to produce a 50% majority rule consensus tree, which was rooted in FigTree v1.4.3 (Rambaut 2010). Individuals in the COI phylogeny were labelled based on their clade membership in the SNP phylogeny.

2.2.5 *Cluster-based analyses of SNPs*

Bayesian clustering analysis was performed on 1477 SNPs for 152 ingroup individuals using the program STRUCTURE 2.3.4 (Pritchard et al. 2000). Ten independent replicates were run for K-values 2 through 6, with 500,000 burn-in iterations and 5 million MCMC iterations each, using the admixture model and the *nolocprior* option. Because the dataset contained multiple species, hierarchical analysis of the

clusters recovered in the STRUCTURE analysis was also performed, to detect any substructure within species that may be present in the data. For each of these hierarchical analyses, 2 million MCMC iterations were run with 200,000 burn-in iterations for K-values 2 through 4. In these hierarchical analyses the *P. batesii* and *P. pulchella* clusters were analyzed together because there were only six individuals of the former species and the *P. batesii* individuals appeared to be admixed with *P. pulchella* (see 2.3 Results). Individuals that appeared to be F1 hybrids were included in substructure analyses for both of their parental species. STRUCTURE HARVESTER v0.6.94 (Earl & vonHoldt 2012) was used to collate results and obtain summary statistics. Collated results were submitted to the beta CLUMPAK web server (Kopelman et al. 2015) which summarized results using a Markov clustering algorithm. The optimal value of K was selected based on the likelihood of the data (Ln Pr(X|K)) (Pritchard et al. 2010) and comparison to the phylogenetic tree to assess biological plausibility. The Evanno method was also applied (Evanno et al. 2005), though this method often only detects the highest level of structure in a dataset (Janes et al. 2017).

Principal component analysis (PCA) was performed on 1477 SNPs for 152 ingroup individuals using the FactoMineR package (Lê et al. 2008) in R 4.0.1 (R Core Team 2020). For visualization of plots, ggplot2 (Wickham 2016) and ggforce (Pedersen 2022) packages were used. Results were analysed for five axes and individuals were coloured based on their species identification in the main STRUCTURE analysis.

2.3 Results

2.3.1 *SNP sequencing statistics*

The unfiltered dataset yielded 12,172 loci. The average read depth was 30 for both individuals and sites with a range of 11-73 for individuals and 7-320 for sites. Filtering for phylogenetic and cluster-based analyses retained 2360 and 1477 single-SNP loci, respectively.

2.3.2 *SNP phylogeny*

The maximum likelihood tree produced three main well-supported species groupings (≥95% UFBoot) that correspond to *P. pulchella*, *P. cocyta*, and *P. tharos* (Figure 2.2A). The monophyletic *P. pulchella* clade branches off first and the *P. cocyta* and *P. tharos* groupings are sister to one another with one putative *P. tharos* individual appearing in the *P. cocyta* grouping. Seven specimens form an intermediate stepwise 'grade' of individuals that appears paraphyletic with respect to *P. tharos* and *P. cocyta*. Six of these individuals putatively represent *P. batesii.* Other than within the *P. batesii* 'grade', the relationships between the species groupings are all well-supported.

Most of the tree was fully resolved, except for several relationships in the *P. cocyta* and *P. tharos* clades. Within the species groupings, strong support was limited to small groupings of 2-4 specimens and a few basal relationships. Other than some specimens from the United States, there is little evidence of geographic structuring; individuals from distant localities are found together in the tree, sometimes with high support.

2.3.3 *Cluster-based analyses of SNPs*

In the STRUCTURE analysis of all individuals, the optimal value selected for K was 4 (Figure 2.2B; Appendix 3) and higher values did not produce meaningful clusters (Appendix 4). The ΔK-value was highest for K = 2 (Appendix 3a) but this result lumped *P. tharos* with *P. cocyta* and *P. batesii* with *P. pulchella* and can be considered an artefact of the Evanno method (Appendix 4; Janes et al. 2017). The ΔK-value was also somewhat high for K = 3 and was low for K = 4 and 5 (Appendix 3a). The likelihood of the data (Ln Pr(X|K)) began to plateau at K = 3 and increased marginally for K = 4 (Appendix 3b).

Overall, the STRUCTURE clusters for K = 3 and 4 were consistent with the ML tree (Figure 2.2A-B). The K = 3 plot recognized *P. pulchella*, *P. tharos*, and *P. cocyta* as separate entities and assigned the six *P. batesii* individuals as admixed individuals of *P. pulchella* and *P. cocyta*, with a small proportion of *P. tharos* ancestry in four individuals.

The K = 4 plot recognized the same three groups but assigned the six *P. batesii* individuals to a fourth distinct cluster with 12.51-27.68% admixture from *P. pulchella*. This result is more biologically plausible because *P. pulchella* is absent in Manitoba, where two of the *P. batesii* specimens were collected (Figure 2.3). The low increase in likelihood for K = 4 (and correspondingly low ΔK-value; Appendix 3a-b) is likely due mostly to the low sample size of *P. batesii*. For both K = 3 and 4, the seventh individual in the intermediate 'grade' had an admixed genotype intermediate between *P. batesii* and *P. cocyta* (Figure 2.2A-B).

Most individuals (129/152) had genotypes that were at least 95% consistent with the cluster associated with their position in the phylogenetic tree (Figure 2.2A-B). All *P. batesii* individuals had 12-28% of their ancestry consistent with *P. pulchella.* Admixture between *P. tharos* and *P. cocyta* was evident in several individuals, mostly in low levels. The *P. tharos* individuals from the southern United States and one individual from Alberta in the *P. tharos* clade each had more than 20% of their ancestry consistent with *P. cocyta* (Figure 2.3). An additional individual from Alberta that appeared in the *P. cocyta* grouping of the tree similarly had 61.7% of its genotype consistent with *P. tharos* and 37.9% with *P. cocyta* (Figures 2.2A-B, 2.3). Two individuals from Alberta in the *P. cocyta* cluster appear to be F1 hybrids – one between *P. tharos* and *P. cocyta* and the other between *P. batesii* and *P. cocyta*. The individual that is between the *P. batesii* grade and the *P. cocyta/P. tharos* phylogenetic clusters appears to be a *P. cocyta* individual with introgression from both *P. batesii* and *P. pulchella*.

All substructure analyses of individual species had an optimal K-value of 2 and higher values did not produce meaningful clusters (Appendix 5). In the combined analysis of *P. batesii* and *P. pulchella*, the two clusters corresponded to each species. The *P. batesii* individual with the most *P. pulchella* ancestry in the main analysis exhibited mixed ancestry in the substructure analysis as well. In the *P. cocyta* analysis, the smaller cluster appeared to represent variation that is present to some extent in nearly all individuals. The SNPs associated with this cluster were only prevalent (>70%) in five individuals – four that had mixed ancestry in the main analysis and one *P. cocyta* individual from BC. Other individuals had up to 49% membership to this cluster without

any apparent association with the main STRUCTURE results or geography. The two individuals that were included in both the *P. batesii/P. pulchella* and *P. cocyta* analyses were each found to belong entirely to *P. batesii* in the former analysis and mostly to the smaller *P. cocyta* cluster in the latter. In the *P. tharos* substructure analysis, the smaller cluster was geographic and was made up of three specimens from North Carolina and Texas. Four Alberta specimens also had ancestry partially consistent with this cluster. The two individuals that were included in both the *P. cocyta* and *P. tharos* analyses belonged mostly to the smaller *P. cocyta* cluster in the former analysis and the main *P. tharos* cluster in the latter.

The PCA of SNP data for all species produced four clear clusters, supporting the K = 4 STRUCTURE result (Figure 2.4). The first dimension clearly separated *P. pulchella* from the rest of the species, with *P. batesii* specimens appearing intermediate between *P. pulchella* and *P. cocyta*/*P. tharos* (Figure 2.4A-D). The second dimension separated *P. tharos* from *P. cocyta* (Figure 2.4A). The third dimension showed the six *P. batesii* specimens as distinct from the other three species (Figure 2.4B). The two putative F1 hybrid individuals appear between their parental species. Principal components 4 and 5 did not show any additional meaningful clustering, except for the southern and western *P. pulchella* individuals (Figure 2.4C-D).

2.3.4 *COI sequence information*

There were few missing data in both the original and the trimmed data sets (<0.04%). Over half of the missing data was from two outgroup individuals from the Wahlberg et al. (2003a) dataset. In the trimmed data set that was used for analysis (633 bp), there were 87 informative sites, 34 singleton sites, and 512 constant sites. There were 79 unique 633 bp haplotypes across 209 individuals. Identical haplotypes were shared by many individuals, in some cases from distant localities or in individuals from the same locality that belong to different lineages in the SNP analyses.

2.3.5 *COI phylogeny*

The maximum likelihood tree of COI sequences produced a tree that was less resolved and only partly corresponded to the SNP groupings (Figures 2.2C, 2.5). Several individuals exhibited mitonuclear discordance with COI haplotypes that are inconsistent with their membership in the genomic SNP phylogenetic and STRUCTURE analyses (Figure 2.2). The *P. batesii* individuals were found throughout the tree and did not form a distinct grouping (Figure 2.5). There was strong support (≥95% UFBoot) for many of the groupings, but not for the relationships between the main groupings. Overall, the phylogeny was consistent with Wahlberg et al. (2003a) and the lettered clades from that study are marked on the nearest corresponding nodes or branches in Figure 2.5 (as in Proshek & Houghton (2012)). Three individuals branched off before all other *P. tharos* species group individuals to form a polytomy. This includes two *P. cocyta* individuals from British Columbia, which were recovered as sister taxa, and a *P. pulchella* specimen from California from clade A in Wahlberg et al. (2003a). Clade B includes all the *P. tharos* individuals, ten *P. cocyta* individuals (8 from this study), and three *P. batesii* individuals (1 from this study). Three clades that correspond to Wahlberg's C, D, & E, represent most of the *P. cocyta* and *P. batesii* individuals and 3 *P. pulchella* individuals (2 from this study). Two of these clades (C & D) formed a polytomy with one another and the third and largest clade (E) was sister to the *P. pulchella* group of clades. Thus, *P. cocyta*/*P. batesii* appears to be paraphyletic with respect to *P. pulchella* based on the barcoding region of COI. The remaining *P. pulchella* specimens formed a polytomy of three clades and one *P. batesii* individual, that together correspond to Wahlberg's clades F, G, and H. The two *P. pulchella* individuals that correspond to clade H in the former study form a clade, but the *P. batesii* individual associated with clade H appears outside of it.

2.4 Discussion

2.4.1 *Phylogenetic relationships and mitonuclear discordance*

The number of species in the *Phyciodes tharos* species group has remained uncertain due to a high level of variation in nearly every trait used to describe the species

(Scott 1994, 1998, 2006) and, more recently, the discovery of shared mitochondrial COI haplotypes between species (Wahlberg et al. 2003a; Proshek & Houghton 2012). The phylogenetic tree based on genome-wide SNPs largely recovered all four described species in Alberta and across their ranges (Figure 2.2A). Geographic distance had little influence on phylogenetic relationships within species, other than *P. tharos* and *P. pulchella* from the United States. Each species formed a well-supported clade, except for *P. batesii* and a single *P. cocyta* individual which together formed a paraphyletic grade with respect to *P. cocyta* and *P. tharos*. One *P. tharos* individual with evidence of gene flow from *P. cocyta* appeared in the *P. cocyta* clade. Despite the lack of clarity of the relationships with *P. batesii*, this tree lends support to the existence of four divergent lineages in this group.

The clear results of the SNP tree provided a more reliable measure for assessing the mitochondrial results. The COI tree demonstrated mitonuclear discordance with respect to the SNPs and did not clearly resolve any of the species (Figures 2.2C, 2.5). As in Wahlberg et al. (2003a) and Proshek & Houghton (2012), only *P. tharos* and *P. pulchella* occurred entirely in mutually exclusive clusters, all other species pairs had individuals that shared identical or similar haplotypes, and *P. batesii* did not form its own unique grouping. Each of the clades that represented *P. tharos*, *P. cocyta*, or *P. batesii* included individuals from at least three species, rendering COI useless for diagnosing species in this group. Regardless of the exact phylogenetic relationships, the SNP and COI trees both indicate that *P. tharos* and *P. pulchella* are the most distantly related to one another and that *P. batesii* is the least genomically-distinct species.

In addition to resolving four relatively clear species clusters, the SNP tree, which was rooted to outgroup species *P. mylitta*, suggests that *P. pulchella* may be the earliest diverging species in the *P. tharos* group, followed by *P. batesii*, *P. cocyta*, and then *P. tharos* (Figure 2.2A). This contrasts with the proposal by Scott (1994) that *P. tharos* may be the earliest branching species based on similarities with *P. mylitta* in wingspan, and genitalic and pupal characters. The COI tree in this study (Figure 2.5) and that by Wahlberg et al. (2003a) supported Scott's assertion, other than two aberrant *P. pulchella* haplotypes from Wahlberg that grouped with outgroup species *P. phaon*. In their higher-

level phylogeny of the Melitaeini, Long et al. (2014) also recovered relationships in support of Scott's hypothesis using the genes COI, EF1α, and wingless, based on one individual of each species. A genomic study of all *Phyciodes* species with more extensive sampling would be useful for clarifying these relationships.

2.4.2 *Cluster-based analysis of species boundaries*

The STRUCTURE and PCA analyses were consistent with the species groupings in the SNP tree (Figure 2.2A). Clear clusters for all four species were recovered in the K = 4 STRUCTURE plot and on the first three dimensions of the PCA (Figures 2.2B, 2.4A-B), with little apparent genotypic variation within species. Other than the genomic differences between geographically distant individuals, there was not any obvious substructuring within species (Figure 2.2A-B, Appendix 5).

The unclear *P. batesii* species boundaries found in the SNP tree were reflected in the K = 3 STRUCTURE plot and on the first two dimensions of the PCA (Figures 2.2A-B, 2.4A) which each showed *P. batesii* as intermediate between *P. pulchella* and *P. cocyta*, as in the SNP tree. This suggests that the *P. batesii* individuals may be F1 hybrids between *P. cocyta* and *P. pulchella*; however, two of the individuals are from Manitoba which is far outside the *P. pulchella* geographic range (Figures 2.1, 2.3). The third dimension of the PCA (Figure 2.4B) and K = 4 STRUCTURE plot (Figure 2.2B) demonstrate that the *P. batesii* cluster is unique and not simply intermediate. Yet, all six *P. batesii* individuals, including the two from Manitoba, still exhibit genomic material consistent with *P. pulchella* in the K = 4 STRUCTURE plot (Figures 2.2B, 2.3). Though contemporary introgression with *P. pulchella* is possible in the western *P. batesii* range where there is potential sympatry, this would not account for the similar genotypes in Manitoba. It is unlikely that the putative *P. pulchella* SNPs are from unsampled "ghost populations" (Beerli 2004; Slatkin 2005) on the edge of the sampling area because there are no other *Phyciodes* species that occur near Manitoba (Brock & Kaufman 2006).

Genomic material may be shared between closely related species based on shared ancestry (Lavretsky et al. 2019), but speciation can also involve, or even be driven by,

gene flow between diverging lineages (Mallet 2007; Sousa & Hey 2013; Dupuis & Sperling 2015). Thus, it is possible that *P. batesii* and *P. pulchella* share retained ancestral genomic material or, alternatively, that the evolutionary history of the *P. batesii* lineage involved regular introgression with *P. pulchella* during divergence. However, insufficient sample sizes can affect accurate estimates of genetic diversity and a more comprehensive sample of *P. batesii* may yield additional SNPs that are unique to this species (Puechmaille 2016; Lawson et al. 2018).

The only individuals that showed evidence of geographic differentiation in the STRUCTURE analysis were the *P. tharos* from North Carolina and Texas, which had genomic material consistent with *P. cocyta* (Figures 2.2B, 2.3). Two Alberta *P. tharos* specimens had a similar proportion of *P. cocyta* SNPs but in the substructure analysis of *P. tharos*, the southern individuals clearly formed a distinct cluster (Appendix 5). It is unlikely that this is due to introgression with *P. cocyta* because this species does not occur in Texas or in the sampled region of North Carolina (Figures 2.1, 2.3). It is more likely due to either subspecific divergence, retained ancestral polymorphism, or introgression with an unsampled *Phyciodes* species that occurs in that region and is not otherwise represented in the STRUCTURE analysis (Beerli 2004; Slatkin 2005). *Phyciodes phaon* is common in the southern United States, including North Carolina and Texas (Lotts & Naberhaus 2020); however, Oliver (1982) found that courtship behaviour would probably prevent mating between *P. tharos* and *P. phaon* in nature. Further, the individuals in this study each have *P. tharos* COI haplotypes (Figure 2.2C), and Oliver found that F1 hybrids and backcrosses involving female *P. tharos* and male *P. phaon* were completely inviable.

2.4.3 *Contemporary introgression and genomic integrity*

The genomic integrity species definition considers species as divergent lineages that maintain their genomic integrity upon contact (Sperling 2003). The number of species in the *P. tharos* group has been uncertain for decades due to morphological ambiguity (Scott 1994), incomplete reproductive isolation (Oliver 1972, 1978, 1979a,b, 1980; Scott 1986b), and shared COI haplotypes (Wahlberg et al. 2003a; Proshek &

Houghton 2012). Genome-wide data produced four clear clusters with evidence of low levels of gene flow (Figure 2.2A-B). Thus, the designation of the four lineages as species is consistent with the genomic integrity definition. Despite the phylogenetic limitations of COI for this group (Figures 2.2C, 2.5), this gene can be used in conjunction with genome-wide SNP data to shed light on patterns of hybridization and introgression.

Based on both genome-wide SNPs and mitochondrial COI, most interaction appears to occur between *P. tharos* and *P. cocyta*. There is evidence of one F1 hybrid between these species and individuals with varying levels of recent gene flow in areas of sympatry in Alberta (Figures 2.1, 2.3). The *P. cocyta* individuals from Montana and New York also show evidence of introgression from *P. tharos* in areas of sympatry, indicating that these species may interact somewhat regularly across their range. Gene flow does not appear to be biased in one direction based on SNPs, but there seems to be unidirectional movement of COI from *P. tharos* to *P. cocyta*. This may have to do with the timing of broods, particularly in the spring. Male butterflies tend to emerge before females (Scott 1977; Wiklund & Fagerström 1977). In Alberta, *P. tharos* is the first species to emerge (Bird et al. 1995) and so female *P. tharos* may overlap with the emergence of *P. cocyta* males, who might mate opportunistically.

There was evidence of occasional hybridization between *P. cocyta* and *P. batesii* in Alberta. One individual appears to be an F1 hybrid of the two species (Figure 2.2B, 2.3). It was caught at the same locality on the same date as five *P. cocyta* and two *P. pulchella* individuals (Appendix 1). Despite this and though all individuals in the *P. batesii* grade include some *P. pulchella* SNPs, there were not any *P. pulchella* SNPs evident. The *P. pulchella* individuals from the same locality did, however, exhibit a small amount of *P. batesii* DNA (1.5-2.2%). The individual that appeared in the paraphyletic grade between *P. batesii* and the *P. cocyta*/*P. tharos* clades appears to be a *P. cocyta* individual with introgression from *P. batesii* and *P. pulchella*. The intermediacy of this individual may partly explain the lack of clarity between *P. cocyta* and *P. batesii* in the tree. Given that *P. batesii* does not have a unique COI haplotype and instead exhibits haplotypes consistent with either *P. cocyta* or *P. tharos*, it is likely that there has been a long history of introgression of *P. batesii* with these species. Both *P. tharos* and *P. cocyta*

tend to emerge prior to *P. batesii* in Alberta (Acorn 1993; Bird et al. 1995), and the timing of emergence may be a factor in the movement of COI from *P. tharos* and *P. cocyta* to *P. batesii*. However, *P. batesii* may be the first species to emerge in Manitoba (Klassen et al. 1998). Only one *P. batesii* in this study had a *P. tharos* haplotype but interestingly, it was in the most northern locality, distant from the *P. tharos* range. Wahlberg et al. (2003a) also found a *P. batesii* individual from a somewhat northern latitude (as well as one individual from the US) with a *P. tharos* haplotype.

The prevalence of genomic introgression between *P. tharos* and *P. cocyta* and the scarcity of detected introgression between *P. cocyta* and *P. batesii* are in contrast with the COI haplotypes in this study (Figures 2.2, 2.5) and those of Wahlberg et al. (2003a). In both studies, *P. cocyta* and *P. batesii* shared indistinguishable COI haplotypes, while *P. tharos* and *P. cocyta* were mostly distinct from one another with limited evidence of introgression. On the other hand, these results were consistent with Proshek & Houghton (2012) who found that, on a local scale, *P. cocyta* had COI haplotypes consistent with either *P. tharos* or *P. batesii* depending on the proximity of *P. cocyta* individuals to populations of each species. Thus, individuals may incidentally mate with other species where they are abundant. In Alberta, though the ranges of *P. cocyta* and *P. batesii* share a larger range of sympatry (Figure 2.1), *P. tharos* appears to be much more abundant than *P. batesii*, based on the specimens collected. Thus, there may be more opportunity for interaction between *P. tharos* and *P. cocyta* despite their limited range of sympatry.

2.5 Conclusions

This is the first treatment of the *Phyciodes tharos* group species boundaries using genome-wide SNP data. Phylogenetic and cluster-based analyses confirm that there are four species in Alberta based on the maintenance of genomic integrity in the presence of occasional hybridization and low levels of gene flow. Mitonuclear discordance obscures the phylogenetic signal from COI but provides evidence of unidirectional mitochondrial introgression. More comprehensive sampling of *P. batesii* is needed to better understand its boundaries, especially with *P. pulchella* and *P. cocyta*. Future sampling across North

America may also provide insight regarding subspecific relationships and the mechanisms of speciation in this group.

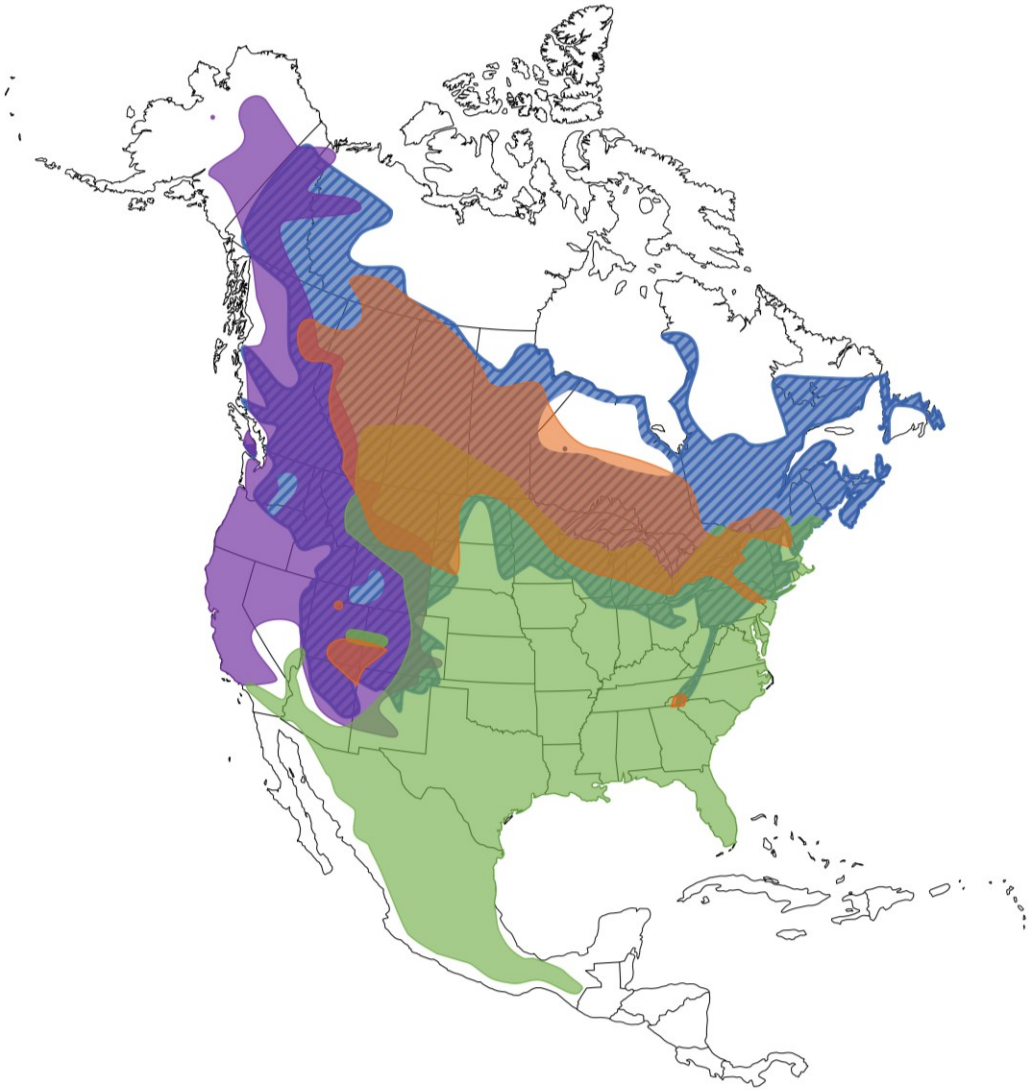


Figure 2.1 Range map for the *Phyciodes tharos* species group of butterflies. Distributions shown are based on Layberry et al. (1998), Wahlberg et al. (2003a), Brock & Kaufman (2006), iNaturalist (2020) (research grade data only), and Lotts & Naberhaus (2020). Green = *P. tharos*; blue pattern = *P. cocyta*; orange = *P. batesii*; purple = *P. pulchella*.

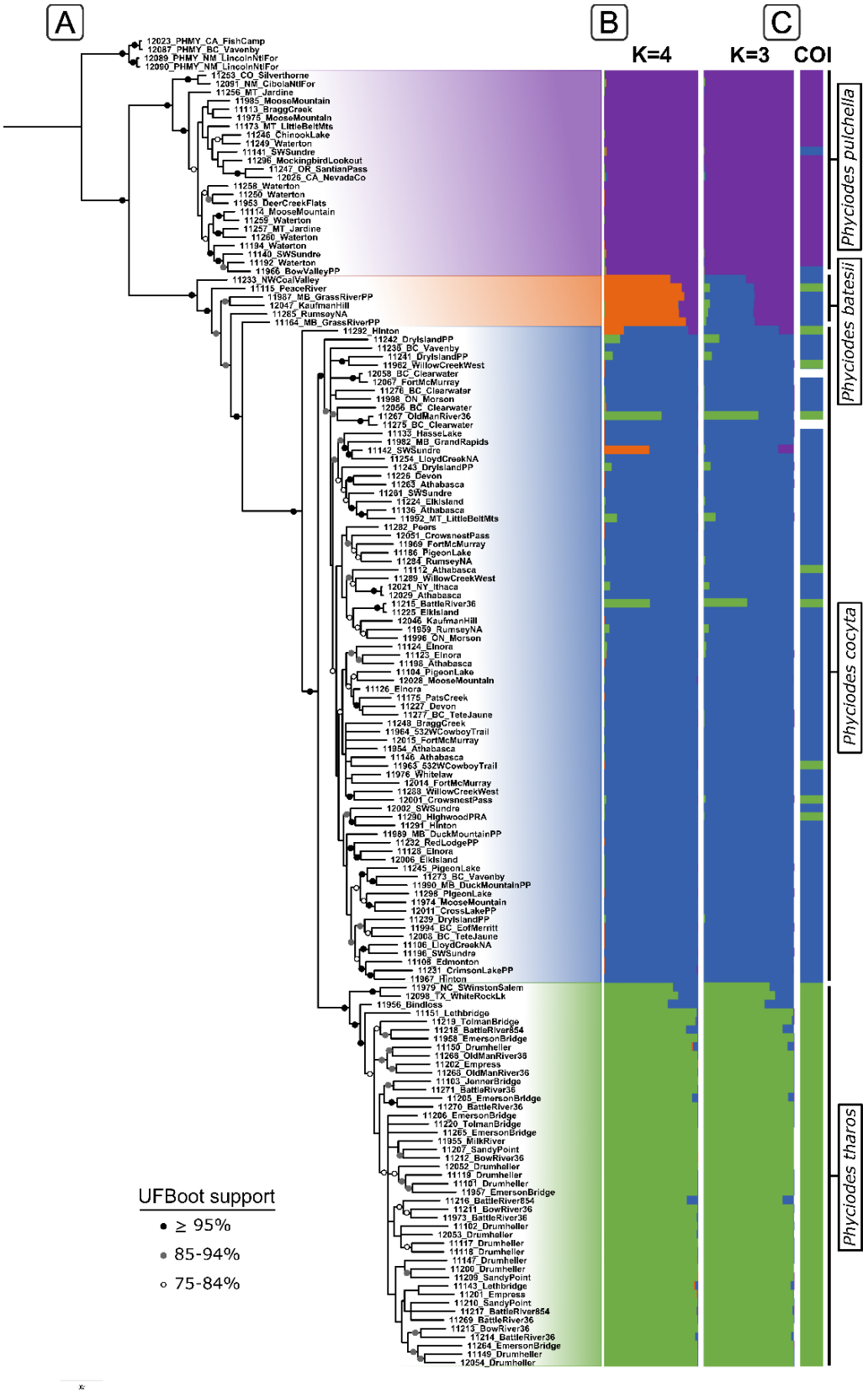

A
B
C
K=4
K=3
COI
12023_PHMY_CA_FishCamp
12087_PHMY_BC_Vavenby
12089_PHMY_NM_LincolnNtlFor
12090_PHMY_NM_LincolnNtlFor
11253_CO_Silverthorne
12091_NM_CibolaNtlFor
11256_MT_Jardine
11985_MooseMountain
11113_BraggCreek
11975_MooseMountain
11173_MT_LittleBeltMts
11246_ChinookLake
11249_Waterton
11141_SWSundre
11296_MockingbirdLookout
11247_OR_SantianPass
12026_CA_NevadaCo
11258_Waterton
11250_Waterton
11953_DeerCreekFlats
11114_MooseMountain
11259_Waterton
11257_MT_Jardine
11260_Waterton
11194_Waterton
11140_SWSundre
11192_Waterton
11966_BowValleyPP
11233_NWCoalValley
11115_PeaceRiver
11987_MB_GrassRiverPP
12047_KaufmanHill
11265_RumseyNA
11164_MB_GrassRiverPP
11292_Hinton
11242_DrylslandPP
11238_BC_Vavenby
11241_DrylslandPP
11962_WillowCreekWest
12058_BC_Clearwater
12067_FortMcMurray
11276_BC_Clearwater
11998_ON_Morson
12056_BC_Clearwater
11267_OldManRiver36
11275_BC_Clearwater
11133_HasseLake
11982_MB_GrandRapids
11142_SWSundre
11254_LloydCreekNA
11243_DrylslandPP
11226_Devon
11263_Athabasca
11291_SWSundre
11224_Elkisland
11136_Athabasca
11992_MT_LittleBeltMts
11282_Peers
12051_CrowsnestPass
11969_FortMcMurray
11186_PigeonLake
11284_RumseyNA
11112_Athabasca
11289_WillowCreekWest
12021_NY_Ithaca
12029_Athabasca
11215_BattleRiver36
11225_Elkisland
12046_KaufmanHill
11959_RumseyNA
11996_ON_Morson
11124_Elnora
11123_Elnora
11198_Athabasca
11194_PigeonLake
12028_MooseMountain
11126_Elnora
11175_PatsCreek
11227_Devon
11277_BC_TeteJaune
11248_BraggCreek
11964_532WCowboyTrail
12015_FortMcMurray
11954_Athabasca
11146_Athabasca
11963_532WCowboyTrail
11976_Whitelaw
12014_FortMcMurray
11288_WillowCreekWest
12001_CrowsnestPass
12002_SWSundre
11290_HighwoodPRA
11291_Hinton
11989_MB_DuckMountainPP
11232_RedLodgePP
11128_Elnora
12006_Elkisland
11245_PigeonLake
11273_BC_Vavenby
11990_MB_DuckMountainPP
11295_PigeonLake
11974_MooseMountain
12011_CrossLakePP
11239_DrylslandPP
11994_BC_EotMerritt
12008_BC_TeteJaune
11106_LloydCreekNA
11196_SWSundre
11108_Edmonton
11231_CrimsonLakePP
11967_Hinton
11979_NC_SWinstonSalem
12098_TX_WhiteRockLk
11956_Bindloss
11151_Lethbridge
11219_TolmanBridge
11216_BattleRiver854
11958_EmersonBridge
11150_Drumheller
11268_OldManRiver36
11202_Empress
11268_OldManRiver36
11103_JennerBridge
11271_BattleRiver36
11205_EmersonBridge
11270_BattleRiver36
11206_EmersonBridge
11220_TolmanBridge
11265_EmersonBridge
11955_MilkRiver
11207_SandyPoint
11212_BowRiver36
12052_Drumheller
11119_Drumheller
11101_Drumheller
11957_EmersonBridge
11216_BattleRiver854
11211_BowRiver36
11973_BattleRiver36
11102_Drumheller
12053_Drumheller
11117_Drumheller
11118_Drumheller
11147_Drumheller
11200_Drumheller
11209_SandyPoint
11143_Lethbridge
11201_Empress
11210_SandyPoint
11217_BattleRiver854
11269_BattleRiver36
11213_BowRiver36
11214_BattleRiver36
11264_EmersonBridge
11149_Drumheller
12054_Drumheller
Phyciodes pulchella
Phyciodes batesii
Phyciodes cocyta
Phyciodes tharos
UFBoot support
≥ 95%
85-94%
75-84%

Figure 2.2 Maximum likelihood SNP tree and STRUCTURE plots aligned with COI haplotype memberships. (previous page) **(A)** Maximum likelihood consensus tree for 2360 SNPs resolves well-supported clades for *P. tharos*, *P. cocyta*, and *P. pulchella*. *Phyciodes batesii* appears as an intermediate grade between *P. pulchella* and *P. cocyta*/*P. tharos*. Branches with <50% UFBoot support were collapsed and supports ≥75% are indicated on the branches. **(B)** STRUCTURE analyses of 1477 SNPs with individuals aligned to (A). When K=3, the *P. batesii* individuals appear to be F1 hybrids between *P. cocyta* and *P. pulchella*. When K=4, the *P. batesii* individuals form a distinct cluster, but exhibit some SNPs consistent with *P. pulchella*. Several individuals show evidence of introgression, including two F1 hybrids. **(C)** Clade membership in ML analysis of 633 bp of COI (Figure 2.5) with individuals aligned to (A). The comparison of SNP and COI data provides additional evidence of introgression between *P. tharos* and both *P. cocyta* and *P. batesii*, with unidirectional movement of mtDNA to the latter to species. The two individuals without COI membership indicated are each from British Columbia (11275 & 12058) and appear at the base of the COI tree along with a *P. pulchella* individual from Wahlberg et al. (2003a). Province and state abbreviations are included for all individuals collected outside of Alberta.

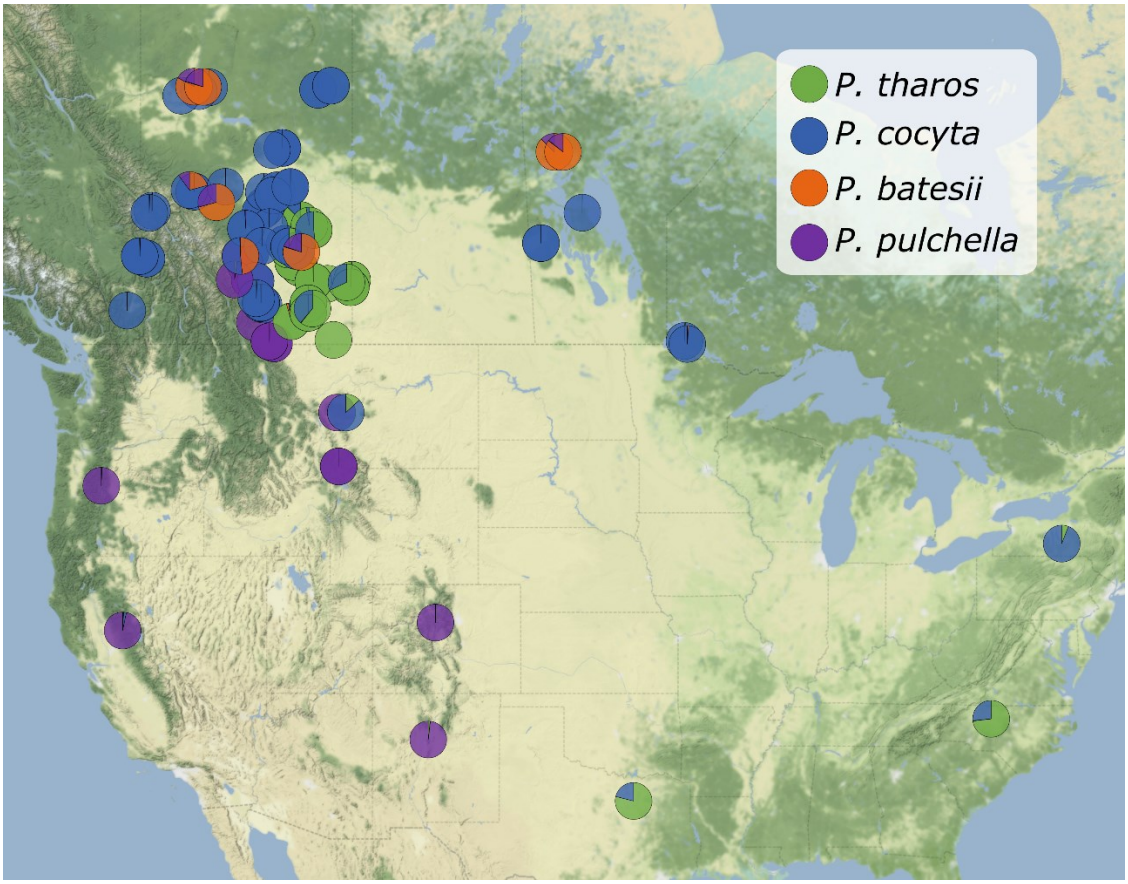


Figure 2.3 K=4 STRUCTURE results over a map of North America. Each plot represents the genotype of one individual specimen. *Phyciodes batesii* individuals exhibit a consistent genotype that includes genomic information that clusters with *P. pulchella* despite the Manitoba specimens occurring far outside the *P. pulchella* range (Figure 2.1). Introgression between *P. batesii* and *P. cocyta* is evident in two individuals in Alberta, including one hybrid. Introgression between *P. tharos* and *P. cocyta* is evident in Alberta and Montana, including one hybrid near where their ranges meet in Alberta. *Phyciodes tharos* individuals in North Carolina and Texas are outside of the *P. cocyta* range and their genotypes may reflect introgression from unsampled species in those areas. *Phyciodes cocyta* and *P. pulchella* each exhibit a consistent genotype across their ranges.

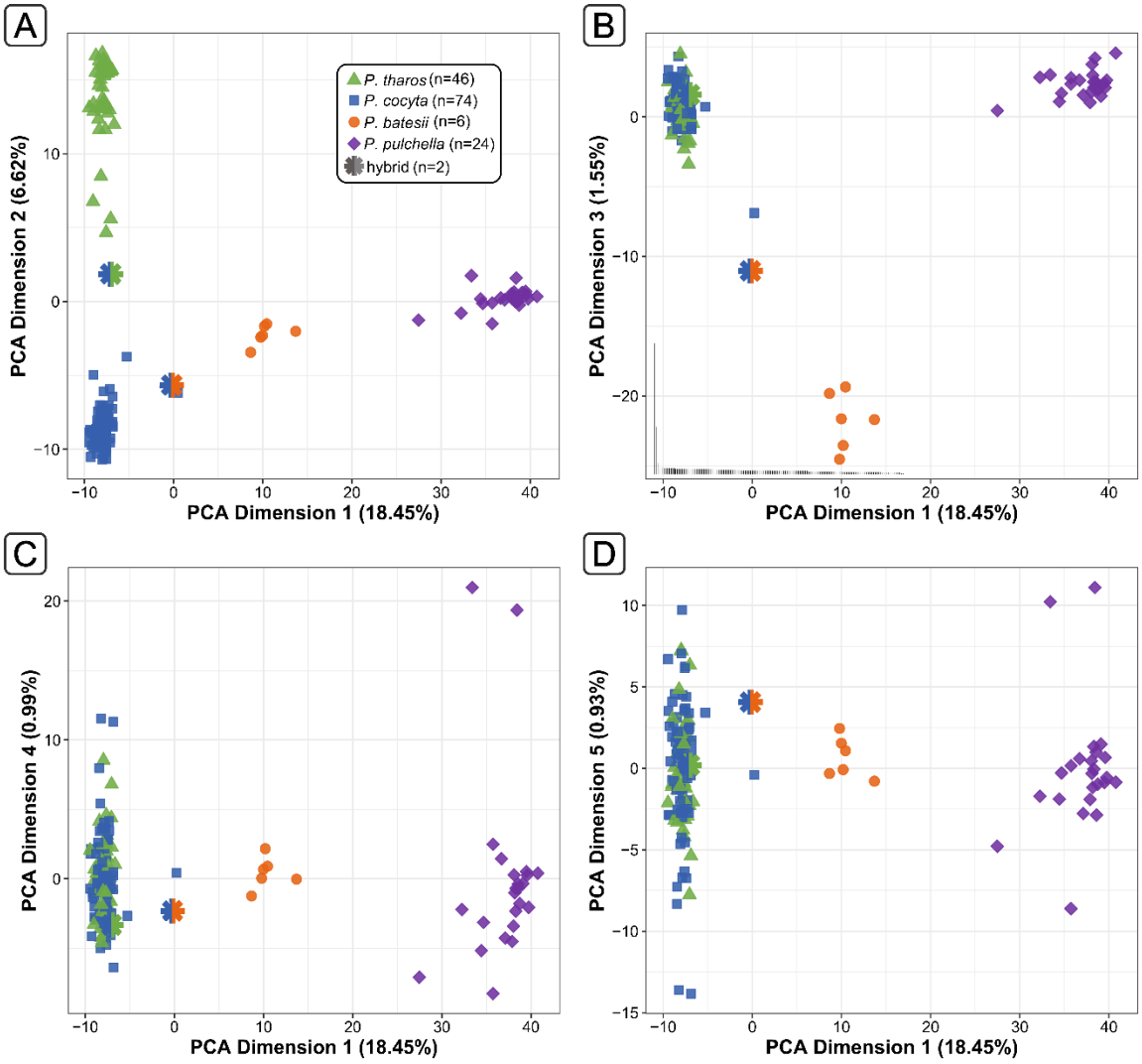


Figure 2.4 Principal component analysis of 1477 SNPs for 152 individuals. **(A)** The first two dimensions indicate that *P. pulchella* is the most genetically distinct and suggests that *P. batesii* is intermediate between *P. cocyta* and *P. pulchella*, as in the SNP tree (Fig. 2.2A) and K=3 STRUCTURE plot (Fig. 2.2B). The two hybrid individuals each appear between their parental species. **(B)** The third dimension demonstrates that *P. batesii* is genetically distinct from the other three species, as in the K=4 STRUCTURE plot (Fig. 2.2B). **(C&D)** The fourth and fifth dimensions separate the *P. pulchella* individuals from the United States from the rest of the species cluster. Two *P. cocyta* from British Columbia (11275 & 11273) are also slightly separated.

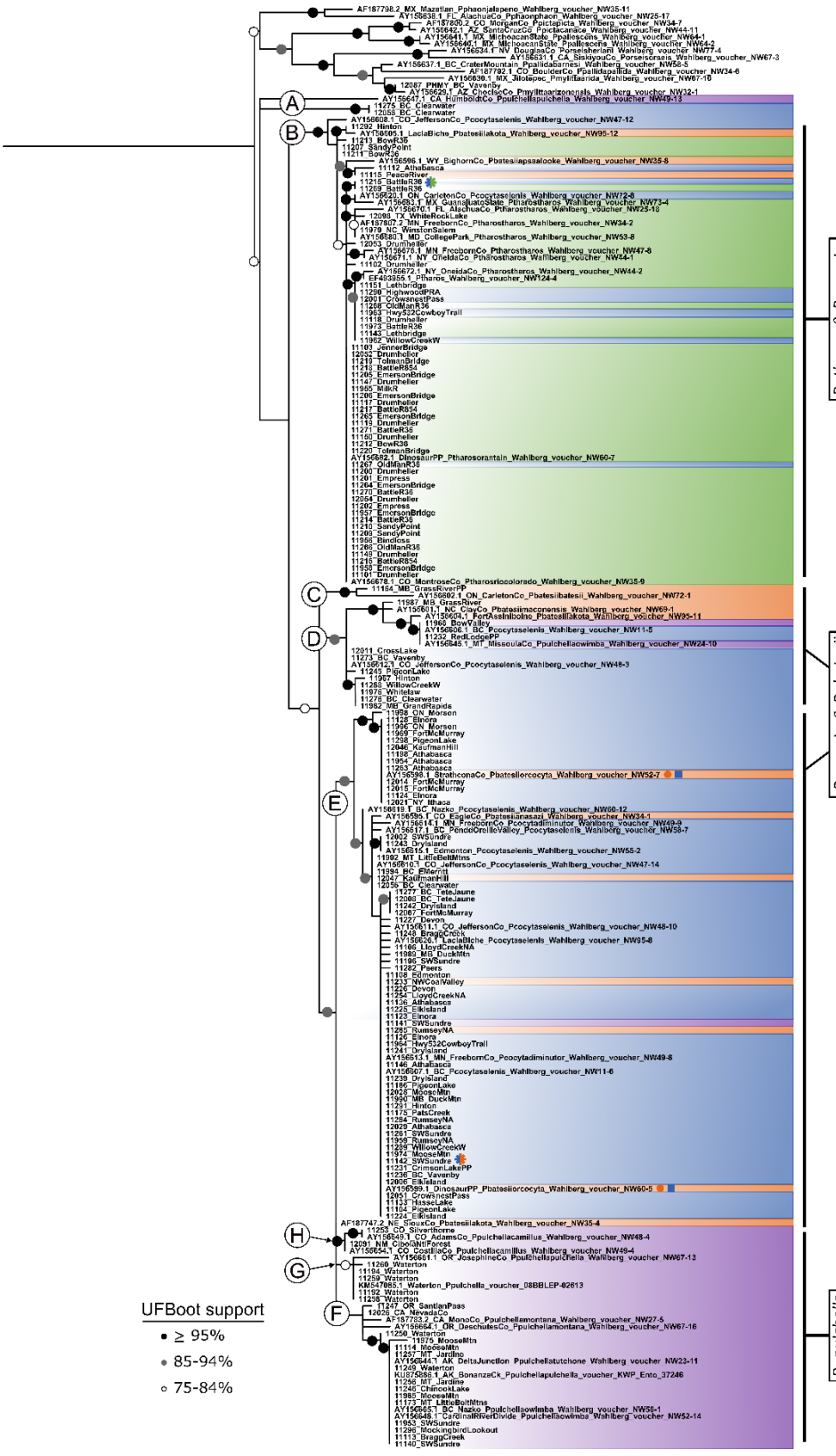

A
B
C
D
E
F
G
H
P. tharos & P. cocyta
P. cocyta & P. batesii
P. pulchella
UFBoot support
≥ 95%
85-94%
75-84%

Figure 2.5 Maximum likelihood tree of 633 bp COI for 270 individuals. (previous page) The 152 novel individuals from this study are coloured based on their species identity in K=4 STRUCTURE analysis (Fig. 2.2B) and the two hybrid individuals are indicated with symbols. Individuals retrieved online have longer names that include their unique identifiers from GenBank and their authors (Appendix 2). They are coloured based on their morphological IDs from these studies. The two individuals from Wahlberg et al. (2003a) that were identified as either *P. batesii* or *P. cocyta* are indicated with symbols for both species. The clade letters on the branches correspond to those identified by Wahlberg et al. (2003a). Branches with <50% UFBoot support were collapsed and supports ≥75% are indicated on the branches.

Chapter 3

Morphological variation among genetic lineages of *Phyciodes* butterflies in Alberta

3.1 Introduction

For centuries, systematists have relied on morphological characters and natural history information to delimit the units of life, thereby linking species delimitation and identification (Balakrishnan 2005; Ahrens et al. 2021). More recently, increasing focus on molecular techniques for delimitation has led to neglect of classical taxonomic data in many groups (Lee 2004), with the disconnection between delimitation and identification leading to some species being described based on molecular information with little or no reference to morphology (e.g., Hebert et al. 2004; Brower 2010; Meierotto et al. 2019; Sharkey et al. 2021). This is problematic because molecular characters can often only be generated by researchers with access to expensive sequencing technology, while species continue to primarily be perceived and identified based on their morphological characters (Lee 2004; Balakrishnan 2005; Zamani et al. 2021).

Citizen science and other biodiversity research approaches commonly depend on observations in nature and so morphological information remains essential to conservation and systematic study (Dunn 2003; Balakrishnan 2005; Acorn 2017; Ríos-Saldaña et al. 2018). Yet relying on morphology alone to distinguish taxonomic units is not without problems; processes like cryptic speciation (Kozlov et al. 2017; Alda et al. 2021), convergence (Gebiola et al. 2012; Li et al. 2021), intraspecific variation (Gebiola et al. 2012; Campbell et al. 2020), and introgression (Coster et al. 2018) can all result in inaccurate or obscure species designations. Molecular taxonomic studies present an opportunity to reexamine classical taxonomic characters in the context of independently derived molecular lineages or clusters (Balakrishnan 2005; Stech et al. 2013). Associating patterns in molecular and morphological data can allow tests of classical taxonomic hypotheses (e.g., Bourguignon et al. 2013; Sistrom et al. 2013; Stech et al. 2013; Gratton et al. 2016), discovery of new species (e.g. Bakkes et al. 2020; Reis et al. 2020), discovery of morphological differences between presumed cryptic species (e.g., Shaklee & Tamaru 1981; Tan et al. 2009; Rittmeyer & Austin 2014; Karanovic et al.

2016; Korshunova et al. 2017; Li 2019; Oliver et al. 2020; Li et al. 2021), and improved diagnostic morphological characters for recognized taxa (Dupuis et al. 2017b; Riva et al. 2020; Smith et al. 2022).

The four currently recognized butterfly species of the *Phyciodes tharos* species group have historically been difficult to identify due to intraspecific variability and overlapping interspecific phenotypes in areas of sympatry (Scott 1994; Wahlberg et al. 2003a; Proshek & Houghton 2012; see Warren et al. 2017 and Lotts & Naberhaus 2020 for example images). Scott (1994) has described morphological variation of the group as a transformational series with incremental specific and subspecific differences progressing from *P. tharos* (Drury, 1773) to *P. cocyta* (Cramer, 1777), *P. batesii* (Reakirt, 1865), and then *P. pulchella* (Boisduval, 1852). Adult females are particularly difficult to distinguish (Layberry et al. 1998; Douglas & Douglas 2005; Hall et al. 2014; Schweitzer et al. 2018) and, in some cases, can only be definitively identified if their eggs are reared to produce a family series that exhibits a range of variation (Scott 2006).

Morphological similarities and overlapping variation between species have resulted in ongoing uncertainty about the number of species in the *P. tharos* species group (Scott 1994; Wahlberg et al. 2003a; Proshek & Houghton 2012). *Phyciodes tharos* and *P. cocyta* were considered conspecific until recently (Oliver 1980; Opler & Krizek 1984; Scott 1994) and were distinguished by differences in their ranges, morphology, ecology, and phenology, along with evidence of hybrid breakdown. *Phyciodes batesii* has also been difficult to distinguish from both *P. tharos* and *P. cocyta* (Layberry et al. 1998; Glassberg 2017), which is particularly problematic because this species has declined drastically in the eastern part of its range (Opler & Krizek 1984; Schweitzer et al. 2018). Although these four species are generally accepted, their boundaries have remained unclear. The use of mitochondrial COI gene sequences did not detect clear species lineages in the *P. tharos* species group (Wahlberg et al. 2003a; Proshek & Houghton 2012), especially between *P. tharos*, *P. cocyta*, and *P. batesii*.

One of the few geographic regions where all four species occur is Alberta, Canada (Brock & Kaufman 2006). This makes Alberta particularly well suited to a comprehensive assessment of their species boundaries (Chapter 2) and the morphological

variation associated with those boundaries. NatureServe (2022) has treated *P. batesii* as "apparently secure" in Alberta. Yet because identifications for this species are almost always tentative, it is difficult to properly assess its conservation status. Genome-wide single-nucleotide polymorphism (SNP) variation in *Phyciodes* sampled primarily in Alberta has now confirmed the presence of four distinct clusters representing the four species in this group (Chapter 2). This presents an opportunity to examine their morphological characters in a quantitative framework using voucher specimens with confirmed genomic identities.

This chapter examines discrete morphological character states that are observable in the field and could potentially be used to sort individuals into their respective species clusters. The aim is to better understand morphological variation in this group and improve identification resources. I ask: 1) does multivariate analysis of morphological data distinguish separate population clusters; 2) are clusters of individuals from morphology congruent with clusters based on molecular variation; and 3) which morphological characters are most consistent with molecular species boundaries based on SNPs. By grounding morphological work in known genomic identifications, this work links species delimitation with species identification. It is the first quantitative assessment of morphological characters in an ordination framework for *Phyciodes* and the first explicit association of morphological characters with molecular data for the group.

3.2 Materials and methods

3.2.1 *Specimen collection and preparation*

I collected *Phyciodes* specimens by aerial net in Alberta and British Columbia during 2016 and 2017 (Appendix 1). Several collaborators provided specimens from other parts of Canada and the United States, as outlined in Chapter 2. Older specimens were available from prior collections by Felix Sperling and local lepidopterists. In total, 213 *Phyciodes* individuals (47 *P. tharos*, 134 *P. cocyta*, 8 *P. batesii*, and 24 *P. pulchella*) were used in this chapter. The 61 additional specimens that were not in Chapter 2 were included here to capture as much morphological variation as possible during character scoring. I assigned species identifications based on STRUCTURE analysis of 1652

informative genome-wide single nucleotide polymorphisms (SNPs) using the sequencing and analysis methods outlined in Chapter 2 (Appendix 6).

Detached wings for each specimen were photographed using a DSLR camera mounted on a copy stand, with wings placed flat under a piece of glass to reduce handling of specimens during character scoring. Voucher wings were stored in glassine envelopes and deposited as vouchers in the E. H. Strickland Entomological Museum as outlined in Chapter 2 (Appendix 1). Antennae were stored with head and abdomen tissues in small vials in a Sperling lab freezer as outlined in Chapter 2.

3.2.2 *Character selection and scoring*

I examined all available butterfly books, guides, and primary literature to compile morphological characters that have been used for species identification in the *Phyciodes tharos* species group. All characters commonly found in the literature that had potentially definable, discrete states were used. Only characters that are observable in the field were considered; thus, genitalic characters and microfeatures of the antennae, such as those described by Scott (1994), were not assessed. Eighteen morphological characters, each with 2-4 states, were selected for character scoring (Figure 3.1, Table 3.2). Fourteen of these characters have been used by multiple authors and four novel characters were selected based on my observations of variation in specimen wings and differences between species. Character states were demarcated by differences in presence, size, completeness, count, clarity, colour, and/or shape. Characters with continuous variation were coded in terms of discrete categories for use in correspondence analyses.

Antennal club colour and wing characters were scored visually (Appendix 7). Wing characters were scored from digital photographs; each specimen was scored for all eighteen characters and then the scoring for each individual character was checked across all specimens to ensure consistency. External genitalia were examined to determine the sex of each specimen.

3.2.3 *Analysis*

Of 4,047 character states (including sex) in 213 individuals, thirteen states across ten individuals were not scorable due to damaged or missing parts of the specimen. These states were imputed using the missMDA package (Josse & Husson 2016) in R 4.0.1 (R Core Team 2020) because subsequent discriminant correspondence analysis (DiCA) does not permit missing data (Appendix 7).

Multiple correspondence analyses (MCA) were performed on the character data using the FactoMineR package (Lê et al. 2008) in R to evaluate the effectiveness of these characters for separating species without known genomic species identities. Multiple correspondence analysis was performed for seven sets of data: all specimens (213 individuals), males only (113 individuals: 31 *P. tharos*, 67 *P. cocyta*, 3 *P. batesii*, 12 *P. pulchella*), females only (100 individuals: 16 *P. tharos*, 67 *P. cocyta*, 3 *P. batesii*, 12 *P. pulchella*, 1 *P. tharos*/*P. cocyta* hybrid, 1 *P. cocyta*/*P. batesii* hybrid), and four two-species analyses (*P. tharos* & *P. cocyta*, *P. tharos* & *P. batesii*, *P. cocyta* & *P. batesii*, *P. batesii* & *P. pulchella*). The proportion of males and females for each species was even, except for *P. tharos*, which had nearly twice as many males as females, and for the two female hybrids. Pairwise analyses were performed for species pairs that are likely to be confused in nature or were not well-separated in analyses of all individuals. Thus, pairwise comparisons were not performed for *P. tharos* & *P. pulchella* or *P. cocyta* & *P. pulchella*. Character state contributions and vtest scores for each of the datasets were obtained using the package factoextra (Kassambara & Mundt 2020).

The imputed dataset was analysed using discriminant correspondence analysis (DiCA) using the ade4 package (Chessel et al. 2004) in R. DiCA maximizes variance between known groups while minimizing variance within them and was used to examine whether the scored morphological characters would separate the species more effectively with known identities. The individuals were assigned to groups based on sex and species identities determined in the STRUCTURE analysis of genomic SNPs. The two individuals identified as hybrids in Chapter 2 were each considered as their own independent groups. MCA and DiCA plots were visualized using the ggplot2 (Wickham 2016) and ggforce (Pedersen 2022) packages in R.

3.3 Results

3.3.1 *Literature survey*

A summary of the morphological character traits used in previous publications to identify members of the *Phyciodes tharos* species group is provided in Table 3.1. This literature review includes information from 34 books, guides, and journal articles that represent a variety of conceptions of this species group. Notably, almost none of the character states used in these publications are considered fixed for any species and several characters have regionally contingent character states due to morphological variation throughout each species' geographic range (see Scott 1994, 1998, 2006 for detailed subspecific information). For instance, antennal club colour (Char. 1 in Table 3.2) is commonly cited, but is highly variable for each species, with major regional differences in *P. tharos*.

The three most cited characters – the ventral hindwing marginal patch (Char. 17 in Table 3.2), the ventral hindwing crescent (Char. 18), and the dorsal forewing median band colour (Char. 3) – are generally used to distinguish *P. tharos* and *P. cocyta* from *P. batesii* and *P. pulchella* (Table 3.1). A paucity of dark patches on the ventral forewing is considered to distinguish *P. pulchella* (Chars. 12, 15, & 16 in Table 3.2) and the stronger dorsal light submarginal crescents on both wings are often used to identify *P. tharos* (Chars. 6 & 10), but each of these characters is also subject to intraspecific variation. The more complete postmedian and median lines on the dorsal forewing are sometimes used to distinguish *P. tharos* from *P. cocyta* (Chars. 4 & 5).

3.3.2 *Scored character state distributions*

In the examined specimens, both sexes of each species exhibited multiple states for most morphological characters (Figure 3.2). Several states were constant in one or both sexes of *P. batesii* or *P. pulchella*. Two states were constant for all specimens of a species: the pale median band on the dorsal forewing (3-1) in *P. batesii* & *P. pulchella* and the pale bars present both in the cell and distal to the cell on the ventral forewing (14-2) in *P. batesii*. The only state that was unique to one species was the complete lack of a

median dark line on the dorsal forewing (5-0) in a few *P. cocyta* individuals. Nearly half of the characters exhibited sexual dimorphism in at least one species and the proportions of many character states were similar between *P. tharos* & *P. cocyta* and/or *P. batesii* & *P. pulchella*. Both sexes of all four species exhibited both states of character 17 (VHW dark patch colour), although the proportions differed slightly.

3.3.3 *Multiple correspondence analyses*

Multiple correspondence analysis (MCA) of character states for all specimens did not produce distinct morphological clusters consistent with each of the four identified genetic species clusters. The first dimension accounted for 12.54% of the variation and separated female *P. batesii* and most *P. pulchella* from *P. tharos* and *P. cocyta* (Figure 3.3A). It also separated most males and females of *P. cocyta* and *P. batesii*. The second and third dimensions accounted for 7.28% and 5.72% of the variation, respectively, and helped further distinguish males from females, *P. pulchella* from *P. batesii*, and *P. tharos* from *P. cocyta* (Figure 3.3A-B). In general, few *P. tharos* individuals appeared outside of the *P. cocyta* range of variation. On the third dimension, the *P. cocyta*/*P. batesii* hybrid grouped with female *P. cocyta* and the *P. tharos*/*P. cocyta* hybrid clustered with female *P. tharos*. Specimens from outside Alberta did not cluster geographically (Appendix 8); however, the male *P. batesii* individual from Manitoba grouped with male *P. pulchella* on the second dimension and the female *P. pulchella* individual from California appeared near female *P. cocyta* and *P. tharos*.

The MCA of males showed slightly more differentiation between species. The first two dimensions accounted for 15.88% and 7.44% of the variation, respectively, and clearly separated *P. tharos* & *P. cocyta* from *P. batesii* & *P. pulchella*, with the latter two species forming distinct clusters (Figure 3.3C). The second dimension separated some additional *P. tharos* and *P. cocyta*, but much overlap remained; approximately half of the *P. tharos* individuals were distinguished from *P. cocyta* while approximately three quarters of *P. cocyta* were distinguished from *P. tharos*. The third dimension accounted for 6.23% of the variation but did not further separate the species (Figure 3.3D).

The MCA of females exhibited the least separation of species. The first and second dimensions accounted for 10% and 7.5% of the variation, respectively, and separated *P. tharos* & *P. cocyta* from *P. batesii* & *P. pulchella* with major overlap in both groups and little space between them (Figure 3.3E). A similar proportion of *P. tharos* were distinguishable on the second dimension compared to the males, but only approximately one third of *P. cocyta* females were distinguishable from *P. tharos*. The third dimension accounted for 5.77% of the variation and distinguished slightly more *P. cocyta* but fewer *P. tharos*. The two hybrid individuals clustered on the third dimension as they did in the analysis of all individuals (Figure 3.3F).

Only a few of the pairwise analyses separated the species more clearly (Figures 3.4, 3.5). The first two dimensions of the *P. tharos*/*P. cocyta* analysis were slightly more effective for separating females of the two species and male *P. tharos* from female *P. cocyta* (Figure 3.4A). The third dimension of the *P. tharos*/*P. batesii* analysis more clearly separated male *P. batesii* from female *P. tharos* (Figure 3.4D). Finally, the first dimension of the *P. batesii*/*P. pulchella* analysis (Figure 3.5C-D) more clearly separated male and female *P. pulchella*. Otherwise, the two-species analyses produced similar or worse results than the analyses of all species.

A summary of character states that may be useful for identifying each species is provided in Table 3.3. None of these characters are diagnostic but were selected based on the proportion of individuals they occurred in for each species (Figure 3.2) as well as their contributions to the MCA analyses (Appendix 9-18). Many character states were useful for separating *P. tharos* & *P. cocyta* from *P. batesii* & *P. pulchella*, but few were helpful within these groups. Some character states were useful for separating species when only males, females, or a pair of species was considered.

3.3.4 *Discriminant correspondence analyses*

The discriminant correspondence analyses (DiCA) produced similar results to the MCA, despite the inclusion of known genomic species identities. In the DiCA of all individuals (Appendix 19A-B), the first two dimensions explained 37.94% and 23.33% of

the variation, respectively, and grouped the species as in the first two dimensions of the main MCA. There was greater overlap between some groups and although several male *P. cocyta* grouped more distinctly from *P. tharos*, there was more overlap between these species overall than in the MCA. The third dimension explained 15.77% of the variation and, along with the first dimension, exhibited the best separation of male *P. tharos* and *P. cocyta* in all the analyses, but demonstrated increased overlap in other groups. The first two dimensions of the male DiCA (Appendix 19C) explained 56.46% and 32.79% of the variation, respectively, and separated *P. tharos* and *P. cocyta* slightly more effectively than the male MCA (Figure 3.3C-D). The third dimension explained the remaining 10.75% of the variation but did not provide additional clarity (Appendix 19D). The first three dimensions of the female DiCA (Appendix 19E-F) explained 45.04%, 25.53%, and 16.19% of the variation, respectively. Compared to the female MCA (Figure 3.3E-F), this analysis better distinguished *P. batesii* from *P. pulchella* on all three dimensions and *P. cocyta* from *P. tharos* on the first two dimensions.

3.4 Discussion

The findings of this study are consistent with historical perceptions of the *Phyciodes tharos* species group based on morphology and the genomic relationships found using SNPs (Chapter 2). They also confirm that the morphological characters are only weakly associated with the species boundaries and that visual identifications are not always possible. Finally, they demonstrate the approximate proportions of individuals that are indistinguishable and provide insights into which combinations of characters are best for attempting identifications.

3.4.1 *Morphological variation and historical perceptions of the* P. tharos *species group*

The continuity of morphological variation, within and between the *Phyciodes tharos* species group genomic lineages, partly resembles the transformational series described by Scott (1994). He described incremental morphological change from *P. tharos* through *P. cocyta*, *P. batesii*, and finally *P. pulchella*. This pattern was found by correspondence analyses of both sexes, but with *P. cocyta* at one end of the continuum

rather than *P. tharos* (Figure 3.3; Appendix 19). The results also support the observation that males are generally more reliably separated than females; this has been emphasized by many authors who suggest that the identification of females is sometimes not possible (Layberry et al. 1998; Douglas & Douglas 2005; Scott 2006; Hall et al. 2014; Schweitzer et al. 2018). The intraspecific morphological similarities between all four species can, at least in part, be attributed to relatively recent divergence (Wahlberg & Freitas 2007; Long et al. 2014) and both ancient and ongoing introgression (Wahlberg et al. 2003a; Proshek & Houghton 2012; Chapter 2).

The clearest distinction was between the species pairs *P. batesii* & *P. pulchella* and *P. tharos* & *P. cocyta* on the first dimension of the ordination plots, especially in the sex-specific analyses (Figure 3.3; Appendix 19). This reflects how the most highly cited characters in the literature are primarily used to separate these two groups (Table 3.1). It is also consistent with the genetic relatedness found between these species (Figures 2.2B, 2.4). Yet, some individuals between these groups were also similar (Figure 3.3A-B; Appendix 19A-B,E-F). The overlap of male *P. batesii* with female *P. tharos* and female *P. cocyta* reflects how difficult it can be to distinguish these species. Some sources state that records of *P. batesii* may be misidentifications of *P. tharos* or *P. cocyta* and vice versa (e.g., Layberry et al. 1998; Glassberg 1999, 2017). On the other hand, the overlap of female *P. pulchella* with female *P. cocyta*, which only occurred in the DiCA (Appendix 19A-B), was not expected because confusion between these species is rarely mentioned, likely due to their limited overlapping geographic range (Brock & Kaufman 2006). The morphological overlap of *P. tharos* and *P. cocyta* with *P. batesii* and *P. pulchella* occurred at the edge of the range of variation of each species and thus only affects a minority of individuals.

Distinctions within each pair of species were less evident. Males of *P. batesii* and *P. pulchella* were mostly distinct while females of the two species overlapped (Figures 3.3, 3.5C-D), except in the DiCA (Appendix 19E-F). This is consistent with the consideration of these species as having an overall darker appearance with a pale median band (Scott 1994; Bird et al. 1995). A large proportion of same-sex *P. tharos* and *P. cocyta* were also indifferentiable (Figures 3.3, 3.4A-B; Appendix 19). These species were

long considered conspecific subspecies due to their morphological similarities and were only recently separated (Oliver 1980; Opler & Krizek 1984). However, it is surprising that the males were nearly as indistinguishable as the females because they have typically been considered more diagnosable (Scott 1994; Layberry et al. 1998; Cech & Tudor 2005; Douglas & Douglas 2005; Hall et al. 2014).

3.4.2 *Effectiveness of characters for species identification*

Despite partial congruence of the ordination analyses with the species boundaries, none of the character states can be considered diagnostic. Most characters were highly variable for each species, thus limiting their utility as identification tools (Figure 3.2). Though some were consistent for one or both sexes of *P. batesii* or *P. pulchella*, this was likely due in part to these species' low sample sizes. The only character that was unique to one species was the lack of a median dark line on the dorsal forewing in *P. cocyta* (5-0), but this was limited to a small number of individuals. The high level of variability is consistent with that found in the literature (Table 3.1). Even in recent literature, the character states proposed for discerning species are quite varied in their usage and their descriptions.

The ineffectiveness of some characters for identification in this study, including some that were highly cited, may also be due, in part, to their simplification into discrete states. For instance, the VHW dark marginal patch (Char. 17) was simplified to presence or absence which does not capture all potential variation in colour, size, and strength (Table 3.1). These two states should have at least recovered+ a difference between the main species pairs (*P. tharos* & *P. cocyta*; *P. batesii* & *P. pulchella*) based on several authors' accounts, but instead this was one of the worst-performing characters.

3.4.3 *Implications for identification & monitoring*

Ordination analyses of character states did not reveal reliable diagnostic characters for any of the species, but the results can be used as a resource for examining and monitoring these species in Alberta and neighbouring areas. As always, the best

practice for identification includes consideration of sex, locality, and time of year. In addition, one should observe the combination of character states exhibited by an individual and consider the likelihood of different species identifications with reference to Figures 3.1 & 3.2 and Tables 3.2 & 3.3. When consulting Figure 3.2, attention should be paid to the sample size for each species. Some character states will provide more certainty than others while individuals on the edge of each species' morphological variation will likely exhibit strongly conflicting characters that will shed doubt on any identification.

Phyciodes pulchella should usually be recognizable based on a combination of characters, including a straighter wing margin (2-0) a darker overall appearance on the dorsal side with broader black markings (4-3, 5-3), a single submarginal crescent on the dorsal forewing in males (6-0), and few or small dark patches on the ventral side (12-0/1, 15-0/1, 16-0/1) (Figure 3.2; Table 3.3). Locality should also be helpful, though there are areas of sympatry with the other species at lower elevations. *Phyciodes batesii* can have a similar appearance to *P. pulchella* but may be more likely to exhibit a black antennal club (1-0), an incomplete or narrow postmedian dark line on the dorsal forewing (4-1/2), a medium or large subapical costal patch on the ventral forewing (12-2/3), and a moderate to large posterior median spot on the ventral forewing (16-2/3).

For confident identifications of *P. tharos*, *P. cocyta*, and *P. batesii*, an effort should be made to look at males and females separately. Male *P. batesii* should be differentiable from male *P. tharos* and *P. cocyta* based on a combination of characters, including a black or brown antennal club (1-0/1), a pale median band (3-1) and a complete postmedian dark line (4-2/3) on the dorsal forewing, obscured anterior & posterior postmedian spots on the dorsal hindwing (7-2), and two pale bars on the ventral forewing (14-2). Male *P. cocyta* should be differentiable in most cases based on an incomplete median dark line on the dorsal forewing (5-1). Laterally detached medial dark loops on the dorsal forewing are also common in this species (8-1). Finally, male *P. tharos* is more likely to exhibit a white or translucent crescent (18-1) on the ventral hindwing than *P. cocyta* and *P. batesii* and is more likely to have a complete median dark line on the dorsal forewing (5-2/3) than *P. cocyta*.

Ideally, females of all species should be identified based on association with males. A population approach to identification can be used by observing several individuals from a single locality on a single collection date, thus observing the typical character states of both sexes. This approach should be used with caution in areas of sympatry and may not always be useful for *P. batesii* since this species does not appear to be common in large numbers. All the *P. batesii* in this study were collected as singletons except the two Manitoba specimens.

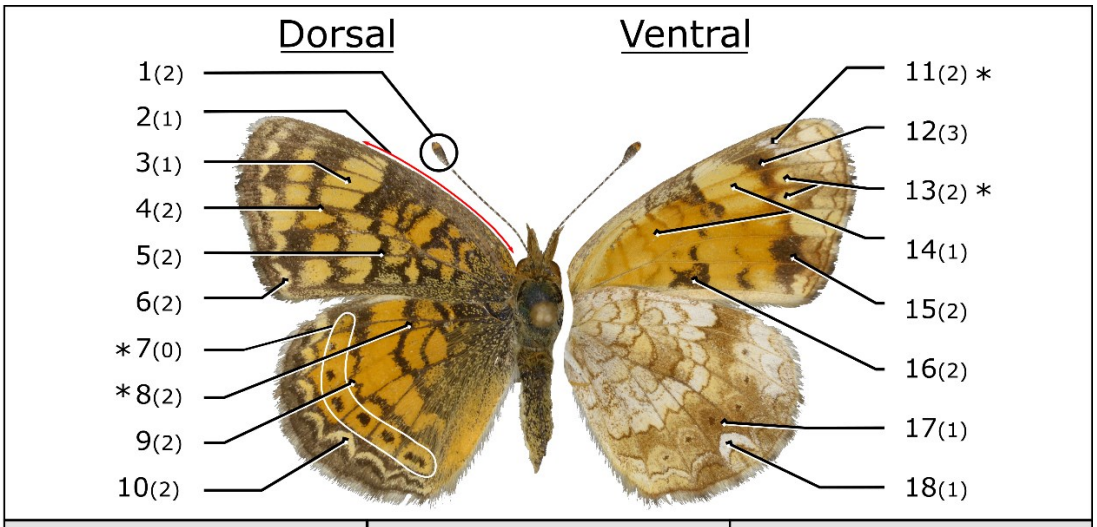
Based on the individuals examined in this study, *P. batesii* is the most difficult species to discern because its morphological variation almost entirely falls within that of all three of the other species. This is based on a low sample size – six individuals surely do not represent the amount of variation found in these species, even when restricted to Alberta and Manitoba. Yet these findings are consistent with the literature. *Phyciodes batesii* also occurs entirely within the geographic range and phenological flight times of *P. cocyta* (Acorn 1993; Bird et al. 1995; Layberry et al. 1998; Brock & Kaufman 2006) which makes monitoring difficult. At present there is no sign of decline in Alberta for *P. tharos*, *P. cocyta*, or *P. pulchella* (NatureServe 2022). *Phyciodes batesii* is considered "apparently secure" in Alberta (NatureServe 2022) but the scarcity of individuals in this study indicates that it may not be. Given this species' decline in the eastern part of its range (Douglas & Douglas 2005; Hall et al. 2014; Acorn & Sheldon 2017; Schweitzer et al. 2018) and "special concern" status in British Columbia (Guppy & Shepard 2001; Acorn & Sheldon 2006), it should be monitored. Molecular information may be important for tracking the status of *P. batesii* and a closer look at areas where the *P. batesii* genotype is confirmed in this study could help further our understanding of the morphological variation, phenology, and rate of occurrence.

3.5 Conclusions

This study is the first to associate genomic species delimitation with morphological characters in a quantitative framework for the *Phyciodes tharos* species group. It confirms that morphology does not fully correspond with the species boundaries and further exemplifies the limits of morphology for this group. No truly diagnostic

characters were identified, which serves as a caution for designating species identities based on morphology. However, I provided the proportion of character states found for each species, which can be used to assess the likelihood of potential identifications. Scientific understanding of the *P. tharos* species group would benefit from more extensive sampling across the ranges of all four species and similar work at a subspecific level. Morphological features that are not necessarily detectable in the field may also elucidate patterns useful for reliable identifications and should be considered in future work. Finally, I recommend that molecular taxonomic studies incorporate classical taxonomic data, to maintain the link between delimitation and identification, and to ensure their results are useful to a broad audience.

a)


Dorsal
Ventral
1(2)
2(1)
3(1)
4(2)
5(2)
6(2)
*7(0)
*8(2)
9(2)
10(2)
11(2) *
12(3)
13(2) *
14(1)
15(2)
16(2)
17(1)
18(1)

b)

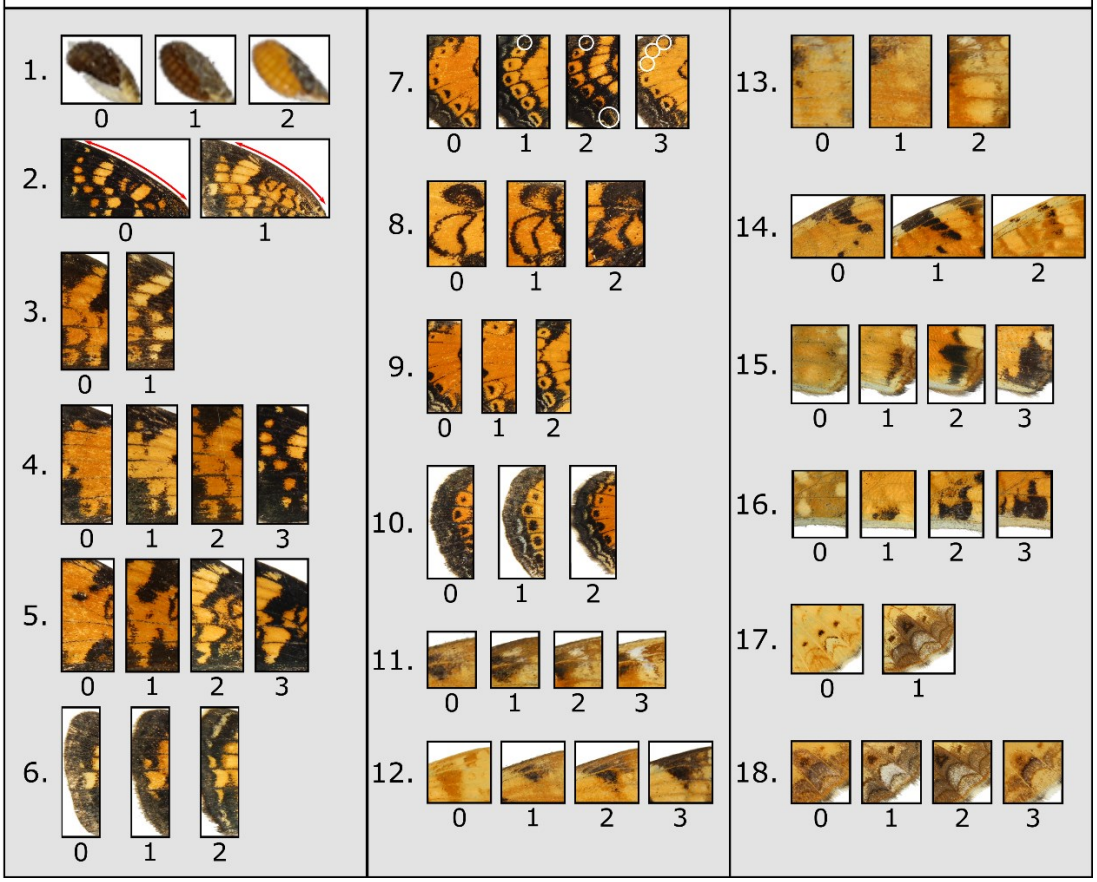

1.
0 1 2
2.
0 1
3.
0 1
4.
0 1 2 3
5.
0 1 2 3
6.
0 1 2
7.
0 1 2 3
8.
0 1 2
9.
0 1 2
10.
0 1 2
11.
0 1 2 3
12.
0 1 2 3
13.
0 1 2
14.
0 1 2
15.
0 1 2 3
16.
0 1 2 3
17.
0 1
18.
0 1 2 3

Figure 3.1 Diagram of characters and states selected for scoring. (previous page) **(a)** Antennal and wing characters selected for visual scoring are shown on a *P. tharos* individual. Asterisks indicate novel characters used in this study. Character numbers are shown with the corresponding character states for the example individual in parentheses. **(b)** Example images of character states are shown. Corresponding character state descriptions are provided in Table 3.2.

Whole *P. tharos* and antennal images (Char. 1) are courtesy of Norbert Kondla. Images of wing character states were taken of study specimens by BDW.

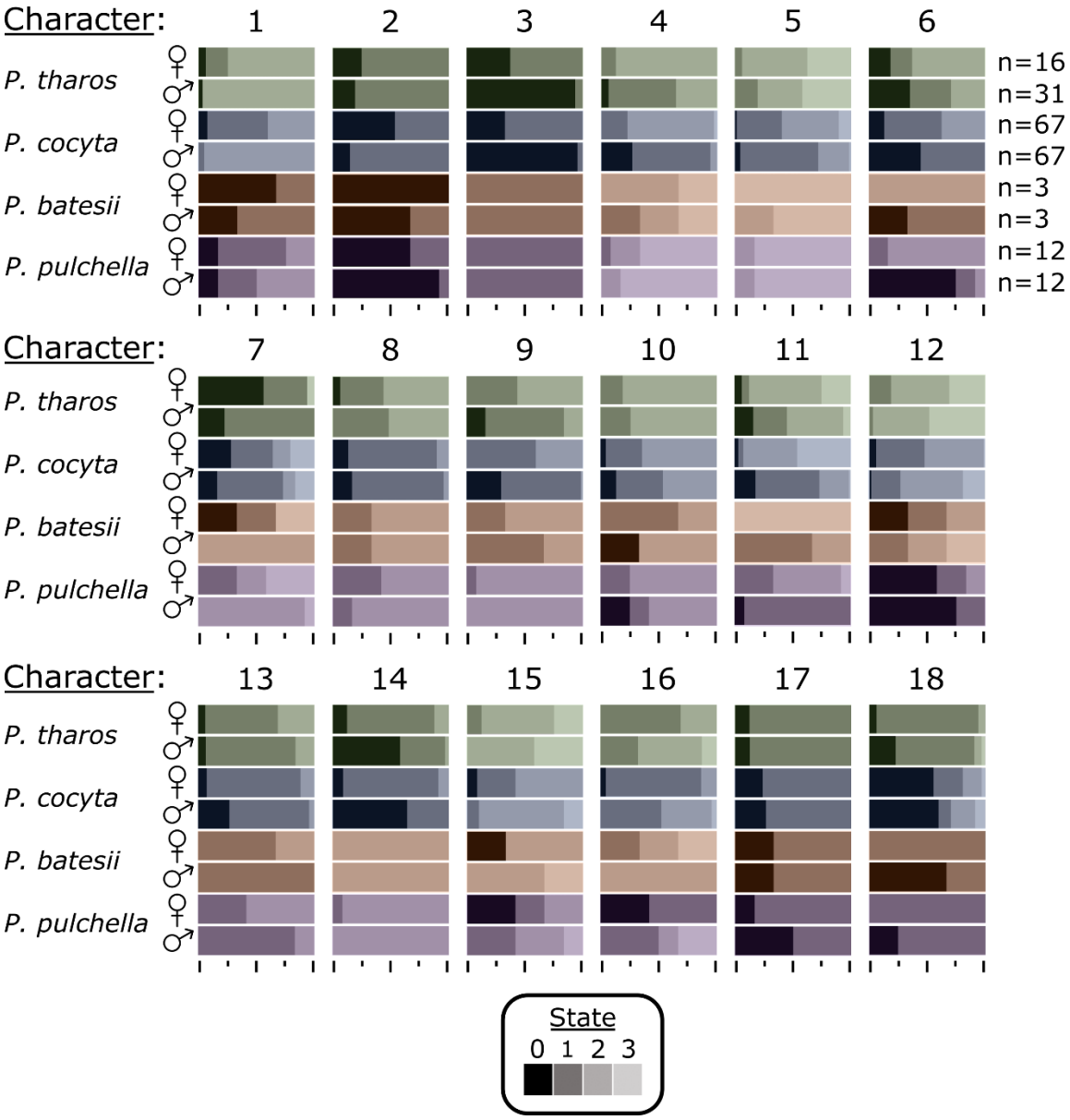


Figure 3.2 Distribution of morphological character states. Distributions are separated by species and sex. Hybrids are excluded from this diagram. Character and state numbers correspond to those in Figure 3.1 and Table 3.2.

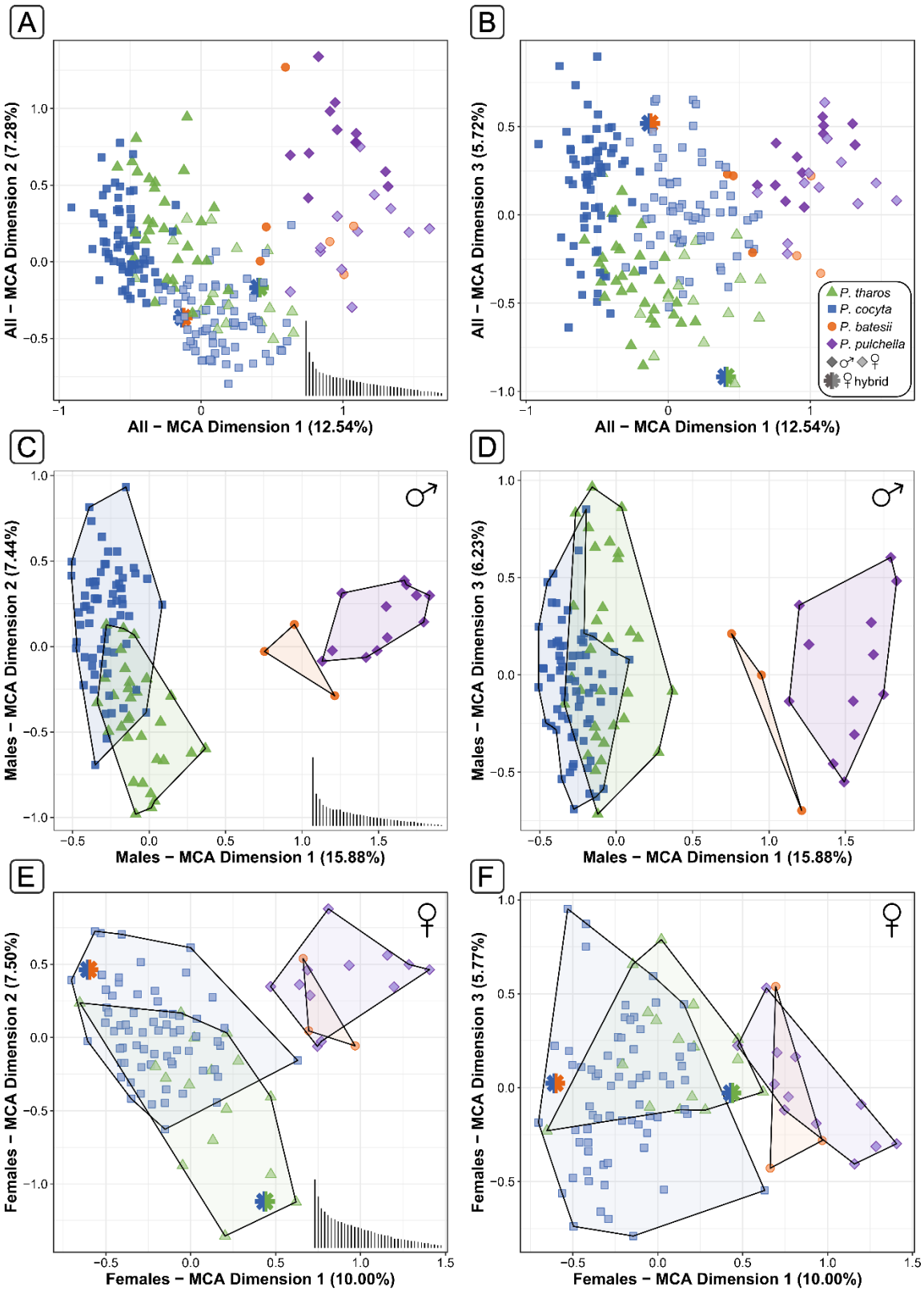

A
All − MCA Dimension 2 (7.28%)
All − MCA Dimension 1 (12.54%)
B
All − MCA Dimension 3 (5.72%)
All − MCA Dimension 1 (12.54%)
P. tharos
P. cocyta
P. batesii
P. pulchella
♂ ♀
♀ hybrid
C
Males − MCA Dimension 2 (7.44%)
Males − MCA Dimension 1 (15.88%)
D
Males − MCA Dimension 3 (6.23%)
Males − MCA Dimension 1 (15.88%)
E
Females − MCA Dimension 2 (7.50%)
Females − MCA Dimension 1 (10.00%)
F
Females − MCA Dimension 3 (5.77%)
Females − MCA Dimension 1 (10.00%)

Figure 3.3 Multiple correspondence analyses for morphological character data. (previous page) **(A)** The first two dimensions for all 213 individuals largely separate *P. batesii* and *P. pulchella* from *P. tharos* and *P. cocyta*, and males and females of the latter two species. **(B)** The first and third dimensions show the hybrid individuals clustering with females of one of their parental species. **(C)** The first two dimensions of the analysis of male individuals exhibits the most clarity between species, with overlap only occurring between some *P. tharos* and *P. cocyta*. **(D)** The third dimension of the male analysis does not provide additional clarity. **(E&F)** The first three dimensions of the analysis of female individuals provide little clarity except between *P. tharos*/*P. cocyta* and *P. batesii*/*P. pulchella.*

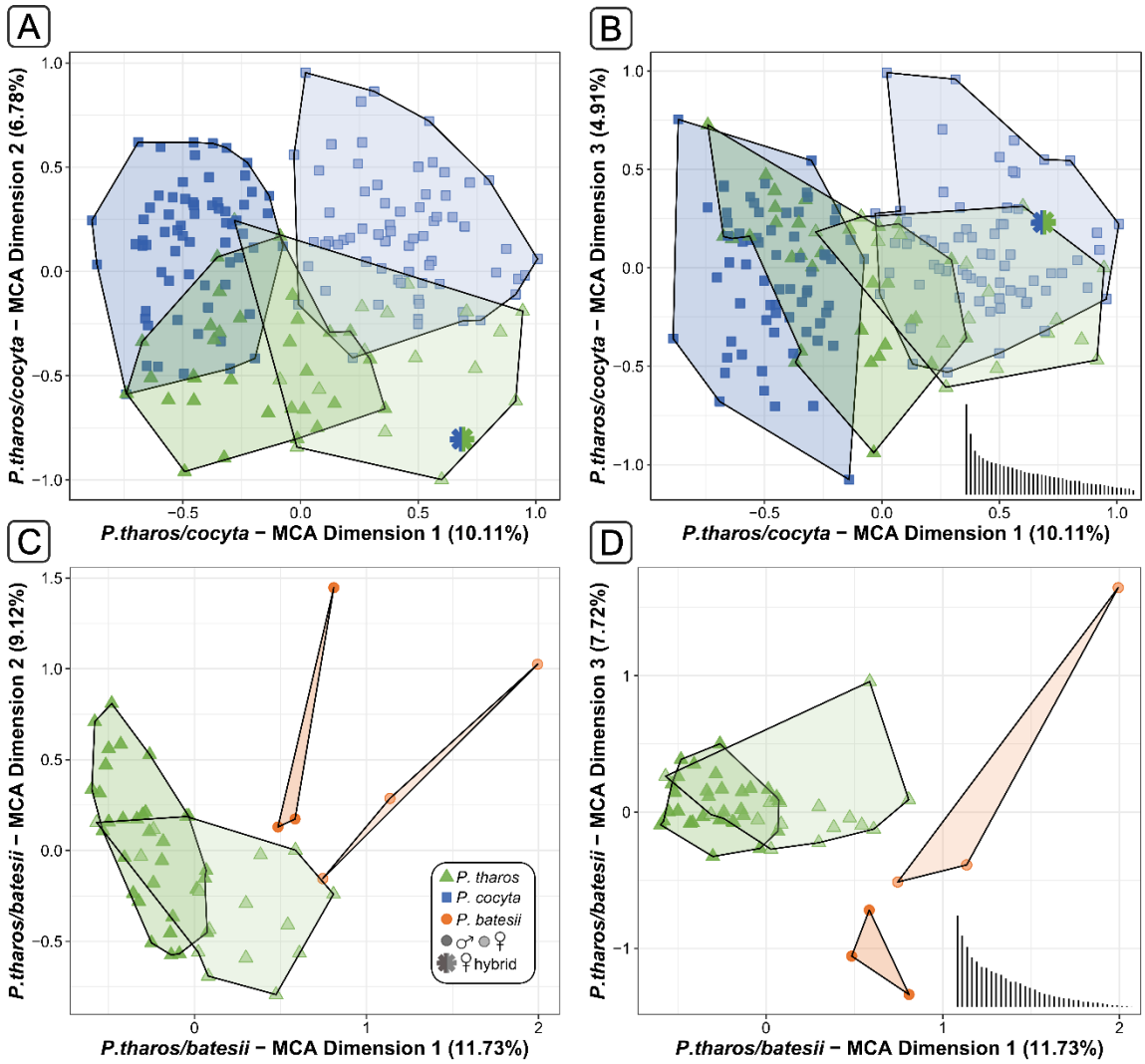


Figure 3.4 Two-species MCA plots for *P. tharos*, *P. cocyta*, and *P. batesii*. **(A)** Dimensions 1 and 2 for *P. tharos* and *P. cocyta*. **(B)** Dimensions 1 and 3 for *P. tharos* and *P. cocyta*. **(C)** Dimensions 1 and 2 for *P. tharos* and *P. batesii*. **(D)** Dimensions 1 and 3 for *P. tharos* and *P. batesii*.

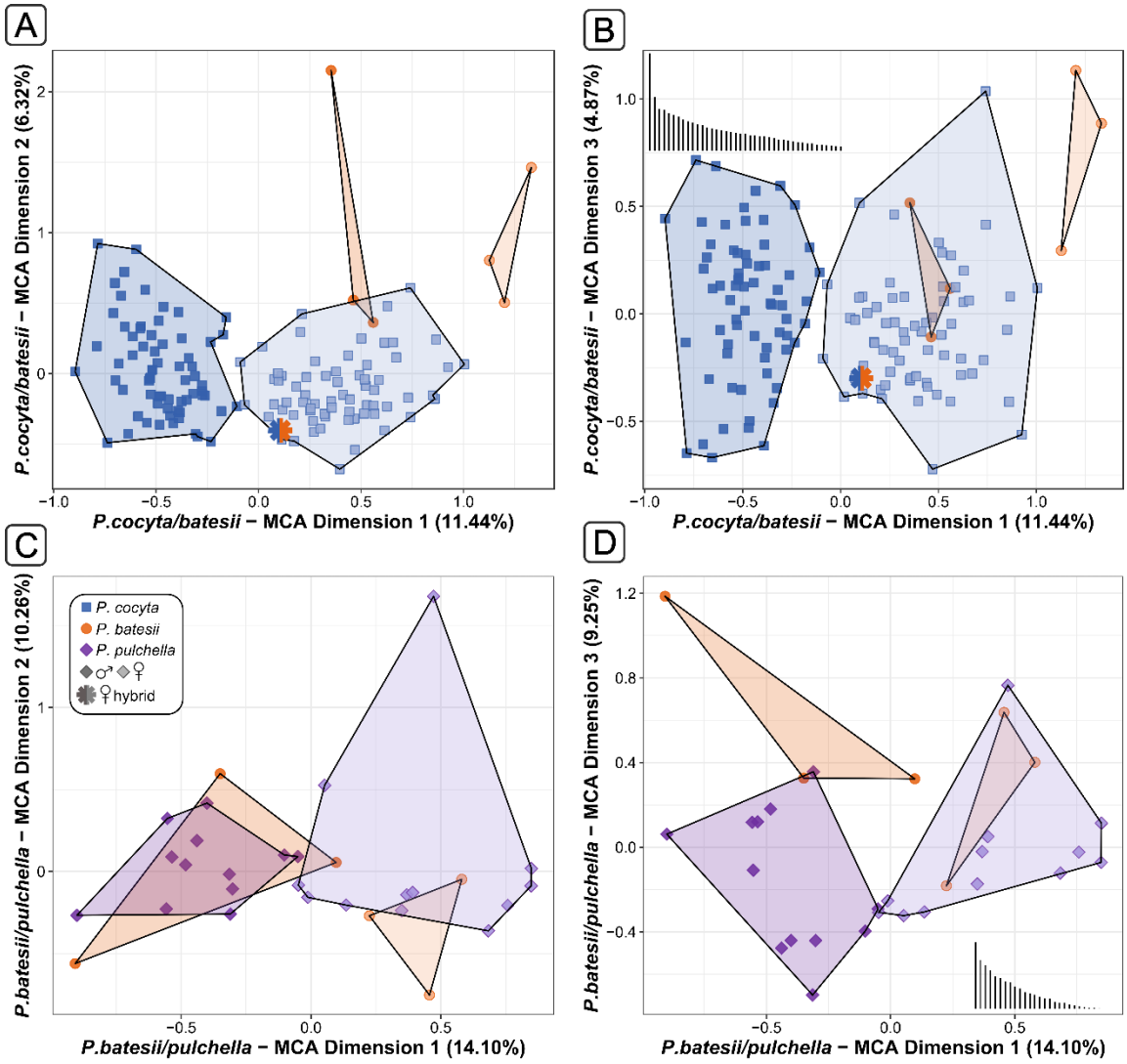


Figure 3.5 Two-species MCA plots for *P. cocyta*, *P. batesii*, and *P. pulchella*. **(A)** Dimensions 1 and 2 for *P. cocyta* and *P. batesii*. **(B)** Dimensions 1 and 3 for *P. cocyta* and *P. batesii*. **(C)** Dimensions 1 and 2 for *P. batesii* and *P. pulchella*. **(D)** Dimensions 1 and 3 for *P. batesii* and *P. pulchella*.

Table 3.1 Morphological characters used to delimit and identify *Phyciodes tharos* group butterflies in the literature. Band and line names are based on Ehrlich & Ehrlich (1961).

Character	*P. tharos*	*P. cocyta*	*P. batesii*	*P. pulchella*
Antennal club colour	Orange (N&W)[1,3,5,8,15-20,22,28-31]; dark grey-black or black & white (S&E)[3,8,11,13,18,22,24,29]; ♀ orange & black (E)[15,16]	Orange[1-3,5,8,11,14-16,18-20,22,26,28-30]; some ♀ brown[14,30]	Black[2,3,5,11,17,19,20,22,28,29]; brown-black[30]; rarely orange[32]; orange (CO)[14]	Orange[5,26,27,31]; brown[7,26,27]; dark[2,30]; black[7]; some ♀ orange-brown[30]
Costal margin curvature	Fairly rounded[32]; gently to strongly curved[5]	Fairly rounded[32]; gently to strongly curved[5]	Fairly rounded[32]	Avg. straighter[32]; straight to gently curved[5]
DFW – Median band	Orange, sometimes ♀ pale[30]; ♀ more uniformly orange than *co*/*ba*[7,15,16,19,20,29]; often slightly yellow (E)[8]	Orange[30]; ♀ often pale[2,3,7,15,16,18,19,22,28,30]; ♂ usually more uniform than *ba*[29]; ♂ creamier (W CO)[14]	Pale[6,7,12,14,20-22,25,28,30,34]; yellowish[8,11]	Usually pale[7,10,12,19,24,30], esp. ♀[10]; ♂ less apparent in some areas[26,31]; dark yellow[4]; ♀ yellowish orange, ♂ orange[5]
DFW – Postmedian line	Usually complete, narrow[5,29,30]; often present[19]	Incomplete/absent[5]	Black lines crossing orange[5,20]	Complete, wide, divides wing[5]
DFW – Median line	Present in ♂[19]	Extensive orange areas[5,9,10,19,20,29]; ♂ usually incomplete[19]	Black lines crossing orange areas[5,20]	Orange in patches and interrupted bands[10]
DFW – Submarginal crescents	Many small, white crescents[1] in ♂[11]; pale whitish jagged line in ♀[19,20]; prominent crescent in cell M_3[12]; often creamy[29]	Wide, solid black margins[5,20,22,28,34], esp. in ♂[9-11,19]; few, if any, small white crescents[1,29]; prominent in cell M_3[12]; ♀ fw only[19]	Wide, black margins[5,20,25]; dark borders like *th*/*co*[29]; crescents absent or indistinct in ♂, except in cell M_3[12]	Crescents absent or indistinct in ♂, except in cell M_3[12]
DHW – Postmedian line	Divides orange center, but may be interrupted[14,30,31]	Orange center not divided[14,30]	Orange center usually divided[30]	Divided[30]
DHW – Submarginal crescents	Strong pale wavy band[5,20,31] of crescents[30]; often creamy[29]; many small white crescents[1] in ♂[11]; ♀ with pale whitish jagged line[19,20]	Weak or absent band of crescents[1,29,30]; wide, solid black margins[5,20,22,28,34], esp. in ♂[9-11,19]; ♀ seldom have jagged line (if so, not whitish)[19,20]	Wide, black margins[5,20,25], like *th*/*co*[29]; weak, rarely strong, band of crescents[30]; ♀ lack thin whitish line[20]	Weaker band of crescents[30]
VFW – Black subapical costal patch	Large[30]; larger than median[28]	Large[30]; larger than median[28]; patches reduced (CO)[14]	Small[8] to large[14,30,32]	Patches reduced[5,12,13,22,25,29,34]; orange-brown[12]; orangish[30]
VFW – Pale/yellow bar	Lacking[12,13,30] or reduced[26]; if present in ♀, becomes orange[19]	Lacking[12,13,27,30] in ♀[26]; if present in ♀, becomes orange[19]	Absent, weak, or present[30]; light[25]; if present in ♀, becomes orange[19]; ♂ often yellow[19]	Often present[7]; yellow[19,29,30]; pale[13,14,26,27]; cream coloured[21]

Character	*P. tharos*	*P. cocyta*	*P. batesii*	*P. pulchella*
VFW – Black tornus spot	Large, rarely giant, narrower in ♀[30]	Large, narrower in ♀[30]; patches reduced (CO)[14]	Small to giant[14,30,32]; narrower in ♀[30]	Patches reduced[5,12,13,22,25,29,34]; narrow dash[30,31], some larger[31]; narrower in ♀[30]; orange-brown[12]
VFW – Posterior median black spot	Small or large[30]	Small or large[30]; patches reduced (CO)[14]; not connected to anterior spot (SK)[19]	Present[5]; equal[30] to or larger than[20,21,28,29] subapical; very large to very small[30,32]; larger[14,25] than *th*[21]; connects to anterior[12,28,29,34] in ♂[19]; some reduced (CO)[14]	Patches reduced[5,12,13,22,25,29,34]; absent to large[31]; small[30]; conspicuous, rectangular[9]; orange-brown[12]
VHW – Dark marginal patch	Strong[15,16,28,30,33]; dark[1,13,21]; brown[7,12,15,16,25,30,33]; black[3,24] in ♂ (ON)[18]; purplish[25] in ♂[9,17]; larger than *ba*/*pu*[1]; often obscures crescent[7]	Strong[8,10,26,27,30]; dark[1,5,13,18,34]; not as dark as *th*[28]; not black[24]; brown[3,5,8,10,12,25-28,30,34]; gray-purple[2]; purplish[25] in ♂[9]; larger than *ba*/*pu*[1,18]; encompasses crescent[5,34]; less extensive in ♀[4,27]	Absent to small[7,8,11,13,18,21,22,25,30] to modest[8,30] to large[30]; markings reduced[15]; "clinal" variation[30]; smaller than *th*/*co*[1]; present in some, brown[16]; always present (AB)[30]	Vague/weak[5,25,30]; usually lacking[21]; smaller[1] and paler[31] than *th*/*co*; some larger[29] or stronger[31]; dark or pale[13]; brown[5,20,25,29,30]; orange-brown, if present[31]; ♂ lacks purple flush[17]
VHW – Crescent	Pale/light[7,15,16,25,33]; silvery/pearlescent[8,11,18,22]; ♀ white[17], ♀ usually cream[30]; ♂ inconsistent/absent[3,30], yellow, or few white[30], obscured by purple flush[17]	Pale/light[16,25]; some pearl/opalescent[19,20,26,27]; pale purplish to dark brown[18]; ♀ usually cream[14,30] to white[2,3,14]; ♂ obscure or absent[2,3,14,30], yellow or few white[30]	Ground colour[17]; tawny[2] (not all[19]); some silver (E)[8]; pale yellow (ON)[18]; ♀ usually cream[30]; inconspicuous[25,34] in ♂[3]; ⅔ present in ♂, pale[30], yellow, cream[14,30], or brown[14]	Ground colour[17]; light/pale[5,20,23,25]; white[4]; some yellower[31]; darkish[2]; ♀ cream, ♂ weak[30]

[1] Acorn (1993); [2] Acorn & Sheldon (2006); [3] Acorn & Sheldon (2017); [4] Angel (2005); [5] Bird et al. (1995); [6] Bouseman & Sternburg (2001); [7] Brock & Kaufman (2006); [8] Cech & Tudor (2005); [9] Christensen (1981); [10] Dornfeld (1980); [11] Douglas & Douglas (2005); [12] Ehrlich & Ehrlich (1961); [13] Ferris & Brown (1981); [14] Fisher (2006); [15] Glassberg (1993); [16] Glassberg (1999); [17] Guppy & Shepard (2001); [18] Hall et al. (2014); [19] Hooper (1973); [20] Klassen et al. (1998); [21] Klots (1951); [22] Layberry et al. (1998); [23] Neill (2001); [24] Opler & Krizek (1984); [25] Pyle (1981); [26] Pyle (2002); [27] Pyle & LaBar (2018); [28] Royer (1988); [29] Scott (1986a); [30] Scott (1994); [31] Scott (1998); [32] Scott (2006); [33] Spencer (2006); [34] Tilden & Smith (1986)

Abbreviations:
D – dorsal; V – ventral; FW – forewing; HW – hindwing; Avg. = average; esp. = especially
N – North; E – East; S – South; W – West
AB – Alberta; CO – Colorado; ON – Ontario; SK – Saskatchewan
ba – P. batesii; *co – P. cocyta*; *pu – P. pulchella*; *th – P. tharos*

Table 3.2 Descriptions of numbered morphological character states shown in Figure 3.1.

1. *Antennal club colour*: 0: black; 1: brown; 2: orange
2. *FW costal margin*: 0: straight to slight curve; 1: strong curve
3. *DFW median band*: 0: ground colour; 1: paler
4. *DFW postmedian dark line*: 0: absent; 1: incomplete; 2: complete & narrow; 3: complete & wide
5. *DFW median dark line*: 0: absent; 1: incomplete; 2: complete & narrow; 3: complete & wide
6. *DFW submarginal crescents*: 0: single spot; 1: 2-3 spots; 2: 4+ spots
7. *DHW postmedian dark spots*: 0: 7 spots; 1: anterior spot obscured; 2: anterior & posterior spots obscured; 3: anterior spots reduced
8. *DHW connection of medial dark loops*: 0: fully detached; 1: laterally detached; 2: continuous/fused
9. *DHW postmedian dark line*: 0: absent; 1: incomplete; 2: complete or nearly so
10. *DHW submarginal crescents*: 0: absent; 1: 1-3 present; 2: 4+ apparent
11. *VFW anterior light spots*: 0: none; 1: yellow; 2: small, white; 3: extensive, white
12. *VFW subapical costal patch*: 0: absent; 1: small; 2: medium; 3: large
13. *VFW lateral yellow spots*: 0: none; 1: diffuse; 2: distinct
14. *VFW pale bar*: 0: absent; 1: median; 2: median and in cell
15. *VFW tornus spot*: 0: absent; 1: narrow dash; 2: moderate; 3: large
16. *VFW posterior median spot*: 0: absent; 1: small; 2: moderate; 3: large
17. *VHW dark patch surrounding crescent*: 0: absent; 1: present
18. *VHW crescent colour*: 0: ground/surrounding colour; 1: white/translucent; 2: mix of ground colour/white; 3: darker

Table 3.3 Character states for identifying *P. tharos* group species. These character states are not diagnostic but were selected based on their relative occurrence in each species (Figure 3.2) and performance in the MCA analyses (Appendices 9-18). Some of these states only marginally contributed to the MCA analyses because they were present in the taxon that appears in the centre of the plot.

	Species	Character states					
Overall	*th/co*	14-0/1	3-0	2-1	7-0/1	1-2	
	th	12-3	15-3				
	co	5-0/1	8-0/1				
	ba/pu	14-2	9-2	5-3	2-0	7-2/3	1-0/1
	ba	1-0					
	pu	12-0	4-3	16-0	15-0/1		
Males-only	*th/co*	4-0/1	9-0				
	th	6-2					
	co	8-0	13-0	5-1			
	ba/pu	3-1	1-0/1	10-0	4-2/3	7-2	
	ba						
	pu	9-2					
Females-only	*th/co*	5-2	4-2	7-0			
	th						
	co	18-0					
	ba/pu	15-0					
	ba						
	pu	13-2	11-1				
th* vs. *co	*th*	18-1	5-2/3	8-2			
	co	12-1	7-2/3	15-1			
th* vs. *ba	*th*	3-0	7-0/1	4-2	11-0/2	14-0/1	1-2
	ba	3-1	7-2/3	4-3	11-1/3	14-2	1-0/1
co* vs. *ba	*co*	3-0	9-0	14-0/1	5-1	1-2	
	ba	3-1	18-1	14-2	5-3	8-2	
ba* vs. *pu	*ba*	4-1/2	12-2/3	16-2/3	1-0		
	pu	4-3	12-0/1	16-0/1	6-0		

Chapter 4

General conclusions

4.1 Thesis overview

Species delimitation is often challenging, a fact that is reflected in the numerous techniques and concepts that have been developed for the task (de Queiroz 1998, 2005, 2007; Lee 2004; Luo et al. 2018). Recently, molecular information has become a primary tool for systematic and taxonomic research; in particular, NGS techniques allow for the derivation of genome-wide markers in non-model organisms that can provide powerful insights into species limits (Metzker 2010; Peterson et al. 2012; Andrews et al. 2016; Roe et al. 2017). However, the increasing focus on molecular methods in general has led to the neglect of classical taxonomic data in some groups (Lee 2004). To provide comprehensive assessments of species boundaries while maintaining a link between delimitation and identification, an integrative (Dayrat 2005; Will et al. 2005) or iterative (Yeates et al. 2011) approach that includes morphological data has been suggested.

The four currently recognized species of the *Phyciodes tharos* group have been considered distinct based on many criteria including morphological characters (Table 3.1), phenology (Klots 1951; Ehrlich & Ehrlich 1961; Ferris & Brown 1981; Pyle 1981), ecological traits (Klots 1951; Hooper 1973; Christensen 1981), chemical analysis (Rawson 1968), and hybrid breakdown (Oliver 1979a, 1980). However, there has been continued uncertainty regarding the level of divergence and amount of introgression between the putative species in this group (Opler & Krizek 1984; Scott 1986b, 1994; Glassberg 1999, 2017; Brock & Kaufman 2006; Acorn & Sheldon 2006). The discordance of mitochondrial COI with the traditional taxonomic identifications has added to this uncertainty (Wahlberg et al. 2003a; Proshek & Houghton 2012). In this thesis, I used an iterative approach to examine the species limits and their diagnostic characters using molecular and morphological data.

It is widely understood that single-locus molecular methods are not reliable for species delimitation due to discordant signals between individual genes (Maddison 1997;

Slowinski & Page 1999; Dupuis et al. 2012). Mitochondrial genes are greatly affected by introgression and can be particularly misleading (Chan & Levin 2005). Genome-wide multilocus sequencing techniques such as ddRADseq provide data that largely overcomes the problems of single-locus methods. In **Chapter 2**, I used phylogenetic and cluster-based methods to examine the species boundaries in the *P. tharos* species group in Alberta using genomic SNPs. I found clear, well-supported, groupings for *P. tharos*, *P. cocyta*, and *P. pulchella.* The *P. batesii* individuals also formed a unique grouping and exhibited genomic information consistent with *P. pulchella* in the STRUCTURE analysis, likely due to ancient introgression or incomplete lineage sorting. Evidence of distinct genomic lineages despite infrequent contemporary hybridization and low levels of introgression supports the designation of these four lineages as species according to the genomic integrity species definition (Sperling 2003). I examined discordance between the genomic species boundaries and COI haplotypes and found additional evidence of introgression, with unidirectional mitochondrial gene flow from *P. tharos* to *P. cocyta* & *P. batesii*, and from *P. cocyta* to *P. batesii*, likely due to brood timing and opportunistic mating between species.

Effective biodiversity and conservation research relies on accurate species identification which, in turn, relies on accurate species delimitation (Balakrishnan 2005). Genomic species identities can provide a robust measure against which to assess diagnostic characters. In **Chapter 3**, I tested whether discrete morphological characters correspond with the *P. tharos* group species lineages based on the genomic SNP clusters from Chapter 2. I found partial correspondence with extensive intraspecific morphological variation that broadly overlaps between species, even in analyses subset by sex or species. None of the character states were diagnostic for any of the species, but a diagram of the proportions of character states exhibited for each species is provided along with the character states' contributions to each analysis. These can be used as resources for uncertain identifications.

This is the first study to iteratively address the delimitation and identification challenges in the *P. tharos* species group by utilizing both molecular and morphological data. This approach provides not only a comprehensive assessment of the group but also

strong support for previous research. Traditional taxonomic methods correctly distinguished four species lineages despite the ambiguous species boundaries. This highlights the importance of testing species limits against several criteria and demonstrates the utility of the genomic integrity definition as a delimitation criterion for taxa with semi-permeable species boundaries.

4.2 Future research

Opportunity remains for future work on the *P. tharos* species group. One major focus should be to obtain a better sample of *P. batesii* in Alberta and across its range. Sample size affects which loci are called and retained during *de novo* catalog construction and filtering which can have downstream effects on phylogenetic and cluster-based analyses (Nabhan & Sarkar 2012; Mastretta-Yanes et al. 2015; Huang & Knowles 2016). It is likely that the intermediacy of this species in the phylogenetic tree and the K = 3 STRUCTURE analysis and the evidence of a large proportion of apparent *P. pulchella* SNPs in the K = 4 analysis are at least partly due to an insufficient number of unique *P. batesii* loci in the catalog. Additional sampling would allow for more representative genotyping and thus help clarify the species boundaries and patterns of introgression. It would also help confirm the presence of this species in other parts of its range and identify populations that should be monitored.

The geographic scope of this thesis in Alberta allowed examination of the four recognized species without the need for considering subspecific relationships. According to Scott (2006), the four species of the *P. tharos* species group consist of 21 subspecies. North America-wide sampling of all subspecies would allow examination of whether there is a genomic basis for each of these taxa and whether any have diverged enough to be considered full species. For instance, Scott (1998) described *P. cocyta diminutor* from southeastern Canada and the northeastern United States. This taxon has since been treated as a potential full species (Scott 2006, 2008, 2014) based on sympatry with both *P. cocyta* and *P. tharos*. Another unclear lineage is *P. incognitus* which was described as a full species in the southern Appalachian Mountains by Gatrelle 2004. Scott (2006) suggests that this is instead a subspecies or variety of '*P. cocyta/diminutor*'. Genomic

analysis would help bring taxonomic stability to these taxa and other subspecies of this group and may further validate past work.

Evidence of hybridization and introgression between *P. tharos*, *P. cocyta*, and *P. batesii* warrants further attention. In Alberta, I found that adult hybrids are rare, but it is unknown whether this is due to pre- or post-zygotic barriers. Evidence of introgression between *P. tharos* and *P. cocyta* in the province indicates that there is at least occasional interaction between these species. This may vary regionally. In Colorado, Scott (1986b) found that *P. tharos* and *P. cocyta* act as conspecifics without evidence of hybrid breakdown, so work in this area would be particularly interesting. As in Wahlberg et al. (2003a), I found that most *P. cocyta* and *P. batesii* were found together in their own set of clades in the COI tree, but some individuals appeared in the *P. tharos* clade. Proshek & Houghton (2012) found *P. cocyta* from Michigan and Ohio grouped primarily with *P. tharos*, and sometimes with *P. batesii*, depending on proximity to populations of these species. In my study, *P. cocyta* individuals with *P. tharos* COI haplotypes did not necessarily occur near *P. tharos* range, but all individuals with genomic evidence of introgression were found in areas of sympatry. All three studies indicate unidirectional movement of COI from *P. tharos* to *P. cocyta* and *P. batesii*. Examination of these patterns may shed light on the mechanisms behind the development and maintenance of species boundaries in this group.

More broadly, phylogenetic study of the entire *Phyciodes* genus using genome-wide data is needed. So far, molecular phylogenies have been limited to one or a few genes and have resolved the *P. tharos* group as paraphyletic and closely related to *P. phaon* (Wahlberg & Zimmerman 2000; Wahlberg et al. 2005; Wahlberg & Freitas 2007). However, Wahlberg et al. (2003a) found two aberrant *P. pulchella* haplotypes that grouped with *P. phaon* at the base of the *P. tharos* species group clade. The order of branching within the species group is also unclear, with the most basal species appearing to be *P. tharos* according to COI and *P. pulchella* according to genome-wide SNPs. These relationships may be more clearly resolved with genomic analysis of the entire genus.

Morphology remains an integral part of taxonomy and should be included in molecular studies, even for the most challenging taxa. The search for reliable diagnostic morphological characters for the *P. tharos* group may seem futile, at least in Alberta, but it should not discourage future researchers. Regional studies that utilize genomic data for species delimitation should attempt to improve identification resources by relating morphological data to discovered lineages. Geometric morphometrics may prove helpful for discovering species-specific patterns that are too subtle for detection by eye (Lawing & Polly 2009; Zelditch et al. 2012).

Overall, I hope that this work is found useful, or at least validating, to lepidopterists who have long been troubled by these cute but mischievous butterflies. While I could not provide truly diagnostic field characters, I was able to confirm that the classically delimited species are supported by genomic information. A huge amount of credit is due to the dedicated lepidopterists and naturalists who spent countless hours observing these butterflies in nature, studying the finest details of their morphology for all life stages, collecting eggs, rearing, and hybridizing butterflies in the lab. My work would not have been nearly as interesting without such a strong foundation to build it upon. Our understanding of the *P. tharos* species group has come a long way in the last fifty years, but plenty of work is left for future students of crescentspot taxonomy.

"The 'Curse of *Phyciodes*' continues."

-James A. Scott (2006)

Biography

I was born on July 2, 1988, in Regina, Saskatchewan, to Anna Marie Wingert. Unlike most entomologists, my origin story does not include a love for insects, but rather a fear of them, except perhaps for ladybugs, caterpillars, and butterflies. I recall rearing a caterpillar with my mom once, only to be disappointed that it turned into a moth and not a butterfly. I went on summer camping trips throughout my childhood with my mom, grandparents, John and Adeline, aunts, uncles, and cousins. I loved picking chokecherries with my grandma, catching frogs with my cousins, and collecting rocks. I also enjoyed sorting things – especially my coin collection by year, and decks of cards by suit and number – and occasionally did math for fun in my free time. Many of my evenings and weekends between the ages of four and fifteen were spent practicing ballet, jazz, and tap in the dance studio.

After high school, I dabbled in university courses, then decided to take some time off to decide on a career path. I stumbled into an accounting job, which seemed like a good fit given my natural inclination for working with numbers. Over time, however, I became interested in animal conservation. This led me to the Environmental and Conservation Sciences program in the Renewable Resources Department at the University of Alberta.

I discovered my fascination with insects during John Acorn's wildlife course. I would stay in the lab as late as I was allowed to, taking photographs of specimens under the microscope. I loved learning about the biodiversity around me and the taxonomic classification system. The following summer I landed my first job as a field assistant and got over my fear of spiders. I later took Dr. Felix Sperling's advanced entomology course which included an imaging and databasing project in the E. H. Strickland Entomological Museum. The idea of working with collections appealed to me and so I pursued a curation internship in the Diptera Unit at the Smithsonian Institution. Under the guidance of Drs. Torsten Dikow and Allen Norrbom, and (what I now consider my bible) the Manual of Nearctic Diptera, I learned to identify many families and some genera of flies. I quickly realized that the flies were my favourite group of insects, due to their enormous diversity, and varied life histories.

In the final year of my undergraduate degree, I worked for the Sperling lab, extracting DNA from *Speyeria* butterflies and rearing spruce budworm moths. That summer, I began field work for my MSc, with two potential projects in mind – the first on a group of asilid flies, the second on *Phyciodes* butterflies. I didn't find a single asilid from my intended study group but found *Phyciodes* in great abundance, and the rest was history. I have been fortunate to have other opportunities to work on Diptera over the last few years. I have done fly identifications for pollinator ecology studies in Dr. Carol Frost's lab and attended Fly School in California in 2019. Recently, I went to my first North American Dipterists Society field meeting in New Jersey.

It has been a long and winding road to get to where I am today, and I am looking forward to what is around the next turn. Onward.

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Appendix 1 Collection information for *Phyciodes* specimens used in this study. Species identifications are based on SNP analyses. Individuals with an asterisk were only included in Chapter 3. DNA# is a unique extraction identifier and UASM# is a unique identifier for the voucher collection deposited in the University of Alberta E. H. Strickland Entomological Museum.

Species	DNA#	UASM#	Coll. Date	Sex	Region	Locality	Latitude	Longitude	Collector(s)
P. mylitta	12023	413001	1995-vi-25	M	US:CA	Fish Camp	37.478	-119.639	FAH Sperling
P. mylitta	12087	413002	2018-vii-24	M	CA:BC	Vavenby	51.579	-119.718	TD Nelson
P. mylitta	12089	413003	2018-v-19	M	US:NM	Lincoln National Forest	32.999	-105.776	N Grishin
P. mylitta	12090	413004	2018-v-19	M	US:NM	Lincoln National Forest	32.999	-105.776	N Grishin
co/ba hybrid	11142	413005	2016-vii-21	F	CA:AB	60 km SW of Sundre	51.658	-115.281	BD Wingert
th/co hybrid	11215	413006	2017-vi-03	F	CA:AB	Battle R & Hwy 36	52.412	-111.820	BD Wingert & G Carscallen
P. tharos	11101	413007	2016-v-29	M	CA:AB	Drumheller	51.480	-112.796	BD Wingert
P. tharos	11102	413008	2016-v-29	M	CA:AB	Drumheller	51.480	-112.796	BD Wingert
P. tharos	11103	413009	2000-v-19	M	CA:AB	Jenner Bridge	50.839	-111.176	GG Anweiler
P. tharos	11117	413010	2016-v-29	M	CA:AB	Drumheller	51.480	-112.796	BD Wingert
P. tharos	11118	413011	2016-v-29	M	CA:AB	Drumheller	51.480	-112.796	BD Wingert
P. tharos	11119	413012	2016-v-29	F	CA:AB	Drumheller	51.480	-112.796	BD Wingert
P. tharos	11143	413013	2016-vii-25	F	CA:AB	Lethbridge	49.704	-112.873	BD Wingert
P. tharos	11147	413014	2016-vii-26	F	CA:AB	Drumheller	51.414	-112.637	BD Wingert
P. tharos	11149	413015	2016-vii-26	F	CA:AB	Drumheller	51.480	-112.796	BD Wingert
P. tharos	11150	413016	2016-vii-26	M	CA:AB	Drumheller	51.480	-112.796	BD Wingert
P. tharos	11151	413017	2016-vii-25	F	CA:AB	Lethbridge	49.704	-112.873	BD Wingert
P. tharos	11200	413018	2016-vii-26	F	CA:AB	Drumheller	51.316	-112.501	BD Wingert
P. tharos	11201	413019	2016-vii-26	M	CA:AB	Empress	50.953	-110.009	GJ Hilchie
P. tharos	11202	413020	2016-vii-27	M	CA:AB	Empress	50.953	-110.009	GJ Hilchie
P. tharos	11205	413021	2017-v-31	M	CA:AB	Emerson Bridge Cg.	50.916	-111.904	BD Wingert & G Carscallen
P. tharos	11206	413022	2017-v-31	M	CA:AB	Emerson Bridge Cg.	50.916	-111.904	BD Wingert & G Carscallen

Species	DNA#	UASM#	Coll. Date	Sex	Region	Locality	Latitude	Longitude	Collector(s)
P. tharos	11207	413023	2017-vi-01	M	CA:AB	Sandy Point	50.730	-110.075	BD Wingert & G Carscallen
P. tharos	11209	413024	2017-vi-01	F	CA:AB	Sandy Point	50.730	-110.075	BD Wingert & G Carscallen
P. tharos	11210	413025	2017-vi-01	F	CA:AB	Sandy Point	50.730	-110.075	BD Wingert & G Carscallen
P. tharos	11211	413026	2017-vi-02	F	CA:AB	Bow R & Hwy 36	50.247	-112.076	BD Wingert & G Carscallen
P. tharos	11212	413027	2017-vi-02	M	CA:AB	Bow R & Hwy 36	50.247	-112.076	BD Wingert & G Carscallen
P. tharos	11213	413028	2017-vi-02	M	CA:AB	Bow R & Hwy 36	50.247	-112.076	BD Wingert & G Carscallen
P. tharos	11214	413029	2017-vi-03	M	CA:AB	Battle R & Hwy 36	52.412	-111.820	BD Wingert & G Carscallen
P. tharos	11216	413030	2017-vi-03	M	CA:AB	Battle R & Hwy 854	52.697	-112.449	BD Wingert & G Carscallen
P. tharos	11217	413031	2017-vi-03	F	CA:AB	Battle R & Hwy 854	52.697	-112.449	BD Wingert & G Carscallen
P. tharos	11218	413032	2017-vi-03	M	CA:AB	Battle R & Hwy 854	52.697	-112.449	BD Wingert & G Carscallen
P. tharos	11219	413033	2017-vi-11	F	CA:AB	Tolman Bridge	51.842	-113.009	JR Dupuis
P. tharos	11220	413034	2017-vi-11	M	CA:AB	Tolman Bridge	51.842	-113.009	JR Dupuis
P. tharos	11264	413035	2017-v-31	M	CA:AB	Emerson Bridge Cg.	50.916	-111.904	BD Wingert & G Carscallen
P. tharos	11265	413036	2017-v-31	M	CA:AB	Emerson Bridge Cg.	50.916	-111.904	BD Wingert & G Carscallen
P. tharos	11266	413037	2017-vi-02	M	CA:AB	Old Man R & Hwy 36	49.960	-112.085	BD Wingert & G Carscallen
P. tharos	11267	413038	2017-vi-02	M	CA:AB	Old Man R & Hwy 36	49.960	-112.085	BD Wingert & G Carscallen
P. tharos	11268	413039	2017-vi-02	M	CA:AB	Old Man R & Hwy 36	49.960	-112.085	BD Wingert & G Carscallen
P. tharos	11269	413040	2017-vi-03	F	CA:AB	Battle R & Hwy 36	52.412	-111.820	BD Wingert & G Carscallen
P. tharos	11270	413041	2017-vi-03	F	CA:AB	Battle R & Hwy 36	52.412	-111.820	BD Wingert & G Carscallen
P. tharos	11271	413042	2017-vi-03	M	CA:AB	Battle R & Hwy 36	52.412	-111.820	BD Wingert & G Carscallen
P. tharos	11955	413043	2017-v-26	M	CA:AB	along Milk River	49.131	-110.894	ZG MacDonald
P. tharos	11956	413044	2017-v-29	M	CA:AB	6 km NW of Bindloss	50.879	-110.275	ZG MacDonald
P. tharos	11957	413045	2017-v-31	F	CA:AB	Emerson Bridge Cg.	50.916	-111.904	BD Wingert & G Carscallen
P. tharos	11958	413046	2017-v-31	M	CA:AB	Emerson Bridge Cg.	50.916	-111.904	BD Wingert & G Carscallen
P. tharos	11973	413047	2000-vi-05	M	CA:AB	Battle R & Hwy 36	52.412	-111.820	B & J Beck
*P. tharos**	11978	413048	2000-vii-30	F	US:NC	S of Winston Salem	36.044	-80.241	FAH Sperling
P. tharos	11979	413049	2000-vii-30	F	US:NC	S of Winston Salem	36.044	-80.241	FAH Sperling

Species	DNA#	UASM#	Coll. Date	Sex	Region	Locality	Latitude	Longitude	Collector(s)
P. tharos	12052	413050	2016-vii-26	M	CA:AB	Drumheller	51.316	-112.501	BD Wingert
P. tharos	12053	413051	2016-vii-26	M	CA:AB	Drumheller	51.480	-112.796	BD Wingert
P. tharos	12054	413052	2016-vii-26	M	CA:AB	Drumheller	51.480	-112.796	BD Wingert
P. tharos	12098	413053	2018-vii-09	M	US:TX	White Rock Lake	32.846	-96.718	N Grishin
P. cocyta	11104	413054	2001-vii-21	F	CA:AB	Pigeon Lake	53.071	-114.078	FAH Sperling
*P. cocyta**	11105	413055	2015-vii-01	M	CA:AB	Pigeon Lake	53.055	-114.178	FAH & T Sperling
P. cocyta	11106	413056	2012-ix-09	M	CA:AB	nr. Lloyd Creek NA	52.939	-114.330	FAH Sperling
*P. cocyta**	11107	413057	2013-vii-07	M	CA:AB	Bragg Creek	50.921	-114.565	FAH Sperling
P. cocyta	11108	413058	1999-vi-26	M	CA:AB	Edmonton	53.539	-113.560	FAH Sperling
*P. cocyta**	11109	413059	2012-vii-01	F	CA:AB	nr. Lloyd Creek NA	52.939	-114.330	FAH Sperling
*P. cocyta**	11110	413060	2013-vi-30	F	CA:AB	nr. Lloyd Creek NA	52.939	-114.330	FAH Sperling
*P. cocyta**	11111	413061	2009-vii-12	M	CA:AB	Pigeon Lake	53.071	-114.078	FAH Sperling
P. cocyta	11112	413062	2001-vii-15	F	CA:AB	Athabasca	54.710	-113.304	FAH Sperling
P. cocyta	11123	413063	2016-vii-02	M	CA:AB	N of Elnora	52.006	-113.196	FAH Sperling
P. cocyta	11124	413064	2016-vii-02	F	CA:AB	N of Elnora	52.006	-113.196	FAH Sperling
*P. cocyta**	11125	413065	2016-vii-02	F	CA:AB	N of Elnora	52.006	-113.196	FAH Sperling
P. cocyta	11126	413066	2016-vii-02	F	CA:AB	N of Elnora	52.006	-113.196	FAH Sperling
*P. cocyta**	11127	413067	2016-vii-02	M	CA:AB	N of Elnora	52.006	-113.196	FAH Sperling
P. cocyta	11128	413068	2016-vii-02	M	CA:AB	N of Elnora	52.006	-113.196	FAH Sperling
*P. cocyta**	11129	413069	2016-vii-09	F	CA:AB	Opal	53.987	-113.304	BD Wingert
*P. cocyta**	11130	413070	2016-vii-09	M	CA:AB	Opal	53.987	-113.304	BD Wingert
*P. cocyta**	11132	413071	2016-vi-23	M	CA:AB	Opal	53.987	-113.304	BD Wingert & V Romanyshyn
P. cocyta	11133	413072	2016-vii-04	F	CA:AB	Hasse Lake	53.493	-114.181	BD Wingert & EO Campbell
*P. cocyta**	11134	413073	2016-vii-12	F	CA:AB	Athabasca	54.710	-113.304	BD Wingert
*P. cocyta**	11135	413074	2016-vii-12	F	CA:AB	Athabasca	54.710	-113.304	BD Wingert
P. cocyta	11136	413075	2016-vii-12	F	CA:AB	Athabasca	54.710	-113.304	BD Wingert

Species	DNA#	UASM#	Coll. Date	Sex	Region	Locality	Latitude	Longitude	Collector(s)
*P. cocyta**	11137	413076	2016-vii-21	M	CA:AB	60 km SW of Sundre	51.658	-115.281	BD Wingert
*P. cocyta**	11138	413077	2016-vii-21	M	CA:AB	60 km SW of Sundre	51.658	-115.281	BD Wingert
*P. cocyta**	11139	413078	2016-vii-21	M	CA:AB	60 km SW of Sundre	51.658	-115.281	BD Wingert
*P. cocyta**	11145	413079	2016-vii-23	F	CA:AB	Athabasca	54.710	-113.304	GJ Hilchie
P. cocyta	11146	413080	2016-vii-12	F	CA:AB	Athabasca	54.710	-113.304	BD Wingert
*P. cocyta**	11174	413081	2014-vii-31	F	CA:AB	Kaufman Hill	56.228	-117.252	JR Dupuis
P. cocyta	11175	413082	2014-viii-01	F	CA:AB	Pat's Creek	56.216	-116.967	JR Dupuis
*P. cocyta**	11176	413083	2014-viii-01	F	CA:AB	Pat's Creek	56.216	-116.967	JR Dupuis
*P. cocyta**	11178	413084	2014-viii-01	F	CA:AB	Pat's Creek	56.216	-116.967	JR Dupuis
P. cocyta	11186	413085	2015-vii-01	M	CA:AB	Pigeon Lake	53.055	-114.178	FAH & T Sperling
P. cocyta	11196	413086	2016-vii-21	M	CA:AB	16 km SW of Sundre	51.751	-114.805	BD Wingert
P. cocyta	11198	413087	2016-vii-23	F	CA:AB	Athabasca	54.710	-113.304	BDW, J Leggo, A Maston, P Evans
*P. cocyta**	11221	413088	2017-vi-08	M	CA:AB	Edmonton	53.539	-113.565	JH Acorn
P. cocyta	11224	413089	2017-vi-23	M	CA:AB	nr. Elk Island	53.632	-112.926	TD Nelson
P. cocyta	11225	413090	2017-vi-23	M	CA:AB	nr. Elk Island	53.632	-112.926	TD Nelson
P. cocyta	11226	413091	2017-vi-24	M	CA:AB	trail near Devon	53.393	-113.741	S Bishop
P. cocyta	11227	413092	2017-vi-24	M	CA:AB	trail near Devon	53.393	-113.741	BD Wingert
*P. cocyta**	11229	413093	2017-vii-03	M	CA:AB	Gull Lake	52.495	-113.921	JH Acorn
*P. cocyta**	11230	413094	2017-vii-04	M	CA:AB	Crimson Lake PP	52.449	-115.028	RLK French
P. cocyta	11231	413095	2017-vii-04	M	CA:AB	Crimson Lake PP	52.449	-115.028	BD Wingert
P. cocyta	11232	413096	2017-vii-04	M	CA:AB	Red Lodge PP	51.949	-114.245	RLK French
*P. cocyta**	11235	413097	2017-vii-06	M	CA:BC	Vavenby	51.579	-119.718	RLK French
P. cocyta	11236	413098	2017-vii-06	M	CA:BC	Vavenby	51.579	-119.718	BD Wingert
*P. cocyta**	11238	413099	2017-vii-07	M	CA:BC	Tete Jaune	52.963	-119.457	TD Nelson
P. cocyta	11239	413100	2017-vii-09	M	CA:AB	Dry Island PP	51.935	-112.981	FAH Sperling
*P. cocyta**	11240	413101	2017-vii-09	M	CA:AB	Dry Island PP	51.935	-112.981	FAH Sperling
P. cocyta	11241	413102	2017-vii-09	F	CA:AB	Dry Island PP	51.935	-112.981	FAH Sperling

Species	DNA#	UASM#	Coll. Date	Sex	Region	Locality	Latitude	Longitude	Collector(s)
P. cocyta	11242	413103	2017-vii-09	F	CA:AB	Dry Island PP	51.935	-112.981	FAH Sperling
P. cocyta	11243	413104	2017-vii-09	F	CA:AB	Dry Island PP	51.935	-112.981	FAH Sperling
*P. cocyta**	11244	413105	2017-vii-10	M	CA:AB	nr. Cross Lake PP	54.631	-113.853	EO Campbell
P. cocyta	11245	413106	2000-vii-03	F	CA:AB	Pigeon Lake	53.105	-114.173	FAH Sperling
P. cocyta	11248	413107	2003-vii-07	F	CA:AB	Bragg Creek	50.921	-114.565	FAH Sperling
*P. cocyta**	11252	413108	2012-vii-01	F	CA:AB	nr. Lloyd Creek NA	52.939	-114.330	FAH Sperling
P. cocyta	11254	413109	2013-vi-30	F	CA:AB	nr. Lloyd Creek NA	52.939	-114.330	FAH Sperling
*P. cocyta**	11255	413110	2013-vii-01	F	CA:AB	Pigeon Lake	53.105	-114.172	FAH Sperling
P. cocyta	11261	413111	2016-vii-21	F	CA:AB	16 km SW of Sundre	51.751	-114.805	BD Wingert
*P. cocyta**	11262	413112	2016-vii-21	M	CA:AB	16 km SW of Sundre	51.751	-114.805	BD Wingert
P. cocyta	11263	413113	2016-vii-23	F	CA:AB	Athabasca	54.710	-113.304	BDW, J Leggo, A Maston, P Evans
*P. cocyta**	11272	413114	2017-vii-06	M	CA:BC	Vavenby	51.579	-119.718	BD Wingert
P. cocyta	11273	413115	2017-vii-06	M	CA:BC	Vavenby	51.579	-119.718	BD Wingert
*P. cocyta**	11274	413116	2017-vii-06	F	CA:BC	Vavenby	51.579	-119.718	TD Nelson
P. cocyta	11275	413117	2017-vii-07	M	CA:BC	Clearwater	51.651	-120.037	BD Wingert
P. cocyta	11276	413118	2017-vii-07	M	CA:BC	Clearwater	51.651	-120.037	BD Wingert
P. cocyta	11277	413119	2017-vii-07	-	CA:BC	Tete Jaune	52.963	-119.457	TD Nelson
*P. cocyta**	11278	413120	2017-vii-07	F	CA:BC	Tete Jaune	52.963	-119.457	BD Wingert
*P. cocyta**	11279	413121	2017-vii-10	F	CA:AB	nr. Cross Lake PP	54.631	-113.853	BD Wingert
*P. cocyta**	11280	413122	2017-vii-13	F	CA:AB	nr. Elk Island	53.632	-112.926	TD Nelson
*P. cocyta**	11281	413123	2017-vii-15	F	CA:AB	Lessard Lake	53.780	-114.622	BD Wingert
P. cocyta	11282	413124	2017-vii-15	M	CA:AB	nr. Peers	53.638	-115.989	BD Wingert
*P. cocyta**	11283	413125	2017-vii-15	M	CA:AB	nr. Peers	53.638	-115.989	BD Wingert
P. cocyta	11284	413126	2017-vii-15	M	CA:AB	nr. Rumsey NA	51.773	-112.627	D Lawrie
*P. cocyta**	11286	413127	2017-vii-16	F	CA:AB	Bragg Creek	50.921	-114.565	FAH Sperling
*P. cocyta**	11287	413128	2017-vii-20	F	CA:AB	nr. J. J. Collett NA	52.550	-113.641	S Bishop
P. cocyta	11288	413129	2017-vii-21	F	CA:AB	Willow Creek West Cg.	50.253	-114.336	EO Campbell

Species	DNA#	UASM#	Coll. Date	Sex	Region	Locality	Latitude	Longitude	Collector(s)
P. cocyta	11289	413130	2017-vii-21	M	CA:AB	Willow Creek West Cg.	50.253	-114.336	EO Campbell
P. cocyta	11290	413131	2017-vii-22	F	CA:AB	nr. Highwood PRA	50.399	-114.528	S Bishop
P. cocyta	11291	413132	2017-vii-26	F	CA:AB	Hinton	53.502	-117.464	BD Wingert
P. cocyta	11292	413133	2017-vii-26	M	CA:AB	Hinton	53.502	-117.464	BD Wingert
*P. cocyta**	11297	413134	2015-vi-21	M	CA:AB	Pigeon Lake	53.071	-114.078	FAH Sperling
P. cocyta	11298	413135	2015-vi-21	F	CA:AB	Pigeon Lake	53.071	-114.078	FAH Sperling
*P. cocyta**	11299	413136	2016-vii-21	F	CA:AB	16 km SW of Sundre	51.751	-114.805	BD Wingert
*P. cocyta**	11300	413137	2016-vii-21	M	CA:AB	16 km SW of Sundre	51.751	-114.805	BD Wingert
*P. cocyta**	11952	413138	2016-vii-21	F	CA:AB	16 km SW of Sundre	51.751	-114.805	BD Wingert
P. cocyta	11954	413139	2016-vii-23	F	CA:AB	Athabasca	54.710	-113.304	BDW, J Leggo, A Maston, P Evans
P. cocyta	11959	413140	2017-vii-15	M	CA:AB	nr. Rumsey NA	51.773	-112.627	FAH Sperling
*P. cocyta**	11960	413141	2017-vii-20	F	CA:AB	nr. J. J. Collett NA	52.550	-113.641	S Bishop
*P. cocyta**	11961	413142	2017-vii-21	F	CA:AB	Willow Creek West Cg.	50.253	-114.336	EO Campbell
P. cocyta	11962	413143	2017-vii-21	F	CA:AB	Willow Creek West Cg.	50.253	-114.336	BD Wingert
P. cocyta	11963	413144	2017-vii-21	F	CA:AB	W of Cowboy Trail	50.269	-114.299	BD Wingert
P. cocyta	11964	413145	2017-vii-21	F	CA:AB	W of Cowboy Trail	50.269	-114.299	BD Wingert
*P. cocyta**	11965	413146	2017-vii-22	F	CA:AB	Willow Creek West Cg.	50.253	-114.336	EO Campbell
P. cocyta	11967	413147	2017-vii-26	M	CA:AB	Hinton	53.502	-117.464	BD Wingert
*P. cocyta**	11968	413148	2017-vii-26	M	CA:AB	Hinton	53.502	-117.464	BD Wingert
P. cocyta	11969	413149	2017-vii-04	F	CA:AB	nr. Fort McMurray	56.371	-111.010	F Riva
P. cocyta	11974	413150	2000-vii-08	F	CA:AB	Moose Mountain	50.939	-114.837	FAH Sperling
P. cocyta	11976	413151	2000-vii-09	M	CA:AB	nr. Whitelaw	56.109	-118.083	A Mitchell
*P. cocyta**	11980	413152	2001-vii-02	F	CA:AB	Pigeon Lake	53.071	-114.078	FAH Sperling
P. cocyta	11982	413153	2002-viii-25	M	CA:MB	S of Grand Rapids	52.906	-99.138	FAH Sperling
*P. cocyta**	11984	413154	2003-vii-01	F	CA:AB	Pigeon Lake	53.018	-114.130	FAH Sperling
*P. cocyta**	11986	413155	2005-vii-31	F	CA:AB	Pigeon Lake	53.071	-114.078	FAH Sperling
P. cocyta	11989	413156	2014-vi-24	M	CA:MB	N of Duck Mtn PP	52.030	-101.091	JR Dupuis

Species	DNA#	UASM#	Coll. Date	Sex	Region	Locality	Latitude	Longitude	Collector(s)
P. cocyta	11990	413157	2014-vi-24	M	CA:MB	N of Duck Mtn PP	52.030	-101.091	JR Dupuis
P. cocyta	11992	413158	2014-vii-23	F	US:MT	Little Belt Mts	46.838	-110.718	FAH & T Sperling, S Ferguson
P. cocyta	11994	413159	2015-vii-13	M	CA:BC	16 km SE of Merritt	50.030	-120.627	FAH Sperling
P. cocyta	11996	413160	2016-vi-15	M	CA:ON	Morson	49.098	-94.315	ZG MacDonald
P. cocyta	11998	413161	2016-vii-18	M	CA:ON	Morson	49.098	-94.315	ZG MacDonald
*P. cocyta**	11999	413162	2016-vii-18	M	CA:ON	Morson	49.098	-94.315	ZG MacDonald
*P. cocyta**	12000	413163	2016-vii-20	F	CA:AB	N of Crowsnest Pass	50.212	-114.194	EO Campbell
P. cocyta	12001	413164	2016-vii-20	F	CA:AB	N of Crowsnest Pass	50.212	-114.194	BJ Campbell
P. cocyta	12002	413165	2016-vii-21	F	CA:AB	60 km SW of Sundre	51.658	-115.281	BD Wingert
*P. cocyta**	12005	413166	2016-vii-21	M	CA:AB	60 km SW of Sundre	51.658	-115.281	BD Wingert
P. cocyta	12006	413167	2017-vii-03	M	CA:AB	nr. Elk Island	53.632	-112.926	TD Nelson
P. cocyta	12008	413168	2017-vii-07	M	CA:BC	Tete Jaune	52.963	-119.457	BD Wingert
*P. cocyta**	12009	413169	2017-vii-07	F	CA:BC	Tete Jaune	52.963	-119.457	BD Wingert
*P. cocyta**	12010	413170	2017-vii-10	M	CA:AB	nr. Cross Lake PP	54.631	-113.853	EO Campbell
P. cocyta	12011	413171	2017-vii-10	F	CA:AB	nr. Cross Lake PP	54.631	-113.853	BD Wingert
*P. cocyta**	12012	413172	2017-vii-15	M	CA:AB	nr. Rumsey NA	51.773	-112.627	FAH Sperling
*P. cocyta**	12013	413173	2017-vii-21	F	CA:AB	Willow Creek West Cg.	50.253	-114.336	S Bishop
P. cocyta	12014	413174	2017-vi-29	F	CA:AB	nr. Fort McMurray	56.266	-111.617	F Riva
P. cocyta	12015	413175	2017-vi-29	F	CA:AB	nr. Fort McMurray	56.266	-111.617	F Riva
P. cocyta	12021	413176	1989-vi-25	M	US:NY	Ithaca	42.440	-76.500	FAH Sperling
P. cocyta	12028	413177	2000-vii-08	M	CA:AB	Moose Mountain	50.939	-114.837	FAH Sperling
P. cocyta	12029	413178	2001-vii-15	M	CA:AB	Athabasca	54.710	-113.304	FAH Sperling
*P. cocyta**	12034	413179	2003-vii-01	F	CA:AB	Pigeon Lake	53.055	-114.178	FAH Sperling
P. cocyta	12046	413180	2014-vii-31	M	CA:AB	Kaufman Hill	56.228	-117.252	JR Dupuis
P. cocyta	12051	413181	2016-vii-20	M	CA:AB	N of Crowsnest Pass	50.212	-114.194	BJ Campbell
P. cocyta	12056	413182	2017-vii-07	M	CA:BC	Clearwater	51.651	-120.037	RLK French
P. cocyta	12058	413183	2017-vii-07	F	CA:BC	Clearwater	51.651	-120.037	RLK French

Species	DNA#	UASM#	Coll. Date	Sex	Region	Locality	Latitude	Longitude	Collector(s)
*P. cocyta**	12059	413184	2017-vii-09	M	CA:AB	Dry Island PP	51.935	-112.981	FAH Sperling
*P. cocyta**	12060	413185	2017-vii-15	M	CA:AB	Lessard Lake	53.780	-114.622	BD Wingert
*P. cocyta**	12061	413186	2017-vii-15	F	CA:AB	nr. Peers	53.638	-115.989	BD Wingert
P. cocyta	12067	413187	2017-vii-04	M	CA:AB	nr. Fort McMurray	56.371	-111.010	F Riva
P. batesii	11115	413188	2002-vii-21	F	CA:AB	Peace River	56.234	-117.272	B Hood
P. batesii	11164	413189	2013-vii-11	F	CA:MB	nr. Grass River PP	54.583	-100.502	JR Dupuis
P. batesii	11233	413190	2017-vii-05	M	CA:AB	NW of Coal Valley	53.078	-116.829	BD Wingert
P. batesii	11285	413191	2017-vii-15	M	CA:AB	nr. Rumsey NA	51.773	-112.627	FAH Sperling
P. batesii	11987	413192	2013-vii-11	M	CA:MB	nr. Grass River PP	54.583	-100.502	JR Dupuis
P. batesii	12047	413193	2014-vii-31	F	CA:AB	Kaufman Hill	56.228	-117.252	JR Dupuis
P. pulchella	11113	413194	2013-vii-07	F	CA:AB	Bragg Creek	50.921	-114.565	FAH Sperling
P. pulchella	11114	413195	2009-vii-04	M	CA:AB	Moose Mountain	50.939	-114.837	FAH Sperling
P. pulchella	11140	413196	2016-vii-21	F	CA:AB	60 km SW of Sundre	51.658	-115.281	BD Wingert
P. pulchella	11141	413197	2016-vii-21	F	CA:AB	60 km SW of Sundre	51.658	-115.281	BD Wingert
P. pulchella	11173	413198	2014-vii-23	F	US:MT	Little Belt Mts	46.838	-110.718	FAH Sperling
P. pulchella	11192	413199	2016-vii-21	M	CA:AB	Waterton Park lookout	49.085	-113.914	BJ Campbell
P. pulchella	11194	413200	2016-vii-21	F	CA:AB	Waterton Park lookout	49.085	-113.914	BJ Campbell
P. pulchella	11246	413201	2001-vii-06	M	CA:AB	Chinook Lake	49.669	-114.605	W Sperling
P. pulchella	11247	413202	2001-vii-25	M	US:OR	nr. Santiam Pass	44.422	-121.855	FAH Sperling
P. pulchella	11249	413203	2005-vii-10	M	CA:AB	nr. Crandell	49.022	-113.698	FAH Sperling
P. pulchella	11250	413204	2005-vii-10	M	CA:AB	nr. Crandell	49.022	-113.698	FAH Sperling
P. pulchella	11253	413205	2012-vii-24	F	US:CO	Silverthorne	39.630	-106.072	FAH Sperling, D Rubinoff, T Gilligan
P. pulchella	11256	413206	2014-vii-22	F	US:MT	Jardine	45.070	-110.640	FAH Sperling
P. pulchella	11257	413207	2014-vii-22	M	US:MT	Jardine	45.070	-110.640	FAH Sperling
P. pulchella	11258	413208	2016-vii-21	F	CA:AB	Waterton Park lookout	49.085	-113.914	EO Campbell
P. pulchella	11259	413209	2016-vii-21	M	CA:AB	Waterton Park lookout	49.085	-113.914	EO Campbell

Species	DNA#	UASM#	Coll. Date	Sex	Region	Locality	Latitude	Longitude	Collector(s)
P. pulchella	11260	413210	2016-vii-21	F	CA:AB	Waterton Park lookout	49.085	-113.914	BJ Campbell
P. pulchella	11296	413211	2002-vii-24	M	CA:AB	Mockingbird Lookout	51.425	-115.072	T Pike
P. pulchella	11953	413212	2016-vii-21	M	CA:AB	Deer Creek Flats	51.650	-115.119	BD Wingert
P. pulchella	11966	413213	2017-vii-23	F	CA:AB	Bow Valley PP Cg.	51.085	-115.092	BD Wingert, S Bishop
P. pulchella	11975	413214	2000-vii-08	F	CA:AB	Moose Mountain	50.939	-114.837	FAH Sperling
P. pulchella	11985	413215	2005-vii-07	M	CA:AB	Moose Mountain	50.939	-114.837	FAH Sperling
P. pulchella	12026	413216	1996-vii-26	F	US:CA	Nevada Co.	39.339	-120.844	FAH Sperling
P. pulchella	12091	413217	2018-v-20	M	US:NM	Cibola National Forest	35.247	-106.413	N Grishin

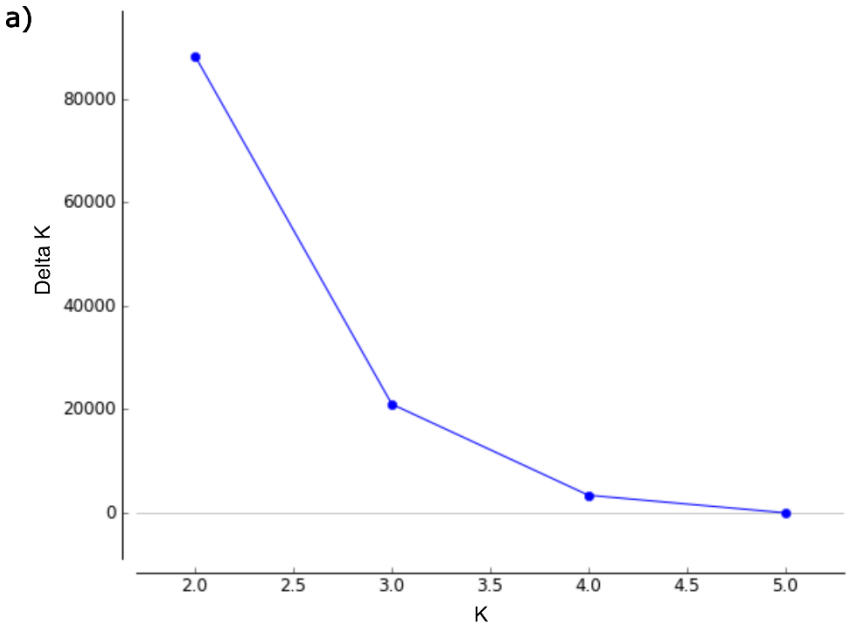
Appendix 2 Collection and publication information for *Phyciodes* COI sequences retrieved from GenBank (Clark et al. 2016). GenBank accession numbers and identification numbers from original publications are included.

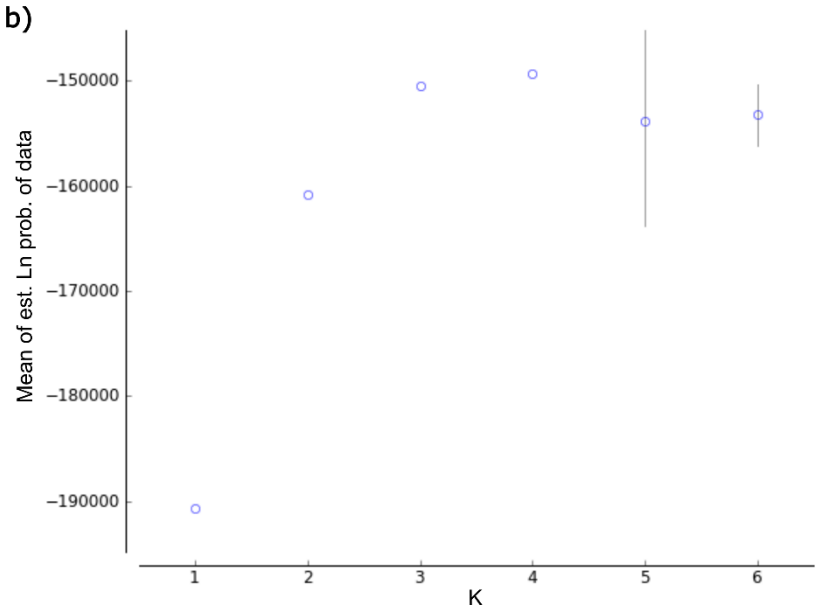
Species	Accession#	ID#	Publication	Coll. Date	Country	Region	Locality
P. mylitta arida	AY156630.1	NW67-10	Wahlberg et al. (2003a)	2001-iv-01	Mexico	MX	Jilotepec
P. mylitta arizonensis	AY156629.1	NW32-1	Wahlberg et al. (2003a)	1998-v-20	USA	AZ	Chocise Co.
P. orseis herlani	AY156634.1	NW77-4	Wahlberg et al. (2003a)	2001-vi-18	USA	NV	Douglas Co.
P. orseis orseis	AY156631.1	NW67-3	Wahlberg et al. (2003a)	2001-iv-24	USA	CA	Siskiyou Co.
P. pallescens	AY156640.1	NW64-2	Wahlberg et al. (2003a)	1996-vii-26	Mexico	MC	Uruapan
P. pallescens	AY156641.1	NW64-1	Wahlberg et al. (2003a)	1997-viii-27	Mexico	MC	Ziracuaretiro
P. pallida barnesi	AY156637.1	NW58-5	Wahlberg et al. (2003a)	2000-vi-27	Canada	BC	Crater Mountain
P. pallida pallida	AF187792.1	NW34-6	Wahlberg et al. (2003a)	1994-vi-08	USA	CO	Boulder Co.
P. phaon jalapeno	AF187798.2	NW35-11	Wahlberg et al. (2003a)	1991-v-26	Mexico		Mazatlan
P. phaon phaon	AY156638.1	NW25-17	Wahlberg et al. (2003a)	1998-v-30	USA	FL	Alachua Co.
P. picta canace	AY156642.1	NW44-11	Wahlberg et al. (2003a)	1997-viii-25	USA	AZ	Santa Cruz Co.
P. picta picta	AF187800.2	NW34-7	Wahlberg et al. (2003a)	1995-vii-28	USA	CO	Morgan Co.
P. tharos	EF493955.1	NW124-4	Wahlberg & Freitas (2007)	-	USA	NC	-
P. tharos orantain	AY156682.1	NW60-7	Wahlberg et al. (2003a)	2000-vii	Canada	AB	Dinosaur PP
P. tharos riocolorado	AY156678.1	NW35-9	Wahlberg et al. (2003a)	1993-ix-10	USA	CO	Montrose Co.
P. tharos tharos	AY156683.1	NW73-4	Wahlberg et al. (2003a)	2001-iii-30	Mexico	GJ	Pénjamo
P. tharos tharos	AY156670.1	NW25-18	Wahlberg et al. (2003a)	1998-v-30	USA	FL	Alachua Co.
P. tharos tharos	AY156680.1	NW53-8	Wahlberg et al. (2003a)	2000-vi-23	USA	MD	College Park
P. tharos tharos	AF187807.2	NW34-2	Wahlberg et al. (2003a)	1998-vi-25	USA	MN	Freeborn Co.
P. tharos tharos	AY156676.1	NW47-8	Wahlberg et al. (2003a)	1998-vi-25	USA	MN	Freeborn Co.
P. tharos tharos	AY156671-1	NW44-1	Wahlberg et al. (2003a)	1998-ix-03	USA	NY	Oneida Co.
P. tharos tharos	AY156672.1	NW44-2	Wahlberg et al. (2003a)	1998-ix-03	USA	NY	Oneida Co.

Species	Accession#	ID#	Publication	Coll. Date	Country	Region	Locality
P. cocyta diminutor	AY156614.1	NW49-9	Wahlberg et al. (2003a)	1999-vii-21	USA	MN	Freeborn Co.
P. cocyta diminutor	AY156613.1	NW49-8	Wahlberg et al. (2003a)	1999-vii-21	USA	MN	Freeborn Co.
P. cocyta selenis	AY156615.1	NW55-2	Wahlberg et al. (2003a)	2000-vii-24	Canada	AB	Edmonton
P. cocyta selenis	AY156626.1	NW95-8	Wahlberg et al. (2003a)	2002-vii-05	Canada	AB	Lac La Biche
P. cocyta selenis	AY156606.1	NW11-5	Wahlberg et al. (2003a)	1997-vii-12	Canada	BC	
P. cocyta selenis	AY156607.1	NW11-6	Wahlberg et al. (2003a)	1997-vii-12	Canada	BC	
P. cocyta selenis	AY156619.1	NW60-12	Wahlberg et al. (2003a)	2000-vii-12	Canada	BC	Nazco
P. cocyta selenis	AY156617.1	NW58-7	Wahlberg et al. (2003a)	2000-vii-01	Canada	BC	Pend d'Oreille Valley
P. cocyta selenis	AY156620.1	NW72-8	Wahlberg et al. (2003a)	2001-vi-12	Canada	ON	Carleton Co.
P. cocyta selenis	AY156608.1	NW47-12	Wahlberg et al. (2003a)	1998-vi-29	USA	CO	Jefferson Co.
P. cocyta selenis	AY156610.1	NW47-14	Wahlberg et al. (2003a)	1998-vi-29	USA	CO	Jefferson Co.
P. cocyta selenis	AY156611.1	NW48-10	Wahlberg et al. (2003a)	1998-vii-02	USA	CO	Jefferson Co.
P. cocyta selenis	AY156612.1	NW48-3	Wahlberg et al. (2003a)	1999-vi-11	USA	CO	Jefferson Co.
P. batesii or *P. cocyta*	AY156598.1	NW52-7	Wahlberg et al. (2003a)	2000-vii-03	Canada	AB	Strathcona Co.
P. batesii or *P. cocyta*	AY156599.1	NW60-5	Wahlberg et al. (2003a)	2000-vii	Canada	AB	Dinosaur PP
P. batesii anasazi	AY156595.1	NW34-1	Wahlberg et al. (2003a)	1996-viii-24	USA	CO	Eagle Co.
P. batesii apsaalooke	AY156596.1	NW35-8	Wahlberg et al. (2003a)	1995-ix-15	USA	WY	Bighorn Co.
P. batesii batesii	AY156602.1	NW72-1	Wahlberg et al. (2003a)	2001-vi-12	Canada	ON	Carleton Co.
P. batesii lakota	AY156605.1	NW95-12	Wahlberg et al. (2003a)	2002-vii-05	Canada	AB	Lac La Biche
P. batesii lakota	AY156604.1	NW95-11	Wahlberg et al. (2003a)	2002-vii-06	Canada	AB	Fort Assiniboine
P. batesii lakota	AF187747.2	NW35-4	Wahlberg et al. (2003a)	1994-viii-11	USA	NE	Sioux Co.
P. batesii maconensis	AY156601.1	NW69-1	Wahlberg et al. (2003a)	2001-v-18	USA	NC	Clay Co.
P. pulchella	KM547085.1	08BBLEP-02613	Hebert et al. (2016)	-	Canada	AB	Waterton Lakes NP
P. pulchella camillus	AY156649.1	NW48-4	Wahlberg et al. (2003a)	1998-ix-02	USA	CO	Adams Co.
P. pulchella camillus	AY156654.1	NW49-4	Wahlberg et al. (2003a)	1998-ix-04	USA	CO	Costilla Co.
P. pulchella montana	AF187783.2	NW27-5	Wahlberg et al. (2003a)	1994-vii-09	USA	CA	Mono Co.
P. pulchella montana	AY156664.1	NW67-16	Wahlberg et al. (2003a)	2000-viii-21	USA	OR	Deschutes Co.
P. pulchella owimba	AY156648.1	NW52-14	Wahlberg et al. (2003a)	2000-vii-22	Canada	AB	Cardinal River Divide
P. pulchella owimba	AY156665.1	NW56-1	Wahlberg et al. (2003a)	2000-vii-12	Canada	BC	Nazko

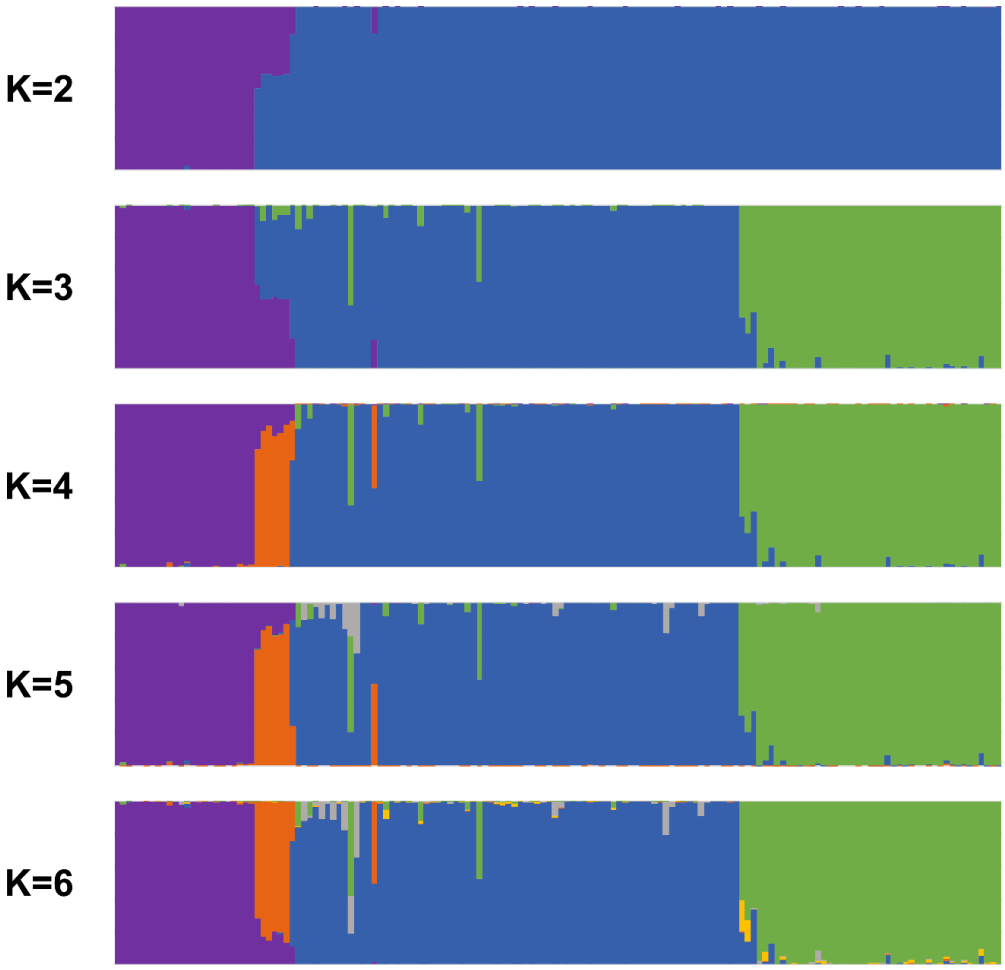
Species	Accession#	ID#	Publication	Coll. Date	Country	Region	Locality
P. pulchella owimba	AY156645.1	NW24-10	Wahlberg et al. (2003a)	1992-vi-08	USA	MT	Missoula Co.
P. pulchella pulchella	KU875886.1	KWP_Ento_37246	Sikes et al. (2017)	2010-viii-31	USA	AK	Bonanza Creek
P. pulchella pulchella	AY156647.1	NW49-13	Wahlberg et al. (2003a)	1998-x-21	USA	CA	Humboldt Co.
P. pulchella pulchella	AY156661.1	NW67-13	Wahlberg et al. (2003a)	2000-vi-25	USA	OR	Josephine Co.
P. pulchella tutchone	AY156644.1	NW23-11	Wahlberg et al. (2003a)	1996-vi-23	USA	AK	Delta Junction

Appendix 3 STRUCTURE HARVESTER outputs for the STRUCTURE analysis of 152 *Phyciodes* individuals. **(a)** Delta K values for K=2-5. **(b)** Mean of Ln prob. of data for K=2-6.

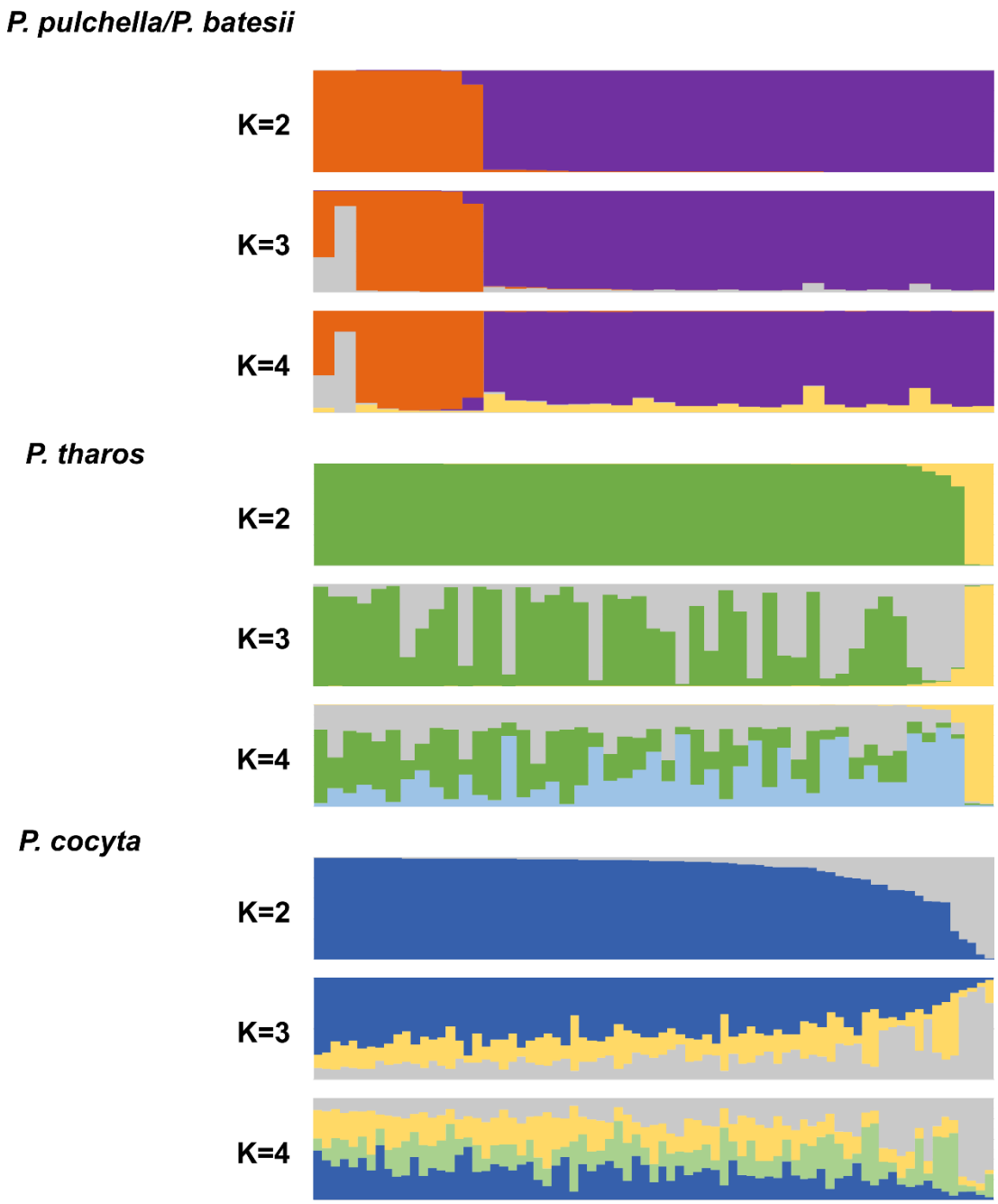




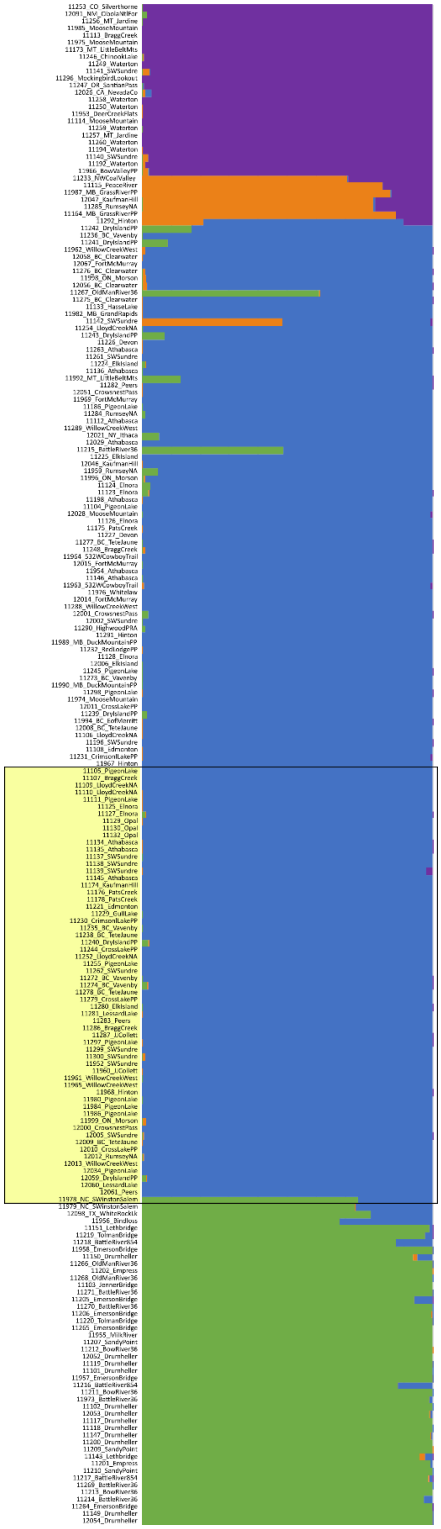
Appendix 4 STRUCTURE results for K=2-6 for 152 *Phyciodes* individuals.



Appendix 5 Subsampled STRUCTURE results for K = 2-4.



Appendix 6 STRUCTURE analysis of 1652 SNPs for 213 individuals. Species identities based on these results were used for Chapter 3 analyses. Individuals highlighted in yellow were only included in Chapter 3.



Appendix 7 Scored morphological character states for 213 *Phyciodes* individuals. Characters and states are shown and described in Figure 3.1 and Table 3.2. The 13 missing character states that were imputed are highlighted yellow.

				Character																
DNA#	**Species**	**Region**	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
11101	*P. tharos*	AB	1	2	1	0	1	3	1	1	1	1	1	2	2	1	0	2	2	1	1
11102	*P. tharos*	AB	1	2	1	0	2	2	0	0	1	1	1	0	3	1	1	2	2	1	0
11103	*P. tharos*	AB	1	2	0	0	2	3	2	1	1	1	2	2	2	2	1	2	2	1	2
11104	*P. cocyta*	AB	2	1	0	1	2	1	0	0	1	1	1	3	1	2	1	2	1	1	2
11105	*P. cocyta*	AB	1	2	0	0	1	1	0	3	1	0	2	1	2	1	0	2	1	1	0
11106	*P. cocyta*	AB	1	2	0	0	1	1	0	1	1	1	0	1	2	0	0	2	1	1	0
11107	*P. cocyta*	AB	1	2	1	0	0	0	1	1	1	0	1	1	2	0	0	1	1	1	0
11108	*P. cocyta*	AB	1	2	1	0	2	1	0	3	1	1	2	2	1	2	0	2	1	1	0
11109	*P. cocyta*	AB	2	1	0	1	2	1	0	1	1	2	1	3	2	2	1	2	1	1	2
11110	*P. cocyta*	AB	2	1	0	0	1	2	0	1	1	1	1	2	1	1	1	2	1	0	1
11111	*P. cocyta*	AB	1	2	1	0	0	0	1	0	1	1	1	0	2	1	0	2	1	1	0
11112	*P. cocyta*	AB	2	1	1	0	2	2	2	0	2	1	2	**2**	2	1	1	2	1	1	1
11113	*P. pulchella*	AB	2	1	0	1	2	3	2	3	1	2	2	2	2	1	2	0	0	1	1
11114	*P. pulchella*	AB	1	1	0	1	3	3	0	2	2	2	1	1	0	1	2	2	1	0	1
11115	*P. batesii*	AB	2	0	0	1	2	3	2	3	1	1	1	3	0	1	2	0	1	1	1
11117	*P. tharos*	AB	1	2	1	0	2	3	0	1	2	1	2	2	1	2	0	2	2	1	1
11118	*P. tharos*	AB	1	2	1	0	1	1	0	0	2	1	2	0	2	1	0	2	1	0	1
11119	*P. tharos*	AB	2	2	1	1	2	3	2	3	0	1	2	0	2	1	1	2	2	1	1
11123	*P. cocyta*	AB	1	2	1	0	1	1	1	1	1	1	1	2	1	0	0	2	2	0	0
11124	*P. cocyta*	AB	2	1	0	1	2	2	2	0	1	2	2	3	1	1	0	2	1	1	2
11125	*P. cocyta*	AB	2	2	0	1	3	3	1	1	1	2	2	3	0	2	0	2	1	1	0
11126	*P. cocyta*	AB	2	2	0	0	2	2	2	0	1	1	2	3	2	2	0	1	1	1	0
11127	*P. cocyta*	AB	1	2	1	0	0	1	0	2	1	1	1	2	1	0	1	2	1	1	2
11128	*P. cocyta*	AB	1	2	1	0	1	1	0	1	1	1	0	1	2	1	1	2	2	1	0

				Character																
DNA#	**Species**	**Region**	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
11129	*P. cocyta*	AB	2	1	0	0	1	1	1	3	1	2	1	2	2	1	0	1	1	1	0
11130	*P. cocyta*	AB	1	2	1	0	1	2	0	2	0	0	1	0	1	1	0	2	2	0	0
11132	*P. cocyta*	AB	1	2	1	0	1	1	1	1	2	1	1	2	1	1	0	3	1	1	0
11133	*P. cocyta*	AB	2	1	0	0	2	2	1	2	1	2	2	2	1	1	1	0	1	1	0
11134	*P. cocyta*	AB	2	2	0	0	2	2	2	1	1	2	2	2	1	1	1	2	1	0	0
11135	*P. cocyta*	AB	2	1	0	0	1	1	0	1	2	1	0	3	1	1	1	1	1	0	1
11136	*P. cocyta*	AB	2	1	0	0	1	0	1	3	0	1	2	2	1	1	1	2	1	0	1
11137	*P. cocyta*	AB	1	2	1	0	1	1	0	2	1	1	1	1	3	0	0	2	3	1	0
11138	*P. cocyta*	AB	1	2	1	0	1	1	1	3	1	0	2	1	1	0	0	2	1	1	1
11139	*P. cocyta*	AB	1	2	1	0	1	2	0	3	0	1	1	1	3	0	1	2	2	1	2
11140	*P. pulchella*	AB	2	1	1	1	3	3	1	3	2	2	1	2	1	1	2	0	0	0	1
11141	*P. pulchella*	AB	2	1	0	1	3	3	2	1	1	2	2	2	0	2	2	0	0	1	1
11142	*hybrid*	AB	2	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	0	0
11143	*P. tharos*	AB	2	2	0	1	2	2	2	0	2	2	2	2	2	1	1	1	1	1	1
11145	*P. cocyta*	AB	2	0	0	0	2	1	1	3	1	1	1	2	2	1	1	1	1	1	2
11146	*P. cocyta*	AB	2	2	0	1	2	1	0	1	1	1	2	2	2	1	1	1	1	1	0
11147	*P. tharos*	AB	2	1	1	0	2	2	0	1	1	1	1	2	3	2	0	2	2	1	1
11149	*P. tharos*	AB	2	2	0	1	2	3	2	1	1	2	2	3	3	2	1	3	2	1	1
11150	*P. tharos*	AB	1	2	0	0	2	3	0	1	2	1	2	1	3	0	0	3	3	1	1
11151	*P. tharos*	AB	2	2	1	1	2	3	0	1	2	2	1	2	2	1	1	3	2	1	0
11164	*P. batesii*	MB	2	0	0	1	3	3	2	0	2	2	1	3	2	2	2	2	2	1	1
11173	*P. pulchella*	MT	2	0	0	1	3	3	2	3	2	2	2	2	0	2	2	2	1	1	1
11174	*P. cocyta*	AB	2	1	1	1	2	1	2	0	1	1	2	2	1	1	1	1	1	1	1
11175	*P. cocyta*	AB	2	2	1	1	2	2	1	0	0	2	1	2	1	1	1	1	0	0	0
11176	*P. cocyta*	AB	2	2	0	1	2	1	1	1	1	1	2	2	1	1	1	1	1	1	0
11178	*P. cocyta*	AB	2	1	0	1	1	1	**1**	2	1	1	1	2	1	1	1	0	1	1	**0**

				Character																
DNA#	**Species**	**Region**	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
11186	*P. cocyta*	AB	1	2	1	0	2	2	1	1	1	1	1	2	3	2	0	2	2	1	2
11192	*P. pulchella*	AB	1	2	1	1	3	3	0	2	1	2	2	0	0	1	2	2	1	0	1
11194	*P. pulchella*	AB	2	1	0	1	3	3	2	2	2	2	1	2	0	1	2	2	1	1	1
11196	*P. cocyta*	AB	1	2	1	0	1	2	1	1	1	1	2	0	2	1	1	3	2	0	1
11198	*P. cocyta*	AB	2	2	1	1	2	2	1	1	1	1	1	2	1	1	1	2	1	1	0
11200	*P. tharos*	AB	2	2	1	0	1	1	1	1	2	1	1	2	1	0	1	2	1	1	2
11201	*P. tharos*	AB	1	2	1	0	2	3	1	1	1	2	2	1	3	1	0	2	2	0	3
11202	*P. tharos*	AB	1	2	1	1	2	3	1	1	1	2	2	1	3	1	1	3	2	1	1
11205	*P. tharos*	AB	1	2	0	0	2	2	2	1	2	0	2	2	3	1	0	2	2	1	1
11206	*P. tharos*	AB	1	2	1	0	1	2	1	1	2	1	1	2	3	1	0	2	3	1	1
11207	*P. tharos*	AB	1	2	1	0	1	1	0	1	1	1	2	2	3	1	0	2	1	1	1
11209	*P. tharos*	AB	2	2	0	1	2	2	1	1	1	1	2	3	2	1	1	2	1	0	1
11210	*P. tharos*	AB	2	0	0	1	2	3	2	0	1	2	2	3	2	1	1	2	1	1	1
11211	*P. tharos*	AB	2	2	1	0	2	2	2	0	2	1	2	2	2	1	1	2	2	0	1
11212	*P. tharos*	AB	1	2	0	0	1	2	2	1	2	0	2	2	2	1	1	2	2	1	0
11213	*P. tharos*	AB	1	2	1	0	1	3	1	1	2	1	2	2	3	1	1	3	3	1	1
11214	*P. tharos*	AB	1	2	0	0	0	3	0	0	1	1	1	0	3	0	0	3	2	1	0
11215	*hybrid*	AB	2	2	0	1	2	2	2	1	1	2	2	3	3	2	1	3	1	1	1
11216	*P. tharos*	AB	1	2	1	0	1	3	0	1	2	1	1	0	2	1	0	3	2	1	0
11217	*P. tharos*	AB	2	1	1	1	2	2	2	0	2	2	2	2	3	1	1	2	1	1	1
11218	*P. tharos*	AB	1	2	1	0	0	2	2	0	2	0	2	1	2	1	1	2	1	1	1
11219	*P. tharos*	AB	2	2	1	0	2	2	2	0	2	2	2	2	2	1	1	2	1	1	1
11220	*P. tharos*	AB	1	2	1	1	1	2	1	1	1	1	2	2	2	1	2	2	2	1	1
11221	*P. cocyta*	AB	1	2	1	0	0	2	0	1	0	0	2	2	2	1	1	2	1	1	0
11224	*P. cocyta*	AB	1	2	1	0	0	2	1	1	1	0	2	2	0	0	1	2	1	1	2
11225	*P. cocyta*	AB	1	2	1	0	0	1	0	1	1	1	1	1	2	1	0	2	2	1	0

				Character																
DNA#	**Species**	**Region**	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
11226	*P. cocyta*	AB	1	2	1	0	0	1	0	0	1	1	2	1	2	1	1	2	1	1	0
11227	*P. cocyta*	AB	1	2	1	0	0	1	0	1	1	0	2	2	2	1	0	2	1	1	0
11229	*P. cocyta*	AB	1	2	1	0	1	1	0	1	1	1	2	2	2	1	0	1	1	0	0
11230	*P. cocyta*	AB	1	2	1	0	1	1	0	2	0	1	1	1	3	1	0	2	2	1	0
11231	*P. cocyta*	AB	1	2	1	0	0	1	1	1	1	1	2	1	1	1	1	1	1	0	2
11232	*P. cocyta*	AB	1	2	1	0	1	1	1	1	1	1	1	1	1	1	1	2	1	1	0
11233	*P. batesii*	AB	1	1	0	1	1	2	1	2	2	2	2	1	1	1	2	2	2	1	0
11235	*P. cocyta*	BC	1	2	1	0	2	1	0	1	1	1	0	0	3	1	0	3	2	1	3
11236	*P. cocyta*	BC	1	**2**	1	0	1	2	0	1	1	1	2	**2**	2	1	1	3	1	1	2
11238	*P. cocyta*	BC	1	2	0	0	1	1	1	1	1	1	**2**	1	3	2	1	2	3	1	**1**
11239	*P. cocyta*	AB	1	2	0	0	1	1	0	1	1	1	1	0	2	1	0	3	1	1	0
11240	*P. cocyta*	AB	1	2	0	0	1	1	1	1	1	0	2	1	2	1	0	2	1	0	0
11241	*P. cocyta*	AB	2	1	1	1	2	2	1	3	1	1	2	2	2	1	1	2	1	1	2
11242	*P. cocyta*	AB	2	2	0	1	1	3	2	3	1	1	2	3	1	1	1	2	1	1	0
11243	*P. cocyta*	AB	2	1	0	1	2	3	2	0	1	2	1	3	1	1	1	2	1	1	1
11244	*P. cocyta*	AB	1	2	0	0	1	1	1	0	1	1	2	1	1	1	0	1	1	1	0
11245	*P. cocyta*	AB	2	**1**	1	1	1	1	2	0	2	1	2	3	1	2	1	1	0	1	1
11246	*P. pulchella*	AB	1	1	0	1	3	3	2	2	2	2	2	1	0	1	2	1	1	1	1
11247	*P. pulchella*	OR	1	1	0	1	3	3	1	2	2	2	2	1	0	2	2	1	1	0	1
11248	*P. cocyta*	AB	2	1	0	1	2	1	1	2	1	1	1	2	2	2	1	1	1	1	0
11249	*P. pulchella*	AB	1	2	0	1	3	3	0	2	1	2	0	1	0	1	2	1	2	1	1
11250	*P. pulchella*	AB	1	1	0	1	3	3	0	2	2	2	0	1	1	2	2	2	3	1	0
11252	*P. cocyta*	AB	2	1	0	1	2	2	1	0	1	1	1	2	1	1	1	2	1	1	0
11253	*P. pulchella*	CO	2	0	0	1	2	3	2	1	1	2	2	1	1	2	2	2	1	1	1
11254	*P. cocyta*	AB	2	2	0	1	2	2	0	1	1	2	0	2	2	1	1	2	2	1	1
11255	*P. cocyta*	AB	2	0	1	1	2	2	2	1	1	1	2	1	1	1	1	2	1	0	0

				Character																
DNA#	**Species**	**Region**	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
11256	*P. pulchella*	MT	2	2	1	1	3	3	2	2	2	2	2	2	1	1	2	1	1	1	1
11257	*P. pulchella*	MT	1	0	0	1	3	3	0	2	2	2	2	1	0	1	2	1	2	1	0
11258	*P. pulchella*	AB	2	2	1	1	3	3	1	2	2	2	1	1	0	2	2	0	1	1	1
11259	*P. pulchella*	AB	1	2	0	1	2	2	0	2	2	2	1	1	0	1	2	2	3	1	0
11260	*P. pulchella*	AB	2	1	1	1	3	3	2	1	1	2	2	2	2	2	2	2	1	1	1
11261	*P. cocyta*	AB	2	1	1	1	2	1	1	3	0	1	1	1	0	1	1	0	2	1	0
11262	*P. cocyta*	AB	1	2	1	0	0	1	0	1	1	0	1	0	2	0	0	2	2	1	0
11263	*P. cocyta*	AB	2	1	1	0	2	2	1	2	1	2	2	0	1	0	1	1	1	0	1
11264	*P. tharos*	AB	1	2	1	0	1	2	0	1	2	0	2	1	2	1	1	2	2	1	1
11265	*P. tharos*	AB	1	**2**	1	0	1	2	2	1	1	0	2	2	2	2	0	2	1	1	1
11266	*P. tharos*	AB	1	2	1	0	1	1	0	1	1	2	2	1	3	1	0	3	1	1	1
11267	*P. tharos*	AB	1	2	1	0	1	2	2	1	1	2	2	3	3	2	0	3	2	1	0
11268	*P. tharos*	AB	1	2	0	0	2	2	1	1	1	1	2	2	3	1	0	3	2	1	1
11269	*P. tharos*	AB	2	2	1	1	2	3	2	0	1	1	2	2	1	1	2	1	1	1	1
11270	*P. tharos*	AB	2	1	1	0	1	3	2	0	2	1	2	2	1	1	1	2	1	1	1
11271	*P. tharos*	AB	1	2	1	0	1	1	1	0	2	1	2	2	2	1	1	2	1	0	0
11272	*P. cocyta*	BC	1	2	1	0	0	2	1	1	1	0	2	2	3	0	0	3	1	0	3
11273	*P. cocyta*	BC	1	2	1	0	1	2	1	1	1	1	0	2	3	1	0	3	2	0	0
11274	*P. cocyta*	BC	2	1	1	1	1	2	1	3	2	1	2	0	2	1	1	2	1	0	0
11275	*P. cocyta*	BC	1	2	1	0	1	2	1	0	1	1	2	0	2	1	0	2	2	1	3
11276	*P. cocyta*	BC	1	2	1	0	1	2	1	0	1	1	2	1	2	1	0	2	2	1	2
11277	*P. cocyta*	BC	**2**	1	0	0	2	3	1	1	1	1	2	3	2	1	2	2	1	1	0
11278	*P. cocyta*	BC	2	2	1	1	2	2	1	1	1	1	2	3	2	1	1	2	1	1	2
11279	*P. cocyta*	AB	2	1	0	1	2	1	1	0	1	1	1	3	2	1	1	1	1	1	3
11280	*P. cocyta*	AB	2	2	1	1	2	3	1	1	1	1	2	1	2	1	1	1	1	0	0
11281	*P. cocyta*	AB	2	2	1	1	2	1	2	3	0	2	2	3	1	2	0	2	1	1	1

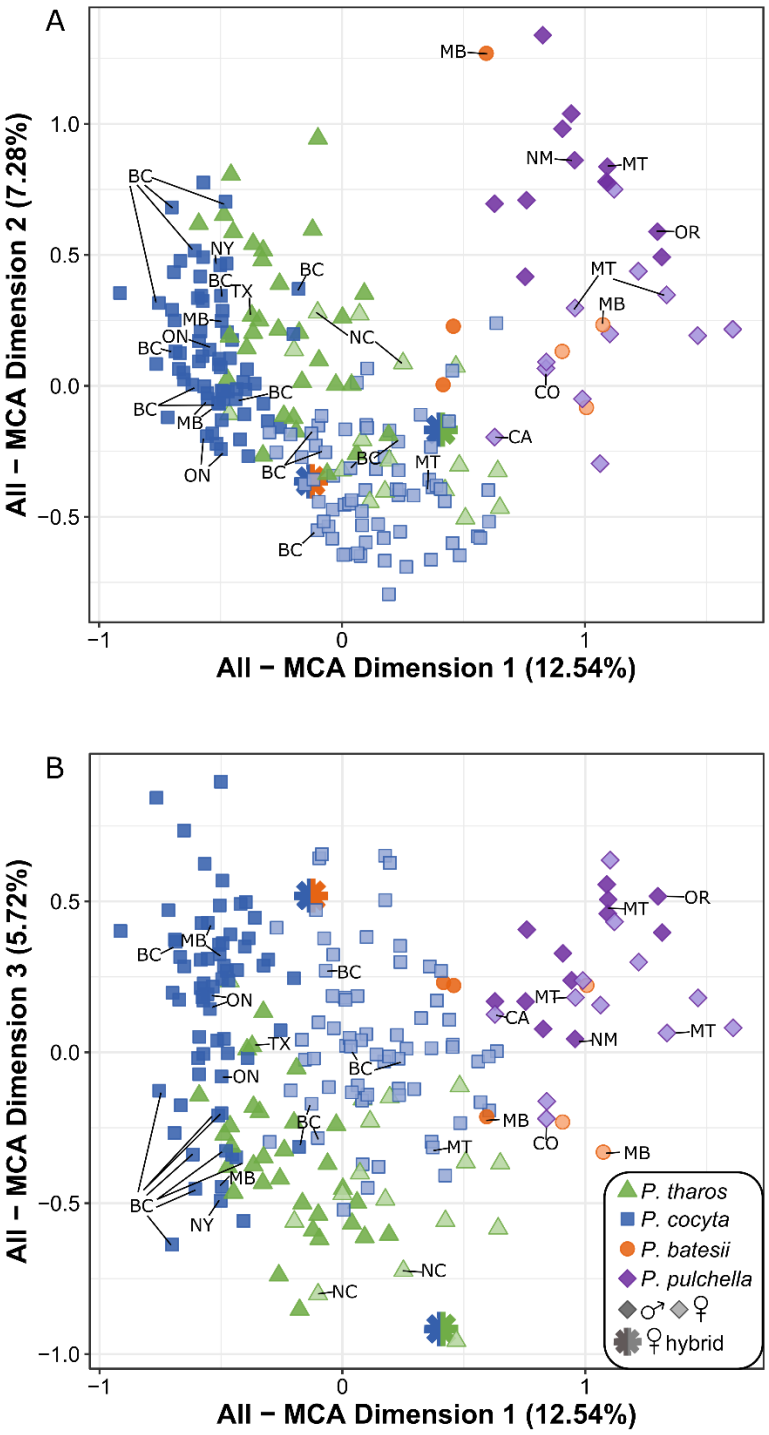
				Character																
DNA#	**Species**	**Region**	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
11282	*P. cocyta*	AB	1	2	0	0	1	1	0	3	1	1	2	1	2	1	1	2	1	1	0
11283	*P. cocyta*	AB	1	2	1	0	1	1	0	3	0	0	2	1	2	1	1	2	2	1	2
11284	*P. cocyta*	AB	1	1	1	0	1	1	1	3	1	1	2	1	2	1	0	2	2	1	0
11285	*P. batesii*	AB	1	1	0	1	2	3	1	2	1	1	2	2	2	1	2	2	2	0	0
11286	*P. cocyta*	AB	2	1	1	1	2	2	0	2	1	2	1	2	2	1	2	2	1	1	2
11287	*P. cocyta*	AB	2	1	1	0	1	1	1	2	1	1	2	3	2	0	1	2	1	1	0
11288	*P. cocyta*	AB	2	0	1	0	1	1	1	2	1	1	1	3	1	0	1	0	1	1	0
11289	*P. cocyta*	AB	1	2	0	0	1	2	0	1	1	1	1	0	2	0	0	3	2	1	0
11290	*P. cocyta*	AB	2	2	1	1	2	2	1	1	1	1	2	3	1	1	1	2	1	0	2
11291	*P. cocyta*	AB	2	1	0	0	2	1	1	3	1	1	2	3	2	1	1	2	1	1	0
11292	*P. cocyta*	AB	1	2	1	1	1	1	0	3	2	1	2	1	2	0	1	2	2	0	1
11296	*P. pulchella*	AB	1	2	0	1	3	3	0	2	2	2	2	1	0	1	2	1	1	0	1
11297	*P. cocyta*	AB	1	2	1	0	1	1	0	2	1	1	1	1	2	1	0	2	1	1	0
11298	*P. cocyta*	AB	2	0	1	1	2	1	2	0	0	1	2	3	2	1	1	1	1	1	2
11299	*P. cocyta*	AB	2	2	0	1	2	1	2	0	1	1	2	3	0	1	1	0	1	1	2
11300	*P. cocyta*	AB	1	2	1	1	0	0	1	0	1	0	2	2	2	1	0	2	1	1	1
11952	*P. cocyta*	AB	2	2	1	0	1	2	1	0	1	2	2	3	2	1	0	2	2	1	0
11953	*P. pulchella*	AB	1	2	0	1	3	3	0	2	2	2	0	1	1	1	2	3	3	0	1
11954	*P. cocyta*	AB	2	2	1	1	2	1	0	1	1	1	2	3	1	1	1	2	1	1	0
11955	*P. tharos*	AB	1	2	1	0	2	3	2	1	2	1	2	3	2	1	0	2	2	1	1
11956	*P. tharos*	AB	1	2	1	0	2	3	2	0	2	1	2	2	2	2	1	2	2	1	1
11957	*P. tharos*	AB	2	2	1	1	2	2	2	0	2	2	2	3	2	2	2	2	1	1	1
11958	*P. tharos*	AB	1	2	1	0	2	2	1	1	1	2	2	2	2	1	0	3	1	1	1
11959	*P. cocyta*	AB	1	2	1	1	0	1	1	2	1	1	2	0	2	1	1	2	2	0	0
11960	*P. cocyta*	AB	2	2	0	0	2	1	1	1	1	2	1	2	2	1	1	2	1	1	0
11961	*P. cocyta*	AB	2	2	0	1	2	1	1	0	1	2	1	2	2	1	1	2	1	1	0

				Character																
DNA#	**Species**	**Region**	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
11962	*P. cocyta*	AB	2	2	1	1	1	2	2	0	1	2	2	2	1	1	1	2	1	1	0
11963	*P. cocyta*	AB	2	1	0	1	2	2	2	1	0	2	2	3	1	2	1	2	1	1	0
11964	*P. cocyta*	AB	2	2	1	1	2	3	2	2	1	2	2	2	1	1	1	2	1	1	0
11965	*P. cocyta*	AB	2	0	0	1	2	2	1	2	1	2	1	2	2	1	1	1	2	0	1
11966	*P. pulchella*	AB	2	1	0	1	2	2	2	3	2	2	2	1	0	1	2	1	1	0	1
11967	*P. cocyta*	AB	1	1	1	0	1	1	1	2	0	1	1	1	2	1	0	2	1	1	2
11968	*P. cocyta*	AB	1	2	1	0	0	1	1	3	1	0	2	1	1	0	0	2	1	1	0
11969	*P. cocyta*	AB	2	2	1	1	2	2	2	0	1	1	2	3	2	1	1	2	2	1	2
11973	*P. tharos*	AB	1	2	1	0	1	2	2	0	2	1	2	2	2	1	1	2	1	1	1
11974	*P. cocyta*	AB	2	1	1	1	2	2	2	3	1	2	2	3	3	1	1	1	2	1	1
11975	*P. pulchella*	AB	2	1	0	1	3	3	2	1	2	2	2	3	0	2	2	0	0	1	1
11976	*P. cocyta*	AB	1	2	1	0	0	3	1	3	0	0	2	0	1	0	0	1	1	0	0
11978	*P. tharos*	NC	2	**2**	1	0	2	2	0	1	1	2	2	2	3	2	0	3	1	1	1
11979	*P. tharos*	NC	2	2	1	1	2	2	1	0	2	2	2	1	3	2	1	3	1	1	1
11980	*P. cocyta*	AB	2	1	1	0	1	1	2	3	1	1	2	2	1	1	1	1	1	1	0
11982	*P. cocyta*	MB	1	2	1	0	1	1	1	0	0	1	2	1	1	0	0	1	2	0	2
11984	*P. cocyta*	AB	2	1	0	1	3	2	2	1	1	2	2	3	2	1	1	1	1	1	0
11985	*P. pulchella*	AB	1	2	0	1	2	2	0	2	2	2	2	1	0	1	2	2	1	0	1
11986	*P. cocyta*	AB	2	1	1	1	2	1	1	3	0	1	2	2	2	1	1	1	0	1	0
11987	*P. batesii*	MB	1	0	1	1	3	3	0	2	2	1	0	1	3	1	2	3	2	1	1
11989	*P. cocyta*	MB	1	2	1	0	2	2	0	0	1	1	0	1	2	1	0	2	2	1	3
11990	*P. cocyta*	MB	1	2	1	0	1	1	0	0	1	0	2	1	2	1	1	2	1	0	0
11992	*P. cocyta*	MT	2	2	0	1	2	2	2	1	0	1	2	3	2	1	2	2	1	1	1
11994	*P. cocyta*	BC	1	2	1	0	0	1	0	1	1	0	1	1	2	1	1	2	2	0	2
11996	*P. cocyta*	ON	1	2	1	0	1	1	1	0	1	1	1	2	3	1	1	2	1	1	2
11998	*P. cocyta*	ON	1	2	1	0	0	1	1	1	0	0	2	3	3	1	1	2	1	1	0

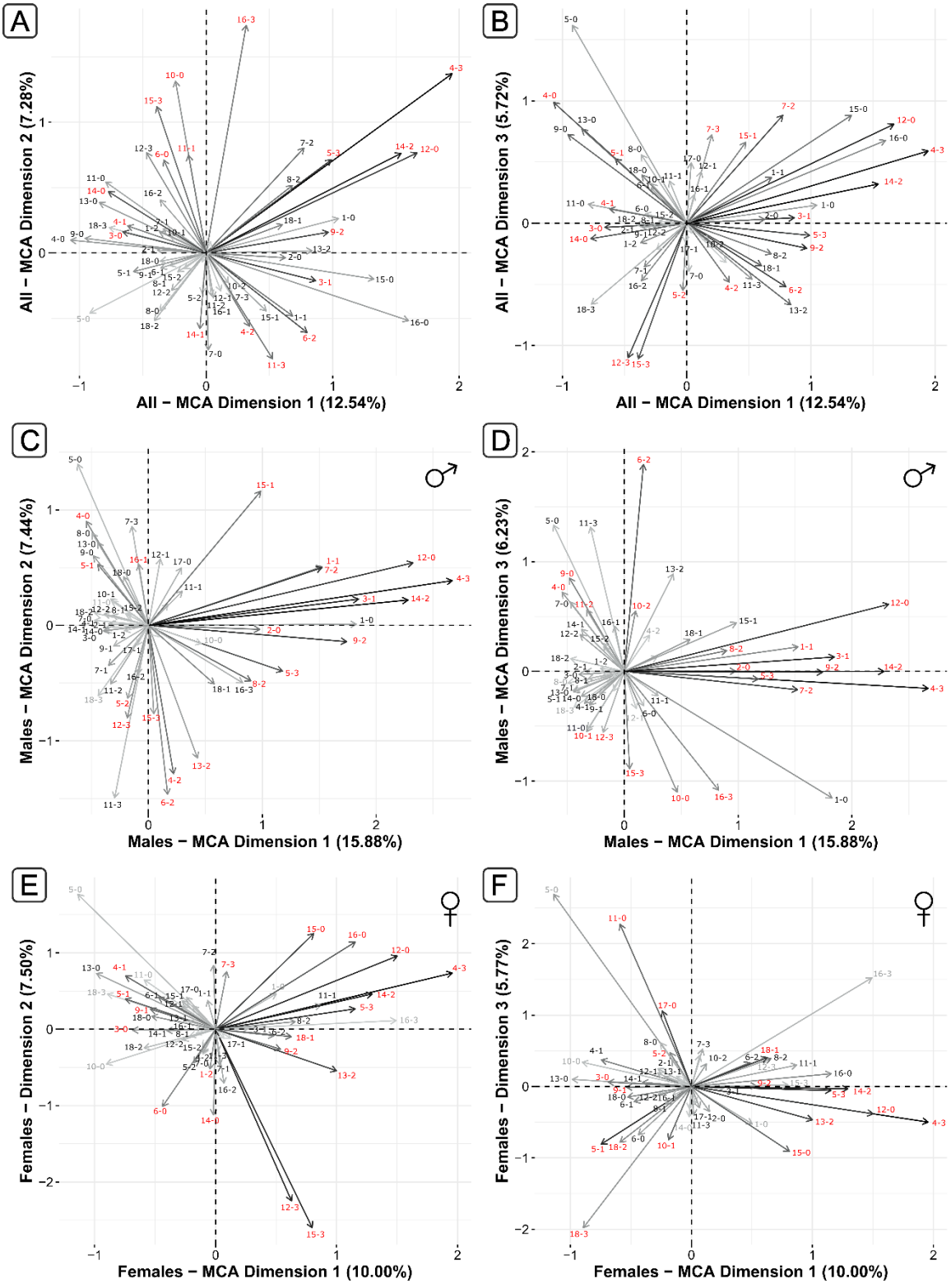
				Character																
DNA#	**Species**	**Region**	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
11999	*P. cocyta*	ON	1	1	1	0	1	1	1	1	1	0	1	1	3	1	1	2	2	1	0
12000	*P. cocyta*	AB	2	2	0	0	2	2	1	1	1	2	2	2	2	1	2	2	1	1	0
12001	*P. cocyta*	AB	2	1	0	0	1	2	2	0	1	1	0	2	2	0	1	2	2	1	**0**
12002	*P. cocyta*	AB	2	1	1	1	2	2	1	2	1	2	2	2	2	1	1	1	1	0	1
12005	*P. cocyta*	AB	1	2	1	0	1	1	1	0	1	1	1	2	2	1	1	2	2	1	0
12006	*P. cocyta*	AB	1	2	1	0	1	1	1	1	1	1	1	0	2	1	0	2	1	1	0
12008	*P. cocyta*	BC	1	2	1	0	1	1	0	1	1	1	1	1	3	1	1	2	2	1	1
12009	*P. cocyta*	BC	2	2	1	0	2	1	0	1	1	2	1	3	2	1	1	2	1	1	1
12010	*P. cocyta*	AB	1	2	1	0	1	2	1	1	1	1	2	1	2	0	1	2	2	0	3
12011	*P. cocyta*	AB	2	1	1	1	1	2	2	3	1	1	2	2	0	1	1	1	1	0	1
12012	*P. cocyta*	AB	1	2	1	0	1	1	1	1	1	0	1	1	1	0	1	1	1	1	0
12013	*P. cocyta*	AB	2	1	0	1	2	3	1	1	1	2	2	2	2	0	2	2	2	0	1
12014	*P. cocyta*	AB	2	1	0	1	2	2	2	0	2	2	2	3	1	1	1	1	2	0	0
12015	*P. cocyta*	AB	2	2	0	1	2	2	1	1	2	2	1	3	1	1	1	2	1	1	0
12021	*P. cocyta*	NY	1	2	1	0	1	2	1	1	1	2	1	1	3	1	0	3	2	1	2
12026	*P. pulchella*	CA	2	2	0	1	1	2	2	3	1	1	2	1	0	2	1	1	0	1	1
12028	*P. cocyta*	AB	1	2	1	0	1	1	1	1	1	1	1	1	2	0	0	2	1	0	1
12029	*P. cocyta*	AB	1	2	1	0	1	1	0	1	0	1	2	1	2	1	0	2	1	1	0
12034	*P. cocyta*	AB	2	1	1	1	2	2	2	1	0	1	1	3	2	1	1	2	1	1	0
12046	*P. cocyta*	AB	1	2	1	0	1	1	1	3	1	1	0	1	1	1	0	2	3	1	0
12047	*P. batesii*	AB	2	1	0	1	2	3	2	1	2	2	2	3	1	1	2	2	3	0	1
12051	*P. cocyta*	AB	1	2	1	0	1	2	1	1	0	1	0	0	2	1	0	3	2	1	0
12052	*P. tharos*	AB	1	2	1	0	1	1	1	1	2	1	2	1	3	1	0	3	2	1	1
12053	*P. tharos*	AB	1	2	1	0	1	3	1	1	1	1	1	1	3	1	0	3	3	1	0
12054	*P. tharos*	AB	1	2	1	0	1	3	0	1	2	1	1	1	3	1	1	3	1	1	2
12056	*P. cocyta*	BC	1	2	1	0	1	2	0	1	2	1	0	1	1	1	0	3	2	1	0

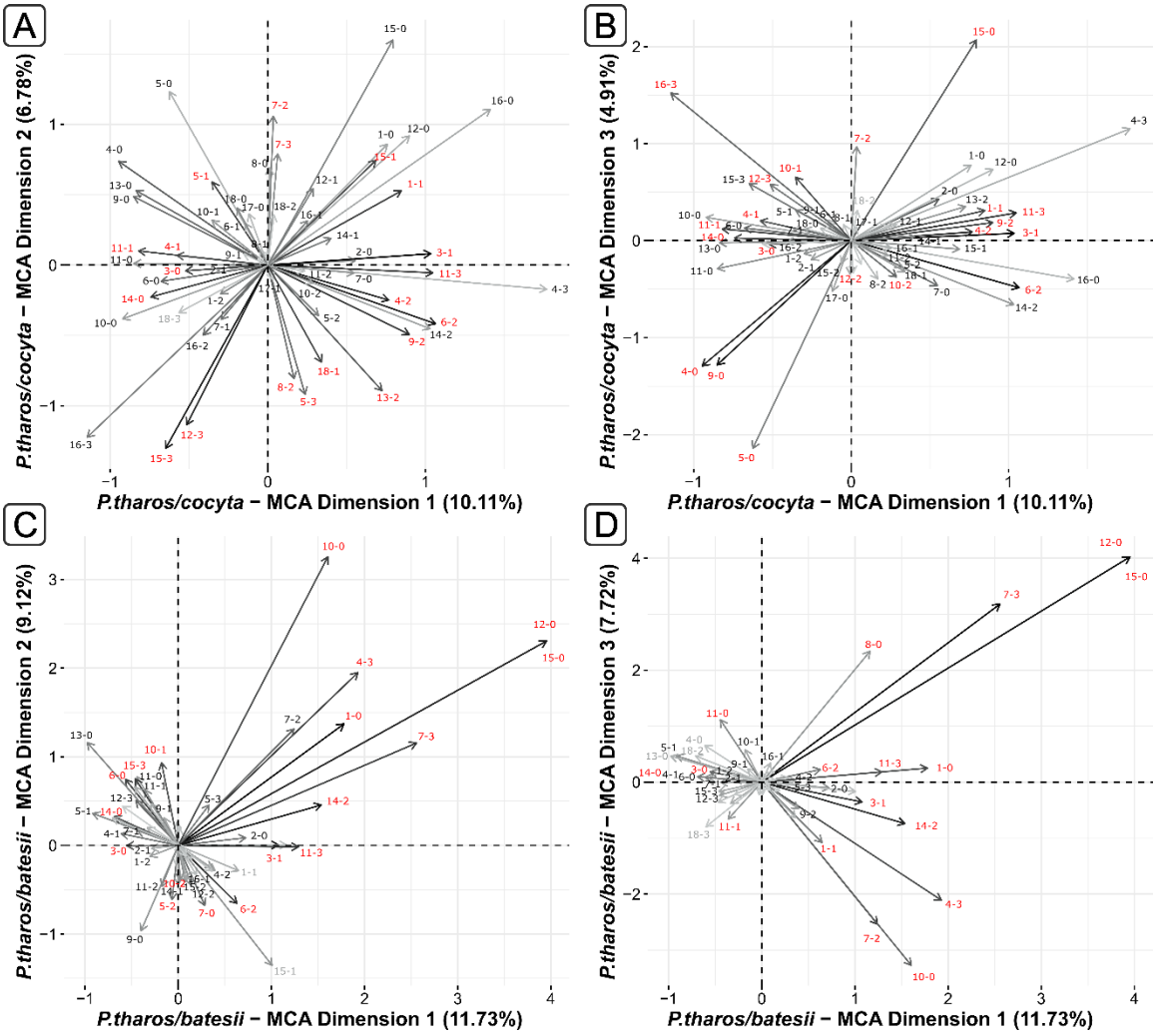
				Character																
DNA#	**Species**	**Region**	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
12058	*P. cocyta*	BC	2	2	1	0	2	2	2	3	1	1	2	2	2	1	2	2	1	0	0
12059	*P. cocyta*	AB	1	2	0	0	1	1	1	1	1	1	1	2	1	1	0	2	2	1	2
12060	*P. cocyta*	AB	1	**2**	0	0	1	2	1	1	1	1	2	1	1	1	0	2	1	0	3
12061	*P. cocyta*	AB	2	2	0	0	2	1	1	0	2	1	2	3	2	1	1	0	1	1	3
12067	*P. cocyta*	AB	1	2	1	0	0	1	0	1	1	0	0	2	2	1	0	2	1	0	0
12091	*P. pulchella*	NM	1	0	0	1	3	3	1	3	2	2	2	1	1	1	2	3	3	1	1
12098	*P. tharos*	TX	1	0	1	0	1	1	0	1	1	1	1	0	2	1	1	3	1	0	1

Appendix 8 MCA plots from Fig. 3.3A-B with non-Alberta individuals labelled.

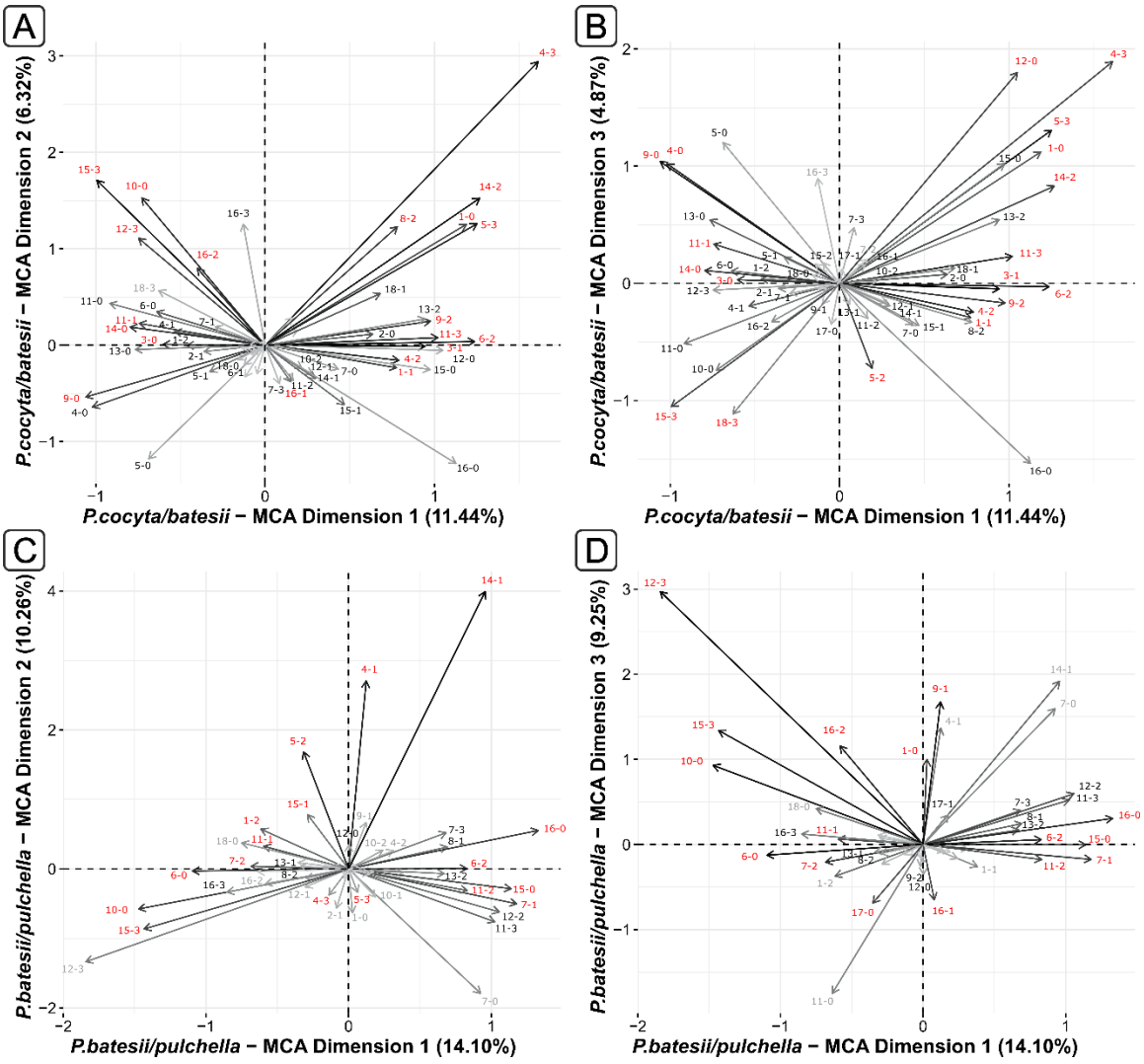


Appendix 9 Character state contributions for the MCA plots in Figure 3.3. (following page) Darker lines indicate higher combined contribution for the two dimensions for each plot. Character state labels are red for top-ten contributors for either dimension, black for states that contributed significantly to either dimension (vtest $\geq$ 1.96), and grey for states that did not contribute significantly to either dimension. **(A)** Dimensions 1 and 2 for all individuals. **(B)** Dimensions 1 and 3 for all individuals. **(C)** Dimensions 1 and 2 for males. **(D)** Dimensions 1 and 3 for males. **(E)** Dimensions 1 and 2 for females. **(F)** Dimensions 1 and 3 for females.


A
All − MCA Dimension 2 (7.28%)
All − MCA Dimension 1 (12.54%)
B
All − MCA Dimension 3 (5.72%)
All − MCA Dimension 1 (12.54%)
C
Males − MCA Dimension 2 (7.44%)
Males − MCA Dimension 1 (15.88%)
D
Males − MCA Dimension 3 (6.23%)
Males − MCA Dimension 1 (15.88%)
E
Females − Dimension 2 (7.50%)
Females − MCA Dimension 1 (10.00%)
F
Females − Dimension 3 (5.77%)
Females − MCA Dimension 1 (10.00%)



Appendix 10 Character state contributions for two-species MCA plots in Figure 3.4. Darker lines indicate higher combined contribution for the two dimensions for each plot. Character state labels are red for top-ten contributors for either dimension, black for states that contributed significantly to either dimension according to (vtest $\geq$ 1.96), and grey for states that did not contribute significantly to either dimension. **(A)** Dimensions 1 and 2 for *P. tharos* and *P. cocyta*. **(B)** Dimensions 1 and 3 for *P. tharos* and *P. cocyta*. **(C)** Dimensions 1 and 2 for *P. tharos* and *P. batesii*. **(D)** Dimensions 1 and 3 for *P. tharos* and *P. batesii*.



Appendix 11 Character state contributions for two-species MCA plots in Figure 3.5. Darker lines indicate higher combined contribution for the two dimensions for each plot. Character state labels are red for top-ten contributors for either dimension, black for states that contributed significantly to either dimension according to (vtest $\geq$ 1.96), and grey for states that did not contribute significantly to either dimension. **(A)** Dimensions 1 and 2 for *P. cocyta* and *P. batesii*. **(B)** Dimensions 1 and 3 for *P. cocyta* and *P. batesii*. **(C)** Dimensions 1 and 2 for *P. batesii* and *P. pulchella*. **(D)** Dimensions 1 and 3 for *P. batesii* and *P. pulchella*.

Appendix 12 Character state coordinates, contributions, and vtest scores for the first three components of the MCA analysis for all individuals. vtest scores ≥ 1.96 indicate significance.

	Dimension 1			Dimension 2			Dimension 3		
Char_State	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**
1_0	1.046	1.400	4.041	0.263	0.152	1.016	0.147	0.061	0.569
1_1	0.683	2.387	5.941	-0.481	2.037	-4.183	0.374	1.566	3.250
1_2	-0.370	1.788	-7.700	0.163	0.594	3.384	-0.161	0.740	-3.346
2_0	0.627	2.906	7.150	-0.037	0.017	-0.419	0.032	0.016	0.362
2_1	-0.385	1.783	-7.150	0.023	0.011	0.419	-0.019	0.010	-0.362
3_0	-0.658	4.789	-10.994	0.161	0.494	2.690	-0.034	0.028	-0.563
3_1	0.866	6.299	10.994	-0.212	0.649	-2.690	0.044	0.036	0.563
4_0	-1.071	2.097	-5.022	0.098	0.030	0.460	0.988	3.910	4.632
4_1	-0.621	2.923	-7.225	0.207	0.560	2.410	0.116	0.225	1.353
4_2	0.344	0.948	4.197	-0.560	4.343	-6.844	-0.482	4.091	-5.887
4_3	1.943	7.582	9.600	1.370	6.493	6.770	0.590	1.536	2.918
5_0	-0.918	0.308	-1.850	-0.459	0.133	-0.925	1.617	2.095	3.258
5_1	-0.574	2.380	-6.422	-0.142	0.252	-1.591	0.526	4.373	5.879
5_2	-0.031	0.007	-0.341	-0.307	1.156	-3.398	-0.549	4.706	-6.076
5_3	0.989	4.649	8.188	0.712	4.146	5.893	-0.100	0.104	-0.826
6_0	-0.335	0.644	-3.157	0.710	4.995	6.701	0.075	0.070	0.704
6_1	-0.286	0.679	-3.595	-0.097	0.136	-1.224	0.286	1.489	3.595
6_2	0.798	3.432	7.193	-0.608	3.431	-5.481	-0.521	3.201	-4.691
7_0	0.017	0.001	0.135	-0.740	4.041	-5.729	-0.393	1.455	-3.046
7_1	-0.341	1.051	-4.626	0.186	0.540	2.526	-0.357	2.523	-4.841
7_2	0.773	1.855	4.905	0.803	3.443	5.093	0.882	5.295	5.597
7_3	0.202	0.123	1.258	-0.332	0.572	-2.071	0.722	3.440	4.499
8_0	-0.365	0.256	-1.758	-0.441	0.642	-2.123	0.559	1.312	2.690
8_1	-0.224	0.624	-4.336	-0.144	0.445	-2.792	0.017	0.008	0.329

	Dimension 1			Dimension 2			Dimension 3		
Char_State	Coord.	Cont. (%)	vtest	Coord.	Cont. (%)	vtest	Coord.	Cont. (%)	vtest
8_2	0.681	2.372	5.923	0.516	2.342	4.485	-0.251	0.705	-2.181
9_0	-0.956	2.088	-5.078	0.112	0.049	0.595	0.726	2.638	3.855
9_1	-0.359	1.399	-5.879	-0.115	0.249	-1.892	-0.034	0.027	-0.550
9_2	0.965	5.873	9.731	0.159	0.273	1.598	-0.205	0.581	-2.068
10_0	-0.242	0.086	-1.005	1.314	4.344	5.453	-0.214	0.147	-0.888
10_1	-0.284	0.494	-2.802	0.129	0.176	1.274	0.323	1.403	3.190
10_2	0.176	0.369	3.211	-0.228	1.065	-4.160	-0.140	0.512	-2.557
11_0	-0.793	1.206	-3.819	0.542	0.971	2.611	0.155	0.101	0.748
11_1	-0.139	0.118	-1.372	0.748	5.896	7.379	0.345	1.594	3.400
11_2	0.041	0.013	0.476	-0.326	1.368	-3.753	-0.082	0.109	-0.939
11_3	0.525	1.083	3.846	-0.809	4.428	-5.926	-0.457	1.801	-3.350
12_0	1.667	5.583	8.238	0.765	2.026	3.781	0.809	2.886	4.000
12_1	0.116	0.072	1.036	-0.289	0.764	-2.578	0.421	2.058	3.750
12_2	-0.273	0.658	-3.630	-0.285	1.241	-3.797	-0.027	0.014	-0.362
12_3	-0.472	0.731	-3.097	0.767	3.331	5.037	-1.100	8.715	-7.221
13_0	-0.844	1.690	-4.581	0.385	0.606	2.091	0.769	3.079	4.176
13_1	-0.031	0.014	-0.746	-0.068	0.113	-1.618	0.009	0.002	0.206
13_2	0.837	2.049	5.126	0.016	0.001	0.101	-0.667	2.848	-4.082
14_0	-0.772	3.758	-7.784	0.469	2.384	4.725	-0.126	0.221	-1.274
14_1	-0.049	0.024	-0.717	-0.578	5.575	-8.382	-0.033	0.022	-0.471
14_2	1.540	8.233	10.451	0.762	3.470	5.171	0.320	0.780	2.172
15_0	1.326	1.926	4.717	-0.199	0.075	-0.707	0.882	1.871	3.140
15_1	0.475	0.824	3.325	-0.443	1.233	-3.100	0.662	3.509	4.634
15_2	-0.174	0.357	-3.141	-0.122	0.301	-2.199	-0.012	0.004	-0.221
15_3	-0.389	0.442	-2.382	1.119	6.303	6.852	-1.109	7.879	-6.789
16_0	1.600	1.869	4.601	-0.517	0.336	-1.487	0.677	0.733	1.946

	Dimension 1			Dimension 2			Dimension 3		
Char_State	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**
16_1	0.058	0.039	1.032	-0.343	2.348	-6.067	0.232	1.369	4.106
16_2	-0.364	0.798	-3.549	0.406	1.711	3.962	-0.470	2.921	-4.587
16_3	0.317	0.110	1.127	1.740	5.714	6.191	-0.321	0.247	-1.142
17_0	0.036	0.006	0.295	0.088	0.062	0.721	0.493	2.485	4.030
17_1	-0.011	0.002	-0.295	-0.028	0.020	-0.721	-0.155	0.782	-4.030
18_0	-0.361	1.083	-4.540	-0.063	0.057	-0.791	0.393	2.814	4.943
18_1	0.602	2.813	7.143	0.217	0.629	2.575	-0.348	2.060	-4.128
18_2	-0.408	0.425	-2.309	-0.517	1.175	-2.927	-0.008	0.000	-0.047
18_3	-0.767	0.483	-2.346	0.194	0.053	0.593	-0.662	0.791	-2.026

Appendix 13 Character state coordinates, contributions, and vtest scores for the first three components of the MCA analysis for males. vtest scores ≥ 1.96 indicate significance.

	Dimension 1			Dimension 2			Dimension 3		
Char_State	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**
1_0	1.826	1.905	3.701	0.010	0.000	0.019	-1.154	1.939	-2.339
1_1	1.527	3.000	4.755	0.508	0.709	1.582	0.223	0.163	0.694
1_2	-0.210	0.633	-6.178	-0.046	0.065	-1.354	0.026	0.025	0.766
2_0	0.979	3.968	6.085	-0.035	0.011	-0.218	0.001	0.000	0.006
2_1	-0.338	1.370	-6.085	0.012	0.004	0.218	0.000	0.000	-0.006
3_0	-0.397	2.093	-9.056	-0.049	0.068	-1.113	-0.028	0.027	-0.641
3_1	1.845	9.731	9.056	0.227	0.314	1.113	0.131	0.124	0.641
4_0	-0.546	0.853	-2.681	0.900	4.946	4.419	0.721	3.786	3.539
4_1	-0.350	1.121	-4.234	0.013	0.004	0.162	-0.291	1.974	-3.521
4_2	0.220	0.125	1.016	-1.285	9.059	-5.917	0.329	0.709	1.515
4_3	2.669	11.200	9.277	0.387	0.502	1.345	-0.155	0.097	-0.540
5_0	-0.621	0.165	-1.085	1.395	1.782	2.439	1.333	1.939	2.329
5_1	-0.446	1.448	-4.278	0.528	4.339	5.069	-0.187	0.650	-1.797
5_2	-0.182	0.156	-1.238	-0.630	4.001	-4.285	0.224	0.603	1.523
5_3	1.177	5.147	6.809	-0.397	1.249	-2.296	-0.071	0.048	-0.411
6_0	0.172	0.216	1.654	0.184	0.524	1.762	-0.314	1.830	-3.014
6_1	-0.201	0.301	-1.968	0.101	0.162	0.988	-0.055	0.056	-0.534
6_2	0.168	0.041	0.555	-1.462	6.522	-4.822	1.885	12.936	6.216
7_0	-0.473	0.577	-2.181	0.043	0.010	0.198	0.632	2.614	2.910
7_1	-0.350	1.087	-4.086	-0.347	2.276	-4.048	-0.107	0.260	-1.251
7_2	1.523	6.958	7.699	0.498	1.592	2.520	-0.168	0.215	-0.848
7_3	-0.145	0.036	-0.529	0.856	2.679	3.121	-0.100	0.044	-0.364
8_0	-0.495	0.385	-1.721	0.798	2.136	2.773	-0.106	0.045	-0.370
8_1	-0.316	1.015	-4.352	0.086	0.161	1.186	-0.066	0.112	-0.904

	Dimension 1			Dimension 2			Dimension 3		
Char_State	Coord.	Cont. (%)	vtest	Coord.	Cont. (%)	vtest	Coord.	Cont. (%)	vtest
8_2	0.900	3.589	5.857	-0.481	2.183	-3.127	0.188	0.400	1.225
9_0	-0.483	0.833	-2.724	0.602	2.761	3.394	0.851	6.594	4.802
9_1	-0.305	0.915	-4.038	-0.179	0.675	-2.373	-0.306	2.356	-4.059
9_2	1.742	8.237	8.287	-0.142	0.116	-0.673	-0.008	0.000	-0.037
10_0	0.465	0.401	1.773	-0.159	0.100	-0.607	-1.100	5.724	-4.197
10_1	-0.331	0.580	-2.445	0.228	0.587	1.684	-0.549	4.062	-4.055
10_2	0.099	0.088	1.172	-0.101	0.196	-1.201	0.549	6.924	6.527
11_0	-0.320	0.263	-1.472	0.208	0.238	0.959	-0.524	1.799	-2.414
11_1	0.297	0.745	3.289	0.296	1.575	3.272	-0.221	1.048	-2.444
11_2	-0.330	0.514	-2.245	-0.507	2.584	-3.444	0.561	3.785	3.815
11_3	-0.297	0.038	-0.519	-1.492	2.038	-2.608	1.314	1.886	2.297
12_0	2.323	7.711	7.660	0.542	0.895	1.786	0.611	1.359	2.015
12_1	0.105	0.035	0.547	0.575	2.220	2.993	-0.338	0.917	-1.761
12_2	-0.388	1.117	-3.788	0.099	0.155	0.965	0.335	2.124	3.273
12_3	-0.186	0.143	-1.154	-0.800	5.662	-4.975	-0.555	3.247	-3.449
13_0	-0.446	0.569	-2.189	0.719	3.156	3.530	-0.173	0.217	-0.847
13_1	0.056	0.037	0.978	-0.035	0.031	-0.614	-0.065	0.129	-1.149
13_2	0.431	0.265	1.421	-1.149	4.027	-3.789	0.887	2.864	2.925
14_0	-0.376	1.234	-4.312	-0.045	0.038	-0.515	-0.227	1.139	-2.596
14_1	-0.376	0.727	-2.721	-0.021	0.005	-0.154	0.384	1.934	2.780
14_2	2.280	11.888	9.801	0.219	0.234	0.942	-0.001	0.000	-0.004
15_1	0.986	1.668	3.598	1.161	4.938	4.237	0.443	0.857	1.616
15_2	-0.177	0.332	-2.583	0.090	0.185	1.318	0.252	1.709	3.672
15_3	0.047	0.009	0.280	-0.764	4.808	-4.531	-0.887	7.737	-5.261
16_1	-0.084	0.051	-0.807	0.528	4.333	5.065	0.412	3.145	3.950
16_2	-0.095	0.065	-0.908	-0.420	2.747	-4.034	-0.178	0.589	-1.709

	Dimension 1			Dimension 2			Dimension 3		
Char_State	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**
16_3	0.828	1.079	2.879	-0.498	0.834	-1.732	-1.083	4.693	-3.762
17_0	0.291	0.350	1.807	0.498	2.195	3.097	0.134	0.191	0.835
17_1	-0.100	0.121	-1.807	-0.172	0.758	-3.097	-0.046	0.066	-0.835
18_0	-0.218	0.352	-2.126	0.420	2.792	4.099	-0.204	0.787	-1.992
18_1	0.578	1.811	4.351	-0.507	2.975	-3.816	0.291	1.171	2.192
18_2	-0.472	0.508	-2.027	0.106	0.055	0.456	0.115	0.077	0.495
18_3	-0.441	0.195	-1.200	-0.609	0.791	-1.655	-0.328	0.275	-0.893

Appendix 14 Character state coordinates, contributions, and vtest scores for the first three components of the MCA analysis for females. vtest scores ≥ 1.96 indicate significance.

	Dimension 1			Dimension 2			Dimension 3		
Char_State	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**
1_0	0.503	0.648	1.667	0.465	0.739	1.542	-0.524	1.221	-1.738
1_1	-0.065	0.051	-0.607	0.377	2.286	3.534	0.120	0.302	1.127
1_2	-0.046	0.023	-0.398	-0.520	3.982	-4.497	-0.010	0.002	-0.083
2_0	0.150	0.300	1.552	0.048	0.041	0.499	-0.349	2.822	-3.619
2_1	-0.162	0.325	-1.552	-0.052	0.045	-0.499	0.379	3.057	3.619
3_0	-0.687	3.390	-4.263	-0.011	0.001	-0.069	0.062	0.047	0.383
3_1	0.267	1.318	4.263	0.004	0.000	0.069	-0.024	0.018	-0.383
4_1	-0.744	2.700	-3.587	0.697	3.159	3.360	0.373	1.176	1.798
4_2	-0.106	0.200	-1.604	-0.305	2.224	-4.633	-0.023	0.016	-0.348
4_3	1.957	10.807	6.846	0.736	2.035	2.573	-0.499	1.216	-1.744
5_0	-1.140	0.333	-1.140	1.767	1.068	1.767	2.693	3.224	2.693
5_1	-0.745	3.988	-4.624	0.396	1.505	2.460	-0.807	8.106	-5.008
5_2	-0.177	0.363	-1.595	-0.440	2.980	-3.961	0.475	4.504	4.271
5_3	1.153	8.868	6.801	0.267	0.633	1.573	-0.056	0.036	-0.329
6_0	-0.438	0.591	-1.610	-1.010	4.189	-3.712	-0.675	2.432	-2.481
6_1	-0.473	2.235	-3.760	0.420	2.352	3.341	-0.212	0.777	-1.684
6_2	0.484	2.938	4.716	-0.087	0.126	-0.847	0.334	2.427	3.256
7_0	-0.104	0.080	-0.661	-0.325	1.048	-2.067	0.064	0.052	0.405
7_1	0.035	0.012	0.268	-0.467	2.756	-3.559	-0.237	0.924	-1.808
7_2	-0.017	0.001	-0.065	0.837	3.114	3.219	-0.311	0.559	-1.197
7_3	0.092	0.046	0.472	0.753	4.073	3.863	0.522	2.547	2.680
8_0	-0.254	0.165	-0.841	0.329	0.370	1.091	0.611	1.660	2.027
8_1	-0.214	0.764	-2.903	-0.089	0.175	-1.202	-0.245	1.730	-3.318
8_2	0.658	2.777	3.780	0.099	0.084	0.568	0.392	1.706	2.251

	Dimension 1			Dimension 2			Dimension 3		
Char_State	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**
9_1	-0.538	3.709	-5.351	0.262	1.172	2.605	-0.009	0.002	-0.091
9_2	0.538	3.709	5.351	-0.262	1.172	-2.605	0.009	0.002	0.091
10_0	-0.903	0.627	-1.579	-0.455	0.213	-0.797	0.339	0.153	0.594
10_1	-0.189	0.274	-1.230	0.305	0.957	1.990	-0.745	7.403	-4.853
10_2	0.125	0.268	1.772	-0.116	0.310	-1.650	0.318	3.020	4.515
11_0	-0.590	0.268	-1.033	0.648	0.431	1.134	2.273	6.891	3.978
11_1	0.871	1.556	2.555	0.313	0.269	0.920	0.300	0.319	0.879
11 _2	-0.123	0.191	-1.201	0.149	0.372	1.454	0.141	0.432	1.374
11_3	0.021	0.005	0.170	-0.294	1.181	-2.387	-0.403	2.887	-3.274
12_0	1.502	6.947	5.520	0.959	3.775	3.524	-0.383	0.782	-1.407
12_1	-0.282	0.732	-2.101	0.293	1.058	2.188	0.187	0.557	1.392
12_2	-0.273	0.860	-2.457	-0.141	0.305	-1.266	-0.106	0.226	-0.957
12_3	0.628	0.708	1.714	-2.248	12.094	-6.136	0.380	0.450	1.039
13_0	-0.985	1.492	-2.475	0.731	1.098	1.839	0.100	0.026	0.250
13_1	-0.224	0.924	-3.570	0.105	0.273	1.681	0.134	0.579	2.146
13_2	1.001	5.651	5.288	-0.544	2.229	-2.876	-0.467	2.135	-2.469
14_0	-0.018	0.001	-0.052	-1.122	3.446	-3.293	-0.424	0.640	-1.245
14_1	-0.405	2.939	-6.150	-0.017	0.007	-0.254	0.059	0.107	0.890
14_2	1.294	9.446	6.837	0.461	1.601	2.438	-0.032	0.010	-0.169
15_0	0.811	2.024	2.980	1.253	6.445	4.605	-0.910	4.412	-3.342
15_1	-0.277	0.549	-1.716	0.372	1.327	2.310	0.248	0.763	1.536
15_2	-0.109	0.167	-1.196	-0.227	0.966	-2.493	0.070	0.121	0.773
15_3	0.799	0.818	1.823	-2.599	11.552	-5.933	0.023	0.001	0.053
16_0	1.155	2.738	3.389	1.143	3.573	3.353	0.179	0.114	0.525
16_1	-0.155	0.468	-2.742	0.018	0.008	0.315	-0.125	0.528	-2.214
16_2	0.069	0.018	0.289	-0.707	2.565	-2.956	0.437	1.272	1.825

	Dimension 1			Dimension 2			Dimension 3		
Char_State	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**
16_3	1.492	0.571	1.492	0.113	0.004	0.113	1.524	1.032	1.524
17_0	-0.241	0.326	-1.271	0.417	1.311	2.206	1.058	10.944	5.590
17_1	0.068	0.092	1.271	-0.118	0.370	-2.206	-0.298	3.087	-5.590
18_0	-0.528	2.784	-4.197	0.170	0.384	1.349	-0.147	0.376	-1.172
18_1	0.627	4.733	5.871	-0.098	0.153	-0.914	0.406	3.438	3.801
18_2	-0.591	1.073	-2.170	-0.246	0.249	-0.905	-0.780	3.249	-2.867
18_3	-0.894	0.410	-1.270	0.461	0.146	0.656	-1.979	3.480	-2.812

Appendix 15 Character state coordinates, contributions, and vtest scores for the first three components of the MCA analysis for *P. tharos* & *P. cocyta*. vtest scores ≥ 1.96 indicate significance.

	Dimension 1			Dimension 2			Dimension 3		
Char_State	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**
1_0	0.760	0.537	2.046	0.859	1.021	2.311	0.778	1.154	2.092
1_1	0.848	3.910	6.152	0.527	2.250	3.823	0.310	1.073	2.247
1_2	-0.299	1.591	-6.725	-0.206	1.125	-4.633	-0.135	0.670	-3.043
2_0	0.557	2.342	5.057	0.032	0.012	0.294	0.429	2.860	3.897
2_1	-0.254	1.068	-5.057	-0.015	0.005	-0.294	-0.196	1.304	-3.897
3_0	-0.521	4.363	-9.881	-0.041	0.040	-0.774	-0.038	0.048	-0.724
3_1	1.034	8.655	9.881	0.081	0.079	0.774	0.076	0.096	0.724
4_0	-0.946	2.373	-4.471	0.737	2.147	3.484	-1.292	9.111	-6.110
4_1	-0.576	3.521	-6.864	0.069	0.075	0.819	0.205	0.915	2.440
4_2	0.768	6.263	9.154	-0.249	0.978	-2.963	0.089	0.175	1.066
4_3	1.770	0.831	2.510	-0.172	0.012	-0.243	1.154	0.726	1.637
5_0	-0.625	0.207	-1.260	1.233	1.201	2.486	-2.142	5.006	-4.320
5_1	-0.352	1.282	-4.102	0.590	5.365	6.874	0.313	2.083	3.646
5_2	0.323	1.008	3.552	-0.358	1.852	-3.944	-0.274	1.490	-3.012
5_3	0.237	0.202	1.332	-0.918	4.499	-5.156	0.153	0.172	0.859
6_0	-0.677	3.222	-5.839	-0.116	0.140	-0.998	0.129	0.241	1.113
6_1	-0.144	0.230	-1.788	0.297	1.460	3.694	0.178	0.730	2.223
6_2	1.065	6.774	8.215	-0.417	1.549	-3.218	-0.485	2.888	-3.741
7_0	0.548	1.832	4.288	-0.056	0.029	-0.441	-0.463	2.685	-3.620
7_1	-0.295	1.075	-4.060	-0.386	2.739	-5.309	0.119	0.361	1.640
7_2	0.035	0.003	0.153	1.058	3.757	4.567	0.967	4.337	4.177
7_3	0.063	0.014	0.348	0.789	3.201	4.335	-0.240	0.410	-1.321
8_0	0.012	0.000	0.060	0.691	1.983	3.359	-0.333	0.634	-1.616
8_1	-0.049	0.040	-0.981	0.109	0.296	2.200	0.165	0.935	3.328

	Dimension 1			Dimension 2			Dimension 3		
Char_State	Coord.	Cont. (%)	vtest	Coord.	Cont. (%)	vtest	Coord.	Cont. (%)	vtest
8_2	0.168	0.130	1.100	-0.807	4.508	-5.300	-0.394	1.483	-2.588
9_0	-0.851	2.401	-4.569	0.487	1.171	2.614	-1.282	11.205	-6.882
9_1	-0.152	0.351	-2.655	0.079	0.141	1.379	0.211	1.387	3.680
9_2	0.899	4.607	6.726	-0.493	2.066	-3.690	0.185	0.403	1.386
10_0	-0.920	1.348	-3.290	-0.382	0.347	-1.366	0.239	0.188	0.856
10_1	-0.353	0.978	-3.294	0.318	1.179	2.963	0.655	6.900	6.102
10_2	0.287	1.216	4.834	-0.128	0.358	-2.148	-0.374	4.233	-6.290
11_0	-0.849	1.914	-4.016	0.011	0.000	0.053	-0.289	0.456	-1.366
11_1	-0.819	4.446	-6.780	0.100	0.098	0.827	0.119	0.194	0.988
11_2	0.234	0.529	2.574	-0.042	0.025	-0.460	-0.156	0.482	-1.713
11_3	1.048	5.679	7.362	-0.056	0.024	-0.390	0.286	0.872	2.012
12_0	0.900	0.537	2.036	0.918	0.833	2.076	0.735	0.737	1.663
12_1	0.289	0.543	2.361	0.543	2.859	4.438	0.167	0.374	1.366
12_2	-0.007	0.001	-0.100	0.091	0.154	1.257	-0.346	3.028	-4.752
12_3	-0.514	1.226	-3.374	-1.135	8.908	-7.450	0.579	3.199	3.800
13_0	-0.836	2.410	-4.592	0.530	1.442	2.909	-0.022	0.004	-0.123
13_1	0.043	0.033	0.969	0.044	0.051	0.984	-0.053	0.104	-1.200
13_2	0.726	1.536	3.620	-0.892	3.463	-4.452	0.352	0.742	1.755
14_0	-0.741	5.020	-7.787	-0.227	0.701	-2.384	0.025	0.012	0.266
14_1	0.402	2.230	6.247	0.190	0.739	2.946	0.040	0.046	0.625
14_2	1.032	1.270	3.165	-0.453	0.365	-1.390	-0.659	1.067	-2.023
15_0	0.795	0.502	1.974	1.599	3.031	3.972	2.068	7.000	5.138
15_1	0.687	1.940	4.187	0.744	3.395	4.538	-0.088	0.065	-0.534
15_2	-0.063	0.060	-1.117	0.044	0.044	0.785	-0.231	1.682	-4.113
15_3	-0.648	1.615	-3.796	-1.303	9.725	-7.630	0.588	2.736	3.445
16_0	1.414	0.795	2.462	1.107	0.726	1.928	-0.396	0.128	-0.689

	Dimension 1			Dimension 2			Dimension 3		
Char_State	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**
16_1	0.253	0.953	4.310	0.314	2.177	5.337	-0.079	0.191	-1.346
16_2	-0.410	1.336	-3.866	-0.498	2.941	-4.699	-0.010	0.002	-0.096
16_3	-1.146	1.219	-3.083	-1.223	2.070	-3.292	1.522	4.425	4.097
17_0	-0.119	0.075	-0.849	0.364	1.045	2.596	-0.520	2.945	-3.710
17_1	0.033	0.021	0.849	-0.102	0.294	-2.596	0.146	0.829	3.710
18_0	-0.194	0.425	-2.447	0.405	2.760	5.105	0.126	0.367	1.586
18_1	0.341	0.928	3.221	-0.690	5.640	-6.507	-0.319	1.664	-3.009
18_2	0.039	0.006	0.225	0.356	0.701	2.041	0.312	0.744	1.790
18_3	-0.564	0.379	-1.729	-0.337	0.202	-1.035	-0.034	0.003	-0.104

Appendix 16 Character state coordinates, contributions, and vtest scores for the first three components of the MCA analysis for *P. tharos* & *P. batesii*. vtest scores ≥ 1.96 indicate significance.

	Dimension 1			Dimension 2			Dimension 3		
Char_State	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**
1_0	1.779	6.525	4.141	1.371	4.986	3.191	0.251	0.197	0.584
1_1	0.648	1.039	1.670	-0.286	0.260	-0.736	-1.079	4.375	-2.780
1_2	-0.304	1.604	-4.289	-0.122	0.334	-1.725	0.124	0.406	1.751
2_0	0.725	3.247	3.283	0.087	0.060	0.393	-0.105	0.104	-0.476
2_1	-0.286	1.282	-3.283	-0.034	0.024	-0.393	0.041	0.041	0.476
3_0	-0.554	4.422	-5.566	-0.003	0.000	-0.025	0.183	0.731	1.836
3_1	1.076	8.598	5.566	0.005	0.000	0.025	-0.355	1.421	-1.836
4_0	-0.612	0.308	-0.873	0.263	0.074	0.376	0.655	0.537	0.935
4_1	-0.611	3.228	-3.567	0.132	0.195	0.772	0.085	0.095	0.497
4_2	0.364	1.528	2.777	-0.257	0.983	-1.963	0.040	0.028	0.307
4_3	1.929	3.068	2.754	1.950	4.037	2.785	-2.110	5.578	-3.014
5_1	-0.919	2.435	-2.584	0.362	0.486	1.017	0.470	0.967	1.321
5_2	-0.068	0.041	-0.411	-0.611	4.362	-3.714	-0.127	0.223	-0.773
5_3	0.330	1.077	2.164	0.455	2.635	2.984	-0.020	0.006	-0.133
6_0	-0.568	1.994	-2.573	0.746	4.432	3.381	0.079	0.058	0.357
6_1	-0.334	0.735	-1.583	0.199	0.335	0.942	-0.381	1.458	-1.809
6_2	0.630	3.599	3.826	-0.653	4.980	-3.968	0.224	0.689	1.359
7_0	0.284	0.566	1.408	-0.677	4.136	-3.356	0.077	0.063	0.381
7_1	-0.441	2.486	-3.775	0.170	0.474	1.453	-0.002	0.000	-0.016
7_2	1.242	1.908	2.194	1.312	2.741	2.318	-2.538	12.103	-4.483
7_3	2.558	5.397	3.653	1.156	1.417	1.650	3.182	12.683	4.544
8_0	1.163	0.558	1.163	0.005	0.000	0.005	2.339	3.426	2.339
8_1	-0.013	0.002	-0.084	0.264	0.852	1.668	0.183	0.482	1.155
8_2	-0.030	0.010	-0.234	-0.210	0.677	-1.663	-0.226	0.925	-1.789

	Dimension 1			Dimension 2			Dimension 3		
Char_State	Coord.	Cont. (%)	vtest	Coord.	Cont. (%)	vtest	Coord.	Cont. (%)	vtest
9_0	-0.400	0.330	-0.932	-0.962	2.457	-2.240	0.135	0.057	0.314
9_1	-0.152	0.294	-1.298	0.310	1.586	2.658	0.240	1.114	2.051
9_2	0.394	1.090	1.954	-0.283	0.723	-1.403	-0.477	2.418	-2.361
10_0	1.605	1.063	1.605	3.256	5.624	3.256	-3.273	6.711	-3.273
10_1	-0.180	0.174	-0.740	0.932	5.990	3.831	0.580	2.738	2.384
10_2	0.019	0.006	0.227	-0.394	3.214	-4.743	-0.109	0.292	-1.317
11_0	-0.445	0.490	-1.147	0.776	1.919	2.000	1.114	4.663	2.870
11_1	-0.360	0.641	-1.405	0.627	2.503	2.446	-0.660	3.269	-2.573
11_2	-0.178	0.338	-1.257	-0.463	2.957	-3.276	-0.016	0.004	-0.112
11_3	1.290	6.172	4.206	-0.016	0.001	-0.053	0.183	0.188	0.595
12_0	3.954	6.445	3.954	2.306	2.822	2.306	4.025	10.147	4.025
12_1	0.364	0.327	0.937	-0.289	0.266	-0.744	-0.637	1.523	-1.640
12_2	0.137	0.194	0.935	-0.450	2.681	-3.063	0.193	0.583	1.315
12_3	-0.455	1.796	-2.661	0.508	2.874	2.967	-0.239	0.754	-1.399
13_0	-0.978	1.182	-1.727	1.161	2.146	2.051	0.463	0.403	0.818
13_1	0.021	0.007	0.250	0.004	0.000	0.051	0.008	0.001	0.094
13_2	0.193	0.169	0.713	-0.332	0.642	-1.224	-0.154	0.164	-0.569
14_0	-0.689	3.918	-3.870	0.325	1.121	1.825	0.096	0.114	0.536
14_1	-0.002	0.000	-0.011	-0.442	2.487	-2.899	0.198	0.592	1.301
14_2	1.536	8.760	5.011	0.456	0.994	1.488	-0.741	3.097	-2.418
15_0	3.954	6.445	3.954	2.306	2.822	2.306	4.025	10.147	4.025
15_1	1.007	0.836	1.438	-1.352	1.941	-1.931	-0.168	0.035	-0.240
15_2	0.073	0.070	0.649	-0.410	2.851	-3.647	-0.022	0.009	-0.193
15_3	-0.461	1.578	-2.385	0.751	5.380	3.881	-0.166	0.312	-0.861
16_1	0.093	0.079	0.565	-0.391	1.781	-2.373	0.340	1.592	2.065
16_2	0.011	0.001	0.079	0.203	0.570	1.439	-0.219	0.781	-1.550

	Dimension 1			Dimension 2			Dimension 3		
Char_State	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**
16_3	-0.467	0.450	-1.088	0.661	1.160	1.539	-0.357	0.398	-0.830
17_0	0.089	0.026	0.269	-0.120	0.061	-0.364	-0.148	0.109	-0.449
17_1	-0.016	0.005	-0.269	0.021	0.011	0.364	0.026	0.019	0.449
18_0	-0.291	0.350	-1.013	0.471	1.178	1.638	-0.225	0.318	-0.783
18_1	0.145	0.336	1.740	-0.156	0.503	-1.876	0.042	0.044	0.508
18_2	-0.709	0.622	-1.252	0.308	0.151	0.545	0.467	0.410	0.825
18_3	-0.597	0.147	-0.597	0.443	0.104	0.443	-0.796	0.397	-0.796

Appendix 17 Character state coordinates, contributions, and vtest scores for the first three components of the MCA analysis for *P. cocyta* & *P. batesii*. vtest scores ≥ 1.96 indicate significance.

	Dimension 1			Dimension 2			Dimension 3		
Char_State	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**
1_0	1.189	1.711	3.451	1.254	3.443	3.639	1.122	3.579	3.257
1_1	0.775	3.820	5.976	-0.234	0.629	-1.803	-0.292	1.272	-2.250
1_2	-0.462	2.943	-7.382	-0.002	0.000	-0.036	0.036	0.042	0.576
2_0	0.636	3.125	5.669	0.114	0.183	1.020	0.077	0.109	0.690
2_1	-0.361	1.771	-5.669	-0.065	0.104	-1.020	-0.044	0.062	-0.690
3_0	-0.603	4.736	-8.927	0.008	0.001	0.115	0.030	0.028	0.447
3_1	0.943	7.405	8.927	-0.012	0.002	-0.115	-0.047	0.044	-0.447
4_0	-1.020	2.831	-4.616	-0.642	2.030	-2.906	1.022	6.670	4.624
4_1	-0.535	2.684	-5.607	0.141	0.338	1.479	-0.193	0.823	-2.027
4_2	0.791	5.391	7.707	-0.157	0.385	-1.532	-0.245	1.216	-2.389
4_3	1.613	1.573	3.260	2.941	9.469	5.946	1.892	5.085	3.825
5_0	-0.687	0.286	-1.390	-1.177	1.516	-2.379	1.201	2.048	2.428
5_1	-0.326	1.158	-3.942	-0.274	1.481	-3.314	0.220	1.236	2.658
5_2	0.192	0.289	1.733	0.155	0.343	1.405	-0.723	9.666	-6.543
5_3	1.252	3.080	4.719	1.260	5.643	4.749	1.307	7.881	4.927
6_0	-0.640	2.478	-4.765	0.348	1.324	2.589	0.106	0.161	0.793
6_1	-0.124	0.170	-1.521	-0.205	0.838	-2.511	-0.047	0.058	-0.578
6_2	1.238	6.489	7.290	0.037	0.011	0.220	-0.029	0.008	-0.172
7_0	0.433	0.877	2.717	-0.247	0.517	-1.551	-0.351	1.360	-2.208
7_1	-0.295	0.843	-3.183	0.192	0.646	2.072	-0.087	0.172	-0.938
7_2	0.163	0.080	0.782	0.285	0.444	1.370	0.204	0.296	0.982
7_3	0.085	0.029	0.481	-0.398	1.125	-2.237	0.476	2.091	2.678
8_0	-0.118	0.042	-0.566	-0.329	0.591	-1.580	0.109	0.084	0.523
8_1	-0.080	0.104	-1.683	-0.099	0.289	-2.087	0.023	0.020	0.482

	Dimension 1			Dimension 2			Dimension 3		
Char_State	Coord.	Cont. (%)	vtest	Coord.	Cont. (%)	vtest	Coord.	Cont. (%)	vtest
8_2	0.781	1.291	3.068	1.229	5.788	4.829	-0.331	0.543	-1.299
9_0	-1.059	3.391	-5.093	-0.536	1.573	-2.579	1.039	7.663	4.997
9_1	-0.101	0.140	-1.593	0.033	0.027	0.523	-0.173	0.957	-2.720
9_2	0.977	4.479	6.139	0.249	0.527	1.566	-0.168	0.310	-1.053
10_0	-0.728	1.041	-2.744	1.527	8.293	5.757	-0.741	2.534	-2.794
10_1	-0.144	0.159	-1.279	-0.027	0.011	-0.244	0.165	0.491	1.466
10_2	0.218	0.553	2.829	-0.240	1.210	-3.110	0.016	0.007	0.209
11_0	-0.917	1.781	-3.602	0.433	0.720	1.703	-0.514	1.313	-2.019
11_1	-0.743	3.507	-5.726	0.227	0.591	1.747	0.336	1.679	2.586
11_2	0.166	0.210	1.460	-0.367	1.847	-3.223	-0.299	1.590	-2.624
11_3	1.021	5.514	6.939	0.079	0.060	0.540	0.230	0.660	1.566
12_0	1.050	1.001	2.620	-0.053	0.005	-0.132	1.800	6.900	4.489
12_1	0.279	0.564	2.368	-0.302	1.197	-2.566	-0.164	0.461	-1.398
12_2	-0.118	0.151	-1.421	-0.026	0.013	-0.311	-0.028	0.021	-0.343
12_3	-0.747	1.267	-3.051	1.110	5.061	4.533	-0.057	0.017	-0.234
13_0	-0.766	2.039	-3.999	-0.044	0.012	-0.230	0.542	2.403	2.833
13_1	0.059	0.056	1.220	-0.021	0.013	-0.436	-0.179	1.209	-3.690
13_2	0.944	1.617	3.406	0.272	0.242	0.980	0.543	1.257	1.960
14_0	-0.797	4.706	-6.881	0.188	0.474	1.624	0.110	0.210	0.948
14_1	0.298	1.073	4.036	-0.344	2.592	-4.663	-0.192	1.045	-2.599
14_2	1.268	2.919	4.577	1.526	7.646	5.507	0.830	2.937	2.996
15_0	0.975	1.006	2.636	-0.253	0.123	-0.684	1.023	2.603	2.767
15_1	0.469	0.998	2.885	-0.615	3.106	-3.784	-0.358	1.369	-2.205
15_2	-0.097	0.132	-1.581	-0.003	0.000	-0.051	0.176	1.015	2.858
15_3	-0.994	1.793	-3.586	1.710	9.597	6.169	-1.052	4.717	-3.797
16_0	1.127	0.577	1.967	-1.226	1.235	-2.139	-1.537	2.515	-2.681

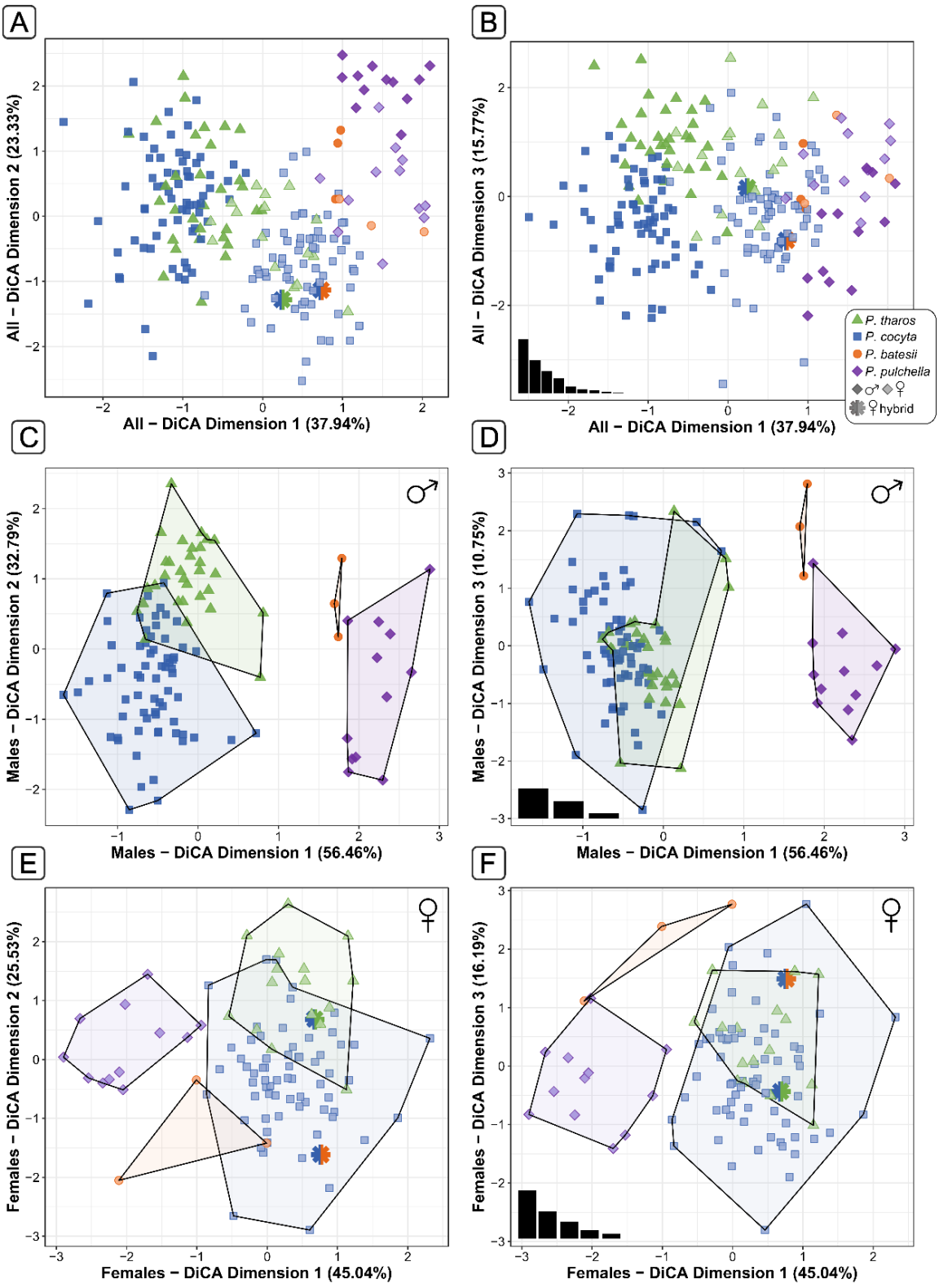
	Dimension 1			Dimension 2			Dimension 3		
Char_State	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**
16_1	0.152	0.320	2.459	-0.379	3.618	-6.146	0.161	0.842	2.603
16_2	-0.400	1.019	-3.086	0.798	7.326	6.153	-0.327	1.595	-2.520
16_3	-0.129	0.010	-0.261	1.256	1.727	2.539	0.894	1.135	1.807
17_0	-0.048	0.013	-0.342	-0.019	0.004	-0.133	-0.349	1.603	-2.465
17_1	0.017	0.005	0.342	0.007	0.001	0.133	0.124	0.570	2.465
18_0	-0.160	0.311	-2.171	-0.151	0.501	-2.049	0.067	0.129	0.913
18_1	0.680	1.958	4.005	0.535	2.195	3.152	0.130	0.167	0.763
18_2	-0.048	0.009	-0.261	-0.297	0.605	-1.634	-0.004	0.000	-0.023
18_3	-0.629	0.479	-1.825	0.568	0.707	1.649	-1.114	3.524	-3.232

Appendix 18 Character state coordinates, contributions, and vtest scores for the first three components of the MCA analysis for *P. batesii* & *P. pulchella*. vtest scores ≥ 1.96 indicate significance.

	Dimension 1			Dimension 2			Dimension 3		
Char_State	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**	**Coord.**	**Cont. (%)**	**vtest**
1_0	0.028	0.004	0.083	-0.630	2.907	-1.870	0.999	8.124	2.968
1_1	0.382	1.557	1.923	-0.054	0.043	-0.271	-0.260	1.100	-1.309
1_2	-0.616	2.601	-2.170	0.573	3.101	2.021	-0.373	1.454	-1.314
2_0	0.021	0.008	0.227	0.141	0.498	1.515	0.039	0.042	0.416
2_1	-0.084	0.033	-0.227	-0.563	1.992	-1.515	-0.155	0.167	-0.416
3_1	0.000	0.000	-Inf	0.000	0.000	Inf	0.000	0.000	-Inf
4_1	0.124	0.024	0.179	2.705	15.337	3.893	1.368	4.351	1.969
4_2	0.315	0.606	1.023	0.266	0.591	0.862	-0.052	0.025	-0.170
4_3	-0.138	0.292	-1.055	-0.377	2.975	-2.869	-0.116	0.312	-0.882
5_2	-0.316	0.380	-0.760	1.684	14.866	4.057	0.110	0.070	0.264
5_3	0.063	0.076	0.760	-0.337	2.973	-4.057	-0.022	0.014	-0.264
6_0	-1.094	9.128	-4.166	-0.029	0.009	-0.112	-0.125	0.183	-0.477
6_1	-0.111	0.056	-0.299	0.038	0.009	0.103	0.076	0.040	0.205
6_2	0.829	7.338	4.176	0.005	0.000	0.023	0.057	0.053	0.287
7_0	0.926	0.654	0.926	-1.792	3.364	-1.792	1.597	2.966	1.597
7_1	1.179	5.297	2.838	-0.502	1.320	-1.209	-0.175	0.179	-0.422
7_2	-0.683	6.046	-4.205	0.038	0.026	0.237	-0.207	0.850	-1.277
7_3	0.684	2.500	2.033	0.521	1.992	1.548	0.401	1.306	1.190
8_1	0.692	3.290	2.441	0.316	0.943	1.115	0.245	0.629	0.865
8_2	-0.297	1.410	-2.441	-0.136	0.404	-1.115	-0.105	0.270	-0.865
9_1	0.123	0.046	0.259	0.668	1.872	1.411	1.676	13.069	3.540
9_2	-0.019	0.007	-0.259	-0.103	0.288	-1.411	-0.258	2.011	-3.540
10_0	-1.472	6.608	-3.109	-0.576	1.392	-1.217	0.930	4.026	1.965
10_1	0.190	0.193	0.564	-0.403	1.190	-1.196	-0.088	0.063	-0.260

	Dimension 1			Dimension 2			Dimension 3		
Char_State	Coord.	Cont. (%)	vtest	Coord.	Cont. (%)	vtest	Coord.	Cont. (%)	vtest
10_2	0.240	0.834	1.698	0.270	1.448	1.909	-0.164	0.591	-1.158
11_0	-0.637	0.309	-0.637	-0.040	0.002	-0.040	-1.750	3.563	-1.750
11_1	-0.595	4.592	-3.665	0.327	1.909	2.016	0.064	0.080	0.391
11_2	0.832	4.225	2.702	-0.311	0.809	-1.009	-0.177	0.292	-0.575
11_3	1.024	3.201	2.164	-0.760	2.420	-1.605	0.522	1.267	1.102
12_0	0.003	0.000	0.018	0.347	2.150	2.139	-0.352	2.446	-2.166
12_1	-0.305	0.566	-0.989	-0.261	0.573	-0.849	0.078	0.056	0.253
12_2	1.058	3.412	2.234	-0.620	1.609	-1.309	0.596	1.654	1.259
12_3	-1.842	2.587	-1.842	-1.336	1.870	-1.336	2.972	10.271	2.972
13_1	-0.335	1.715	-2.554	0.034	0.025	0.261	-0.085	0.169	-0.649
13_2	0.671	3.430	2.554	-0.068	0.049	-0.261	0.171	0.338	0.649
14_1	0.958	0.699	0.958	3.991	16.693	3.991	1.918	4.276	1.918
14_2	-0.033	0.024	-0.958	-0.138	0.576	-3.991	-0.066	0.147	-1.918
15_0	1.140	5.948	3.070	-0.285	0.512	-0.768	-0.003	0.000	-0.008
15_1	-0.288	0.505	-0.934	0.782	5.123	2.539	-0.229	0.487	-0.743
15_2	-0.018	0.003	-0.087	-0.150	0.307	-0.707	-0.167	0.422	-0.787
15_3	-1.433	4.701	-2.573	-0.863	2.344	-1.550	1.340	6.266	2.406
16_0	1.329	6.734	3.200	0.554	1.606	1.333	0.306	0.544	0.737
16_1	0.079	0.066	0.397	0.012	0.002	0.062	-0.650	6.869	-3.272
16_2	-0.582	1.552	-1.568	-0.212	0.282	-0.570	1.158	9.362	3.119
16_3	-0.851	2.760	-2.049	-0.334	0.585	-0.805	0.123	0.088	0.296
17_0	-0.352	0.945	-1.340	0.092	0.089	0.351	-0.689	5.517	-2.623
17_1	0.176	0.472	1.340	-0.046	0.045	-0.351	0.344	2.759	2.623
18_0	-0.749	2.137	-1.803	0.375	0.736	0.902	0.420	1.028	1.012
18_1	0.150	0.427	1.803	-0.075	0.147	-0.902	-0.084	0.206	-1.012

Appendix 19 Discriminant correspondence analyses (DiCA) for scored morphological character data. (following page) DiCA attempts to separate individuals into clusters based on pre-defined identities, whereas MCA treats individuals independently. Despite this, DiCA did not separate the species more clearly than the MCA analyses (Fig. 3.3) **(A)** The first two dimensions for all 213 individuals. **(B)** The first and third dimensions for all 213 individuals. **(C)** The first two dimension for males only. **(D)** The first and third for males only. **(E)** The first two for females only. **(F)** The first and third for females only.


A
All – DiCA Dimension 2 (23.33%)
All – DiCA Dimension 1 (37.94%)
B
All – DiCA Dimension 3 (15.77%)
All – DiCA Dimension 1 (37.94%)
P. tharos
P. cocyta
P. batesii
P. pulchella
♂ ♀
♀ hybrid
C
Males – DiCA Dimension 2 (32.79%)
Males – DiCA Dimension 1 (56.46%)
D
Males – DiCA Dimension 3 (10.75%)
Males – DiCA Dimension 1 (56.46%)
E
Females – DiCA Dimension 2 (25.53%)
Females – DiCA Dimension 1 (45.04%)
F
Females – DiCA Dimension 3 (16.19%)
Females – DiCA Dimension 1 (45.04%)