"Without abundant material it were ridiculous to attempt a wide revision of these insects; and, as our author truly states, a mass of this material causes one's courage to sink at the sight of so uniform and apparently characterless a group."

- C. Morley, 1912

"... I find that nearly all available characters present an alarming degree of variation – even compared to other Ichneumonidae genera which I have studied."

- J.P. Brock, 1982

"He attacked everything in life with a mix of extraordinary genius and naive incompetence, and it was often difficult to tell which was which."

– Douglas Adams

University of Alberta

Systematics and diversity of Ichneumonidae, with an emphasis on the taxonomically neglected genus *Ophion* Fabricius

by

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For my beautiful daughter Keala, who makes every day a joy.

And for my parents, Rita Dahlie and Earl Schwarzfeld.
Thank you for your unending love, support, and encouragement.
This thesis would not exist without you.

Abstract

Ichneumonidae are the most species-rich family of parasitic Hymenoptera and are important for regulating populations of other arthropods. However, with an estimated 75% of species undescribed, we lack fundamental information about their taxonomy, ecology, and distributions. Using two different groups of Ichneumonidae, I address each of these issues in this thesis.

In Chapters 2 – 4, I focus on the taxonomically-neglected genus *Ophion*. I provide the first phylogenetic hypothesis of *Ophion* based on molecular data (COI, ITS2 and 28S) and divide the genus into ten provisional species-groups (Chapter 2). I also describe the secondary structure of ITS2 for the first time in Ichneumonidae, and discuss its potential to inform phylogenetic inference in *Ophion* (Chapters 2, 3). I investigate the diversity of *Ophion* at the species level by comparing quantitative species delimitation methods with each other and with morphologically-defined species (Chapter 3). The total number of delimited species is dependent on the method and parameters used; however all methods agree that there is a wealth of undescribed diversity in Nearctic *Ophion*. Finally, I revise the Nearctic species within the newly defined *Ophion scutellaris* species-group (Chapter 4). An integrative analysis of DNA, geometric wing morphometrics, classical morphometrics and morphology indicates that this species-group contains a minimum of seven species in Canada, although the full diversity of the group has likely not been sampled. *Ophion clave* sp. n., *O. aureus*

sp. n., *O. brevipunctatus* sp. n., *O. dombroskii* sp. n., *O. keala* sp. n., and *O. importunus* sp. n. are described.

Once species have names, it is possible to address other fundamental questions about their distribution and ecology. I conducted a survey of Ichneumonidae in a boreal deciduous forest, with an emphasis on Pimplinae, Poemeniinae, and Rhyssinae. Responses to forest harvesting were weak, but there is evidence that the community composition at the species level is correlated with shrub composition. Even within this relatively well-known group, there is much unexplored diversity.

Any nomenclatural changes or new taxa proposed in this thesis should not be considered valid until published in primary journals as defined by the ICZN (International Commission on Zoological Nomenclature 1999).

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My inability to keep this acknowledgements section to less than four pages is indicative of how much help and support I have received through the course of my PhD program – and if I thanked everybody who deserves to be here, this section would run another four pages!

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List of Abbreviations

28S	28S D2-D3 expansion region of rDNA
AD	area dentiparis
AP	area petiolaris
APE	area poster-externa
APL	area postero-lateralis
ASu	area superomedia
ATC	anterior transverse carina
BMNH	Natural History Museum
CA	Conservation Area
CAW	clypeus apical width
CH	clypeus height
CL	metacoxa length
CM	classical morphometrics
CNC	Canadian National Collection of Insects, Arachnids and Nematodes
COI	cytochrome c oxidase I
CUIC	Cornell University Insect Collection
CW	metacoxa width
ER	Ecological Reserve
F1	first flagellomere length/width
F20	20th flagellomere length/width
FH	face height
FL	hind femur length
Flag	flagellomere
FM	face maximum width
FW	face width
FeW	hind femur width
GI	genal inflection
GM	geometric morphometrics
HW	head width
IOD	interocellar distance
ITS2	internal transcribed spacer 2
LLC	lateral longitudinal carina
ML	maximum likelihood
MLC	median longitudinal carina
MP	maximum parsimony
MS	malar space
MT1	metatarsomere 1
MT2	metatarsomere 2
MTS	midtibial spur ratio
MW	mandible basal width
NF	National Forest
NFRC	Northern Forest Research Centre
NJ	Neighbour-joining
NP	National Park

OC	occipital carina
OL	ocellus length
OOD	ocellar-ocular distance
PC	pleural carina
PCA	principal component analysis
PP	Provincial Park
PRA	Provincial Recreation Area
PTC	posterior transverse carina
RBCM	Royal British Columbia Museum
SEMC	Snow Entomological Museum
SFUC	Simon Fraser University Collection
SL	scutellum length
SW	scutellum width
TA	tergite 1 apical width
TB	tergite 1 basal width
TS	tergite 1 spiracle width
UBCZ	Spencer Entomology Collection, UBC

Chapter 1

General Introduction

Parasitic Hymenoptera

Parasitoids are insects with free-living adults that complete their larval development in or on a single living host, ultimately killing it (Godfray 1994). The parasitoid lifestyle has arisen independently in numerous insect orders (particularly Diptera, but also Coleoptera, Lepidoptera, Neuroptera, and Trichoptera), however the vast majority of parasitoid species are in the order Hymenoptera (Eggleton and Belshaw 1992; Godfray 1994). Within this order, the parasitoid life-history strategy arose within the suborder Symphyta, in the common ancestor of the symphytan family Orussidae and the suborder Apocrita, and thus became the ancestral groundplan for Apocrita (Ronquist *et al*. 1999, Vilhemson 2001, 2003). Several groups of apocritans have since departed from this ancestral condition, becoming predators, pollen and nectar feeders, omnivorous scavengers, or herbivores; however, most of the diversity of Apocrita has arisen while maintaining the parasitoid life-cycle (Goulet and Huber 1993; Godfray 1994).

Parasitic Hymenoptera are extremely diverse, possibly comprising 20 percent of the world's insect species (LaSalle and Gauld 1991). They are perhaps the most important population regulators of herbivorous insects. This is best documented in the numerous instances of introduced herbivores escaping their

native parasitoids and quickly reaching pest status, as well as the suppression of these species with the introduction of appropriate parasitoids (Greathead 1986; Myers *et al*. 1989; De Bach and Rosen 1991). It has also been suggested that parasitoids are important drivers of diversity patterns in other species (LaSalle and Gauld 1991, 1993). However because of their high trophic level, small population sizes and specialized life histories, it is hypothesized that parasitoids are particularly vulnerable to extinction or extirpation (LaSalle and Gauld 1991; Kruess and Tscharntke 1994; Hochberg *et al*. 1998; Shaw and Hochberg 2001; Thies *et al*. 2003; Shaw 2006). Parasitoids may also be disproportionately impacted by climate change which again would have ecological ramifications throughout ecosystems (Shaw 2006; Hance *et al*. 2007). For example, altered seasonal temperatures can disrupt the synchronization of host-parasitoid phenology, as well as cause thermal preferences and geographic ranges of hosts and parasitoids to diverge (Hance *et al*. 2007).

Despite their abundance, diversity, ecological importance, and sensitivity to habitat changes, parasitic Hymenoptera remain very little known both biologically and taxonomically (LaSalle and Gauld 1991; Shaw and Hochberg 2001). It is estimated that 77 – 99 percent of parasitic Hymenoptera are undescribed (LaSalle and Gauld 1991). Even among described species, there is very little known about the ecological or biological requirements of most species. For example, in Britain, where the insect fauna is better known than anywhere on earth, it is estimated that reliable host records exist for less than one quarter of parasitoid species (Shaw 2006). There are similarly limited data regarding habitat

requirements for most species, and even distribution records are mostly a function of collector bias (Shaw 2006).

Ichneumonidae

With an estimated 100,000 species world-wide, Ichneumonidae are the most diverse family of Hymenoptera (Gauld 2002) and may be the most species-rich family of insects on earth (Owen *et al*. 1981; Gauld 1991). The family is one of two monophyletic families (along with Braconidae) that comprise the superfamily Ichneumonoidea (Sharkey and Wahl 1992; Belshaw *et al*. 1998). Ichneumonoidea was previously thought to be the sister group of Aculeata (Rasnitsyn 1988; Dowton and Austin 1994); however, more recent studies suggest that the superfamily is sister to Proctotrupomorpha (Heraty *et al.* 2011; Sharkey *et al.* 2012), or to Aculeata + Proctotrupomorpha (Sharanowski *et al.* 2010). Thirty-eight subfamilies are currently recognized within Ichneumonidae; however, their boundaries and relationships are still incompletely resolved and several subfamilies are currently regarded as paraphyletic (Quicke *et al.* 2009). Ichneumonidae attack a wide variety of hosts, most commonly within Lepidoptera and Symphyta, and life-history strategies vary widely within and between subfamilies (Wahl 1993).

Ichneumonidae are well-known as an example of the anomalous diversity pattern wherein they are more diverse in temperate than in tropical latitudes (Owen and Owen 1974; Janzen 1981). A rich body of literature has attempted to explain this pattern (e.g. Janzen and Pond 1975; Rathke and Price 1976; Janzen

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1981; Gauld *et al.* 1992; Sime and Brower 1998). However recent studies have suggested that the observed pattern is a result of observation bias, with at least some groups of tropical ichneumonids being equally or more diverse than the same groups in temperate areas (Horstmann *et al.* 1999; Sääksjärvi *et al.* 2004). Quicke (2012) recently concluded that the state of knowledge of Ichneumonidae is too preliminary to even recognize, let alone explain, distributional patterns.

Ichneumonidae play an important role in the population regulation of herbivorous insects. While used less often as biocontrol agents compared to other Hymenoptera (e.g. Chalcidoidea or Braconidae) (Greathead 1986), there are numerous instances in both agricultural and forest ecosystems where Ichneumonidae are a dominant parasitoid of pest species (e.g. Nuttall 1980; Kingsley *et al.* 1993; Pair *et al.* 1996; Langor *et al.* 2000; Hoballah *et al.* 2004; Dosdall *et al.* 2011). However the lack of biological and taxonomic information regarding all parasitoid Hymenoptera applies to Ichneumonidae as well. There are approximately 24,000 described species of Ichneumonidae (Yu *et al.* 2012); if Gauld's (2002) estimate is accurate, then three-quarters of Ichneumonidae species are undescribed. Reliable host records, biodiversity surveys, and ecological studies at the species level are also rare, limiting our ability to understand the ecological roles played by Ichneumonidae (Shaw 2006; Schwarzfeld 2013).

The goal of this thesis is to investigate the taxonomy and ecology of Ichneumonidae in order to lessen the deficit of information regarding this important family of parasitoids. In Chapters 2, 3, and 4, I focus on the systematics and diversity of *Ophion*, a taxonomically-neglected ichneumonid genus in the

subfamily Ophioninae. In Chapter 5, I present a biodiversity survey of three comparatively well-known ichneumonid subfamilies (Pimpline, Poemeniinae, and Rhyssinae) in a boreal deciduous forest and investigate the influence of variable retention harvesting on the ichneumonid community.

Systematics and diversity of Ophion Fabricius (Chapters 2 – 4)

The genus *Ophion* consists of large orange or yellowish nocturnal ichneumonids that are frequently observed at lights. *Ophion* was first described by Fabricius in 1798 who included 21 species (Hooker 1912). Since then, the genus as originally defined has been modified, split, and redefined by a series of ichneumonid taxonomists (Hooker 1912; Morley 1912; Cushman 1947; Townes 1971; Gauld 1985). The original genus thus spans much of the diversity of the subfamily Ophioninae (Hooker 1912), which now includes 32 genera (Yu *et al*. 2012). Most ophionine genera are primarily tropical, however the genus *Ophion*, as currently defined, reaches its peak of abundance and diversity in temperate regions. While the genus has a nearly worldwide distribution, in tropical regions it is almost entirely restricted to cooler climates at high elevation (Gauld and Mitchell 1981; Gauld 1988). In comparison, *Ophion* is the dominant ophionine genus in virtually all habitats throughout the Palearctic and Nearctic regions (Gauld 1980, 1988).

Biology

Ophion are koinobiont endoparasitoids of holometabolous larvae, meaning the larvae continue to develop after being parasitized. Almost all host records are from Lepidoptera, primarily from large-sized larvae that feed in exposed sites (Gauld 1985). The most commonly reported hosts are Noctuidae; however, several other Lepidoptera families have also been recorded (Yu *et al*. 2012). One Nearctic species (*O. nigrovarius* Provancher) has been reported from *Phyllophaga* (Coleoptera, Scarabeidae) (Townes 1971). For the vast majority of species, host records are lacking and there is little information regarding host specificity of *Ophion* (Gauld 1985). The most extensive rearing studies of *Ophion* have been conducted in Europe, with at least one verified host record for the majority of species (Brock 1982; Várkonyi *et al*. 2002).

In most species that have been studied in detail, *Ophion* females deposit their eggs into late-instar host larvae (Rohlfs and Mack 1985b; Várkonyi *et al*. 2002). Ophionines usually pupate after the host larva has spun its cocoon or made a pupation chamber, but before the host pupates (Gauld 1985). In seasonal environments, they overwinter within the host's cocoon or pupation chamber, usually as mature larvae (Gauld 1985).

Of the Nearctic species, *O. flavidus* Brullé has been studied most intensively, as it is a parasitoid of the pest species *Spodoptera frugiperda* (JE Smith) in Neotropical and southern Nearctic agricultural systems (Rohlfs and Mack 1983, 1985a, b; Gross and Pair 1991). There are few ecological studies of *Ophion* in natural ecosystems, though there is evidence that one species (*O.*

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luteus) is the primary regulator of extreme population fluctuations of *Xestia* spp. in Europe (Várkonyi *et al*. 2002). However even in this intensively studied system, there are no rearing records that confirm that *O. luteus* parasitizes *Xestia* spp. (Várkonyi *et al*. 2002).

Taxonomic history

Several "species groups", "species complexes", and "close species pairs" have been referred to informally within *Ophion*, such as the *O. luteus* complex and *O. mocsaryi* complex (Gauld 1973); *O. minutus* species group (Gauld 1977); *O. gelus* and *O. inutilis* species groups (Gauld and Mitchell 1981); and *O. pteridis-parvulus* and *O. mocsaryi-costatus* species-pairs (Brock 1982). These were generally discussed in isolation, however, with little reference to the remainder of the genus. As part of a morphological analysis of *Ophion* and related genera, Gauld (1980) suggested several additional species groups (e.g. *O. caudatus* species group, *O. areolaris* species group), some of which were previously classified as separate genera. However since the focus of this study was on determining generic limits, there was little discussion of the overall classification or arrangement of species groups within *Ophion*. The most comprehensive classification of *Ophion* to date is found in Gauld (1985), where eight major species groups are outlined, although there is no discussion of what constitutes a "major" vs. a "minor" species group. Most of these species groups contain relatively few species, were defined by distinctive morphological characters, and were presumed to be monophyletic groupings (Gauld 1985). The

one exception is the *O. luteus* species group, which is a catch-all species group

that contains the majority of Palearctic, Nearctic, and Neotropical species. Gauld

(1985) presumed this group was paraphyletic with respect to the other groups, and

defined it based on plesiomorphic characters. No phylogenetic relationships were

suggested between species groups and the lack of information regarding the

Nearctic species, in particular, contributed to the inability to resolve this *"luteus*

anathema" (Gauld 1985).

With the exception of nine species of *Ophion* that were included as part of

a phylogenetic analysis of Ichneumonidae based on 28S rDNA (Quicke *et al*.

2009), there have been no published molecular studies of *Ophion*. In Chapter 2, I

use molecular data to construct a preliminary phylogeny of the genus, with an

emphasis on Nearctic and Palearctic species. I then use this phylogeny to propose

several new species groups within the "*O. luteus* species group" *sensu* Gauld

(1985). This chapter thus provides the necessary framework for all future studies

of *Ophion* systematics.

Systematics and diversity

Small, tropical species are expected to be at the forefront of the

"taxonomic impediment" that describes the insufficient number of taxonomists

and resources in the face of overwhelming biological diversity (LaSalle and

Gauld 1991; Quicke *et al*. 2012). However, despite being large-bodied, abundant

in temperate habitats and readily attracted to lights, *Ophion* remains very little

known taxonomically, particularly in the Nearctic region. Eleven Nearctic species

are described but Gauld (1985) estimated that the Nearctic fauna consists of approximately 50 species. In the Palearctic region, *Ophion* are better known, with 79 described species (Yu *et al*. 2012) and a comprehensive key to the British species (Brock 1982). However, even in Britain, where *Ophion* have been examined most extensively, the circumscription of some species is not readily apparent (Brock 1982, G. Broad, pers. comm.)

Ophion are a morphologically challenging group, with high levels of morphological convergence across species and high variability within species (Townes 1971; Gauld 1980; Brock 1982). Several authors have proposed that the diversity of such species-rich, little-known taxa can be best investigated with "DNA taxonomy", i.e. automated quantitative methods of delimiting taxonomic units based on DNA sequences (Tautz *et al*. 2003; Blaxter 2004; Pons *et al*. 2006). Alternatively, it has been argued that species delimitation should proceed in an integrative taxonomy framework, using all available character sets to establish and refine species hypotheses (Dayrat 2005; Roe and Sperling 2007; Schlick-Steiner *et al*. 2010)

In Chapter 3, I investigate the diversity of *Ophion* at the species level by comparing multiple quantitative species delimitation methods with each other and with several morphologically-defined species. In Chapter 4, I focus in detail on one newly defined species group (the *O. scutellaris* Thomson species group) and describe six new species based on an integrative analysis of molecular data, morphology, and quantitative morphometrics.

Ichneumonidae diversity in a boreal ecosystem (Chapter 5)

Once species have been named, we are able to explore other fundamental questions about their distributions and ecology. Because of their ability to regulate herbivorous insect populations, Ichneumonidae are an integral part of forest ecosystems; however, as with *Ophion*, many taxa are taxonomically too little-known to serve as a basis for biodiversity inventories. Pimplinae, Poemeniinae, and Rhyssinae are the subfamilies that are most easily identifiable and that have been used most often in ichneumonid biodiversity research (Fraser *et al*. 2007, 2008; Gaston and Gauld 1993; Sääksjärvi *et al*. 2004). Even among these relatively well-known subfamilies, however, there have been few surveys or ecological studies in North American forests that identified specimens to the species level. In Chapter 5, I provide a baseline survey of Pimpline, Poemeniinae, and Rhyssinae in a boreal deciduous forest and investigate the influence of variable retention harvesting and shrub community composition on the ichneumonid community.

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Chapter 2

A preliminary phylogeny of *Ophion* Fabricius (Hymenoptera: Ichneumonidae, Ophioninae), based on 28S, COI, and ITS2 secondary structure

Introduction

The ichneumonid subfamily Ophioninae consists primarily of nocturnal yellowish-brown species that are frequently observed at lights. Ophionines are koinobiont endoparasitoids of holometabolous larvae, particularly Lepidoptera. There are currently 32 recognized genera within the Ophioninae (Yu *et al*. 2012), almost all of which are most diverse in tropical regions (Gauld 1985). The genus *Ophion* Fabricius is the one exception to this pattern. While the genus has a nearly world-wide distribution, the majority of tropical species are restricted to cooler climates at high elevation (Gauld and Mitchell 1981; Gauld 1988). In contrast, *Ophion* is by far the most abundant and diverse ophionine genus in virtually all habitats across the Palearctic and Nearctic regions (Gauld 1980, 1988).

The genus *Ophion* has been revised in several geographical regions (Gauld and Mitchell 1978, 1981; Gauld 1977, 1988; Fernández-Triana 2005, Kim *et al*. 2009); however, most of these regions are tropical, where *Ophion* species form a small component of the ophionine fauna. Despite being a predominately temperate genus, *Ophion* in the Holarctic region has received little taxonomic attention. In the Nearctic region, eleven species are currently described, although it has been estimated that there are approximately 50 Nearctic species (Gauld 1985) and ongoing taxonomic work suggests there are many more (see Chapter 3). In the Palearctic region, *Ophion* is better known, with 79 described species (Yu *et al.* 2012); however, there have been no revisions of the species across the Palearctic. Ophioninae were revised in the Netherlands by Oosterbroek (1978). *Ophion* has been examined most comprehensively in the United Kingdom, with a

number of revisions over the years (Gauld 1973, 1976, 1978), culminating in a thorough revision of the 16 known species by Brock (1982).

Ophion is a morphologically challenging genus, with high levels of morphological convergence between species and few informative characters (Gauld 1980; Brock 1982). Thus far, all attempts to divide *Ophion* into monophyletic species groups have been based on morphology, and have been hampered by the lack of information regarding the eastern Palearctic and Nearctic species (Gauld 1985).

A number of "species groups" and "species-complexes" have been informally referred to within *Ophion*; however, these were often not explicitly defined and were generally discussed in isolation, with little reference to the remainder of the genus (e.g. Gauld 1973, 1977; Gauld and Mitchell 1981; Brock 1982). The most comprehensive classification to date was by Gauld (1985), based in large part on the results of a previous morphological analysis (Gauld 1980). Gauld (1985) outlined eight major species groups, though there was no discussion of what constitutes a "major" versus a "minor" species group. The majority of these groups were presumed to be monophyletic and contained a small number of morphologically distinctive species, whereas one group (the *O. luteus* species group) served as a catch-all group for the remaining species.

All Nearctic and Palearctic species were placed within four of these species groups (Gauld 1985). A fifth group, the *O. bicarinatus* species group, possibly contains the Palearctic species *O. minutus* and is characterized by having the *Rs+2r* vein broadened and slightly curved before reaching the pterostigma. The *O. areolaris* group was previously classified as the genus *Platophion* Hellén (Gauld 1977; Brock 1982) and is characterized by the loss of the occipital carina, among other characters. The *O. similis* species group contains three Palearctic and two undescribed Nearctic species that have a stout body shape, short antennae, small eyes and ocelli, and appear to be diurnal. The *O. dentatus* species group is restricted to the eastern Palearctic region and is characterized by long slender mandibles and long claws. Finally, the *O. luteus* species group is a large group that contains the vast majority of Palearctic and Neotropical species, and all of the

Nearctic species except for the above two species from the *O. similis* group. It can only be defined by the lack of characters that characterize the other species groups, and is thought to be a plesiomorphic assemblage of species (Gauld 1985).

Within this group, Brock (1982) suggested some sister-group relationships between British species, but these have not been tested with molecular characters, and no hypothesis has been proposed for the phylogeny of the genus as a whole. Quicke *et al*. (2009) included nine species of *Ophion* as part of their broad taxonomic sampling to obtain a phylogeny of Ichneumonidae based on 28S rDNA. Otherwise, there have been no published molecular studies of *Ophion*.

Two of the most commonly used molecular markers for hymenopteran phylogenies are the D2-D3 expansion region of 28S ribosomal RNA (28S) and the mitochondrial gene cytochrome oxidase 1 (COI) (Mardulyn and Whitfield 1999; Quicke *et al*. 1999; Dowton and Austin 2001; Whitfield *et al*. 2002; Michel-Salzat and Whitfield 2004; Shi *et al*. 2005; Quicke *et al*. 2005; Laurenne *et al*. 2006; Zaldivar-Riverón *et al*. 2007; Zaldivar-Riverón *et al*. 2008; Quicke *et al*. 2009; Klopfstein *et al*. 2011). COI is often used at the species level, due to its relatively high mutation rate (Monti *et al*. 2005; Li *et al*. 2010; Williams *et al*. 2012); however, it can also be informative at higher phylogenetic levels (Klopfstein *et al*. 2010, Quicke *et al*. 2012). In contrast, 28S is a highly conserved gene that has been widely used for higher-level insect phylogenies (Caterino *et al*. 2000; Heraty *et al*. 2011; Sharkey et *al*. 2012), though it has also proved useful in distinguishing species (e.g. Monaghan *et al*. 2005; Derycke *et al*. 2008; Raupach *et al*. 2010).

The internal transcribed spacer 2 (ITS2) of nuclear ribosomal RNA is another rapidly evolving gene that has been most often used at the species-level in insects (Campbell *et al*. 1993; Gomez-Zurita *et al*. 2000; Alvarez & Hoy 2002; Hung *et al*. 2004; Wagener *et al*. 2006; Li *et al*. 2010). However, the highly conserved secondary structure of this gene, with characteristic paired domains and unpaired regions, also makes this an informative gene at higher phylogenetic levels (Coleman and Vacquier 2002; Coleman 2007). Incorporating secondary structure can improve alignment of rRNA, an otherwise problematic task due to

the presence of numerous indels (Kjer 1995; Coleman and Vacquier 2002; Gillespie *et al*. 2005). As well, since the non-independence of paired regions can mislead phylogenetic analyses, incorporating secondary structure can allow the use of rRNA-specific models for phylogeny estimation (Savill *et al*. 2001; Telford *et al*. 2001; Wolf *et al*. 2008; Letsch and Kjer 2011).

The goal of this study is to obtain a preliminary molecular phylogeny of *Ophion*, with an emphasis on the *O. luteus* species group *sensu* Gauld (1985), based on COI, 28S, and ITS2. The primary focus is on Nearctic species, however representatives of most species known from the UK are included, as well as a few species from Costa Rica, Madagascar, Taiwan and Australia.

Methods

Specimens

A total of 493 specimens of Nearctic *Ophion* were newly sequenced for this study. The majority of these specimens are from Canada, with a much smaller number from the United States. Most were newly collected for this study, however 25 specimens were sequenced from alcohol-preserved material on loan from the Canadian National Collection of Insects, Arachnids and Nematodes. Specimens were selected for sequencing to represent the range of morphological variation observed in over 4000 specimens from a variety of habitats. Eighty specimens from 15 species of Palearctic *Ophion* were also newly sequenced, all of which are from the United Kingdom, except for one specimen from Spain and one from France.

In addition to these newly sequenced specimens, 91 *Ophion* COI sequences from British Columbia were included from the Barcode of Life database (BOLD), courtesy of J. deWaard (project code: ICHBC). Finally, any available *Ophion* sequences from GenBank (Benson *et al*. 2013) were also included. This resulted in an additional 19 COI sequences (including eight sequences from Costa Rica and two from Madagascar) and nine 28S sequences (including one sequence each from *O. zerus* Gauld (Australia) and *O. bicarinatus* Cameron (Taiwan)).

Enicospilus, another genus within the subfamily Ophioninae, was used as an outgroup. I sequenced COI from one specimen and 28S from two specimens of *Enicospilus*. An additional 28S sequence was included from GenBank and two COI sequences were included from BOLD. I was unable to successfully sequence ITS2 from *Enicospilus* (discussed below). I also conducted a preliminary neighbour-joining analysis of 28S rRNA using several additional Ophioninae genera available on GenBank (*Eremotylus*, *Thyreodon*, *Simophion*, *Pycnophion*, *Pamophion*, and *Stauropoctonus*); however the inclusion of additional outgroups had no effect on the root of the tree. Since these sequences were not available for COI and ITS2, I excluded them in the final analyses.

The provenance of all sequenced specimens is listed in Appendix 1. With the exception of sequences obtained from GenBank or BOLD, all Nearctic taxa were identified by MDS and all Palearctic taxa were identified by G. R. Broad, with the exception of *O. forticornis*, which was identified by M. R. Shaw.

Sequencing and alignment

DNA was extracted from a single hind leg using DNeasy Blood & Tissue Kits (Qiagen, Toronto, ON); the final elution volume was 150 µL. I conducted PCR in either 50 µL or 15 µL reactions. The 50 µL reactions contained 4-8 µL genomic DNA, 5 µL 10x PCR buffer (containing 15mmol/ µL $MgCl_2$) (Promega, Madison, WI), 3 µL of 25 mmoles/µL $MgCl_2$ (Promega), 1 µL of 10 mmoles/µL dNTP's ((Roche, Switzerland), 1 µL each of 5pmol/µL forward and reverse primers, 0.5 µL of 5 U/µL *Taq* polymerase (Fermentation Service Unit, University of Alberta) and 30.5-34.5 µL of autoclaved Millipore water. The 15 µL reactions used 4-8 µL DNA, 1.5 µL PCR buffer, 0.9 µL $MgCl_2$, 0.3 µL each of dNTP's and forward and reverse primers, 0.15 µL *Taq* and 3.55-7.55 µL water. All PCR products were purified using ExoSap-IT (USB Corporation, Cleveland, OH), and were sequenced using BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA), followed by ethanol precipitation. Sequencing reactions were run on an ABI Prism 3730 DNA analyser. Sequences

are deposited in NCBI GenBank, and Genbank accession numbers are listed in Appendix 1.

COI: The primers lco hym (5' – CAA ATC ATA AAG ATA TTG G – 3') and hco out (5' – CCA GGT AAA ATT AAA ATA TAA ACT TC – 3') (Schulmeister 2003), were used to produce a 676 base pair fragment equivalent to the "barcode" region (Hebert *et al.* 2003). PCR conditions were: 94° for 2 min, 35 cycles of 94° for 30 s, 45° for 30 s, 72° for 2 min, and a final extension at 72° for 5 min. Alignment was unambiguous, and was confirmed by translating nucleotides to amino acids in Mesquite (Maddison and Maddison 2011). The first character represented the third codon position; for consistency with other analyses, this was removed so that the alignment begins with the first codon position. I sequenced COI for 567 *Ophion* specimens, and 1 specimen of *Enicospilus*. A few sequences were not successfully sequenced in both directions, or were otherwise partially incomplete; these sequences were included with the gaps coded as missing data. The 110 *Ophion* and two *Enicospilus* sequences obtained from Genbank and BOLD were sequenced using slightly different primers (see BOLD website for sequencing protocols) and were 658 base pairs in length.

28S: I sequenced the D2-D3 region of 28S rDNA using the following primers: Forward: 5'-GCG AAC AAG TAC CGT GAG GG-3'; Reverse: 5'-TAG TTC ACC ATC TTT CGG GTC-3' (Laurenne *et al.* 2006). PCR cycling was 94° for 2 min, 30 cycles of 96° for 15 s, 50° for 30 s, 72° for 30 s and a final extension of 75° for 7 min. Alignment was performed by eye in Mesquite; there were occasional small indels, but generally alignment was unambigous. The aligned sequences were 725 base pairs in length. The final dataset included 96 *Ophion* sequences (87 newly sequenced and 9 from Genbank), as well as three sequences of *Enicospilus* (two newly sequenced and one from Genbank).

ITS2: I sequenced this gene using the primers ITS2-F (5'-GGG TCG ATG AAG AAC GCA GC-3') and ITS2-R (5'-ATA TGC TTA AAT TCA GCG GG-3') which anneal to the flanking 28S and 5.8S genes (Navajas *et al.* 1994). PCR cycling was 94° for 2 min, 35 cycles of 94° for 30 s, 55° for 1 min, 72° for 2 min

and a final extension of 75° for 5 min. Excluding the flanking 28S and 5.8S regions, the unaligned sequences ranged from 717 to 1053 base pairs in length. I was unable to obtain complete ITS2 sequences for several specimens, presumably due to the length of the gene and the presence of several microsatellite regions that caused polymerase slippage. Since the analysis of ITS2 secondary structure (described below) was sensitive to missing data, I only included sequences that were complete and that lacked ambiguous regions. The final dataset included 394 ITS2 sequences.

ITS2 secondary structure

There are two main analytical methods to obtain the secondary structure of ITS2 (Schultz and Wolf 2009). The first is to fold sequences using homology modeling based on available structures in the ITS2 database (Schultz *et al*. 2006; Selig *et al*. 2008; Koetschan *et al*. 2010). However template structures are only identified if the sequences are highly similar, with at least 75% of the structural elements able to be transferred from the template to the sequence (Schultz *et al*. 2006). If no sufficiently similar sequences are available, then the sequence can be folded using structure prediction algorithms (Schultz and Wolf 2009). Since the database was not able to identify any sufficiently similar sequences to use as templates, I used two different RNA folding programs to obtain a template secondary structure. Comparative methods that simultaneously fold multiple homologous RNA sequences have been found to improve accuracy over single sequence folding algorithms (Gardner and Giegerich 2004). I therefore initially conducted a multiple sequence folding analysis using the program MXScarna (Tabei *et al*. 2008). I simultaneously folded and aligned six *Ophion* sequences along with ITS2 sequences from six species of Ichneumonidae in 4 subfamilies, obtained from GenBank (Table 2-1). The aligned structure was examined for structural motifs and compared to the conserved structure of ITS2 described by Coleman (2007). I also folded the same six *Ophion* sequences individually using the RNAfold webserver (Hofacker 2003), with the minimum free energy method and default settings. The structure that was obtained from RNAfold was

consistent with the combined structure from MXScarna; I therefore chose to use RNAfold to obtain the template structure for the remaining sequences, as the output from this program facilitated the use of downstream programs.

To obtain the final sequence-structure dataset, I first folded an arbitrarily chosen single sequence in RNAfold. I then imported this structure into the ITS2 database, and used it as a template for folding the remaining sequences. If any sequences had less than 90% similarity to the existing template, one of the anomalous sequences was directly folded in RNAfold, and then added as an additional template to the database. Using this iterative process, the final dataset consisted of eight sequences that were folded directly and 386 sequences that were folded using homology. Twenty-three base pairs of the flanking 28S and 5.8S genes were retained, as this has been shown to improve the accuracy of the secondary structure folding algorithms (Morgan and Blair 1998). For the majority of sequences, the complete 28S flanking region was not successfully sequenced. The missing bases were therefore manually added, as this region of 5.8S was invariant in all Ichneumonidae sequences available on GenBank. Sequences and structures were aligned using 4Sale, a program designed to synchronously align RNA sequences and structures (Seibel *et al*. 2006). The 28S and 5.8S flanking regions were not included in the phylogenetic analyses; the final alignment of ITS2 was 1750 base pairs in length.

Phylogeny estimation

The phylogeny was estimated using maximum likelihood (ML) and maximum parsimony (MP) analyses. Analyses were conducted on each dataset separately (COI, ITS2, and 28S), as well as on a combined dataset using only those specimens that were successfully sequenced for all three genes. *Enicospilus*, another genus within the subfamily Ophioninae, was used as an outgroup for the COI and 28S analyses; however, I was unable to obtain clean sequence for *Enicospilus* for ITS2. The ITS2 and combined trees were therefore rooted with *Ophion minutus* Kriechbaumer, since this was consistently recovered as basal by the other two analyses.

I conducted the MP analyses using MEGA version 5 (Tamura *et al.* 2011). The analyses were run using heuristic searches with tree-bisection-reconnection, search level 5, 10 starting trees, and saving a maximum of 1000 trees. I also conducted MP bootstrap analyses in MEGA, with 1000 replicates and the same parameters as the MP analyses.

Models for the maximum likelihood analyses were determined using PartitionFinder version 1.0.1 (Lanfear *et al.* 2012). This program considers all potential partitions within a dataset, and calculates the best partitioning scheme and best model(s) for each scheme. Only those models able to be implemented in the GARLI web service were included. Models were calculated for COI using the three codon positions as potential partitions, and the "greedy" search algorithm. For the combined analysis, each gene as well as codon position for COI were included, with the "greedy" search algorithm. The 28S and ITS2 datasets were not partitioned, and all included models were assessed ("search = all"). Best models were selected according to the Bayesian Information Criterion (Schwarz 1978). Based on these results, the COI dataset was partitioned by codon position (CP), with the first and third positions using GTR+I+G, and the second position using F81+G. ITS2 was analyzed using SYM+G, while 28S used K80+I+G. The combined analysis used five partitions: 28S: K80+I+G; ITS2: SYM+G; CP1: GTR+G; CP2: F81; CP3: GTR+G.

The ML analyses were conducted using the web service for GARLI 2.0 (Zwickl 2006). This service uses grid computing to rapidly conduct analyses across hundreds of computers (Cummings and Huskamp 2005, Bazinet *et al.* 2007, Bazinet and Cummings 2008, Myers *et al.* 2008, Bazinet and Cummings 2011); post-processing of the results is done using DendroPy (Sukumaran and Holder 2010) and the R system for statistical computing (R Development Core Team 2011).

The ITS2 and COI ML analyses used a neighbour-joining starting tree constructed in MEGA (K2P model, pairwise deletion of missing data). For each gene, I initially ran an analysis with 20 replicates. Further analyses were then run in sets of 10 replicates, until a set of 10 replicates resulted in no improvement in

score. A total of 50 COI replicates and 30 ITS2 analyses were thus conducted. For the much smaller 28S and combined datasets, I ran an initial analysis of 10 replicates, with a stepwise addition starting tree. Following the method developed by Regier *et al*. (2009), it was calculated that 8 and 4 analyses, respectively, were needed to be 95% certain of obtaining the best topology; therefore, no further analyses were conducted. Five hundred bootstrap replicates were calculated for each dataset, using stepwise addition starting trees.

Finally, in order to incorporate the structural information of the ITS2 sequence data, I calculated 100 neighbour-joining (NJ) bootstrap replicates using an ITS2 structure-specific rate matrix in ProfDistS (Wolf *et al*. 2008). The rate matrix was obtained from ProfDistS, where it was calculated by converting the four bases into a 12-letter alphabet, with each base in three possible states (unpaired, paired-right or paired-left), and then determining a GTR (maximum likelihood)-corrected substitution model (Seibel *et al*. 2006; Wolf *et al*. 2008).

Results

Overall, the maximum likelihood (ML) and maximum parsimony (MP) analyses gave very similar topologies, with similar bootstrap support. In general, the MP analyses resulted in slightly higher bootstrap values, though the opposite occurred occasionally. The ITS2-specific NJ bootstrap values from ProfDistS were almost identical to the MP bootstrap values for ITS2, and the analysis did not produce any divergent results. All ML bootstrap values of greater than 50% are shown in Figures 2-1 – 2-4, while the MP and NJ (ITS2) bootstrap values of greater than 50% are only indicated for the nodes of the major divisions of the trees (defined below).

COI and ITS2 were broadly congruent in terms of topology, despite differing greatly in the amount of sequence divergence in different parts of the tree (Figures 2-1, 2-2). This is demonstrated by the graph of pairwise sequence divergences for each gene (Figure 2-5). The ITS2 graph has the vast majority of sequence pairs with very small or no sequence divergence, and highly distinct sequencing gaps between the major portions of the tree (Figure 2-5b). In contrast,

while the COI graph similarly shows sequencing gaps between the major parts of the tree, there are many more sequences with intermediate divergences (Figure 2-5a).

The largest difference between the trees produced by these two genes was the extremely long branch of *O. flavidus* in the ITS2 analysis, discussed below. The 28S tree was the least resolved of any of the analyses, with few clades having strong bootstrap support (Figure 2-3). In contrast, the combined analysis was highly resolved, with most major nodes having high bootstrap values (Figure 2-4). Summary statistics for all analyses are shown in Table 2-2.

The delimitation of taxa above the species level is necessarily subjective. I considered a group of specimens to be a "species group" if there was reasonably strong evidence that the group was monophyletic, if it was well-supported by multiple datasets, and if there was a distinct sequencing gap between adjacent groups. Based on these criteria, I have divided the specimens from this study into ten provisional species groups, with varying levels of support (Figures 2-1 – 2-4, Table 2-3). With the exception of *O. minutus* (possibly part of the *O. bicarinatus* group) and *O. ocellaris* (a member of the *O. areolaris* group), the remaining species were all presumably included within the catch-all *O. luteus* species group (Gauld 1985). While the vast majority of *Ophion* in this study are undescribed (see Chapter 3), there is at least one described species in all except one of the species groups ("species group 1"). There are also a few sequences/species that remain unassigned to any of the species groups. In general, ITS2 had high sequence divergence between the majority of species groups, as defined in this study, but very low sequence divergence within groups (Figures 2-2, 2-5). In comparison, COI had a less distinct separation of within-group and between-group divergence (Figures 2-1, 2-5).

Definition of species groups

Ophion minutus Kriechbaumer species group

According to Gauld (1985), the *Ophion bicarinatus* species group includes *O. bicarinatus*, all of the Australian species (including *O. zerus*), and possibly *O. minutus*, and is characterized by having the *Rs+2r* vein thickened and slightly curved before reaching the pterostigma. *O. ventricosus* Gravenhorst also has a slightly thickened *Rs+2r* vein (Brock 1982), but was not mentioned by Gauld (1985). Similarly thickened *Rs+2r* veins are found in other ophionine genera, such as *Eremotylus*, which lends support to these species being basal within *Ophion*. In contrast, the *O. luteus* species group *sensu* Gauld (1985) has the *Rs+2r* vein straight and of equal thickness along its length.

In this study, *O. minutus, O. ventricosus* and *O. zerus* were all recovered as basal, with *O. minutus* and *O. ventricosus* forming a monophyletic group in both the COI and 28S analyses, though with only moderate bootstrap support (Figures 2-1, 2-3; Table 2-3). I was not able to successfully sequence ITS2 for *O. ventricosus*. The *O. minutus+ventricosus* clade is further supported by biology, as both species have been reared from Geometridae, unlike the majority of *Ophion*, which are parasitoids of Noctuoidea (Brock 1982). Interestingly, in their analysis of 28S across Ichneumonidae, Quicke *et al*. (2009) recovered *O. zerus* and *O. minutus* as a basal clade, but did not recover *O. ventricosus* within *Ophion*, even though they used the same 28S sequence as the current study. The 28S sequence from Genbank identified as *Ophion bicarinatus* was recovered as part of the newly-defined *O. obscuratus* species group in this study (discussed below), and was part of the same clade in Quicke *et al*. (2009). This may indicate a misidentification of this species by Quicke *et al*. (2009), since it would be surprising to find a species with a thickened *Rs+2r* vein within a group with otherwise typical venation. In this study, I have included *O. minutus* and *O. ventricosus* in the *O. minutus* species group, but left *O. zerus* unassociated, pending further study of *O. bicarinatus* and of other members of the *O. bicarinatus* species group. In most cases, I have named each species group after

the earliest-named species within it, however since the term "*O. minutus* species group" has previously been used in the literature (e.g. Gauld 1977), I am retaining it for this group.

O. flavidus Brullé species group

The *O. flavidus* group was weakly supported by COI (ML bootstrap: 56, MP bootstrap: 61), but strongly supported by 28S (ML bootstrap 83, MP bootstrap: 95). Three newly sequenced specimens were part of this group for 28S and COI (*O. flavidus* from Florida plus two undescribed species from Ontario). As well, an additional eight COI sequences were included from Genbank and BOLD, all of which are from Costa Rica (Appendix 1).

I was only able to successfully sequence ITS2 for one specimen within this group (*O. flavidus*). The ITS2 sequence of this specimen was highly divergent from all other species, with large numbers of single base repeats. The secondary structure was equally distinct, though the conserved regions of the structure were maintained (Figure 2-6). The small amounts of clean ITS2 sequence that were obtained from two other specimens within this group (DNA5550 and DNA5556, Appendix 1) indicate that the sequences are similar to that obtained for *O. flavidus*. The extremely long branch length of this anomalous sequence in the ML analysis indicates that it may be a pseudogene, as it greatly exceeds the amount of expected variation in ITS2. (Kita and Ito 2000; Alvarez and Wendel 2003). However another indication of ITS2 pseudogenes (lower GC content) was not observed in this sequence (Buckler *et al*. 1997, Kita and Ito 2000). The *O. flavidus* group had a basal position in all analyses except for ITS2 ML, in which it was found within the *obscuratus* group. The branch was so long, however, that this placement is likely spurious (Kita and Ito 2000).

O. areolaris Brauns species group

The *O. areolaris* species group (Gauld 1985) has been considered by some authors to be the genus *Platophion* (Hellén 1926, Brock 1982), but was synonymized with *Ophion* by Gauld (1980). Only one species from this group (*O.*

ocellaris) was included in the present study; however, all three genes support its inclusion within *Ophion.* Its position within *Ophion* varied depending on which gene was analyzed, though all analyses recovered it as relatively basal. The strongest evidence (from ITS2 and the combined analysis) suggests it may be sister to the *scutellaris* group. This group is also unusual within *Ophion* as it has been reared from Thyatirinae (Drepanidae) (Brock 1982).

O. scutellaris Thomson species group

The *O. scutellaris* group was well-supported in nearly all analyses. The clade as a whole was not recovered by the MP analysis of the 28S dataset, though the two sub-groups that make up this species group were recovered (see Chapter 4). In the 28S ML analysis the clade was recovered with bootstrap support of 62. In all other analyses, this clade was recovered with bootstrap values ranging from 83 (COI MP) to 100 (combined ML, MP). The members of this group are mostly dark reddish, early-season species and can be recognized by a suite of morphological characters (e.g. subequal mid-tibial spurs, carinate scutellum). See Chapter 4 for a complete treatment of this species group.

O. parvulus Kriechbaumer species group

The *O. parvulus* group was recovered by all genes and all analyses, with bootstrap support ranging from 52 (28S ML) to 100 (combined ML and MP). A single specimen identified as *O. parvulus* (G. Broad, BMNH) was also recovered within the *obscuratus* group. I was sent a leg of this specimen, but have not examined the rest of the body, as the specimen remains at BMNH. However, since this group is not closely related, it likely represents a misidentified specimen. The Nearctic specimens in this group were further divided by COI into two strongly supported clades. In one of these clades, however, sequencing appears to have amplified a nuclear mitochondrial pseudogene (numt) (Gellissen *et al.* 1983, Lopez *et al.* 1994), as there is a two base pair deletion at the same location in each sequence that would result in a frameshift in the coding mitochondrial gene.

Morphologically, this group can be recognized by the evenly arched *Rs* vein of the forewing (i.e. not sinuous) and by the unusually short first tergite, with the spiracles in line with the ventral membrane. Thus far all Nearctic specimens in this group have been collected in the fall (August or September), though one of the two Palearctic species was collected in May.

O. slossonae Davis species group

The *O. slossonae* group was monophyletic in the COI and combined analyses, with bootstrap support ranging from 73 (COI ML) to 99 (combined MP). It was also recovered as monophyletic in the ITS2 MP analysis, though with bootstrap support of less than 50. In the ITS2 ML, 28S ML, and 28S MP analyses, it was recovered as a paraphyletic grade with respect to the *O. parvulus* group.

This species group is highly morphologically variable and has been collected from early June to mid-September (with one specimen from Florida in March). As well, this is the only species group to have two distinct ITS2 structures in different species (Figure 2-6). Additional morphological study of this group may reveal characters that are consistent within the group or, conversely, might suggest it should be further subdivided.

O. pteridis Kriechbaumer species group

The *O. pteridis* Kriechbaumer group was recovered in all analyses except for 28S. The clade was well supported in most ITS2 and COI analyses (Table 2-3) and it was strongly supported in the combined analysis (ML: 98, MP: 100). In the 28S ML analyses, it was recovered as two separate clades, only one of which had weak bootstrap support (Figure 2-3). Sequence from the nominate species (*O. pteridis*) was only successfully sequenced for COI.

This is a late-flying species group, with almost all collection records from July – September (Appendix 1). Unlike most *Ophion*, species in this group have strong scutellar carinae.

O. luteus (L.) species group

The *O. luteus* group was recovered in all analyses. It was well-supported by ITS2, 28S and the combined analysis, with bootstrap values ranging from 70 (28S ML, MP) to 100 (combined ML). In the COI MP analysis, it had bootstrap support of 76; however, while the clade was recovered in the COI ML analysis, it had bootstrap support of less than 50.

Species from this group have been collected from May – September, with one species from Arizona in March or April (Appendix 1). While morphologically variable, most species can be recognized by the long trochantellus, the relatively transverse face shape and the weak or absent internal angle of the mandibles.

Species group 1

This species group is a small monophyletic clade that was strongly supported in all analyses, though only four specimens were included in the ITS2 analysis, and three for 28S. Twelve sequences were included in the COI analysis. Within the group, COI had a maximum sequence divergence of only 2.3%, however based on the diverse morphology of these specimens, I predict the group contains a minimum of four species.

O. obscuratus Fabricius species group

The *O. obscuratus* group is a large group containing 56% of all COI sequences and 67% of ITS2 sequences. It was recovered as monophyletic by COI and the combined analysis, but with less than 50% bootstrap support. In the ITS2 ML analysis, it was monophyletic except for the long branch of *O. flavidus* that was recovered within this group and three specimens that were excluded. One of these was the single ITS2 sequence from *O. obscuratus* – perhaps indicating a different species group name should have been selected. However since there is sufficient evidence from COI, 28S, and the combined analysis that *O. obscuratus* is related to species within this group, and no strong conflicting evidence from ITS2, I have chosen to use this name regardless. In the 28S analysis, this group was paraphyletic with respect to the *luteus* group, *pteridis* group, species group 1,

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and *O. nigrovarius*. The single 28S sequence of *O. bicarinatus* from Taiwan (EU378715) was placed within this species group.

Within the *obscuratus* group, COI recovered a well-supported clade consisting of several Palearctic species (*O. crassicornis, O. costatus, O. mocsaryi, O. brevicornis, O. forticornis*, and potentially two species within "*O. obscuratus*"). Two other specimens identified as *O. obscuratus* were not recovered as part of this clade, though they were part of the *obscuratus* group. I was unable to successfully sequence ITS2 from *O. brevicornis* and *O. forticornis*; however, three of the remaining species (*crassicornis, costatus, mocsaryi*) formed a similarly well-supported clade. This Palearctic clade was not recovered in the 28S analysis.

Unplaced species

Several specimens remain unassigned to any of the above species groups. The Nearctic species *O. nigrovarius* was recovered by COI as the sister species to the Palearctic *O. perkinsi,* with an ML bootstrap value of 69 and a MP bootstrap of 100. Together these two species were the sister group of the Palearctic *O. longigena* and the Nearctic species group 1 in the ML analysis but with less than 50% bootstrap support. In the MP analysis, *O. longigena* and species group 1 also formed a clade, but it did not include (*O. nigrovarius+O. perkinsi)*. I was not able to sequence *O. perkinsi* or *O. longigena* for the other two genetic markers. In the ITS2 analysis, *O. nigrovarius* was sister to the *O. pteridis* group, and according to 28S, it was indistinguishable from the *obscuratus* group.

Finally two unidentified specimens from Madagascar were sister to the *pteridis* and *luteus* groups though with less than 50% bootstrap support (Figure 2-1). They likely represent an additional species group, however with such limited sampling from the region, I am leaving them as unassociated.

Overall phylogeny

The relationship between the species groups remains unresolved, with little bootstrap support for the deeper nodes within the phylogeny. Sequencing of

28S was undertaken primarily to resolve these nodes; however, there was even less resolution from this gene than from COI and ITS2. In general, the tree can be separated into two broad divisions (exclusive of the three basal species). One division contains the *scutellaris*, *parvulus*, *slossonae*, *flavidus,* and *areolaris* species groups, though except for the *parvulus+slossonae* clade, the relationships between these groups vary. The second division contains the *pteridis* group, *luteus* group, *obscuratus* group, species group 1 and *O. nigrovarius*, though again the relationships between these groups are analysis-dependent.

ITS2 secondary structure

The internal transcribed spacer 2 (ITS2) of rDNA varied from 717 base pairs to 1056 base pairs in length, with all except two species having greater than 850 base pairs. The secondary structure of all ichneumonid ITS2 folded in MXScarna is congruent with the conserved features of ITS2 observed across a wide range of taxa (Schultz *et al*. 2005; Coleman 2007). In particular, all species have recognizable helices II and III, the hallmark helices of almost all known ITS2 sequences (Coleman 2007). Helix II is relatively short, almost always unbranched, and has a pyrimidine-pyrimidine mismatch bulge near its base. Helix III is long, often branched, and more highly variable, but with a conserved sequence motif near the apex on the 5' side. In all Ichneumonidae examined, this motif is CGGTCGATCGAGTCC. In *Ophion*, there are an additional two to four helices between Helix II and III. The first of these (tentatively labeled Helix IIA) is present in all species and is almost always very long (Figure 2-6).

Within *Ophion*, the secondary structure of ITS2 was quite conserved among many taxa, and highly divergent between others. The vast majority of specimens were successfully modeled from a single template, with a minimum of 90% of the base pairs assigned to the template structure. This includes all specimens from the *obscuratus* group, the *luteus* group, the *pteridis* group, and species group 1, as well as *O. nigrovarius*. The remaining species groups each required a separate template (Figure 2-6). In most cases, these differed only slightly in shape, particularly in the number of short helices between Helix IIa and

Helix III, and in the branching pattern of Helix III. The structures for *O. minutus* and *O. flavidus* were the most distinct. The *O. flavidus* structure was particularly unusual as Helix II was branched, a pattern rarely observed across Metazoa (Coleman 2007); this is further evidence that this sequence may be a pseudogene. The *slossonae* group was the only species group to have two distinct secondary structures. One of these structures (*slossonae* group 2, Figure 2-6) was found in a single undescribed species (3 specimens), and differed from all sequences by having a large deletion in the centre of the sequence, thus lacking most of Helix IIa.

Discussion

This paper presents the first phylogenetic hypothesis for the genus *Ophion*. It is a preliminary analysis due to limited geographical sampling; only three of the eight species groups outlined by Gauld (1985) are included in the analysis, and the sampling is heavily biased towards Canadian material. The species groups were delimited primarily based on distinct sequencing gaps between well-supported clades; however, further sampling may fill in some of these gaps, thus requiring a reanalysis of these species groups. Despite these limitations, this is the first attempt at resolving the "*luteus*-group anathema" (Gauld 1985), and as such provides a valuable framework for understanding the relationships between the vast majority of *Ophion* species.

Despite their different modes of evolution, the ITS2 and COI datasets were remarkably congruent in their division of this broad group into species groups. The 28S analyses provided less resolution than the other two gene regions, but did not conflict with them. This overall congruence between datasets is supported by the combined analysis having very strong support for virtually all major nodes of the tree.

While the species groups were defined primarily based on patterns in the molecular data, in several cases phenological and morphological data further support these groups. The *O. scutellaris* species group is particularly well-characterized and is discussed in detail in Chapter 4. Several other species groups

(e.g. the *O. pteridis* group, the *O. parvulus* group, and the *O. luteus* group) have morphological characters that are apparently consistent within the groups; however, more research is needed to confirm that these characters are unique to these groups. Other groups (e.g. the *O. slossonae* group and species group 1) are well-characterized according to molecular characters, but thus far lack obvious morphological characters.

The status of *O. nigrovarius*, *O. perkinsi*, and *O. longigena* requires further study. All three species are buccate-headed (i.e. have expanded genae), which supports the molecular data linking them. *O. nigrovarius* and *O. perkinsi* in particular look similar, as well as having the strongest support for a sister group relationship. There is some evidence that these species are related to species group 1, which also has some buccate-headed species (though others are more typical); this group should perhaps be expanded to include these species. However, since I only have COI sequences for *O. perkinsi* and *O. longigena*, and since the position of *O. nigrovarius* varies between analyses, I have left these species unassociated. *O. nigrovarius* is a particularly interesting species since it has been recorded from *Phyllophaga* (Coleoptera: Scarabaeidae), the only known record of a coleopteran host for *Ophion* (Townes 1971).

While this study provides much resolution of the species groups within *Ophion*, the *O. obscuratus* group remains an unresolved challenge. This species complex consists of many apparently closely related species, some of which are morphologically distinct, while others are less so. This group may not even be a monophyletic species group, but it is nonetheless a useful categorization pending further study. The group includes an early-season, yellow-marked *Ophion* species that is by far the most abundant species in all Canadian localities that have been well-sampled; however, the precise boundaries of this species are still undetermined (MDS, unpub. data). Within the *obscuratus* group, the majority of the British species form a monophyletic clade according to COI; this clade is less supported by ITS2 and 28S, but further study may warrant designating this clade as a separate species group.

The vast majority of Nearctic species in this study are undescribed. However, the presence of at least five previously unrecognized putative new species within the British material shows that even in well-studied areas there is much work to be done at the species level within *Ophion*. This also suggests that using a single exemplar per species for phylogenetic inference can potentially mislead the analysis if there is any doubt as to the identity of the species involved. Until *Ophion* are better resolved at the species level, we are limited in our understanding of the overall phylogeny.

The amplification of nuclear mitochondrial pseudogenes (non-functional copies of mitochondrial genes that have been incorporated into the nuclear genome, or numts) can mislead phylogenetic analyses based on mitochondrial DNA (Song *et al*. 2008, Buhay 2009, Moulton *et al*. 2010). In this study, the amplification of a presumed numt in some members of the *parvulus* group means the results of the analyses should be viewed cautiously. Since ITS2 and 28S also support the monophyly of this group, it appears that at least at the species group level, the numts remain phylogenetically informative. Their presence, however, limits the utility of COI for species delimitation. These numts were recognized due to a presumably homologous two-base-pair deletion in several otherwise clean sequences. However, not all numts contain indels or stop codons, and may be difficult to recognize (Song *et al*. 2008, Buhay 2009, Moulton *et al*. 2010). Similarly, the amplification of a possible ITS2 pseudogene in the *O. flavidus* species group could also potentially mislead phylogenetic analyses. However, the congruence of COI and 28S with regards to this group provides evidence that the delimitation of this species group is accurate. Both of these examples emphasize the importance of not relying on a single molecular marker for phylogenetic inference (Dupuis *et al*. 2012).

ITS2 secondary structure

The highly variable unpaired regions of ITS2, combined with the non-independence of pairing regions, can potentially mislead phylogenetic inferences (Tillier and Collins 1995; Telford *et al*. 2001; Wolf *et al*. 2008; Letsch and Kjer

2011). However, using an ITS2 specific model that incorporates secondary structure had very little impact on the results of this study. This may be simply because the observed variation in ITS2, namely large differences between species groups and low divergence within them, was a strong enough signal that the choice of model did not significantly affect the results.

Since the ITS2 sequences were folded in silico, further studies are needed to confirm that these structures are accurate (Eddy 2004; Marinho *et al*. 2013). Overall, the ITS2 structures were consistent with the conserved patterns observed by Coleman (2007), loosely corresponding to the four-domain model predicted for ITS2 (Schultz *et al*. 2005). They did, however, differ in significant ways. Most dramatically, ITS2 in *Ophion* is considerably longer than in most other species that have been studied (Schlötterer *et al*. 1994; Gomez-Zurita 2000; Young and Coleman 2004; Coleman 2007; Marinho *et al*. 2011), including many other Ichneumonidae (Ashfaq *et al*. 2005; Wagener *et al*. 2006). The length contributed to difficulties in sequencing this gene, making it less optimal for phylogenetic analyses in this genus compared to other taxa. Much of the additional length was found in the third helix between Helix II and III. I have called this domain Helix IIA, assuming it is equivalent to the IIA of other insect species (Coleman 2007). However the lack of a variable Helix IV in most species indicates that perhaps this domain is equivalent to Helix IV in other species. It is biologically interesting that one species within the *O. slossonae* group lacked this long helix, even though all other specimens within the species group had a more typically-shaped ITS2 (Figure 2-6).

With the exception of the highly divergent *O. flavidus* sequence, *O. minutus* had the most atypically-shaped ITS2, in comparison with other species. This provides additional evidence that *O. minutus* is distinct from the remaining *Ophion*. Determining the ITS2 sequence and structure of other basal *Ophion* would be informative for understanding the root of the *Ophion* phylogeny.

The remaining *Ophion* had an overall similarly-shaped ITS2. The nearly identical structure found in the *pteridis*, *luteus*, and *obscuratus* groups, along with species group 1 and *O. nigrovarius*, further supports the monophyly of this clade.

Conclusion

This study provides an essential framework for future studies of the diverse and morphologically challenging genus *Ophion*. For the first time, the "*luteus* anathema" (Gauld 1985) is divided into more manageable, discrete units that can be examined for morphological characters and biological information. Many species are morphologically very similar between species groups, and there are also highly divergent species within groups. However at least some of the species groups can be recognized morphologically as well as with molecular characters. With this phylogeny as a guide, it will now be possible to conduct more targeted morphological analyses, with the reanalysis of known morphological characters and the discovery of new characters, to better characterize the species groups. This will in turn facilitate the delimitation of species within each group, in order to revise the woefully underdescribed Nearctic *Ophion*, and ultimately to revise *Ophion* worldwide.

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Table 2-1. Ichneumonidae species included in multiple folding of ITS2 in
MXScarna.

Subfamily	Species (species group)	GenBank	Length (base pairs)
Campopleginae	*Diadegma semiclausum* Hellén	AJ885183	624
Campopleginae	*Meloboris* sp.	AJ888025	599
Diplazontinae	*Sussaba aciculata* (Ruthe)	JN626397	709
Diplazontine	*Tymmophorus erythrozonus* (Förster)	JN626423	805
Mesochorinae	*Mesochorus* sp.	AY588968	718
Pimplinae	*Scambus calobatus* (Gravenhorst)	JN243123	667
Ophioninae	*Ophion ocellaris* Ulbricht (*areolaris* grp)	KF616299	821
Ophioninae	*Ophion flavidus* Brullé (*flavidus* grp)	KF616301	865
Ophioninae	*Ophion* sp. (*scutellaris* grp)	KF615947	867
Ophioninae	*Ophion* sp. (*parvulus* grp)	KF615980	977
Ophioninae	*Ophion* sp. (*slossonae* grp)	KF615977	1013
Ophioninae	*Ophion* sp. (*obscuratus* grp)	KF615984	864

Table 2-2. Summary of maximum likelihood (ML) and maximum parsimony (MP) analyses of three genes of *Ophion*. CI = Consistency index; RI = Retention index

Analysis	Statistics	COI	ITS2	28S	Combined
	No. sequences	680	394	99	68
ML	Log likelihood	-12645.19	-8975.60	-2203.09	-16555.56
MP	Tree Length	2457	1255	159	2571
	CI/RI	0.22/0.93	0.76/0.97	0.70/0.89	0.53/0.79

Table 2-3. Summary of bootstrap support values for nine[1] major divisions (species groups) of Nearctic and western Palearctic *Ophion*. n.m. = not monophyletic

Species group	COI		ITS2			28S		Combined	
	MP	ML	MP	ML	NJ	MP	ML	MP	ML
minutus	<50	76	-	-	-	55	73	-	-
scutellaris	83	98	100	95	97	<50	62	100	100
slossonae	95	73	95	n.m.	93	n.m.	n.m.	99	97
parvulus	92	71	100	97	99	78	52	100	100
pteridis	90	54	92	82	89	n.m.	n.m.	100	98
luteus	76	<50	95	87	91	69	70	99	100
obscuratus	<50	<50	<50	<50*	<50	n.m.	n.m.	<50	<50
flavidus	61	56	-	-	-	95	83	-	-
Group 1	100	95	100	100	98	82	75	100	100

[1]Only a single species of the *O. areolaris* species group was included in the analyses, therefore this group is not included in the table.

* monophyletic except for the exclusion of three specimens and the likely spurious inclusion of *O. flavidus*.
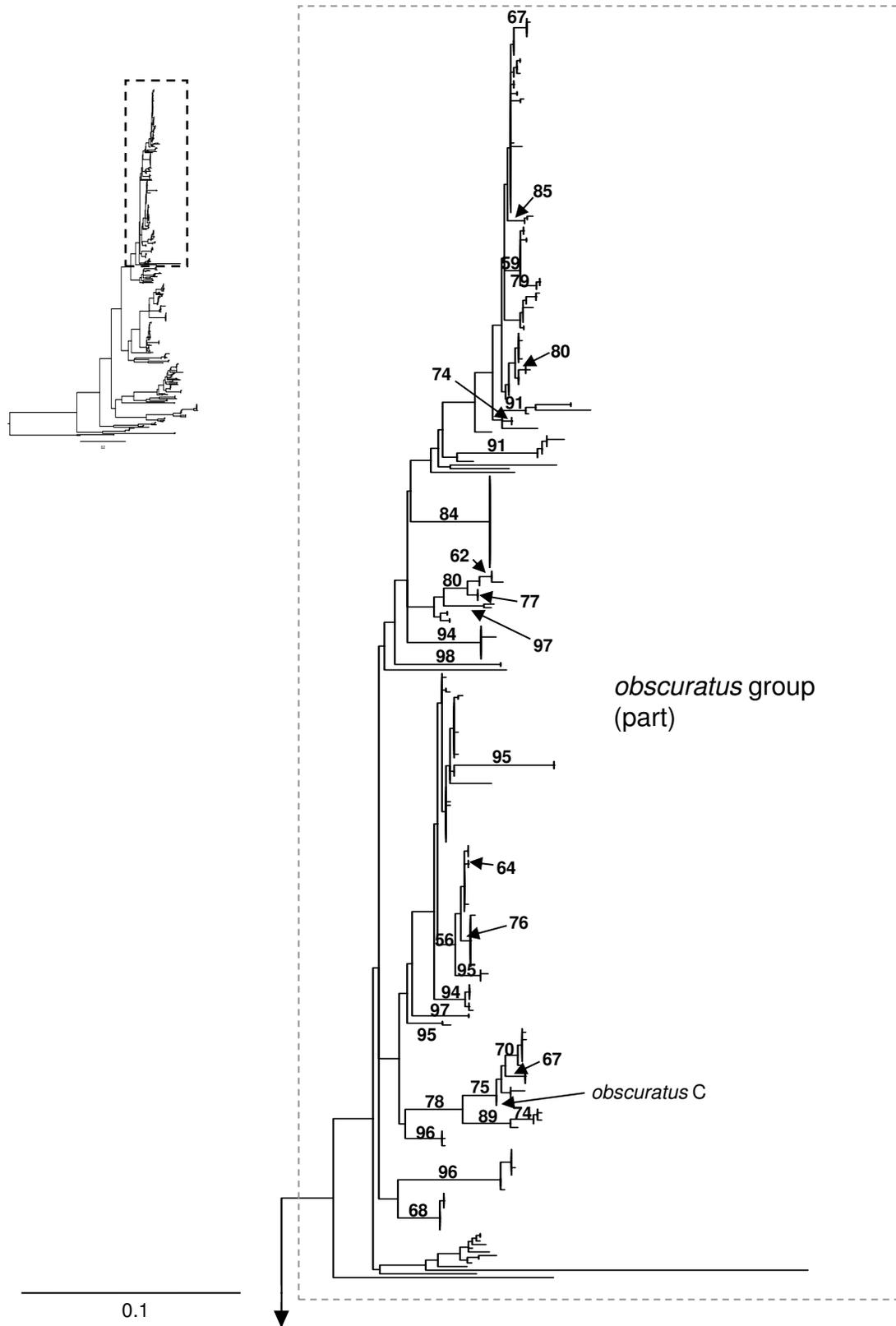
Figure 2-1. ML tree of 677 *Ophion* and 3 *Enicospilus* COI sequences. ML bootstrap values are in bold, MP bootstraps are in italics and are only shown for major nodes.
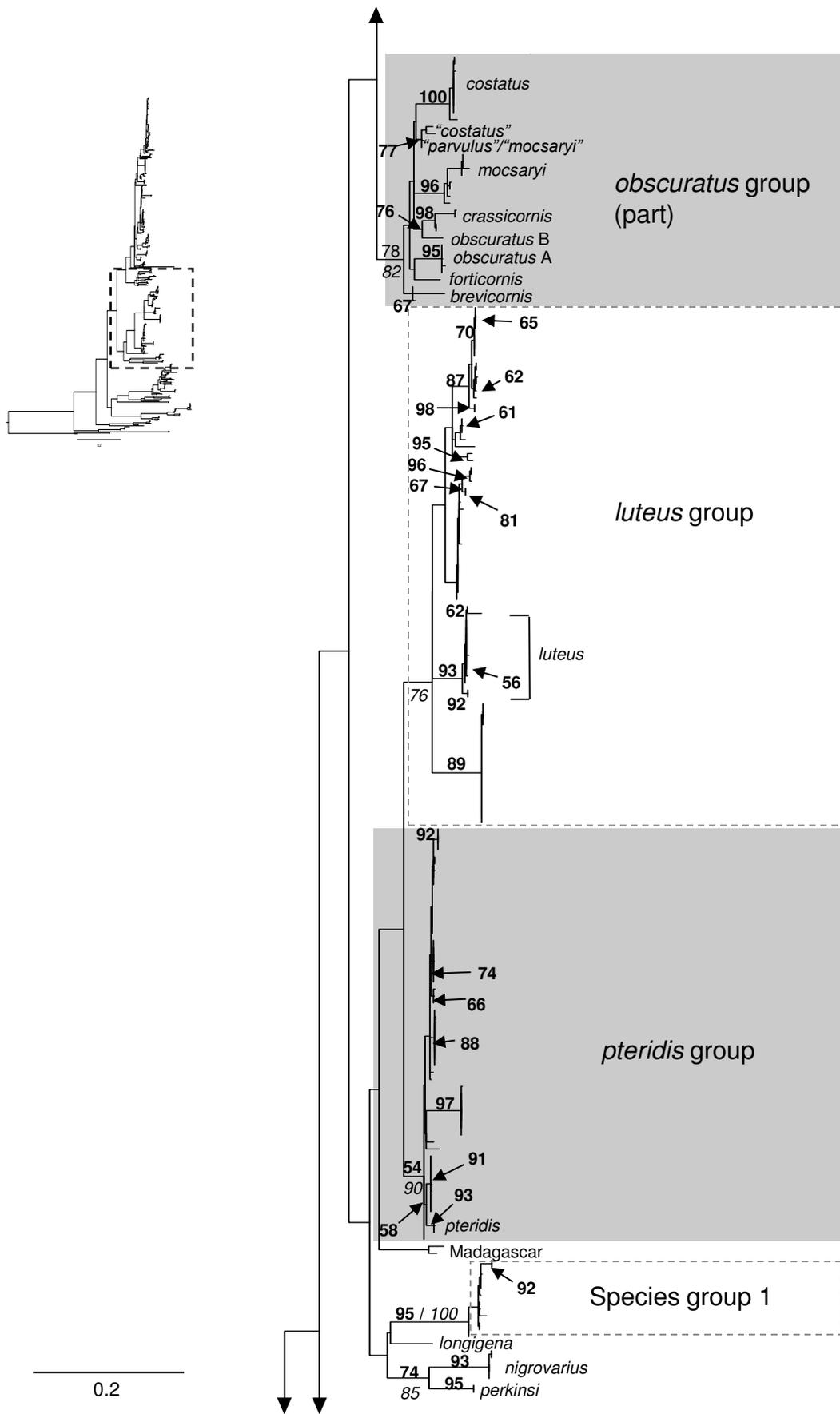
Figure 2-1 cont'd.

Figure 2-1 cont'd.   59
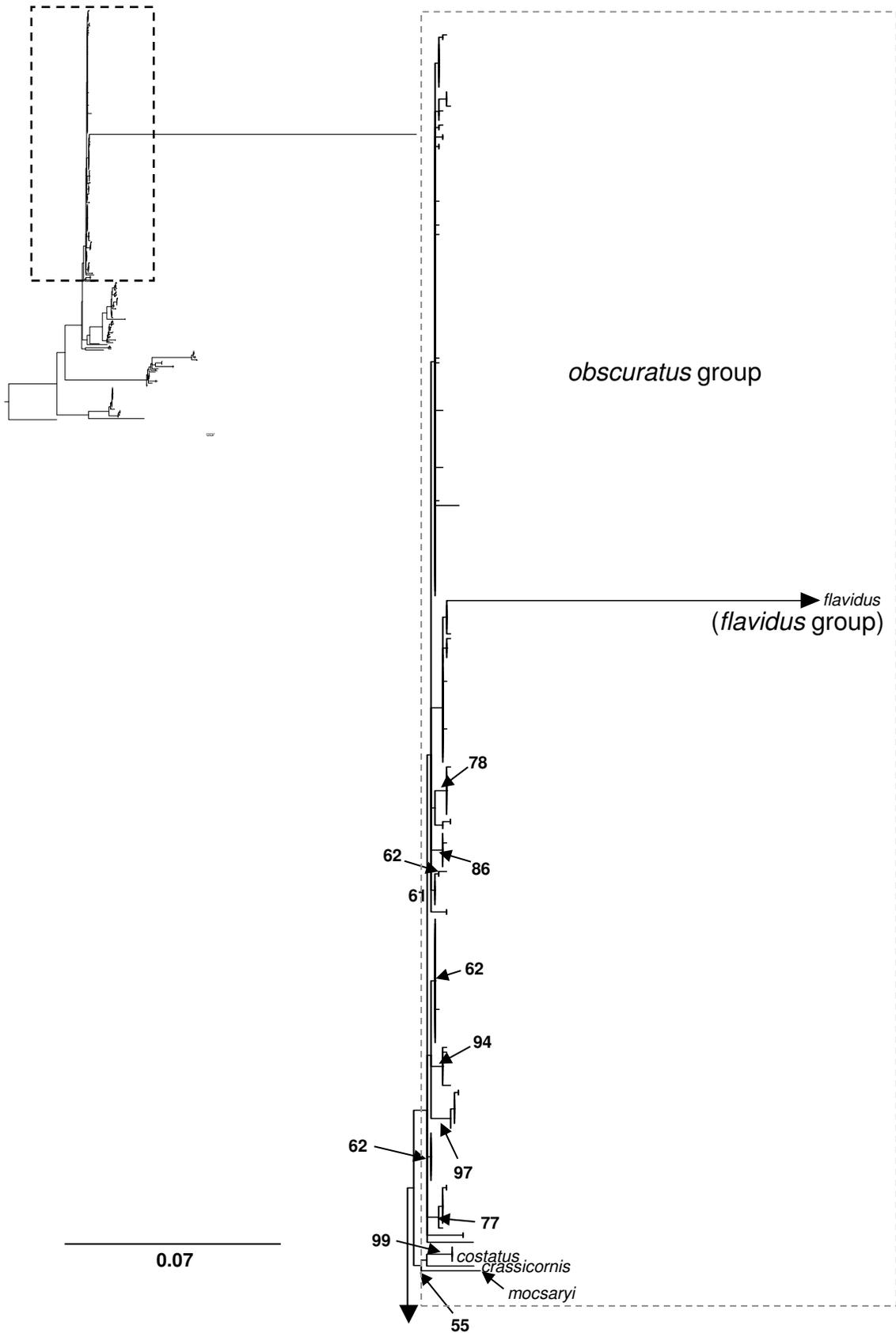
Figure 2-2. ML tree of 394 *Ophion* ITS2 sequences. ML bootstrap values are
in bold, MP bootstraps are in italics, and ITS2-specific NJ bootstraps are
underlined. MP and NJ bootstrap values are only shown for major nodes.
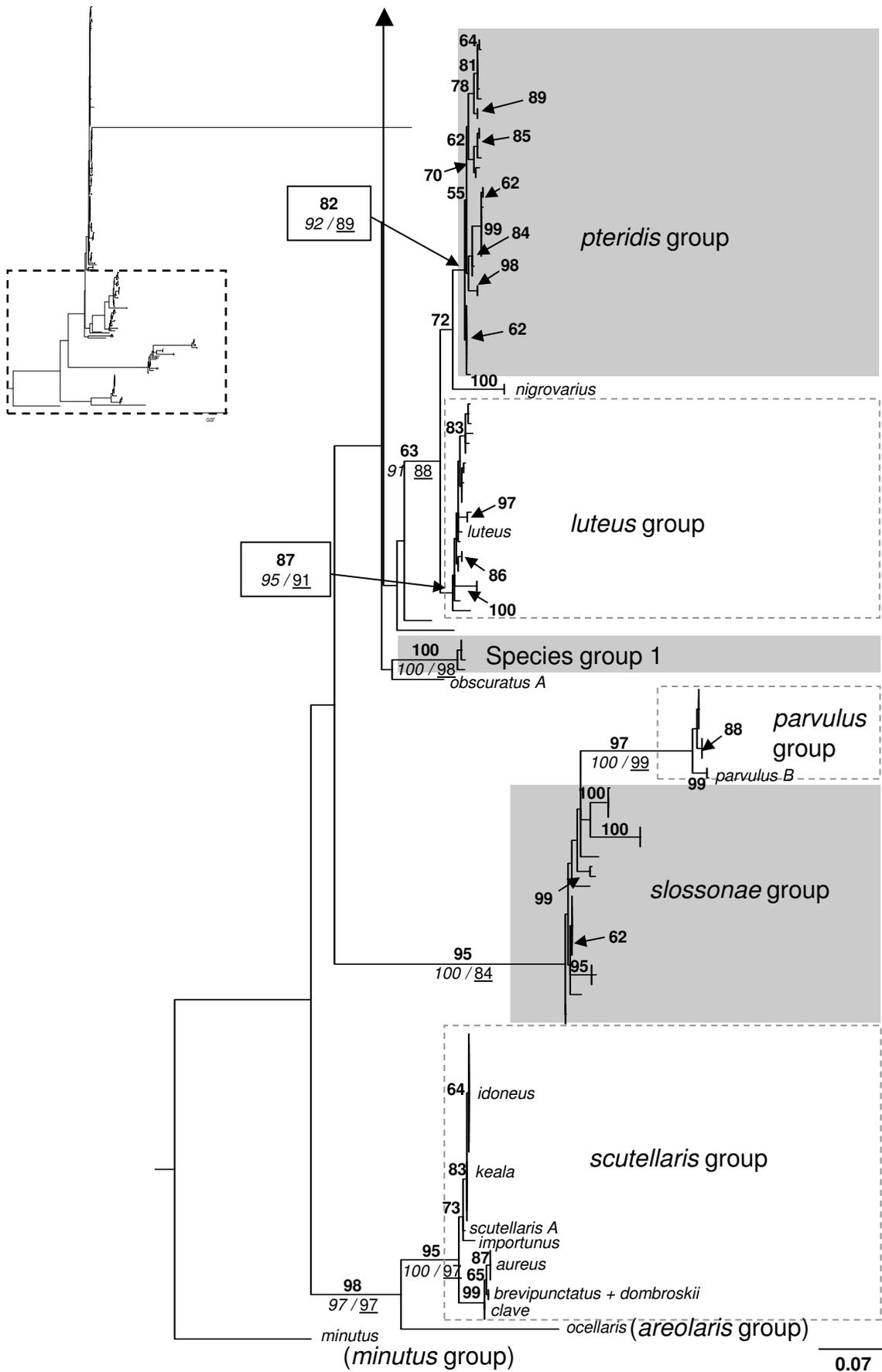
Figure 2-2 cont'd.
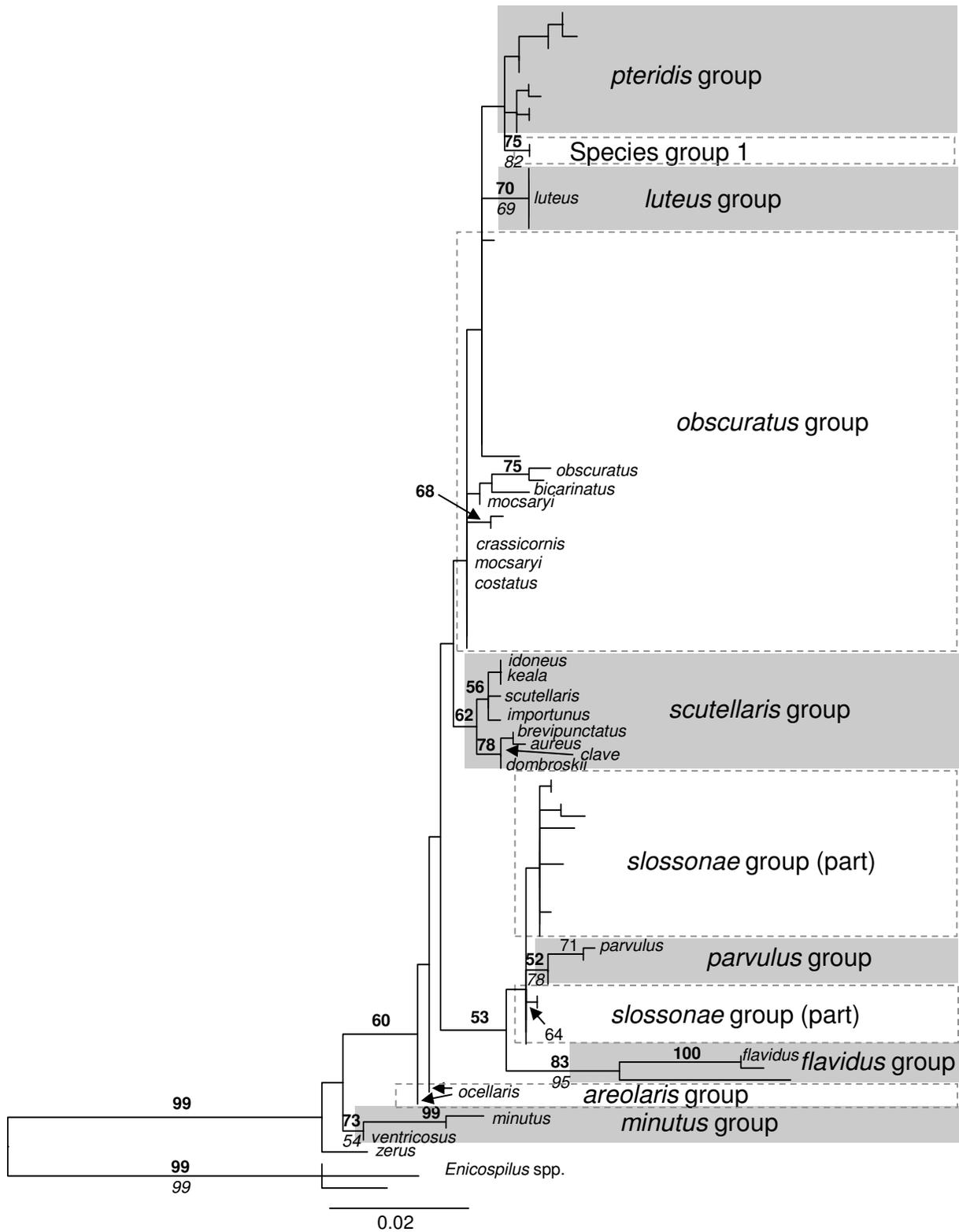
Figure 2-3. ML tree of 96 *Ophion* and 3 *Enicospilus* 28S D2-D3 sequences.
ML bootstrap values are in bold; MP bootstraps are in italics and are only
shown for major nodes.
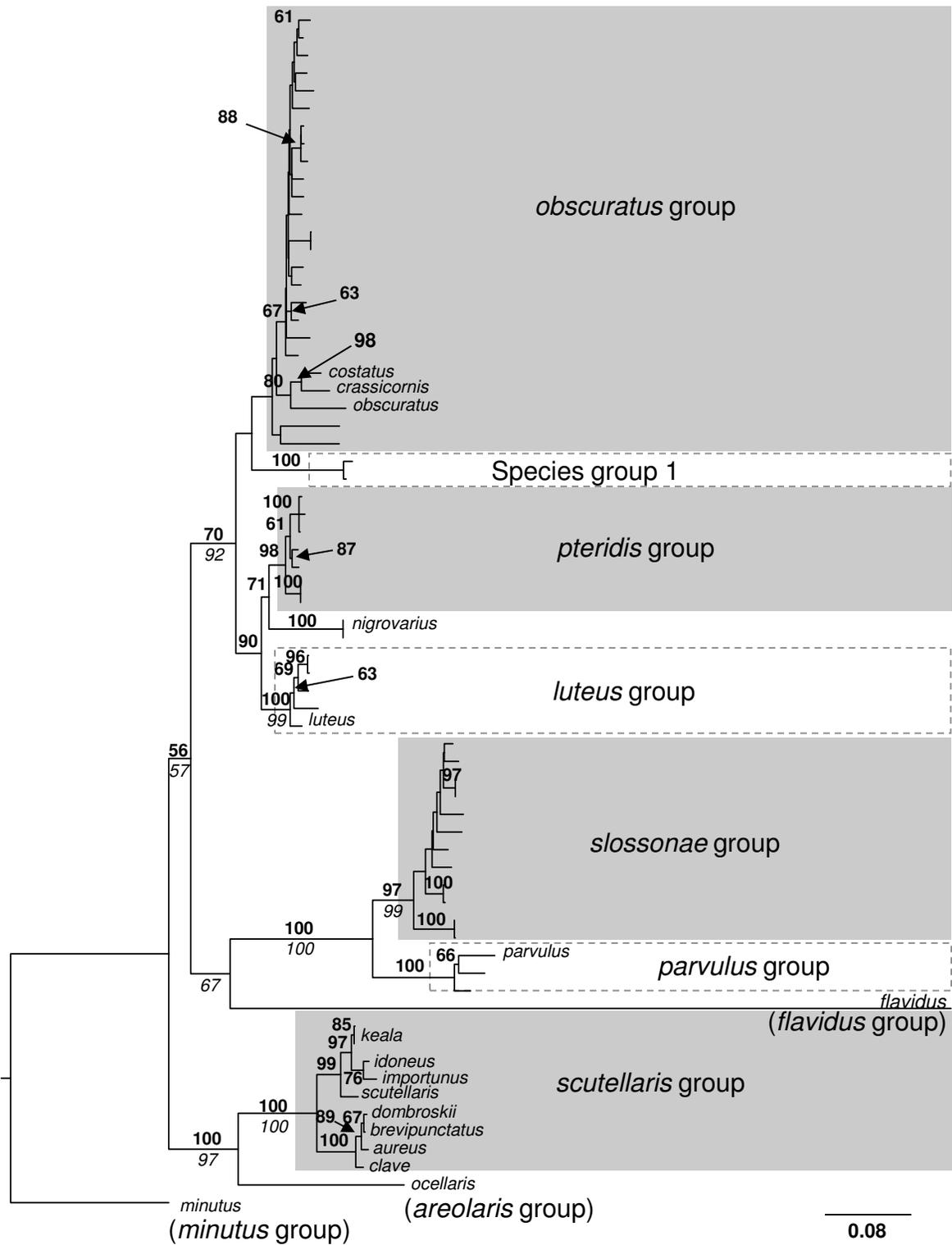
Figure 2-4. ML tree of 68 combined COI, ITS2 and 28S sequences of *Ophion*.
ML bootstrap values are in bold; MP bootstraps are in italics and are only
shown for major nodes.
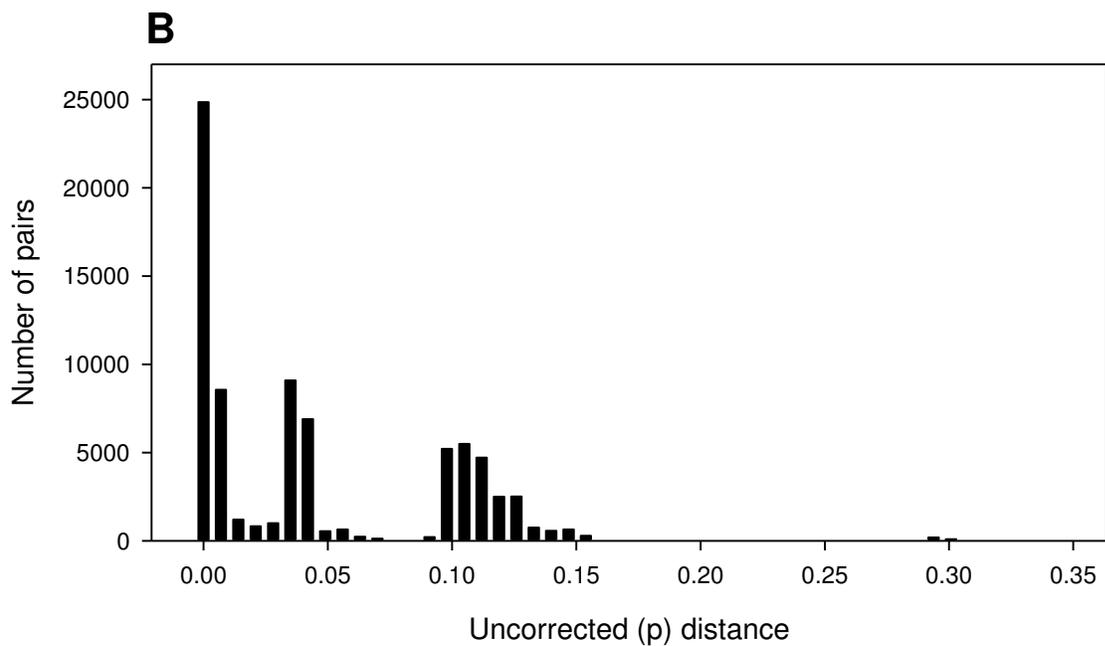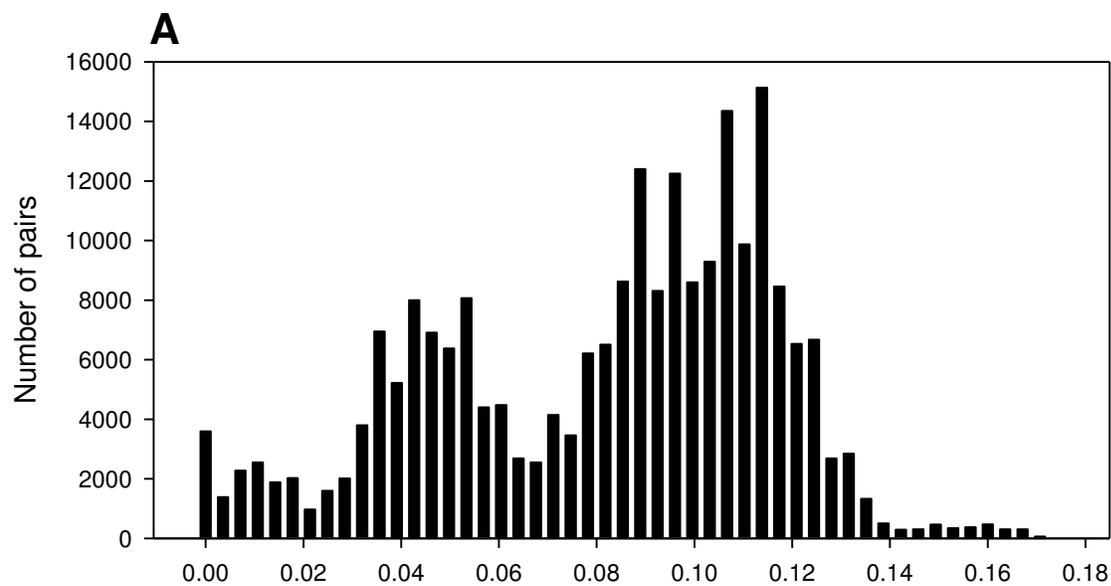
Figure 2-5. Uncorrected pairwise distances between all *Ophion* sequences. **A:** COI; **B:** ITS2
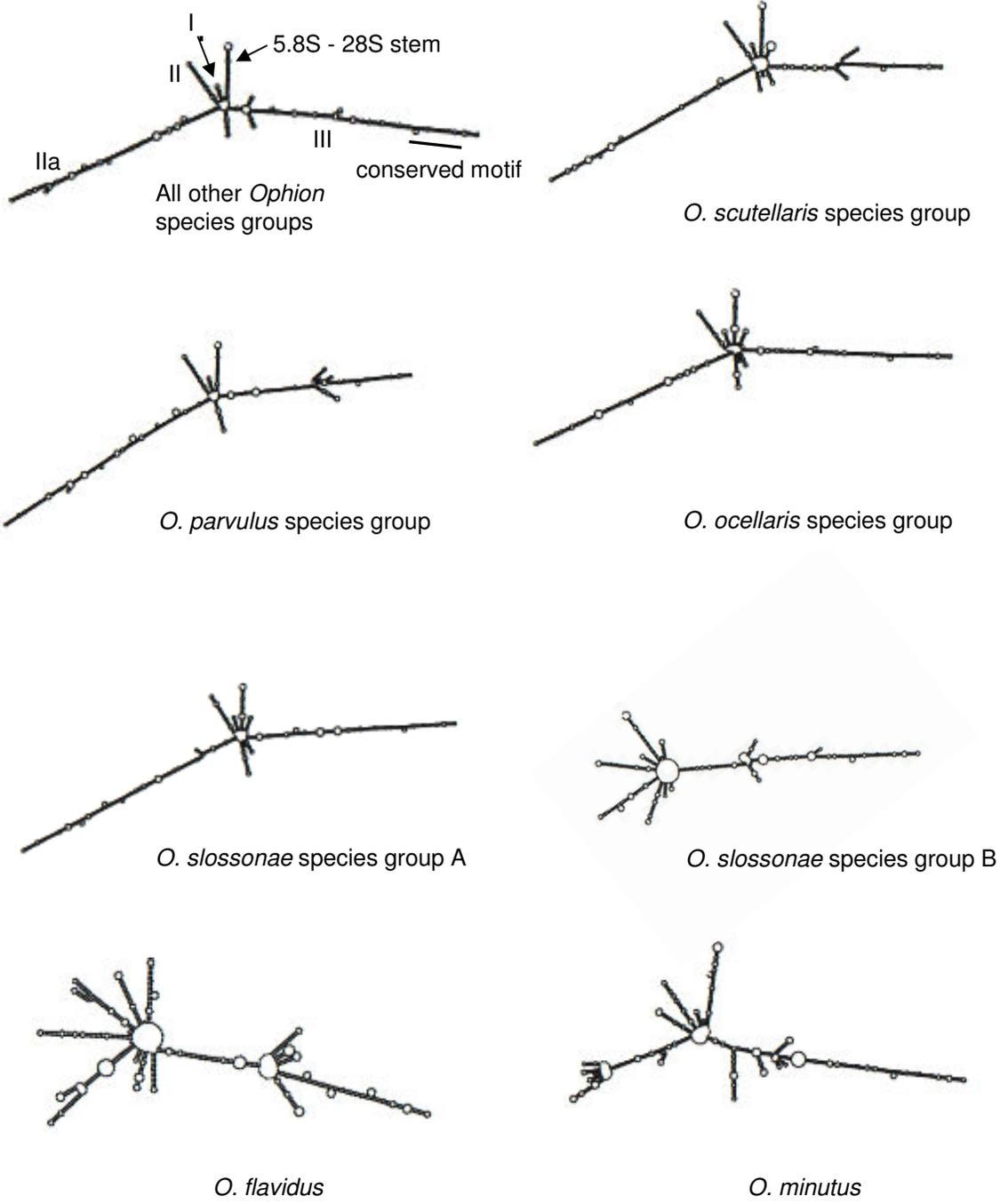
Figure 2-6. Secondary structure of ITS2 in *Ophion* spp., as determined by the free energy method in RNAfold.

Chapter 3

Quantitative species delimitation in *Ophion* Fabricius, a diverse genus of parasitoid Hymenoptera

Introduction

Species are the basic unit for studies of biodiversity, ecology, biogeography and evolution (Claridge *et al*. 1997; Sites and Marshall 2004). They are also essential for applied or management oriented applications such as conservation decisions or the use of biological control agents. However, a large proportion of the earth's species are currently undescribed and it has been argued that the limited number of taxonomists and the pace of traditional methods of taxonomy are insufficient for dealing with this unknown diversity (Brooks and Hoberg 2000; Godfray 2002; Wheeler 2004). With the advent of DNA sequencing, molecular taxonomy has been proposed as a method of quickly assessing species diversity in diverse, little-known taxa (Tautz *et al*. 2003; Blaxter 2004; Pons *et al*. 2006). In particular, the large-scale Barcode of Life project has popularized the use of the 5´ half of the cytochrome oxidase I gene of mitochondrial DNA as a standardized "barcode" for both species identification and species discovery (Hebert *et al*. 2003a, b; Miller 2007).

Many studies incorporating DNA taxonomy, particularly of the COI barcoding gene, have relied on distance-based clustering methods for species delineation. For example, species have been defined as terminal monophyletic clusters on neighbour-joining trees (Hajibabaei *et al*. 2006), or have been delimited based on a standard interspecific threshold, often 1-3% (Hebert *et al*. 2003a, b; Hebert *et al*. 2004; Smith *et al*. 2009; Strutzenberger *et al*. 2011; Tang *et al*. 2012; Smith *et al*. 2013; Stalhut *et al*. 2013), or an interspecific distance of 10X the intraspecific distance (Hebert *et al*. 2004). This last criterion, however, is only practicable with prior knowledge of the genetic diversity within pre-defined species. While it is widely acknowledged that a single threshold divergence will not apply to all taxa (Cognato 2006; Monaghan *et al*. 2009; Hendrich *et al*. 2010),

the use of a pre-defined threshold has nonetheless been either implicitly or explicitly used as a criteria for assessing species diversity in a number of studies (Strutzenberger *et al*. 2011; Smith *et al*. 2009; Smith *et al*. 2013). Other studies have attempted to delimit species based on the presence of a "barcoding gap", i.e. the gap between intraspecific and interspecific pairwise genetic distances (Hebert 2003a; Barrett and Hebert 2005). However the universality of barcoding gaps is controversial, with several studies finding significant overlap between intra- and interspecific divergences (Meyer and Paulay 2005; Wiemers *et al*. 2007). As well, objectively determining this gap can be methodologically challenging. Recently, a method called Automatic Barcode Gap Discovery (ABGD) was developed to automate the search for barcoding gaps, even in the presence overlapping intra- and interspecific divergences (Puillandre *et al*. 2012a).

The use of distance-based methods has been criticized as being purely phenetic, rather than incorporating evolutionary theory (Will and Rubinoff 2004; Will *et al*. 2005; Rubinoff *et al*. 2006; DeSalle 2007). There have thus been a number of attempts to develop coalescence or tree-based methods specifically to address species delimitation of large numbers of species (Carstens and Dewey 2010; Leaché and Fujita 2010; O'Meara 2010; Fujita *et al*. 2012). One of these methods, the generalized mixed Yule coalescent (GMYC) model attempts to determine the point at which coalescent branching patterns (within species) transition to Yule patterns (between species) (Pons *et al*. 2006; Fontaneto *et al*. 2007). This method has shown promise at identifying species boundaries in diverse, little known taxa (Monaghan *et al*. 2009; Ceccarelli *et al*. 2012); however, the results may vary depending on the choice of parameters used to construct the ultrametric tree on which the analysis is based (Ceccarelli *et al*. 2012).

Regardless of which delimitation method is used, the reliance on a single genetic marker may provide misleading results (Dupuis *et al*. 2012). While COI has many advantages for studies of species delimitation, such as its ease of amplification and relatively rapid rate of mutation (Monti *et al*. 2005; Li *et al*. 2010; Williams *et al*. 2012), it may not accurately delimit species due to factors

such as introgression, retained ancestral polymorphisms, or the coamplification of nuclear pseudogenes (numts) ((Funk and Omland 2003; Cognato 2006; Meier *et al*. 2006; Schmidt and Sperling 2008; Dupuis *et al*. 2012). It is therefore important to use additional datasets, such as additional genes or morphology, in an integrative taxonomic analysis to more accurately delimit species (Dayrat 2005; Roe and Sperling 2007; Schlick-Steiner *et al*. 2009).

The internal transcribed spacer 2 (ITS2) of ribosomal RNA is another rapidly evolving genetic marker that has proved useful for phylogenetic analyses at the species level (Campbell *et al*. 1993; Gomez-Zurita *et al*. 2000; Alvarez & Hoy 2002; Hung *et al*. 2004; Wagener *et al*. 2006; Li *et al*. 2010). As with COI, it is present in multiple copies in the cells, and is therefore relatively easy to amplify. However the high rate of mutation and the presence of numerous insertion-deletion events (indels) can make accurate alignment of this gene challenging, particularly between more distantly related species (Coleman and Vacquier 2002). ITS2 has a highly conserved secondary structure, with characteristic paired domains and unpaired regions (Coleman 2007). Incorporating this secondary structure can improve alignment of ITS2 (Coleman and Vacquier 2002; Wiemers *et al*. 2009) and also permits the discovery of compensatory base changes (CBC's). These are base pair changes where two paired nucleotides (often quite far apart in the linear gene) both mutate such that the original pairing is maintained (Gutell *et al*. 1994; Coleman and Vacquier 2002; Coleman 2009). Müller *et al.* (2007) determined that even a single CBC between two individuals indicates enough evolutionary time has passed for reproductive isolation to have arisen, resulting in separate species status. A lack of CBC's is not, however, proof of conspecificity, as closely related species may lack any CBC's (Müller *et al*. 2007; Wiemers *et al*. 2009). CBC's are therefore hypothesized to provide a minimum number of species among the specimens examined.

Ophion is a genus of large nocturnal Ichneumonidae in the subfamily Ophioninae. Unlike the majority of ophionine genera, *Ophion* is most diverse in temperate regions, with few species in tropical habitats (Gauld 1980, 1988).

Members of *Ophion* come readily to lights; however, despite their large size, abundance and ease of collection, Nearctic species have received almost no taxonomic attention. Eleven Nearctic species are currently described, although it has been estimated that the fauna consists of approximately 50 species (Gauld 1985). In the Palearctic region, *Ophion* are better known, with 79 described species (Yu *et al*. 2012), but there have been no revisions of the species across the Palearctic. The fauna has been most comprehensively examined in the United Kingdom, with a number of revisions over the years (Gauld 1973, 1976, 1978), culminating in a comprehensive revision of the 16 known species by Brock (1985).

Gauld (1980, 1985) proposed a number of small, morphologically distinctive species groups within *Ophion*, but attempts to divide the majority of *Ophion* species into monophyletic species groups were limited by the lack of information regarding the Palearctic and Nearctic species. In Chapter 2, I provide a preliminary phylogeny of *Ophion*, with an emphasis on Nearctic and British taxa, and propose several new species groups based primarily on molecular characters. However the species diversity of *Ophion* in the Nearctic region has not been extensively examined. *Ophion* are notoriously difficult to identify as they can be both morphologically homogenous across species, and morphologically variable within species (Gauld 1980, Brock 1982). They are thus well-suited to the application of molecular taxonomy methods as a first step towards species delimitation.

In this chapter, I analyze *Ophion* species diversity using a tree-based method (GMYC), two distance-based methods (ABGD and threshold analysis), and by assessing compensatory base changes of ITS2. The study is primarily based on Canadian specimens, but I have also included some specimens from the United States, as well as all except one described British species.

Methods

Specimen collection

        A total of 672 specimens of *Ophion* were included in this study. The majority of specimens (570) are from Canada, with a much smaller number (19 individuals) from the United States (Figure 3-1). Most specimens were newly collected and sequenced for this study; however, 25 specimens were sequenced from alcohol-preserved material on loan from the Canadian National Collection of Insects, Arachnids and Nematodes, and 92 COI sequences from British Columbia were included from the Barcode of Life database (BOLD), courtesy of J. DeWaard (project code: ICHBC). Specimens were selected for sequencing to represent the range of morphological variation observed in over 4000 specimens from a variety of habitats. Eighty specimens from 15 species of Palearctic *Ophion* were also newly sequenced, all of which are from the United Kingdom except for a single specimen from Spain and one from France. Finally, one sequence of Palearctic *O. obscuratus* Fabricius and two sequences of *O. flavidus* Brullé from Costa Rica were included from Genbank and BOLD.

        The provenance of all specimens is provided in Appendix 1. With the exception of sequences obtained from GenBank or BOLD, all Nearctic taxa were identified by MDS and all Palearctic taxa were identified by G. R. Broad, with the exception of *O. forticornis*, which was identified by M. R. Shaw.

Sequencing

        DNA was extracted from a single hind leg using DNeasy Blood & Tissue Kits (Qiagen, Toronto, ON); the final elution volume was 150 µL. I conducted PCR in either 50 µL or 15 µL reactions. The 50 µL reactions contained 4-8 µL genomic DNA, 5 µL 10x PCR buffer (containing 15mmol/ µL $MgCl_2$) (Promega, Madison, WI), 3 µL of 25 mmoles/µL $MgCl_2$ (Promega), 1 µL of 10 mmoles/µL dNTP's ((Roche, Switzerland), 1 µL each of 5pmol/µL forward and reverse primers, 0.5 µL of 5 U/µL *Taq* polymerase (Fermentation Service Unit, University of Alberta) and 30.5-34.5 µL of autoclaved Millipore water. The 15 µL reactions used 4-8 µL DNA, 1.5 µL PCR buffer, 0.9 µL $MgCl_2$, 0.3 µL each of

dNTP's and forward and reverse primers, 0.15 μL *Taq* and 3.55-7.55 μL water. All PCR products were purified using ExoSap-IT (USB Corporation, Cleveland, OH), and were sequenced using BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA), followed by ethanol precipitation. Sequencing reactions were run on an ABI Prism 3730 DNA analyser. Sequences are deposited in NCBI GenBank, and Genbank accession numbers are listed in Appendix 1.

COI: The primers lco hym (5' – CAA ATC ATA AAG ATA TTG G – 3') and hco out (5' – CCA GGT AAA ATT AAA ATA TAA ACT TC – 3') (Schulmeister 2003), were used to produce a 676 base pair fragment equivalent to the "barcode" region (Hebert *et al.* 2003). PCR conditions were: 94° for 2 min, 35 cycles of 94° for 30 s, 45° for 30 s, 72° for 2 min, and a final extension at 72° for 5 min. Alignment was unambiguous, and was confirmed by translating nucleotides to amino acids in Mesquite (Maddison and Maddison 2011). The first character represented the third codon position; for consistency with other analyses, this was removed so that the alignment begins with the first codon position. I sequenced COI for 565 *Ophion* specimens. A few sequences were not successfully sequenced in both directions, or were otherwise partially incomplete; these sequences were included with the gaps coded as missing data. The 95 *Ophion* sequences obtained from BOLD and Genbank were sequenced using slightly different primers (see BOLD website for sequencing protocols) and were 658 base pairs in length.

ITS2: I sequenced this gene using the primers ITS2-F (5'-GGG TCG ATG AAG AAC GCA GC-3') and ITS2-R (5'-ATA TGC TTA AAT TCA GCG GG-3') which anneal to the flanking 28S and 5.8S genes (Navajas *et al.* 1994). PCR cycling was 94° for 2 min, 35 cycles of 94° for 30 s, 55° for 1 min, 72° for 2 min and a final extension of 75° for 5 min. Excluding the flanking 28S and 5.8S regions, the unaligned sequences ranged from 717 to 1053 base pairs in length. I was unable to obtain complete ITS2 sequences for several specimens, presumably due to the length of the gene and the presence of several microsatellite regions that caused polymerase slippage. Since the analysis of ITS2 secondary structure

was sensitive to missing data, I only included sequences that were complete and that lacked ambiguous regions. The final dataset included 394 ITS2 sequences.

ITS2 secondary structure and CBC's

The complete protocol used to determine the secondary structure of ITS2 is described in Chapter 2. Briefly, I estimated the structure for an arbitrarily chosen sequence using the RNAfold webserver, using the minimum free energy method with default settings (Hofacker 2003). I imported this sequence and structure into the ITS2 database (Schultz *et al*. 2006; Selig *et al*. 2008; Koetschan *et al*. 2010) and used it as a template for folding the remaining sequences. If any sequences had less than 90% similarity to the existing template, one of the anomalous sequences was directly folded in RNAfold and added as an additional template to the database. I repeated this iterative process until all sequences were folded; the final dataset consisted of eight sequences that were folded directly and 386 sequences that were folded using homology. Sequences and structures were aligned using 4Sale, a program designed to perform structural alignments of RNA sequences (Seibel *et al*. 2006) and a matrix of compensatory base changes (CBC's) was exported. The final alignment (excluding the 28S and 5.8S flanking regions) was 1750 base pairs in length. I calculated a neighbour-joining tree based on the CBC distance matrix using the program CBCAnalyzer (Wolf *et al*. 2005).

Barcode accumulation curves

All species delimitation algorithms are dependent on sampling (Meyer and Paulay 2005; Lohse 2009; Papadopoulou *et al*. 2009; Bergsten *et al*. 2012). I assessed the completeness of sampling by calculating "barcode accumulation curves" of the COI sequences, following the procedure outlined by Smith *et al*. (2009). Similar to species accumulation curves used in ecological studies, barcode accumulation curves should approach an asymptote as fewer unique haplotypes are added to the specimen pool, indicating that the majority of the diversity has been sampled. Since the vast majority of specimens were from Canada, I calculated the curve for Canadian material (n = 561), as well as a separate curve

including all Nearctic specimens (n = 580). I also calculated an accumulation curve for specimens from the province of Alberta, since this is the most densely sampled region (n = 342). Finally, I calculated a barcode accumulation curve including only Palearctic specimens (n = 78).

To calculate the curves, I first constructed a neighbour-joining tree for each dataset in MEGA version 5 (Tamura *et al*. 2011), using the K2P model and pairwise deletion of missing data, and imported the tree into the program Conserve (Agapow and Crozier 2008). I excluded all sequences, and then randomly added sequences in sets of 10 (Nearctic, Canada and Alberta datasets) or sets of 5 (UK dataset), and calculated the phylogenetic diversity (PD) of the tree at each successive addition. These results were plotted to assess whether the phylogenetic diversity was approaching an asymptote.

Species delimitation

I analyzed these data using three quantitative methods of species delimitation: generalized mixed Yule coalescent analysis (GMYC) (Pons *et al*. 2006; Fontaneto *et al*. 2007), automatic barcode gap discovery (ABGD) (Puillandre *et al*. 2012a) and threshold analysis using the program jMOTU (Jones *et al*. 2011). Each analysis uses different terminology to refer to the delimited taxa (GMYC = "entities"; ABGD = "groups" or "hypothetical species"; jMOTU = "molecular taxonomic units" or "MOTU"), thus acknowledging that they may not represent biologically meaningful species. However, for clarity and consistency, I am using the term "species" interchangeably with the above terms.

GMYC

I obtained the ultrametric trees required for the GMYC analysis using BEAST version 1.7.5 (Drummond *et al*. 2012) on the CIPRES Science Gateway computing cluster (Miller *et al*. 2010), with ITS2 and COI analyzed separately. Identical haplotypes were removed using the program Alter (Glez-Peña *et al*. 2010), and outgroups were excluded. Models were selected using the program PartitionFinder version 1.0.1 (Lanfear *et al*. 2012), with each gene region

analyzed as a single partition. For the ITS2 dataset, the selected model was TVMef+G, and COI used HKY+I+G. The choice of tree prior can impact the results of the phylogenetic analysis (Ceccarelli *et al*. 2011). For example, the coalescent tree prior is often more appropriate for population-level data and recently-diverged taxa, whereas the Yule prior is recommended for species-level data (Drummond *et al*. 2006). I therefore calculated trees using a random starting tree, the uncorrelated lognormal relaxed clock model, and two different tree models (coalescent and Yule-process) for each marker (ITS2 and COI), for a total of four analyses. Each analysis was run with either four (COI coalescent, COI Yule, ITS2 coalescent) or six (ITS2 Yule) independent threads, and log files and tree files were combined using LogCombiner (part of the BEAST package). The two COI analyses were run for 40 million generations, with trees sampled every 4000 generations and a burnin of 25%. Combined logfiles were examined in Tracer version 1.5 (Rambaut and Drummond 2009) to assess convergence; all combined estimated sample sizes (ESS) were > 200 with the exception of a single parameter using the Yule prior which was 179. The ITS2 analysis with the coalescent prior was run for 80 million generations, sampling every 4000 generations, with a burnin of 25%; all ESS were > 200 except for two parameters that were between 100 and 200. Obtaining convergence in the ITS2 analysis with the Yule prior was more challenging. The final analysis included three threads of 160 million generations and two threads of 80 million generations, sampled every 4000 generations. The first 25 percent of the longer runs, and the first 50 percent of the shorter runs were removed as burnin. Two parameters failed to show convergence (ESS < 100), however the remaining parameters were either ≥ 200 or very nearly so. All analyses were resampled in LogCombiner to give a combined total of approximately 15000 trees. Maximum clade credibility trees (the tree in each analysis with the highest product of posterior clade probabilities) were produced from the combined tree files in TreeAnnotator (part of the BEAST package), using the mean heights option, and used as input for the GMYC analyses.

I conducted GMYC analyses with the SPLITS package (available from http://r-forge.r-project.org/projects/splits/) using the program R (RStudio 2012; R Core Development Team 2013). I analyzed each tree using both single and multiple threshold settings, including all haplotypes (COI_{all} and $ITS2_{all}$). To facilitate comparison between genetic markers, I also ran the GMYC analyses only including taxa that were represented in both datasets (COI_{common} and $ITS2_{common}$). Since in several instances a single COI haplotype was represented by multiple ITS2 haplotypes, or vice versa, the two analyses did not necessarily have the same number of individuals. I obtained the reduced trees by pruning the non-overlapping taxa from the ultrametric trees in R, prior to running the GMYC analyses. Results from the analyses were summarized in R and output as a tree plot.

ABGD

Automatic barcode gap discovery (ABGD) infers clusters of sequences by recursively partitioning the data using a range of prior intraspecific sequence divergences and by calculating a model-based confidence limit for intraspecific divergence at each iteration (Puillandre *et al.* 2011). I first calculated distance matrices for COI and ITS2 in MEGA 5.1 using both K2P corrected distances and uncorrected p-distances. Separate matrices were calculated for all specimens (COI_{all} and $ITS2_{all}$), and only including specimens that were successfully sequenced for both genes (COI_{common}, $ITS2_{common}$). I calculated the number of clusters using the ABGD online tool (Puillandre *et al.* 2012a; available at http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html). For the analyses of COI, I used the default priors (prior intraspecific divergence = 0.001 – 0.1, divided into 10 partitions), since the program is optimized for use with the COI barcoding gene, based on numerous barcoding studies. For ITS2, intraspecific sequence divergence has been much less commonly assessed and is highly taxon-dependent. Within Ichneumonidae, Wagener *et al.* (2006) found the intraspecific sequence divergence of *Diadegma* (subfamily Campopleginae) was 0.2 – 0.6 percent. Within the *Ophion scutellaris* species group (Chapter 4), intraspecific

variation was similarly very low, ranging from a single haplotype per species to a maximum of 0.1 percent. I therefore ran the ABGD analysis using prior intraspecific divergences of 0.0001 – 0.1 (divided into 20 partitions). For each analysis I used both the default relative gap width of 1.5 and a gap width of half the default (0.75) to increase the sensitivity of the analysis (Puillandre *et al*. 2012a)

Threshold Analysis

        COI and ITS2 sequences were clustered into molecular taxonomic units (MOTU) using the program jMOTU (Jones *et al*. 2011). Commonly used thresholds for species delimitation based on COI are 1-3% (Hebert *et al*. 2003a, b; Hebert *et al*. 2004; Smith *et al*. 2009; Strutzenberger *et al*. 2011; Tang *et al*. 2012; Bribiesca-Contreras 2013; Smith *et al*. 2013; Stalhut *et al*. 2013). Since it is not much more computationally expensive to include more thresholds in the jMOTU analysis, I calculated the number of species using a wider range of thresholds, from a single base pair difference (approximately 0.15%) to a 50 base pair threshold (7.5% of the average sequence length). For the analysis of ITS2, I used the same range of thresholds (1-50 base pairs) as in the COI dataset, which is equivalent to 0.1 – 5.7 percent of the average sequence length. Since the ITS2 sequences contain numerous large indels, I used a range of low identity BLAST filters, from 80 – 97, to attempt to obtain the best alignment (Jones *et al*. 2010); however, the results were identical in all cases. I therefore ran the analyses on both the complete datasets (COI_{all} and $ITS2_{all}$) and on the common datasets (COI_{common} and $ITS2_{common}$), using the default settings of a minimum 60% overlap and low identity BLAST filter set to 97.

Comparison with morphology

        Comparative studies of species delimitation methods provide useful information into the behaviour of various algorithms. However, even if multiple methods converge on the same results, there is no guarantee that the clusters represent species. The morphological analysis of *Ophion* is ongoing, with many

species thus far lacking clear morphological characters. Nevertheless, some species are morphologically distinguishable. I therefore selected 11 morphospecies to use as test species to determine whether or at what threshold they were recovered by various analyses. Seven of these species are the Nearctic members of the *O. scutellaris* species group, and were resolved using a combination of morphology, wing morphometrics and classical morphometrics (Chapter 4). The other species include *O. nigrovarius* Provancher, *O. slossonae* Davis, and two undescribed, morphologically recognizable species, sp. A and sp. B. Both undescribed species were collected from a variety of localities; the distinguishing morphological characters are thus not the result of geographic variation. Sp. A is part of the *O. slossonae* species group (Chapter 2), and is most easily recognizable by its extremely well-developed propodeal carinae, with all carinae complete and distinctly higher than all other *Ophion* I have examined (Figure 3-2a). Sp. B is part of the *O. obscuratus* group (Chapter 2) and can be recognized by a faint brown area on the fenestra of the forewing, a pattern not observed in even closely related species (Figure 3-2b). *Ophion nigrovarius* is distinctly black-patterned and buccate-headed (i.e. with a very long malar space and temple, and ocelli that are widely separated from the eyes) (Figure 3-2c). *Ophion slossonae* is by far the largest Nearctic *Ophion* species I have examined, and also has unusually dark infuscate wings (Figure 3-2d); this last species was only successfully sequenced for COI.

To assess each species delimitation analysis, I used the COI_{all} and $ITS2_{all}$ datasets. I considered an analysis to have successfully recovered a species if a single cluster included all of the sequenced individuals and no additional sequences. For the GMYC analyses, I reported if a species was successfully recovered (+), split between more than one cluster (Sp) or lumped with other non-conspecific sequences (L). For the ABGD and jMOTU analyses, I listed the range of prior intraspecific divergences or thresholds, respectively, that successfully recovered the species, or recorded if the species was never recovered (-).

Species delimitation of Palearctic Ophion

Since the Nearctic fauna is almost entirely undescribed, few well-characterized species are available for assessing the performance of species delimitation methods. *Ophion* from the UK have been much more thoroughly studied, with a comprehensive morphological revision and workable key to species (Brock 1985). However, these species have not been assessed with molecular data, and several species present potential taxonomic difficulties (Brock 1985, G. Broad, pers. comm.). I have therefore analyzed the species limits of 15 out of the 16 known British species, based on COI sequence data. I restricted the analysis to COI as I had limited success sequencing these specimens for ITS2. A total of 77 COI sequences were obtained from material determined by G. Broad (Natural History Museum, London) or M. Shaw (*O. forticornis*, National Museums Scotland, Edinburgh). I have examined 46 of these specimens, while for the remaining specimens, I was sent legs for sequencing, but have not examined the specimens. As well, one sequence of *O. obscuratus* was included from Genbank (FN662468). Based on the results of the morphological comparisons described above, I selected a subset of analyses that were most successful at distinguishing Nearctic species. I assessed each Palearctic species using GMYC (coalescent prior, single threshold method), ABGD (relative gap width: 0.75, prior intraspecific divergence 0.0046), and jMOTU (threshold: 7, 14, and 20 base pairs, or approximately 1%, 2%, and 3% sequence divergence). While the 3% threshold had limited success at delimiting the Nearctic test species, I included it because this threshold is commonly used in the barcoding literature (e.g. Hebert 2003a; Tang *et al.* 2012).

Results

Barcode accumulation curves

The three Nearctic barcode accumulation curves (all Nearctic specimens, Canadian only, and Alberta only) showed a similar shape, though as expected, diversity increased with geographic scale of sampling (Figure 3-3). No dataset reached an asymptote, although the Alberta sequences may be approaching one.

The sequences from the UK are still in the steep initial part of the curve; it is therefore not possible to compare the actual diversity with that of the other three datasets. The Nearctic dataset had higher diversity than the Canadian dataset, but since most sequences are included in both curves, with very limited sampling from the United States, this is a biased examination of Nearctic diversity. Every specimen sequenced from the southern United States (ten specimens from Arizona, Florida, Georgia, Kentucky, and New Mexico) almost certainly represents an additional species, based on both genetic divergence and morphology.

CBC analysis

There was a maximum of 5 compensatory base changes (CBC's) between any two specimens. There were no CBC's within any species group (as defined in Chapter 2), except for two specimens from the *obscuratus* group that differed from the rest of the group by one (*O. obscuratus* from the U.K., DNA6917) or two (undescribed species from Arizona, DNA5569) CBC's. The NJ tree derived from the CBC matrix supported most species groups defined in Chapter 2, although *O. nigrovarius* was found within the *luteus* group, and three specimens from the *obscuratus* group (including the above two specimens) were not recovered with the rest of the group (Figure 3-4). Within species groups, however, CBC's were generally uninformative for distinguishing species.

GMYC

The results of the GMYC analyses are found in Table 3-1. Three of the single threshold analyses of the Yule prior trees (ITS2$_{all}$, ITS2$_{common}$, and COI$_{common}$) failed to significantly reject the null hypothesis that the sequences cannot be differentiated from a single cluster. Nonetheless, the estimated numbers of clusters and species (= clusters plus singletons, or "entities") from these analyses were within the range of all other analyses. Across all analyses, the proportion of singletons ranged from 29% to 57% of the total number of GMYC species, with an average of 40%. Excluding the analyses with non-significant

results, the confidence interval differences in the number of species ranged from 3 to 60, with an average of 30.5.

In general, the number of clusters was fairly robust with respect to both tree prior (Yule (Y) or coalescent (C)) and GMYC method (single (S) or multiple (M)). For the COI_{all} data, the number of clusters varied from 72 to 81, while in the smaller $ITS2_{all}$ dataset three of the four analyses resulted in 52 or 53 clusters. The Y-M analysis of the $ITS2_{all}$ data, however, only recovered 35 clusters. When only common taxa were included, the number of clusters was also quite robust between the two genes, with all analyses recovering 45-50 clusters.

The total number of species was more variable. For the COI_{all} dataset, the number of species was fairly similar between three of the analyses (114 - 124), however the C-M analysis recovered 150 species. For the $ITS2_{all}$ analyses the number of species varied from 51 (Y-M) to 95 (C-M). For the common datasets, there was a minimum of 69 species (ITS2 Y-S) and a maximum of 107 (COI C-M). All of the COI analyses recovered more species than did the corresponding ITS2 analyses. Within COI_{common}, the Y-M and C-M analyses recovered far more species (103 and 107, respectively) than did the corresponding single threshold analyses (86 and 80).

ABGD

For the COI datasets, ABGD delimited the same number of species regardless of which distance matrix was used as the input data (K2P or uncorrected p-distance). In the ITS2 analyses, a few intermediate partitions had a maximum of three additional species with the uncorrected distance matrix, compared with the K2P distance matrix. Since the results were so similar, I am only reporting the results of the uncorrected analysis.

The relative gap width had a strong influence on the intermediate partitions of both the COI and ITS2 analyses (Figure 3-5). For example, at a prior intraspecific divergence of 0.0017 (COI) or 0.0013 (ITS2), the more sensitive analysis (gap width = 0.75) recovered over twice as many species as the default gap width (= 1.5).

The number of species recovered by each COI analysis dropped precipitously between the first and second partition (COI priors = 0.0010 and 0.0017, respectively; Figure 3-5a). Over the remaining partitions, the pattern was analysis- and dataset-dependent, though by a prior of 0.036 all analyses were recovering a single cluster. The COI_{all} analysis using the default gap width had a clear plateau in the number of species recovered, ranging from 66 to 82. However the other analyses had a less clear pattern. The more sensitive analyses (gap width = 0.75) had a range of 52 – 168 species (COI_{all}) or 23 – 120 species (COI_{common}) over the intermediate priors, with the second and third partition recovering the same number of species. The COI_{common} analysis with the default gap width (1.5) had only a single intermediate partition, with 48 species.

The $ITS2_{all}$ and $ITS2_{common}$ ABGD analyses gave identical results, except that 0 – 3 fewer species were delimited in each partition for $ITS2_{common}$. For clarity, I have therefore only shown the $ITS2_{all}$ data in Figure 3-5b. Using any prior intraspecific divergences at or below 0.001 resulted in the same number of species (COI_{all}: 120; COI_{common}: 117). With priors above 0.001, the number of species dropped rapidly. With a prior of 0.0013, 27 and 59 species were recovered by the two $ITS2_{all}$ analyses (gap width 1.5 and 0.75, respectively) and with a prior of 0.0078 or greater, fewer than 20 species were recovered by all analyses.

jMOTU

The number of species delimited for both COI and ITS2 initially dropped steeply as the threshold increased, eventually flattening out (Figure 3-6). The curve was more gradual for COI, not reaching a clear plateau until over 5 percent sequence divergence, whereas for ITS2, the curve flattened out by approximately 1 percent divergence. A total of 128, 90, and 47 species were delimited by the analysis of COI_{all} at thresholds of approximately 1, 2, and 3 percent, respectively (7, 13, and 20 base pairs). At these thresholds, the COI_{common} dataset delimited 74, 43, and 20 species. Comparing this to the $ITS2_{common}$ dataset, the most similar numbers of species were found at thresholds of 0.3, 0.8, and 2.6 – 3 percent (3, 7, and 23 – 26 base pairs) .

Test species

The GMYC analysis of COI was generally more successful at correctly delimiting species than was that of ITS2 (Table 3-2). Within the COI analysis, the coalescent or Yule tree priors were equally successful at recovering the test species. The single threshold method was most successful, correctly delimiting all species except *O. brevipunctatus* and *O. dombroskii*, which were recovered as a single cluster. The multiple threshold method also lumped these two species, and in addition failed to recover *O. keala* and Sp. A. The C-S, C-M, and Y-S analyses of the ITS2 data each delimited 5 of the 10 species but which species were successfully delimited varied between the analyses. The Y-M analysis of ITS2 was the least successful GMYC analysis, recovering only two species. *O. nigrovarius* was the only species to be recovered by all GMYC analyses and both markers.

Sp. B was never recovered by ABGD, being either split or lumped, depending on the parameters used (Table 3-2). All other species were recovered by at least one ABGD analysis. Again, the analyses of COI were generally more successful than the ITS2 analyses, with most species being successfully delimited over a range of partitions. The more sensitive analysis of COI (gap width = 0.75) was most successful, with all species except for Sp. B being correctly delimited in at least one, and usually several, partitions. Within this analysis, the prior intraspecific divergence of 0.0046 was the only partition that correctly delimited all species (excluding Sp. B). ABGD of the ITS2 dataset recovered 7 of the 10 species in all partitions with prior intraspecific divergences ≤ 0.001); three of these species were also recovered in additional partitions. The remaining three species were never successfully delimited.

All of the test species were successfully delimited by jMOTU with the exception of Sp. B in the analysis of COI and *O. clave* in the analysis of ITS2 (Table 3-2). In both cases, these species were split at low thresholds, and lumped with other sequences at higher thresholds, but never delimited successfully. In the COI analysis, all remaining species were delimited at a threshold of 1% sequence

divergence, and all except *O. brevipunctatus* and *O. dombroskii* at 2% divergence. Only three species (*O. nigrovarius*, *O. slossonae*, and *O. keala*) were delimited at 3% divergence or higher. For the ITS2 analysis, most species were delimited at very low divergences, with only *O. nigrovarius* and Sp. A being successfully delimited at thresholds of more than 1% divergence (9 base pairs). At thresholds of greater than a single base pair (0.1%), *O. idoneus* and *O. keala* were lumped with each other, and *O. dombroskii* was lumped with a single sequence from *O. clave*.

Species delimitation of Palearctic Ophion

ABGD and GMYC were broadly congruent in their delimitation of Palearctic species, with only slight differences in the number of species within a few clades (Table 3-3). In comparison, while the jMOTU analyses often split species into a similar number of groups, in several instances it lumped these putative species with other sequences. In some cases it combined highly divergent taxa. For example, at thresholds of 2 and 3 percent divergence, it combined *O. pteridis* and *O. luteus*, along with several Nearctic species, into one large group, even though they differ by approximately 6% sequence divergence.

Of the 15 Palearctic *Ophion* species that were analyzed, three (*O. forticornis*, *O. longigena*, *O. ocellaris*) were delimited by all analyses, although two of these species (*O. forticornis* and *O. longigena*) were represented by singletons. *O. ventricosus* was recovered as a single species by all except the 1% jMOTU analysis. *O. perkinsi* and *O. pteridis* were never split into multiple species; however, according to the 2% (*O. pteridis*) and 3% (both species) jMOTU analyses, these species were lumped with other sequences. All other species were split to various extents by the different analyses. *Ophion brevicornis*, *O. crassicornis, O. luteus, O. minutus*, and *O. scutellaris* were recovered as monophyletic by all Bayesian analyses performed with BEAST. The splitting of these species is therefore due to divergence within these monophyletic groups. In comparison, *O. obscuratus* and *O. parvulus* were each split into at least 3 species by all analyses, and according to the phylogenetic analyses, these three taxa are

not monophyletic. In particular, while two species of *O. parvulus* were within the *O. parvulus* species group, one specimen identified as *O. parvulus* was recovered within the *O. obscuratus* species group (Chapter 2), as part of a clade with sequences identified as *O. costatus* and *O. mocsaryi*. All *O. obscuratus* were found within the *O. obscuratus* group; however, while most sequences were within the clade containing most British species (Chapter 2), two sequences were sister to Nearctic taxa (or conspecific with Nearctic taxa, according to jMOTU).

Discussion

Sampling

All quantitative species delimitation methods can be misled if species are not adequately sampled, as they rarely are in diverse, understudied taxa (Lohse 2009; Papadopoulou *et al*. 2009; Lim *et al*. 2010). In particular, it can lead to both underestimation of species numbers (since some species are not sampled) or overestimation (since some of the diversity within species may not be sampled, resulting in apparent genetic structuring) (Papadopoulou *et al*. 2009). Two separate measures indicate that the genetic diversity of *Ophion* is underrepresented in the current study.

First, none of the barcode accumulation curves reached an asymptote. This study is almost entirely restricted to specimens from Canada and the northern United States. Only ten specimens were sequenced from more southern regions of North America (Arizona, Florida, Georgia, Kentucky, and New Mexico) and almost every individual represents an additional species (based on sequence divergence, the species delimitation results from this study, and morphology). While the southern Nearctic region is almost entirely unexamined, the more extensively studied Canadian fauna is also insufficiently sampled. The most densely sampled region is the province of Alberta, which provided 52% of the sequences for this study. The curve representing these sequences appears to be approaching an asymptote, but even here, more sampling is needed to fully assess the genetic diversity of these species. Further sampling is therefore a high priority in order to increase confidence in the results of these delimitation methods.

Secondly, a high proportion of singleton species were recovered in all analyses, a typical result for diverse, understudied taxa (Monagahan *et al*. 2009; Ceccarelli *et al*. 2012; Lim *et al*. 2010). The robustness of species delimitation algorithms with regard to undersampled species is an important consideration as singleton species are abundant in taxonomic and biodiversity studies, and increasing sampling will not necessarily resolve this (Lim *et al*. 2010). The GMYC method has been found to be accurate even with a high proportion of singleton species (Monaghan *et al*. 2009; Ceccarelli *et al*. 2012). In contrast, for accurate results with ABGD, it is recommended to include 3-5 sequences per species (Puillandre *et al*. 2012a). However without *a priori* knowledge of the group being studied, this may be difficult to achieve.

Increased sampling does not always lead to more accurate species delimitation, particularly in threshold-based analyses. An apparently distinct barcoding gap often disappears with increased sampling across the range of genetic variation of a given taxon (Moritz and Cicero 2004; Meyer and Pauley 2005; Wiemers *et al*. 2007). In this study, at the commonly used 2% threshold, the single-linkage clustering algorithm used by jMOTU clustered several distantly-related taxa. For example, *O. luteus*, *O. pteridis*, and a number of Nearctic species, were grouped into a single MOTU, as there were apparently enough intermediate sequences to act as a bridge between them. However; a trial analysis with the dataset restricted to *O. luteus* and *O. pteridis* retained them as separate MOTUs at thresholds of up to 6% divergence.

Comparison of ITS2 and COI as markers for species delimitation of Ophion

ITS2 in *Ophion* has a very different pattern of divergence compared to COI, with high divergence between species groups, but very low intra- and interspecific divergence among closely related species (Chapter 2). Nevertheless, the branching pattern was largely congruent with COI, and at least according to GMYC, a similar number of species were delimited.

Due to its length, the presence of microsatellite regions, and the high number of indels, ITS2 was more logistically challenging to sequence and align

for *Ophion*. As well, COI successfully identified more test species in GMYC, and the choice of priors or thresholds for ABGD and jMOTU is more straightforward, due to the large number of studies focusing on this gene. This contrasts with the results of Wagener *et al*. (2006) who found ITS2 was more effective than COI at distinguishing species of *Diadegma* (Ichneumonidae, Campopleginae). Despite the advantages of COI in this study, relying on this single gene may provide misleading results due to incomplete lineage sorting, introgression, bacterial endosymbionts, or nuclear mitochondrial pseudogenes (numts) (Funk and Omland 2003; Schmidt and Sperling 2008; Song *et al*. 2008; Raychoudhury *et al*. 2010). In at least one species in this study, a numt (Gellissen *et al*. 1983, Lopez *et al*. 1994) was apparently amplified, as there is a homologous 2 base pair deletion in each sequence which would result in a shift in the codon reading frame of the COI gene. However since the GMYC analyses of ITS2 also recovered the same species, it appears the numt is nevertheless phylogenetically informative. It is therefore critical to include an independent molecular marker to verify the results obtained with COI. ITS2 is an important tool for delimiting species in this genus, though the investigation of other relatively rapidly evolving nuclear genes (e.g. CAD) (Wild and Maddison 2008; Sharanowski *et al*. 2011) may provide additional and potentially more tractable species-level genetic markers.

Summary of species delimitation methods

   CBC analysis

        The potential of a single compensatory base change (CBC) to unambiguously identify species as separate is a promising "magic bullet" for species delimitation (Müller *et al*. 2007; Coleman 2009). Müller *et al*. (2007) found that in 97% of the examined taxon pairs, a single CBC in a conserved region of ITS2 indicated enough evolutionary time had passed that the two species were no longer capable of interbreeding. They do provide the caveat, however, that a lack of CBC's does not guarantee that two individuals are conspecific. As well, their study was focused on plants and fungi, but did not assess the utility of CBC's in animal taxa. In an analysis of blue butterflies

(Lycaenidae), Wiemers *et al*. (2009) found CBC's were too rare to distinguish species. The lack of CBC's between closely related *Ophion* species provides further evidence that, at least in insects, CBC's are apparently too scarce to be of any practical use in distinguishing species. They are, however, potentially useful in defining higher taxonomic groups, as can be seen by their support of most species groups defined in Chapter 2.

GMYC

The multiple threshold method of GMYC was developed to improve accuracy over the single threshold method by taking into account the different branching patterns and rates of evolution across the tree (Monaghan *et al*. 2009; Papadopoulou *et al*. 2009). However, Esselstyn *et al*. (2012) and Fujisawa *et al*. (2013) have recently determined it to be consistently less accurate than the single threshold method. This study confirms this finding with fewer test species being correctly delimited with this method in both the analyses of COI (both tree priors) and ITS2 (Yule prior). While the multiple method did not invariably recover higher numbers of species than the respective single analysis, all of the highest estimates were obtained using this method, supporting the conclusion of Esselstyn *et al*. (2012) that the multiple method overestimates the number of species. In most cases, it was the number of singleton species driving this pattern, with the number of clusters being fairly consistent between methods.

Yule priors are generally considered more appropriate for analyses of speciation, whereas coalescent priors are more applicable to intraspecific population level data (Drummond *et al*. 2006). In a recent GMYC analysis of a diverse genus of Braconidae, Ceccarelli *et al*. (2012) found the accuracy of each prior depended on which gene was being analyzed, with the coalescent prior being more consistent with morphology for COI data. In the current study, the estimated number of species and the accuracy of the analyses, were largely unaffected by which tree prior was used to construct the phylogenetic tree, particularly in the analysis of COI. The two priors were not identical, however. In the single threshold analyses, the Yule prior estimated 7 more species than the

coalescent prior for COI, whereas for the ITS2 dataset, the opposite pattern was seen, with the Yule prior estimating 10 fewer species than the coalescent prior.

ABGD

The results of the ABGD analyses were highly dependent on the gap width selected. In most recent studies that have employed this method, the default gap width of 1.5 is either explicitly used (Tang *et al*. 2012), or presumably used (Crawford *et al*. 2012; Puckridge *et al*. 2013). However Puillandre *et al*. (2012b) increased this setting to 10 in order to decrease the sensitivity of their analysis to small gaps. In this study, reducing the gap width doubled the number of delimited species in several partitions for both COI and ITS2. As well, there was no clear plateau in the estimated number of species with the more sensitive gap width, making the choice of prior a subjective but essential task if this analytical method is to be used to assess species diversity. While both gap widths recovered most test species, for the COI analysis more species were recovered over a wider range of priors using the more sensitive gap width. The principal reason for the difference in the number of estimated species between gap widths lies within the *O. obscuratus* species group (Chapter 2). For example, in the analysis of COI using an intraspecific prior of 0.0017, the default analysis estimates 15 species within the *O. obscuratus* group, whereas the more sensitive analysis recovers 81 species in this group. The one test species within this species group (Sp. B) was not accurately delimited in either case, being split with the more sensitive analysis and lumped with the default analysis. The sensitivity of the analysis to the gap width may only be an issue within species complexes or recent radiations, which can confound any species delimitation method (Monaghan *et al*. 2006; Reid and Carstens 2012). Nonetheless, consideration of the impact of gap width should be considered when using this method, since the choice of this parameter is largely arbitrary.

While the gap width also affected the intermediate partitions of the ITS2 analysis this had little impact on the overall results, as only three test species were recovered with any intraspecific divergence above 0.001. This supports the results

seen in the *O. scutellaris* group (Chapter 4) where all species had a maximum of 0.1% intraspecific divergence.

jMOTU

Based on thresholds of 1 – 3%, there was almost a three-fold difference in the number of species estimated by jMOTU, from 47 species at 3% divergence to 128 species at 1%. While 2% is the most commonly used threshold in DNA barcoding studies (e.g. Strutzenberger *et al*. 2011; Bribiesca-Contreras *et al*. 2013; Fernandez-Flores *et al*. 2013; Smith *et al*. 2013), other thresholds are also used, such as 1.6% (Smith *et al*. 2009), 2.3% (Young *et al*. 2012), or 3% (Hendrich *et al*. 2010; Tang *et al*. 2012). While the choice of threshold is often based on empirical studies (e.g. Hebert *et al*. 2003a), there is no reason to believe the same thresholds will apply in different taxa; even within a taxon, there can be highly variable patterns of intra- and interspecific divergence (e.g. Bergmann *et al*. 2013).

Despite this range, with the standard 2% threshold the estimated number of species (90) was within the range of GMYC and the intermediate ABGD analyses, and most test species were recovered. However, due to the greedy algorithm used in the single-linkage clustering employed by this method (Jones *et al*. 2011), the delimitation of species boundaries differed considerably from the other two methods, particularly within the *O. luteus* – *O. pteridis* species groups and within the *O. obscuratus* species group. This had little effect on the test species since most were not found within these groups, but it did affect the delimitation of Palearctic species.

Comparison of methods

ABGD is by far the least computationally demanding of the three methods used, providing results within seconds. jMOTU takes longer, with the analyses from this study requiring approximately 1.5 to 5 hours on a laptop computer. While the GMYC analyses were relatively rapid (particularly using the single threshold method), the construction of the ultrametric trees in BEAST was very

time-consuming, with analyses requiring between approximately 17 hours (COI, coalescent prior) and 55 hours (ITS2, Yule prior) of runtime per replicate on the CIPRES computer cluster.

Despite its computational demands, GMYC offers some distinct advantages over the other two methods of species delimitation. The largest advantage is that this method incorporates evolutionary theory and is therefore much less reliant on the application of arbitrary thresholds. It does, however, rely on the priors and parameters used to construct the ultrametric tree (Ceccarelli *et al*. 2012). Another advantage of this method is that it is more tractable with genetic markers such as ITS2 that have less well-characterized intra- and interspecific patterns of diversity. Using an appropriate model of evolution means this method can easily be applied to any genetic marker. In comparison, choosing an appropriate threshold or range of thresholds for a marker other than COI is particularly arbitrary, since no "standard" divergence has been proposed for species delimitation. Both jMOTU and ABGD provide the flexibility of examining a range of thresholds or priors, respectively, which can be informative in understanding the effects of the algorithms and the diversity within the group. However, the majority of barcoding studies use a single threshold, most often 2%, to estimate species diversity (e.g. Strutzenberger 2010; Bribiesca-Contreras 2013; Fernandez-Flores 2013; Smith *et al*. 2013; but see Laetsch 2012). A range of priors is more often examined with ABGD (Paz and Crawford 2012; Puckridge *et al*. 2013); however, the impact of the relative gap width is rarely considered, even though it can have a significant impact on the results obtained. A further disadvantage of threshold analysis as implemented in jMOTU is the grouping of distantly related species among taxa that lack a clear barcoding gap. Despite the potential advantages of GMYC, however, it has been suggested that this method overestimates species diversity compared to other methods (Paz and Crawford 2012). All methods can thus be informative as a source of species hypotheses; however, caution should be used in relying solely on any one of them for estimates of species diversity.

The species selected to test the accuracy of each species delimitation method varied in the number of sequences per species ($n = 1 – 13$), the phylogenetic position within *Ophion*, and the amount of interspecific divergence between adjacent species. Some species (e.g. *O. nigrovarius*, *O. slossonae*, and Sp. A) were robust to the delimitation methodology used, being delimited in almost all GMYC analyses (Sp. A in the multiple threshold analyses of COI is the exception), and over a wide range of parameters and thresholds by ABGD and jMOTU. Other species (e.g. Sp. B, *O. brevipunctatus*, and *O. dombroskii*) were much more sensitive to the method used. Sp. B is found within the complex of species that makes up the *O. obscuratus* species group (Chapter 2). *O. brevipunctatus* and *O. dombroskii* are sister species, each represented by a single sequence and separated by only 1% sequence divergence, but highly morphologically distinct (Chapter 4). It is therefore not surprising that the analyses would be less successful at delimiting these species. However the varying success of the different analyses with these more challenging species demonstrates the importance of not relying on a single method of species delimitation, and of treating all proposed clusters as hypotheses, to be assessed with other data (e.g. Puillandre *et al*. 2012b).

Palearctic Ophion

The splitting of most described British species may be due to the failure of the delimitation methods at successfully circumscribing "good" species, or alternatively, may indicate the presence of cryptic or previously unrecognized species. In several instances, the putative species were not recovered as a monophyletic clade by phylogenetic analyses, indicating that multiple, previously unrecognized species are almost certainly present. However, in some groups, the putative species represent divergent sequences within a monophyletic group. For these cases, further evidence, such as morphology, is needed to determine if the groups represent genetic structuring within a single species, or are truly distinct species. For at least one such species (*Ophion scutellaris*), a preliminary morphological assessment indicates that two species are indeed present (Chapter

4). Finally, in some instances, a combination of the two scenarios is possible. For example *O. costatus* was split into 3-5 species by the various analyses. One of the species is almost certainly a misidentified *O. mocsaryi*, since this specimen was grouped with *O. mocsaryi* in all analyses, and these species are known to be challenging to distinguish (G. Broad, pers. comm.). A second species was grouped into a cluster with three other specimens, two identified as *O. mocsaryi* and one as *O. parvulus*. I have not examined any of these specimens, but based on the molecular data, they may represent a previously unrecognized species that has been incorrectly assigned to various described species, or alternatively, could be a hybrid between *O. mocsaryi* and *O. costatus*. Finally the remaining specimens were assigned to 1 – 3 species; further study is needed to determine whether this represents variation within *O. costatus* or additional cryptic species. The steep slope of the barcode accumulation curve for the Palearctic species emphasizes the preliminary nature of this study. Nevertheless, these analyses clearly show that even in the relatively well-studied British fauna, there is a strong need for more comprehensive molecular and morphological revisionary work.

So, how many species are there?

I was unable to include ITS2 sequences for several species of *Ophion* in this study, either because sequencing was unsuccessful, or because these species were only represented by COI sequences obtained through BOLD. I have therefore based the overall estimates of species numbers on the analyses of the COI_{all} dataset. These results should thus be treated as preliminary, since the patterns obtained from a single gene may not be representative of the genome as a whole (Dupuis *et al*. 2012). As well, all estimates are of the number of species in the dataset and do not represent estimates of the total number of species in the Palearctic or Nearctic regions.

There was a wide range of estimates depending on the analytical method and parameters used. Based on all plausible results of the quantitative species delimitation analyses (i.e. including all GMYC analyses; ABGD with gap widths of 1.5 and 0.75 and priors of 0.0017 – 0.0077 and 0.0017 – 0.0129 intraspecific

divergence, respectively; and jMOTU thresholds of 1-3% divergence), there are 47 – 168 species of *Ophion* represented in this study. Excluding the more extreme estimates (i.e. including only single threshold GMYC analyses; ABGD analyses with a prior of 0.0046; and jMOTU results with a threshold of 2%) resulted in estimates of 90 – 121 species (64 – 97 of which are from the Nearctic region). In comparison, a qualitative estimate of species diversity, based on an integrative assessment of the clustering patterns of neighbour-joining trees (COI and ITS2, with bootstrap values) and morphology, suggests the dataset consists of 75 – 99 species (55 – 76 Nearctic).

The wide range of estimates emphasizes the point that all methods of molecular species delimitation provide "primary species hypotheses" (Puillandre *et al*. 2012b), which need to be assessed using additional methods. As a first step, these methods can be a helpful tool to delimit hypothetical species; however, for comparative studies relying on species numbers (e.g. for conservation applications or for the analysis of patterns of biodiversity), it is vital that researchers also consider the parameters and limitations of the delimitation method(s) used to obtain the estimates.

Using a variety of analytical methods and parameters can aid in planning subsequent research to assess the hypothetical species obtained with these methods. For example, groups of sequences that are consistently delimited with a variety of quantitative methods are good candidate species to examine in more detail for confirmatory morphology, ecology or other sources of data that either support or refute the molecularly-defined species. In comparison, the species that are inconsistently delimited, while also requiring an integrative taxonomy approach, are good candidates for more fine-scaled analyses, such as quantitative morphometrics (Chapter 4; Lumley and Sperling 2010) or population genetics (Shaffer and Thomson 2007; Lumley and Sperling 2011).

Conclusion

Based on morphology, Gauld (1985) estimated a fauna of approximately 50 Nearctic species of *Ophion*. This study indicates that the true number of

Nearctic species is considerably higher and with more complete sampling will easily double that estimate. The 11 described species of *Ophion* therefore represent just a minute fraction of the total fauna. It is widely acknowledged that there are vast numbers of undescribed species among tropical, small-bodied taxa (e.g. Quicke *et al*. 2012). However, this study shows that even in a large-bodied, primarily temperate genus that is readily attracted to lights and easily collected in large numbers, there is a wealth of diversity waiting to be discovered.

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Table 3-1. Results of generalized mixed Yule coalescent (GMYC) analysis. CI = confidence interval for the number of species (clusters + singletons); L-Null = likelihood of the null model (= all sequences form a single cluster); L-GMYC = likelihood of GMYC model; LRT = p-value of likelihood ratio test; COI_{all} and $ITS2_{all}$ = all haplotypes included in analysis; COI_{common} and $ITS2_{common}$ = only haplotypes represented by both markers were included.

Dataset	Tree prior	Method	Clusters	Species	CI	L-Null	L-GMYC	LRT
COI_{all}	Coalescent	Single	72	114	100-137	3119.398	3135.499	<0.000
		Multiple	83	150	129-167	3119.398	3145.07	<0.000
	Yule	Single	81	121	117-134	3057.645	3064.79	0.003
		Multiple	79	124	88-125	3057.645	3073.966	0.000
$ITS2_{all}$	Coalescent	Single	53	85	85-104	2326.429	2334.213	0.001
		Multiple	52	95	93-96	2326.429	2339.508	0.000
	Yule	Single	53	75	62-81	2162.679	2166.034	0.0818*
		Multiple	35	51	47-104	2162.679	2173.709	0.001
COI_{common}	Coalescent	Single	45	80	67-93	1728.258	1739.615	<0.000
		Multiple	49	107	103-107	1728.258	1744.155	<0.000
	Yule	Single	50	86	2-106	1695.617	1698.06	0.180*
		Multiple	44	103	72-116	1695.617	1704.424	0.024
$ITS2_{common}$	Coalescent	Single	45	76	53-94	2135.929	2143.237	0.002
		Multiple	49	78	78-92	2135.929	2147.648	0.001
	Yule	Single	48	69	59-77	1990.495	1994.305	0.0545 *
		Multiple	48	73	39-99	1990.495	2001.224	0.0181

* not significant

Table 3-2. Summary of congruence between three automated species delimitation methods and eleven species/morphospecies. GMYC = generalized mixed Yule coalescent model: C = coalescent tree prior; Y = Yule tree prior; S = single threshold GMYC method; M = multiple threshold GMYC model; + = species successfully delimited; L = species lumped with other sequences; Sp = species split into > 1 groups. Table continues next page.

Species	COI (*n*)	ITS2 (*n*)	GMYC							
			COI				ITS2			
			C-S	C-M	Y-S	Y-M	C-S	C-M	Y-S	Y-M
O. clave	5	2	+	+	+	+	+	+	+	L
O. aureus	2	4	+	+	+	+	+	L	L	L
O. brevipunctatus	1	1	L	L	L	L	L	L	L	L
O. dombroskii	1	1	L	L	L	L	L	L	L	L
O. keala	7	7	+	Sp	+	Sp	L	Sp	L	L
O. importunus	1	1	+	+	+	+	+	+	+	L
O. idoneus	13	13	+	+	+	+	L	+	L	L
O. nigrovarius	5	2	+	+	+	+	+	+	+	+
O. slossonae	3	0	+	+	+	+	n/a	n/a	n/a	n/a/
sp. A	11	7	+	L	+	L	+	+	+	+
sp. B	9	9	+	+	+	+	Sp	Sp	+	L

Table 3-2 continued. ABGD = automatic barcode gap discovery, analyzed using two gap widths: 1.5 and 0.75; numbers represent the range of prior intraspecific percent divergence that successfully delimited each species. jMOTU = threshold analysis using the program jMOTU; numbers indicate the range of thresholds, as a percentage of the mean sequence length, that successfully delimited each species; for both analyses, - indicates the species was never delimited.

Species	ABGD (%)				jMOTU (%)	
	COI		ITS2		COI	ITS2
	1.5	0.75	1.5	0.75		
O. clave	0.2 – 0.8	0.2 – 1.3	≤ 0.01	≤ 0.01	0.3 – 2.9	-
O. aureus	-	0.5	≤ 0.01	≤ 0.01	1.1 – 2.0	0.1 – 0.3
O. brevipunctatus	0.1	0.1 – 0.5	-	-	≤ 1.1	≤ 0.2
O. dombroskii	0.1	0.1 – 0.5	-	-	≤ 1.1	≤ 0.1
O. keala	0.2 – 0.8	0.5 – 1.3	≤ 0.01	≤ 0.01	0.6 – 4.1	0.1
O. importunus	0.1 – 0.8	0.1 – 1.3	< 0.01 – 0.06	< 0.01 – 0.06	≤ 2.3	≤ 0.8
O. idoneus	0.2 – 0.8	0.2 – 1.3	≤ 0.01	≤ 0.01	0.3 – 2.3	0.1
O. nigrovarius	0.5 – 0.8	0.5 – 1.3	< 0.01 – 1.7	< 0.01 – 1.7	0.5 – 5.2	0.1 – 4.3
O. slossonae	0.2 – 0.8	0.2 – 1.3	n/a	n/a	0.5 – 4.5	n/a
sp. A	0.2 – 0.8	0.2 – 1.3	< 0.01 – 0.4	< 0.01 – 0.4	0.6 – 2.3	0.1 – 2.6
sp. B	-	-	-	-	-	0.5 – 0.8

Table 3-3. Number of putative species delimited for each morphologically identified species of *Ophion*, based on the COI barcode region. All specimens are from the United Kingdom, with the exception of a single specimen of *O. obscuratus* from Spain and one specimen of *O. forticornis* from France. Analyses that successfully delimited a species are identified with +. The GMYC analysis used the single threshold method on an ultrametric tree produced in BEAST, with a relaxed lognormal clock and coalescent tree prior. The ABGD analysis used a relative gap width of 0.75 and a prior intraspecific divergence of 0.0046. The jMOTU analysis used thresholds of 7, 13 and 20 base pairs, or approximately 1%, 2%, and 3% sequence divergence.

				Number of "species"		
					jMOTU	
Species	***n***	**GMYC**	**ABGD**	**1%**	**2%**	**3%**
brevicornis	3	2	+	3^b	3^b	2^b
costatus	13	3^a	4^a	5^c	5^c	$4^{b,c}$
crassicornis	4	2	2	2	2	+
forticornis	1	+	+	+	+	+
longigena	1	+	+	+	+	+
luteus	14	2	2	2	1^b	1^b
minutus	8	2	3	4	2	+
mocsaryi	9	3^a	4^a	5^c	4^c	$2^{b,c}$
obscuratus	7	3	3	5^b	5^b	3^b
ocellaris	3	+	+	+	+	+
parvulus	3	3^a	3^a	3	3	3^b
perkinsi	2	+	+	+	+	1^b
pteridis	3	+	+	+	1^b	1^b
scutellaris	5	2	2	2	2	+
ventricosus	4	+	+	2	+	+

a. 1 sequence identified as *O. parvulus*, 1 identified as *O. mocsaryi*, and 2 identified as *O. costatus* were recovered as a single species by ABGD and as a monophyletic group split into two species by GMYC.

b. At least one of the delimited species lumped with other, presumably non-conspecific sequences

c. One sequence identified as *O. costatus* was consistently found within one of the *O. mocsaryi* species – almost certainly a misidentification of this specimen.
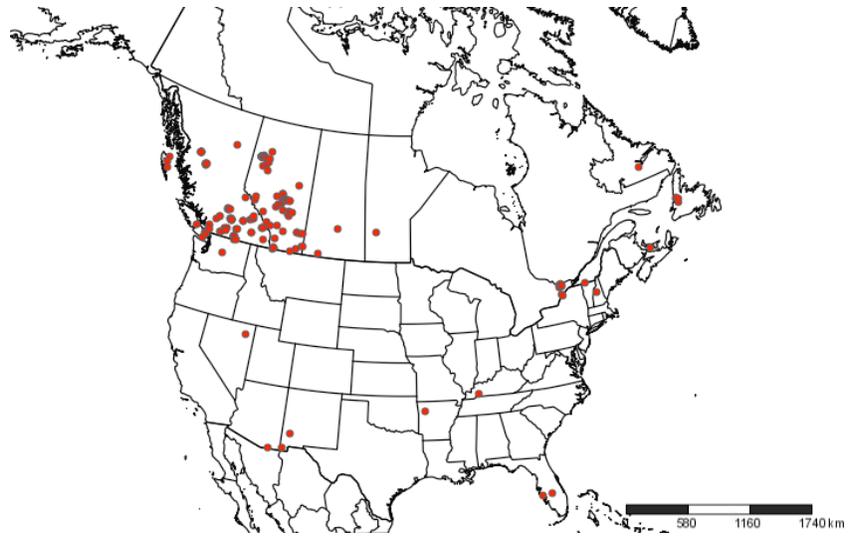
Figure 3-1. Collection localities for all Nearctic specimens sequenced in this study.
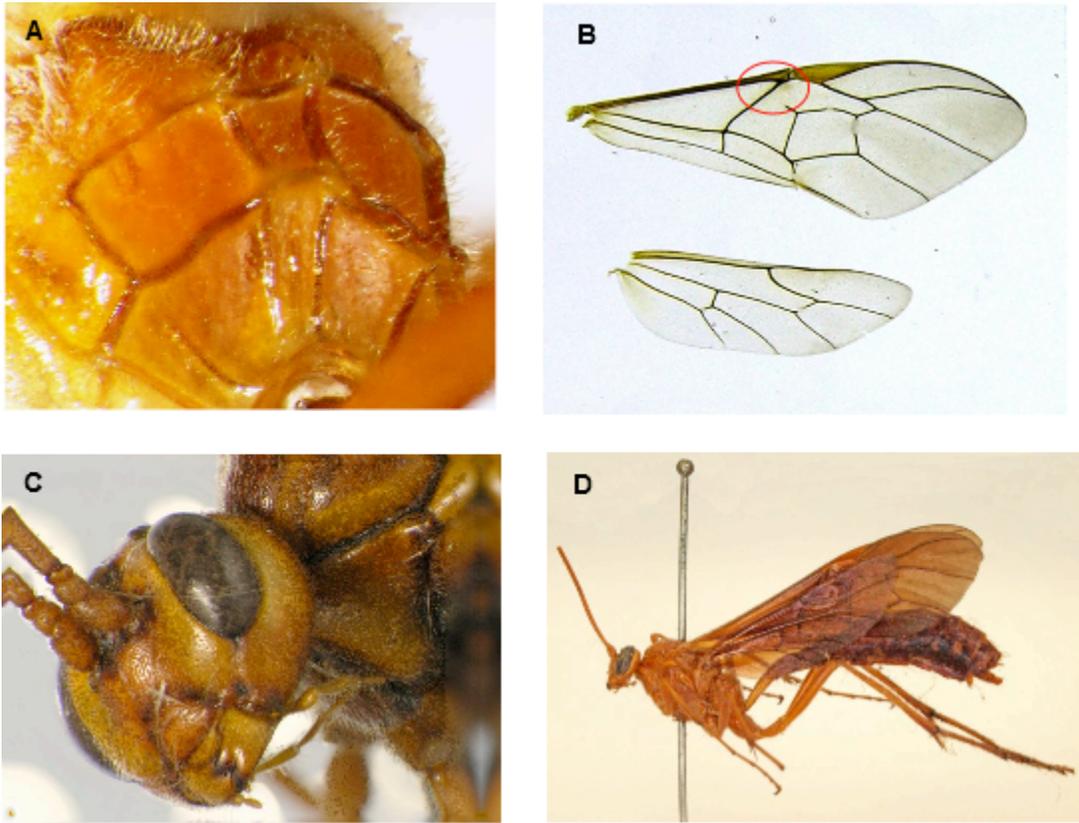
Figure 3-2. Distinguishing characters of four test species. A = Sp. A (propodeum); B = Sp. B (demonstrating brownish patch on forewing); C = *O. nigrovarius*; D = *O. slossonae*
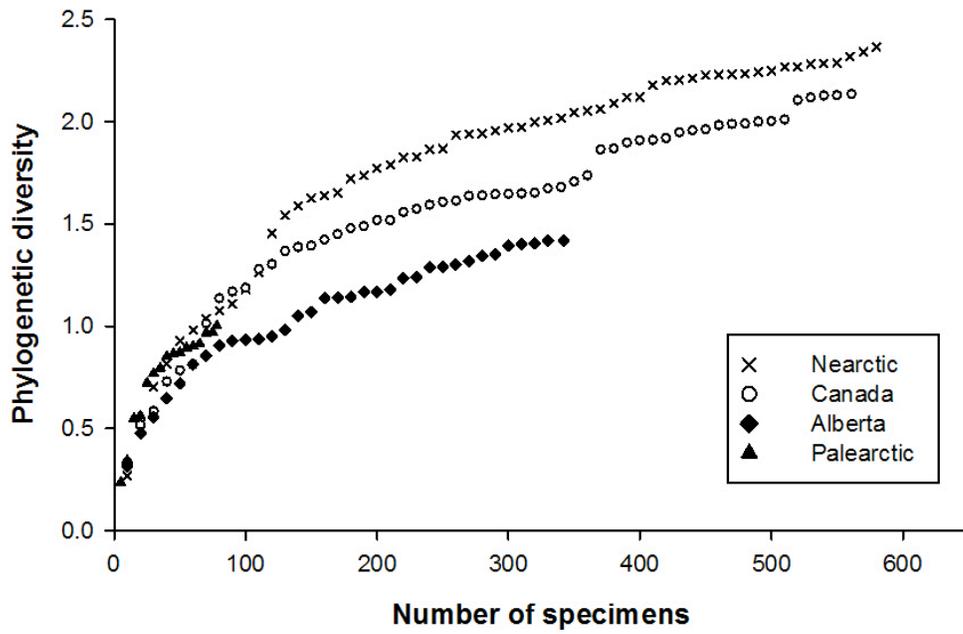
Figure 3-3. Barcode accumulation curves of all sequences from this study.

O. obscuratus

Group 1

O. ocellaris

O. luteus group

O. nigrovarius

O. pteridis group

O. slossonae group

O. parvulus group

O. flavidus

O. scutellaris group

1.0

O. minutus

10
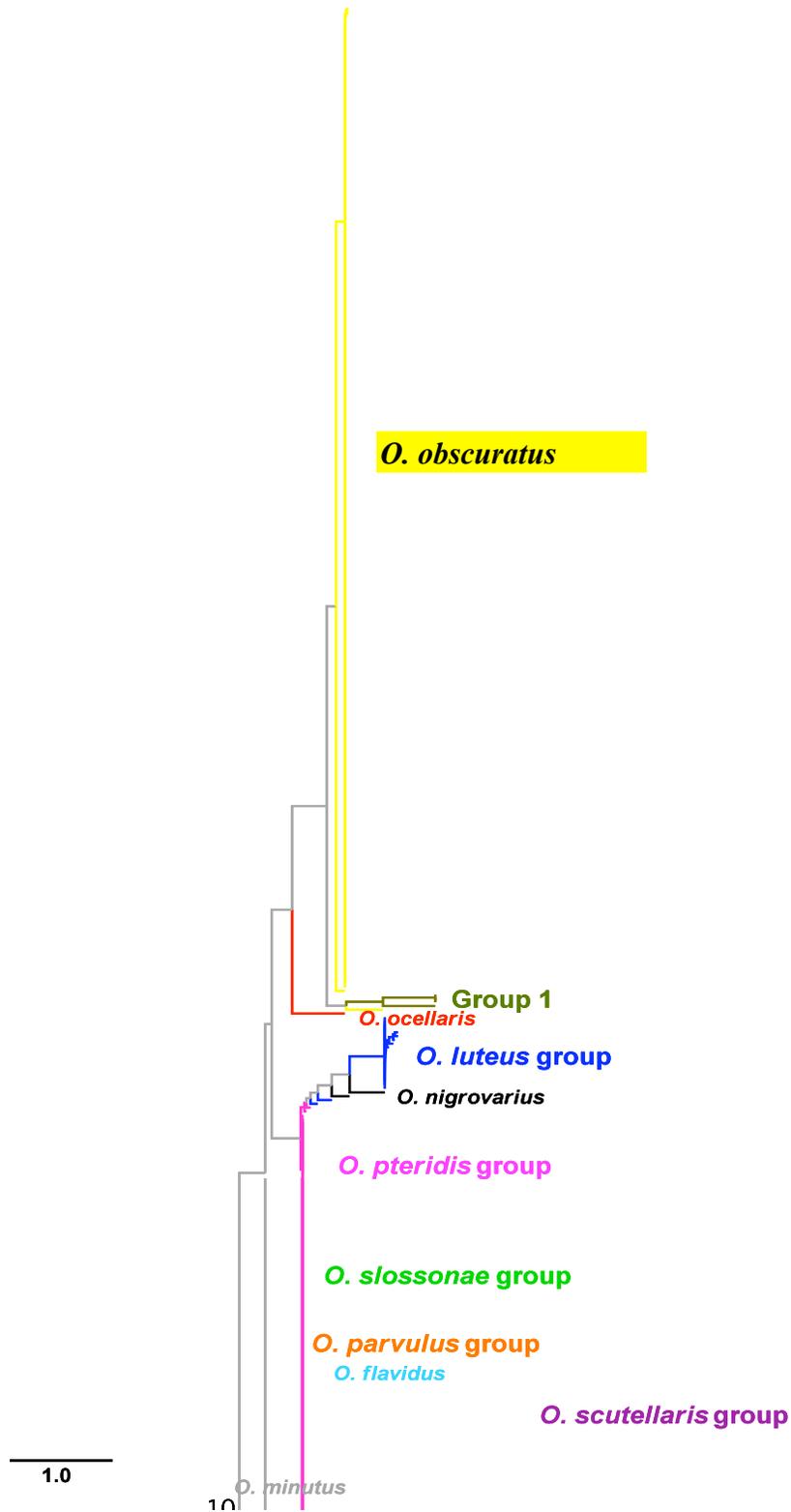
Figure 3-4. Neighbour-joining tree derived from matrix of compensatory base changes (CBC's) from ITS2 structural alignment. Coloured branches represent species groups.
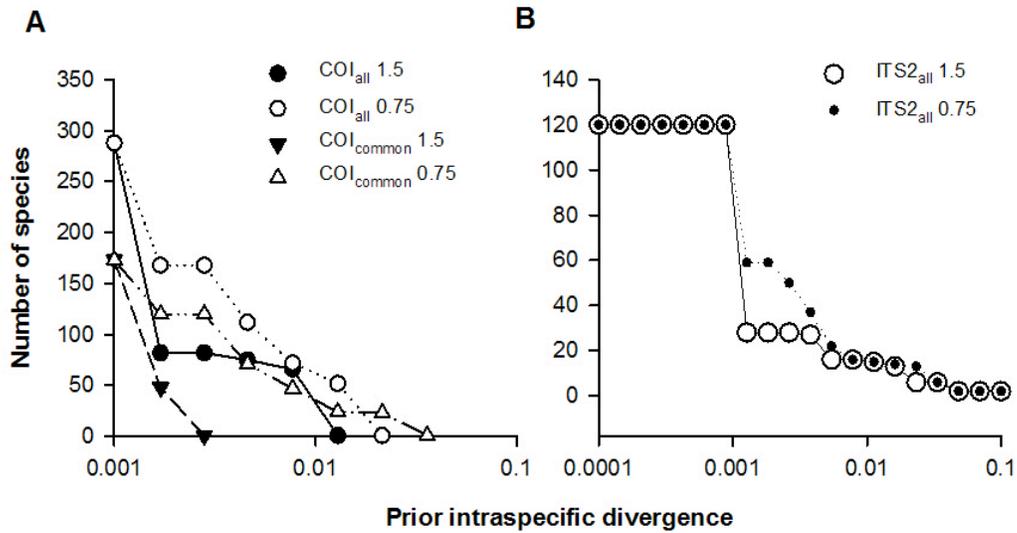
Figure 3-5. Results of ABGD analyses over a range of prior intraspecific divergences with two different gap widths (1.5 and 0.75). **A)** COI: all = all sequences; common = only including sequences in common with ITS2. **B)** ITS2: all sequences (ITS2$_{common}$ not shown, since results are equivalent).
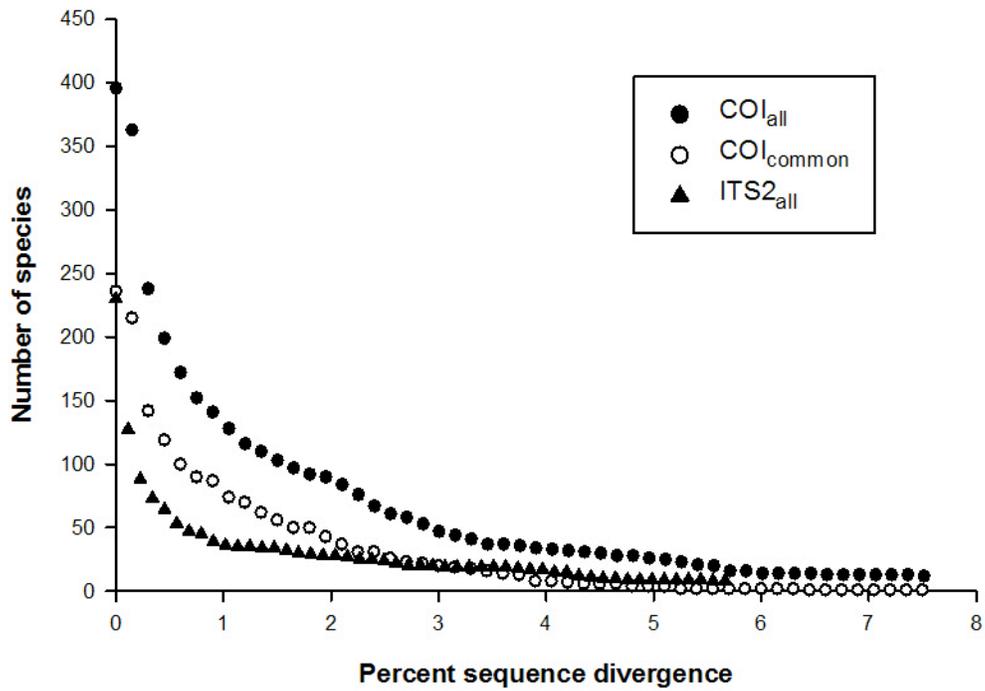
Figure 3-6. Number of species delimited by jMOTU at thresholds of 0 – 50 base pairs (equivalent to 7.5% (COI) or 5.7% (ITS2) sequence divergence); ITS2$_{common}$ not shown as results are equivalent to ITS2$_{all}$

Chapter 4

Species delimitation using morphology, morphometrics and molecules: definition of the *Ophion scutellaris* Thomson species group, with descriptions of six new species (Hymenoptera: Ichneumonidae)

Introduction

    Ophion Fabricius is a genus of large nocturnal Ichneumonidae in the subfamily Ophioninae. Most species parasitize medium to large-sized Lepidoptera larvae, especially Noctuoidea (Townes 1971; Brock 1982). Whereas the Ophioninae are generally more diverse in the tropics, *Ophion* is most diverse in temperate regions (Townes 1971; Gauld 1985). Gauld (1985) estimated that the Nearctic fauna consists of approximately 50 species, and molecular work suggests the number is much higher (Chapter 3). However only eleven Nearctic species of *Ophion* are currently described (Yu 2011), the most recent of which were described in 1912 (Hooker 1912; Morley 1912).

    While several species groups of *Ophion* have been proposed (summarized by Gauld 1985), almost all Nearctic species were included within the "*O. luteus* species group". Gauld (1985) acknowledged that this species group was paraphyletic and defined by plesiomorphies, but not enough was known about the Nearctic species to further subdivide it into monophyletic groupings. In Chapter 2, I provide a preliminary molecular phylogeny of *Ophion* and divide the "*O. luteus* group" *sensu* Gauld (1985) into several additional species groups. One of these groups, the *O. scutellaris* Thomson species group, is the focus of this study.

    Ophion species are difficult to distinguish morphologically and have a great deal of intraspecific variability (Townes 1971; Brock 1982). Molecular taxonomy has been proposed as a method to accurately delimit and identify species that lack useful morphological characters (Caterino *et al*. 2000; Tautz *et al*. 2003; Blaxter 2004). In particular, DNA barcoding, or the sequencing of a standardized 658 bp segment of the mitochondrial cytochrome c oxidase I gene

(COI), has been promoted as a standardized method for species discovery and delimitation (Hebert *et al*. 2003). Among the advantages of COI are its rapid evolution (hence potentially informative at the species level) and presence in multiple copies (hence easily amplified). However, many studies have argued that COI alone is not sufficient to accurately delimit species due to factors such as hybridization, retained ancestral polymorphisms and high intraspecific variability (Funk and Omland 2003; Cognato 2006; Meier *et al*. 2006; Schmidt and Sperling 2008; Dupuis *et al*. 2012).

To avoid relying on a single mitochondrial gene, nuclear genes can provide an additional data source to evaluate species boundaries (Simon *et al*. 2006). The internal transcribed spacer of nuclear ribosomal DNA (ITS2) is another rapidly evolving gene that has been successfully used for species delimitation in insects (e.g. Alvarez and Hoy 2002; Hung *et al*. 2004; Wagener *et al*. 2006). It too, however, can potentially give misleading results due to high intraspecific or even intra-individual variation (Rich *et al*. 1997; Harris and Crandall 2000; Li and Wilkerson 2007). It can also be difficult to align accurately due to the presence of numerous insertion-deletion events. A second nuclear gene, the nuclear ribosomal 28S gene is a highly conserved gene that has often been used for higher level insect phylogeny (Caterino *et al*. 2000), though it has also proved useful in distinguishing species (e.g. Monaghan *et al*. 2005; Derycke *et al*. 2008; Raupach *et al*. 2010). In particular, the D2-D3 expansion region of 28S rDNA is among the most commonly used molecular markers for Hymenoptera phylogenies (e.g. Mardulyn and Whitfield 1999; Dowton and Austin 2001; Laurenne et al. 2006; Quicke et al. 2009; Klopfstein et al. 2011).

Quantitative morphometric analyses have been shown to be at least as accurate as molecular analyses in the delimitation of morphologically challenging species, and possibly more so (Lumley and Sperling 2010). Classical (or traditional) morphometrics involves direct measurements of various characters which are then analyzed using multivariate methods (Marcus 1990; Mutanen and Pretorius 2007). This technique is often successful at separating similar species, even when there is overlap between the individual characters (e.g. Lumley and

Sperling 2010; Buck *et al*. 2012). Alternatively, geometric morphometrics analyzes overall changes in shape by using landmarks of homologous structures (Rohlf and Marcus 1993). Insect wings are an ideal subject for geometric morphometric studies, as they are two-dimensional, easily imaged and the venation provides many points that are clearly homologous and straightforward to landmark accurately. Geometric morphometric analysis of wing venation has been used successfully to discriminate species of several complexes of closely related insect species (e.g. Villemant *et al*. 2007; Tofilski 2008; Francuski *et al*. 2009; Milankov *et al*. 2009), though to our knowledge has not yet been used for Ichneumonidae. Although not using geographic morphometrics, two studies show promise for separating closely related Ichneumonidae species based on overall wing venation (Yu *et al*. 1992; Weeks *et al*. 1997).

The use of multiple lines of evidence to circumscribe species is often referred to as "integrative taxonomy" (Dayrat 2005; Will *et al*. 2005; Sperling and Roe 2009; Schlick-Steiner *et al*. 2010; Dupuis *et al*. 2012). In reality, this study more closely conforms to the concept of "iterative taxonomy" (Yeates *et al*. 2010), as I used morphology, multiple genes, geometric morphometrics and classical morphometrics to iteratively assess and evaluate species hypotheses. Specimens were first chosen for sequencing based on the identification of morphospecies. The molecular work identified the species group as a whole, and presented a framework for the relationships of taxa within the group. Putative species were then analyzed morphometrically and re-examined morphologically to assess the validity of the species hypotheses and to refine morphospecies concepts.

In this paper, I define the *Ophion scutellaris* species group and describe six new species within the group. In total, this study includes seven Nearctic and two Palearctic species; the Nearctic species are: *Ophion idoneus* Viereck 1905, *O. clave* sp. n., *O. aureus* sp. n., *O. reticulatus* sp. n., *O. dombroskii* sp. n., *O. keala* sp. n. and *O. importunus* sp. n. Five specimens remain unplaced, and most likely represent additional undescribed species.

Methods

Depositories of material examined

BMNH: The Natural History Museum, London, UK

CNC: Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada

CUIC: Cornell University, Ithaca, New York, USA

NFRC: Northern Forest Research Centre, Edmonton, Alberta, Canada

RBCM: Royal British Columbia Museum, Victoria, British Columbia, Canada

SEMC: Snow Entomological Museum, University of Kansas, Lawrence, Kansas, USA

SFUC: Simon Fraser University, Burnaby, British Columbia, Canada

UBCZ: Spencer Entomology Collection, University of British Columbia, Vancouver, British Columbia, Canada

Recognition of the O. scutellaris *species group*

This species group was first identified as part of a large-scale taxonomic and phylogenetic study of *Ophion* (Chapters 2, 3). From the sequencing results of several dozen morphospecies, a well-supported clade that included the Palearctic species *O. scutellaris* Thomson was identified (Chapter 2). Morphological comparison showed that several of the characters that define *O. scutellaris*, according to Brock (1985), were characteristic of the group as a whole. Additional characters were recognized based on examination of all specimens recovered within this group. I therefore used this suite of characters (defined in the Taxonomic Part, below) to locate all specimens within this species group from the available unsequenced material. I then used an iterative analysis of quantitative morphometrics, morphology, and molecular data to assess all sequenced and unsequenced specimens and to determine species boundaries within this newly-defined species group.

Specimens and sampling

Most specimens were newly collected as part of a large-scale study of Canadian *Ophion*. Out of more than 4000 specimens that were collected from a range of habitats and localities across Canada, 196 were from the *Ophion scutellaris* species group, and 134 of these (68%) were a single species, *Ophion idoneus* Viereck. I also borrowed 662 specimens of *Ophion* from the following institutions: CUIC, UBCZ, SEMC, RBCM, CNC, NFRC, SFUC; 42 of these were from the *O. scutellaris* group, of which all except for four were *O. idoneus*. Five specimens of *Ophion scutellaris* were sequenced from British material provided by G. Broad (BMNH). I examined three of these, while for two specimens, I was sent legs for sequencing but have not seen the specimens.

DNA sequencing

I sequenced one mitochondrial and two nuclear genes for the *O. scutellaris* group: the cytochrome c oxidase I gene (COI), the internal transcribed spacer 2 (ITS2), and the D2-D3 variable region of 28S rRNA. I also sequenced these three genes for eight species of *Ophion* outside of the target species group; these were chosen to represent the diversity across *Ophion*, based on a large-scale molecular analysis of *Ophion* (Chapters 2, 3). Finally I sequenced one individual of *Enicospilus*, another genus within Ophioninae, as an outgroup. DNA was extracted from a single hind leg using DNeasy Blood & Tissue Kits (Qiagen, Toronto, ON); the final elution volume was 150 μL. I conducted PCR in either 50 μL or 15 μL reactions. The 50 μL reactions contained 4-8 μL genomic DNA, 5 μL 10x PCR buffer (containing 15mmol/ μL $MgCl_2$) (Promega, Madison, WI), 3 μL of 25 mmoles/μL $MgCl_2$ (Promega), 1 μL of 10 mmoles/μL dNTP's (Roche, Switzerland), 1 μL each of 5pmol/μL forward and reverse primers, 0.5 μL of 5 U/μL *Taq* polymerase (Fermentation Service Unit, University of Alberta) and 30.5-34.5 μL of autoclaved Millipore water. The 15 μL reactions used 4-8 μL DNA, 1.5 μL PCR buffer, 0.9 μL $MgCl_2$, 0.3 μL each of dNTP's, forward and reverse primers, 0.15 μL *Taq* and 3.55-7.55 μL water. All PCR products were purified using ExoSap-IT (USB Corporation, Cleveland, OH), and were

sequenced using BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA), followed by ethanol precipitation. Sequencing reactions were run on an ABI Prism 3730 DNA analyser. Sequences are deposited in NCBI GenBank, and genbank accession numbers are listed in Appendix 1.

COI: I used the primers lco hym (5' – CAA ATC ATA AAG ATA TTG G – 3') and hco out (5' – CCA GGT AAA ATT AAA ATA TAA ACT TC – 3') (Schulmeister 2003), which produce a fragment equivalent to the "barcode" region (Hebert 2003); in *Ophion*, this region is 676 base pairs in length. PCR conditions were: 94° for 2 min, 35 cycles of 94° for 30 s, 45° for 30 s, 72° for 2 min, and a final extension at 72° for 5 min. Alignment was unambiguous, and was confirmed by translating nucleotides to amino acids in Mesquite (Maddison and Maddison 2011). The first character represents the third codon position. I sequenced COI for 30 Nearctic and 5 Palearctic specimens within the *O. scutellaris* group (Appendix 1)

ITS2: I analyzed this gene using the primers ITS2-F (5'-GGG TCG ATG AAG AAC GCA GC-3') and ITS2-R (5'-ATA TGC TTA AAT TCA GCG GG-3') which anneal to the flanking 28S and 5.8S genes (Navajas *et al*. 1994). PCR cycling was 94° for 2 min, 35 cycles of 94° for 30 s, 55° for 1 min, 72° for 2 min and a final extension of 75° for 5 min. Sequences were aligned using ClustalW (Larkin *et al*. 2007), and then were modified by eye in Mesquite. Large numbers of indels and highly divergent sequences made alignment of the *scutellaris* group with the non-*scutellaris* group sequences subjective; however within the *scutellaris* group the alignment was unambiguous. I was not able to successfully sequence ITS2 for *Enicospilus*. Instead *O. minutus* was used as the outgroup, since it was recovered as basal by both the COI and 28S datasets, as well as having morphological characters indicating that it is likely basal within *Ophion* (Chapter 2). The final alignment (including partial 28S and 5.8S) was 1307 base pairs in length. However the alignment for the *O. scutellaris* group, excluding all other specimens, was 996 base pairs long, with individual sequences ranging from

979-992 base pairs. I successfully sequenced 29 Nearctic and 2 Palearctic specimens within the *O. scutellaris* group.

28S: I sequenced the D2-D3 region of 28S rDNA using the following primers: Forward: 5'-GCG AAC AAG TAC CGT GAG GG-3'; Reverse: 5'-TAG TTC ACC ATC TTT CGG GTC-3' (Laurenne *et al*. 2006). PCR cycling was 94° for 2 min, 30 cycles of 96° for 15 s, 50° for 30 s, 72° for 30 s and a final extension of 75° for 7 min. Alignment was performed by eye in Mesquite; there were occasional small indels, but generally alignment was unambigous. The aligned sequences were 725 base pairs in length. I sequenced 8 Nearctic and 1 Palearctic specimen(s) within the *O. scutellaris* group, and also included a single sequence of *O. scutellaris* from GenBank.

DNA analysis

All molecular analyses were conducted using MEGA version 5 (Tamura *et al*. 2011). I conducted both maximum likelihood (ML) and maximum parsimony (MP) analyses for the three genes separately. The maximum parsimony analyses were run using heuristic searches with tree-bisection-reconnection, search level 5. I used all sites, 10 starting trees and set max trees to 1000. The best models for the ML analyses were selected in MEGA, using the Bayesian Information Criterion. The following models were selected: COI: TN93+G+I; ITS2: K2+I; 28S: T92+G. The ML search parameters were subtree-pruning-regrafting, with very strong branch swap filter; I used all sites, and the starting tree was obtained using NJ/BioNJ. The trees were tested using both ML and MP bootstrapping in MEGA. The bootstrap analyses used the same parameters as the tree searches, with 1000 replicates for the ML analysis and 10,000 replicates for the MP analysis. I calculated the intra- and interspecific sequence divergences using the Kimura-2-parameter model.

Geometric morphometrics (GM)

I analysed wing morphometrics for 123 specimens (75 female and 48 male; Table 4-1). One sequenced specimen had a missing abdomen, so could not be classified by sex; all other sequenced *O. scutellaris* group specimens were included in this analysis as well as in the classical morphometrics analysis below. The right fore- and hindwings were removed at the base, soaked briefly in 95% ethanol, and then temporarily slide-mounted in 95% ethanol. Slides were placed on the pane of a lightbox, and photographed using either an 8 megapixel Nikon Coolpix 8400 or a 7.2 megapixel Sony Cybershot DSC-W80 digital camera. The Nikon camera was mounted on a camera mount at a distance of 3.3 cm from the in-focus wing, and wings were photographed using the macro setting and manual focus. The Sony camera was placed at a distance of 4.0 cm from the wing and photographed using the macro setting and autofocus. Several wings were photographed using both cameras to ensure that the two methods were comparable and I concluded that variability between cameras was negligible compared to the variability between specimens (data not shown). Once photographed, the wings were glued at the base to a small square of cardstock, and included with the specimen as an extra label.

Only the forewings were used for the geometric morphometrics. A total of 23 landmarks were digitized in tpsDig version 2.16 (Rohlf 2010) (Figure 4-1). The landmark data was analyzed in MorphoJ, version 1.03 (Klingenberg 2011). A preliminary analysis showed differences between male and female wings, therefore the sexes were analysed separately. A Procrustes fit was conducted on the male and female datasets to eliminate the variables of position, size and rotation (Rohlf and Slice 1990). I then calculated a covariance matrix of the Procrustes coordinates, and analyzed it using principal component analysis.

Classical morphometrics (CM)

Eighty-four specimens (52 females and 32 males; Table 4-1) and thirteen morphometric variables were included in this analysis. Eleven of these are ratios of measurements; the other two are forewing length and number of flagellomeres.

I did not use any forewing characters (aside from length) so that there is no character overlap between this analysis and the geometric morphometric analysis. All variables are described in Table 4-2.

I conducted principal component analysis of the morphometric data in Gingko version 1.7 (Bouxin 2005), with sexes analyzed separately.

Results

Summary of molecular analyses

Statistics from the molecular analyses are found in Table 4-3. Maximum likelihood and maximum parsimony analyses gave essentially equivalent results, and there was no conflict between the three genes. The monophyly of the *O. scutellaris* species group was strongly supported in almost all analyses, with bootstrap support ranging from 72% in the 28S ML analysis to 100% in the ITS2 MP analysis (Figure 4-2a – c). Only the MP analysis of the 28S data lacked bootstrap support for the species group. Within the species group there was a further strongly supported division into two subgroups (A and B, Figure 4-2); these two subgroups were recovered by all genes and all analyses, with bootstrap support ranging from 72% (28S MP) to 100% (COI ML). The Palearctic species, *O. scutellaris*, was recovered within subgroup A. The five sequences identified as this species formed a well-supported clade in the COI analysis (ML bootstrap: 99, MP: 95). However there was also a divergence of 3.61% between one specimen (*O. scutellaris* B) and the remaining *O. scutellaris* specimens, which may indicate a previously unrecognized species. Only one of these putative species was sequenced for ITS2.

Sequence divergence within the *O. scutellaris* group was highest in the COI dataset, with a maximum of 10.1% sequence divergence within the species group (16.6% within all included *Ophion)*. While ITS2 had a higher sequence divergence overall (19.9%), the divergence within the *O. scutellaris* species group was only 2.6%. 28S was highly conserved, with a 2.9% divergence within all *Ophion*, and 0.7% within the *O. scutellaris* group.

Summary of morphometric analyses

For the geometric morphometric (GM) analysis, approximately 60% of the variation in wing shape could be summarized in the first three principal components (PC). In the analysis of females, the variation was quite evenly represented by the first two principal components (PC1: 26.5%, PC2: 21.7%, PC3: 10.3%; Figure 4-3a), while in the analysis of males, most of the variation was explained in the first PC (PC1: 35.9%, PC2: 15.4%, PC3: 8.4%; Figure 4-3b).

In the classical morphometric (CM) analysis, the first three principal components represented 77.9% of the total variation in the female dataset (PC1: 52.0%, PC2: 18.9%, PC3: 7.0%). For the analysis of males, they represented 74.8% of the variation (PC1: 41.4%, PC2: 21.1%, PC3: 12.2%). All measurements are summarized in Tables 4-5 and 4-6.

Morphological characterization of subgroups

Based on the recovery of the two strongly supported subgroups by the molecular analyses, I examined all specimens within these subgroups to determine if they could also be characterized morphologically. The most useful character to distinguish these subgroups was found on the propodeum. All specimens in subgroup A have a weakly arched anterior transverse carina (Figures 4-10 – 4-12c), while subgroup B has this carina strongly arched centrally (Figures 4-6 – 4-9c). In addition, the clypeus of species in subgroup A tends to be more highly convex, with small regular punctures (Figures 4-10 – 4-12b), compared to the flatter clypeus, with large, irregularly spaced puntures, in subgroup B (4-6 – 4-9b).

Species discovery and delimitation

Six Nearctic species are described, and one redescribed, based on the integrated results of these analyses (see Taxonomic Part below). Because of the nature of the iterative process, a single process was not followed in determining the species boundaries for all species.

Two species (*O. aureus* sp. n. and *O. keala* sp. n.) were easily recognizable as distinct based on gross morphology. In particular, they are both considerably larger than the remaining species in the group. *O. aureus* is only known from the Peace River region of Alberta, and was recognized based on its golden-orange colour and distinctive propodeal carinae (Figure 4-7). *O. keala* is the largest species in this group (as well as being among the largest species of *Ophion* I have examined) and is a uniformly dark red colour (Figure 4-10). The molecular analyses recovered *O. aureus* within subgroup B and *O. keala* within subgroup A, and the morphology of each is consistent with the respective subgroups. Each species was recovered in the analysis of COI, with bootstrap values of 99 (MP) or 100 (ML). According to ITS2, *O. aureus* was also strongly supported, with bootstrap values of 85 (ML) or 91 (MP); however *O. keala* was recovered as a paraphyletic grade with respect to another species, *O. idoneus*.

Each of these species was highly sex-biased. *O. aureus* was represented entirely by males, except for a single female specimen, while the opposite occurred in *O. keala*. They could therefore only be thoroughly assessed in the morphometric analyses of male or female specimens, respectively. Both species were successfully delimited by both GM and CM analyses, with essentially no overlap with other specimens (Figures 4-3, 4-4). Only the plot of PC1 vs PC3 in the GM analysis failed to recognized *O. keala* as distinct. The singleton specimens of the opposite sex were also included in the respective analyses. While PCA maximizes the spread of variation of all specimens, and therefore is less informative for singletons, these single specimens were also often recovered as distinct from all other clusters, particularly in the CM analysis (Figures 4-4a,b).

O. idoneus Viereck, the only previously described Nearctic species within this species group, was also initially recognized on the basis of morphology, in particular the shape of the propodeal carinae, the lack of a ramellus in the forewing and the small size (Figure 4-12). It was identified to species by comparison to the type specimen. The molecular analyses recovered it within subgroup A, and it was strongly supported by both COI and ITS2, with bootstrap support values ranging from 90 – 99. It was also recovered as distinct in all

morphometric analyses, with only the plot of PC1 vs PC3 in the CM study having significant overlap with any other group (Figures 4-3, 4-4). It was by far the most common species in this species group.

A single female specimen of *O. importunus* sp. n. was first noted as being very similar to *O. idoneus*, but differing slightly in the shape of the propodeal carinae, along with being slightly larger and having a longer ramellus (Figure 4-11). Sequencing of this specimen confirmed it as being related to *O. idoneus*, with all analyses strongly supporting its inclusion within subgroup A. However it was separated from *O. idoneus* by an average of 2.4% divergence according to COI and 0.92% divergence according to ITS2 (compared to 0.046% and 0% divergence, respectively, within *O. idoneus*) (Table 4-4). Two other female specimens were identified morphologically as belonging to subgroup A, and were hypothesized to be conspecific with *O. importunus*. Both GM and CM analyses supported this hypothesis, with the three specimens grouping closely together, while being distinct from the cluster of *O. idoneus* (Figures 4-3a, 4-4a).

O. clave was the only species other than *O. idoneus* that was represented by multiple male and female specimens, though female specimens were more common. It was first identified as a putative species based on the molecular analyses. It was recovered as part of subgroup B by all analyses, and formed a well-supported monophyletic clade in the analysis of COI (ML bootstrap: 100, MP: 99; Figure 4-2a); only two specimens were successfully sequenced for ITS2, however these were also recovered as a clade, with moderate bootstrap support (ML: 59, MP 72; Figure 4-2b). Based on these results, the specimens were examined for morphological characters and a number of additional, unsequenced specimens were hypothesized to be part of this species.

The morphometric analyses generally recovered both males and females of this species as distinct clusters compared to other species. For the analysis of females, this result was strongest in the plots of PC1 vs PC2 of both GM and CM, whereas all plots from the analysis of males recovered this species as a distinct group (Figures 4-3, 4-4). In a few cases, female specimens that were originally unassigned to any species also clustered with this group in both GM and CM

analyses. Based on a qualitative examination of these specimens, I further refined my morphological species concept for this species. In the GM analysis, a single female specimen was not recovered with the rest of the species. However, morphological examination of this specimen supports its assignment to *O. clave*, as does the CM analysis. This indicates that wing shape outliers can exist within species, though misidentification cannot be conclusively ruled out.

O. brevipunctatus sp. n. and *O. dombroskii* sp. n. are each represented by singletons in this study. *O. dombroskii* differs from all other members of the *scutellaris* species group, and from all other known Nearctic *Ophion*, by its distinctive black face and thorax (Figure 4-9). In comparison, *O. brevipunctatus* looks superficially very similar to *O. clave.* However both ITS2 and COI recovered these two specimens as a monophyletic clade, with bootstrap support ranging from 65 (ITS2 ML) to 98 (COI MP). They were separated by only 1.05% sequence divergence according to COI, and by 0.3% divergence in ITS2. Neither morphometric analysis, however, showed any similarity between these two specimens, which supports the view that they are not merely colour morphs of the same species. Qualitatively, *O. dombroskii* has unusually short antennal segments, which, along with the black colouration, suggest this species is active diurnally (Gauld 1980). While PCA will not necessarily distinguish singletons, *O. brevipunctatus* was nevertheless recovered as distinct from all other clusters in the CM study, thus supporting the distinctiveness of this species. Further morphological examination uncovered additional characters. In particular, this species can be distinguished from the apparently more common *O. clave* by the larger size, extremely shallow and sparse facial punctures, and the lack of any yellow on the orbits (Figure 4-8).

Five specimens (3 female and 2 male) remain unassigned to any species. Based on the structure of the propodeal carinae, one of the female specimens can be assigned to subgroup A, while the other two are part of subgroup B. The former specimen (labeled as "A" in figures and tables) was initially identified as *O. idoneus*, but based on its divergent position in the morphometric analyses, particularly in the GM analysis, I re-examined this specimen, and concluded that

it also differs from *O. idoneus* morphologically. The other two female specimens ("B" and "C") cluster near or within *O. clave* in both CM and GM analyses. However they were sufficiently morphologically divergent from *O. clave* and from each other that I suspect this does not indicate conspecifity.

One of the two unplaced male specimens ("D") can be assigned to subgroup B, and the other ("E") is in subgroup A. According to both the GM and CM analyses, these clustered with species from their respective subgroup, i.e. "D" was near *O. clave* and "E" was near *O. idoneus*. However, they are sufficiently different morphologically from these species that this is probably an artifact of the data. It is possible that specimen "E" represents the unknown male of *O. importunus*, whereas I am almost certain that specimen "D" is an additional undescribed species.

Discussion

The genus *Ophion* is often considered a particularly challenging taxonomic puzzle, due to high intraspecific and low interspecific morphological variation (Townes 1971, Brock 1982). I used an iterative analysis of multiple genetic markers, geometric morphometrics, classical morphometrics and morphology to define the *O. scutellaris* species group, and to assess and delimit species within the group. All analyses were broadly congruent, however each provided unique information to aid in understanding this group.

The species group as a whole was very well supported by both mitochondrial and nuclear genetic markers (Chapter 2). Along with strong molecular support, the group is supported by a suite of morphological characters. The full description of the species group is provided in the Taxonomic Part below.

Within the species group, there is a further division into two subgroups, each of which is strongly supported by all three genetic markers. The two subgroups are also supported morphologically, although in some cases very similar-looking species (e.g. *O. clave* and *O. importunus*) are in different subgroups, and highly morphologically divergent species (e.g. *O. idoneus* and *O. keala*) are within the same subgroup. Subgroup A can generally be recognized by

the convex clypeus; the small, regular, dense clypeal punctures; and the weakly convex central part of apical transverse carina of the propodeum. Subgroup B has a flatter clypeus which is less distinctly separated from the face; coarse, irregularly scattered clypeal punctures; and the apical transverse carina of the propodeum is strongly arched (U-shaped) in the centre.

While 28S was useful in supporting the monophyly of the species group as a whole, and of the two subgroups, it was too conserved to distinguish species within each subgroup. COI and ITS2 were both apparently effective at separating species, since most species, as defined through the integrated analysis, were recovered as well supported clades, with greater divergence between species than within species. However, few individuals over a limited geographical range were sequenced for the majority of species; further sequencing is needed to fully assess the ability of these markers to distinguish species across their range (Ekrem *et al.* 2007, Bergsten *et al.* 2012). Nevertheless, at least in the case of *O. idoneus* the haplotypes were almost invariant across Canada.

Despite the apparent utility of these molecular markers, morphology is an essential component to diagnosing species, since clusters of haplotypes are not necessarily equivalent to biological species (Sperling and Roe 2009). As well, DNA cannot be sequenced for all specimens and without morphology, these species would remain largely unknown (Schlick-Steiner *et al.* 2007). Finally at least two species in this study, *O. brevipunctatus* and *O. dombroskii*, would probably be considered a single species based on DNA alone, yet were clearly distinct morphologically.

Qualitative morphology, geometric morphometrics and classical morphometrics provide three additional semi-independent datasets with which to evaluate species diagnoses. Each species in this study is distinguishable morphologically, though again small sample sizes and limited geographical sampling mean the morphological variability within each species has probably not been fully sampled. Four species (*O. clave*, *O. brevipunctatus*, *O. importunus*, and *O. idoneus*) look superficially very similar, however each has qualitative morphological characters that are sufficient to separate them. In comparison, *O.*

aureus, *O. keala*, and *O. dombroskii* are morphologically distinctive species, yet multiple lines of evidence are still needed to confirm that they are not a simply a different phenotypic morph of another species. Both *O. clave* and *O. dombroskii* are described from single specimens. While some taxonomists argue that species should never be described from singletons (e.g. Dayrat 2005), Lim *et al* (2012) counter that rarity is a fact of biodiversity, and that we will consistently underestimate diversity if we ignore singleton species. Finally, while beyond the scope of this study, initial examination of the two putative species of "*O. scutellaris*" supports their distinctiveness, with "*scutellaris* B" being larger and having coarser, denser facial punctures.

Principal component analysis is a one-group method for data exploration, meaning it does not include *a priori* group designations (Strauss 2010). As such, it will not definitely separate groups, even if they are distinct. Discriminant analysis is more effective for separating predefined clusters of species and assigning specimens to groups, but it requires known species, which have been identified based on other criteria (e.g. hosts, pheromones, genetic markers) (Marcus 1990, Mutanen and Pretorius 2007, Strauss 2010). In this case, sequenced specimens would be ideal, but sample sizes were too small for the analysis to be statistically valid. Basing the analysis on specimens that were identified morphologically would be circular, considering some specimens were re-examined and re-classified based on the morphometric analyses. At this time, I have therefore restricted the analysis to the exploratory PCA's. Future studies are needed to increase molecular sampling over a wider geographic range, which could then be used for discriminant analyses of morphometric data.

Both geometric and classical morphometrics were effective at clustering species, though the clusters were often not widely separated. In general, superficially similar species clustered more closely together than they did to morphologically divergent species, indicating congruence between quantitative measures of shape and subjective, qualitative analysis. Specimens that did not cluster with other members of their putative species were flagged for further examination. In some cases these were misidentifications, while in others

morphology and the alternate morphometric analysis supported their placement within a given species. Conversely, inclusion within a cluster did not guarantee that the specimen was a member of that species. These examples further support the advantage of including multiple lines of evidence for accurate species delimitation.

Unplaced specimens may represent undescribed species, or may be currently unrecognized morphological variation within these described species. Further analysis (particularly molecular analysis) is needed to confirm the placement of these specimens.

Conclusion

This is a first attempt at describing species within this newly-defined species group; as such, it should be considered a work in progress. This study was almost entirely limited to Canadian material, most species were from only a few localities, and all species except *O. idoneus* were represented by small numbers of individuals. As well, there are almost certainly additional undescribed species in the material available. All of this indicates that we have just begun to sample the true diversity within this species group. Nonetheless, we have shown that by using an iterative analysis of morphology, molecular analysis and morphometrics, we can delimit and describe species within a particularly morphologically-challenging genus. Furthermore, molecular and morphological recognition of this species group will now allow more targeted specimen collection and museum research, in order to ultimately revise the species group in its entirety.

Taxonomic Part

Terminology follows Brock (1982) and Gauld (1991). Abbreviations defined in the classical morphometrics study above are also used here. Additional abbreviations are: CH/CAW: clypeus height/clypeus apical width; OC/OL: distance between occipital carina and posterior ocellus/posterior ocellus length; GI/MW: length of genal inflection/mandible basal width; F1: first flagellomere length/width; F20: 20th flagellomere length/width; MTS: midtibial spur ratio

(shorter/longer); CL/CW: metacoxa length/width; MT1/MT2: first metatarsomere length/second metatarsomere length.

The carinae of the propodeum are often considered too highly variable to be of much use in species delimitation (Brock 1982, Gauld 1991). At least within this species group, however, I have found them to be quite useful. While they are certainly variable, particularly in the extent to which the various carinae are developed, there are usually at least a few essential elements that are consistent within species. Propodeal carinae and areas follow Gauld (1991), with the exception of the unnamed area apical to the propodeal crest, which I call the area postero-lateralis (Figure 4-5). Other important characters are the sculpture of the face and clypeus, punctation of the metapleuron, number of flagellomeres, length of the ramellus and overall size and colour.

Ophion scutellaris species group

Diagnosis: Most species are reddish-coloured, lacking yellow markings (except for narrowly on orbits, tegula, mesepimeron and rarely mandibles); early season (most collected April to June); mid-tibial spurs nearly equal in length; eyes separated from posterior ocellus by 0.27 to 1.25x ocellar diameter; facial punctures more widely separated in centre of face than on sides; scutellum strongly carinate (at least partially); propodeum short, posterior area abruptly sloping so that it is vertical or nearly so at apex, anterior transverse carina (at least in centre) and lateral part of posterior transverse carina (along APL) strong, otherwise carinae variable, but median longitudinal carinae always convergent apical of area superomedia; petiolar spiracle distinctly anterior to membrane of first metasomal segment.

Description: Head: Eyes convergent to nearly parallel, not strongly bulging (FW/HW: 0.46 – 0.56); eyes moderately indented (FW/FM: 0.69-0.91); face with minute to medium sized punctures, variously separated, but always closer together on sides and under toruli than in centre; clypeus coriaceous, variously punctate,

CH/CAW: 0.49-0.70; mandible with internal angle well-defined, punctate except for impunctate polished flange and tips; stemmaticum slightly to strongly raised, OC/OL: 0.42-1.2x, OOD/OL: 0.11-0.92; IOD/OL: 0.27-1.25; ocellar carina rounded, in some species very slightly rippled or with a small peak at the apex; temple receding, usually approximately equal to width of eye in lateral view; antennae with 51-73 flagellomeres; epicnemial carina with pleurosternal angle obtuse (rarely 90º); propodeum short, posterior area abruptly sloping so that it is vertical or nearly so at apex, anterior transverse carina strong and convex along area superomedia, usually entirely strong, but sometimes obsolete or absent laterally, lateral part of posterior transverse carina (along APL) strong, usually absent or obsolete along APE, obsolete to strong along ASu; median longitudinal carinae variously developed, but always convergent apical of area superomedia, lateral longitudinal carinae mostly absent except strong to absent along APL; pleural carina strong, rarely connected to spiracle by a very weak carina; compare sculpture to other spp; Wings: *Rs* sinuate, ramellus absent to somewhat long, fenestra not extending below prestigma, most (all?) species lacking glabrous area in discocubital cell along *Rs+M;* Legs: metacoxa 1.41-2.37; trochantellus dorsally shorter than width; metafemur 6.3-11.6; midtibial spurs nearly equal in length (MTS: 0.70-0.97). Metasoma: First tergite with spiracles distinctly anterior to membrane; first tergite 1.3-1.6x as long as second; second tergite 2.4-4.5x as long as high.

Colour: Uniformly reddish, without yellow markings except orbit (usually narrowly), tegula, mesepimeron and rarely mandibles; females with ovipositor sheath concolourous with apex of metasoma; one species with head and mesosoma predominately black.

Seasonality: Early-flying species, most dates of capture in Canada are May to mid-June. Unusual dates of captures are one specimen that was collected in July, and one collected in August.

Remarks: The *O. scutellaris* species group can be further divided into two monophyletic subgroups (see text). One group (B) includes *O. clave*, *O. aureus*, *O. brevipunctatus*, and *O. dombroskii*, and can be recognized by the strongly arched anterior transverse carina of the propodeum (so that the base of the area superomedia is U-shaped) and the flatter clypeus, weakly separated from the face, usually with larger, sparser clypeal punctures. The second group (A) includes *O. idoneus*, *O. scutellaris*, *O. keala* and *O. importunus*. It can be recognized by the weakly arched anterior transverse carina of the propodeum (so the base of the area superomedia is only slightly convex) and the more convex and distinctly separated clypeus with smaller, denser punctures.

O. idoneus is by far the most common species within the *O. scutellaris* species group; however, only a small proportion of collected *Ophion* will generally be from this group.

Ophion clave sp. n.
Figure 4-6

Type Material: <u>Holotype</u> ♀ (MS13746, DNA7383, GenBank KF594963): CAN: AB: Jenner Bridge, S. Jenner, riparian willow/sagebrush shrub, 50.844 -111.154, 2 vi 2010, UV trap, G.Anweiler (CNC). Paratypes 13 ♀♀, 3 ♂♂. CAN: AB: 1 ♂, 2 ♀♀ (MS13749, DNA7381, GenBank KF594961; MS13748; MS13752) Same data as holotype; 1 ♀ (MS1689, DNA7391, GenBank KF594970) Jenner rodeo grounds, 50.842 -111.151, 07 vi 2007, Light, G.Anweiler; 1 ♂, 1 ♀ (MS71, DNA7382, GenBank KF594962; MS72) Jenner rodeo grounds, 50.842 -111.151, 09 v 2007, UV trap, M.Schwarzfeld; 1 ♀ (MS1434) Jenner rodeo grounds, 50.842 -111.151, 26 v 2007, UV trap, J.Dombroskie & G.Anweiler; 3 ♀♀(MS12193, DNA6944, GenBank KF594831, KF615972, KF616352; MS12198-99) Spruce Grove, 13 km South, 3.4 -113.9, 28 v-2 vi 1989, Malaise, A.T.Finnamore; 1 ♀ (MS12386) 17 km S of Stettler, Lowden Springs Conserv. Area, aspen/buckbrush/grassland, 822m, 52.154 -112.713; 26 v 2010; UV; C.D.Bird; 1 ♂ (MS11578) 17 km S of Stettler, Lowden Springs Conserv. Area, native prairie,

825m, 52.154 -112.712, 24 v 2009, UV, C.D.Bird. MB: 1 ♀ Brandon, 29.v.49, light trap (NFRC); ON: 2 ♀♀ (MS10758, MS10763) Leeds, Grenville Co. Long Mtn, 44.487 -76.008, 27 iv 2009, B.C.Schmidt; 1 ♀ Waterloo Co., Cambridge, malaise, 18-21.v.1992, Skevington & Cannings (RBCM)

Etymology: "*Clave*" is the Spanish word for key, and refers to the arrangement of the propodeal carinae, which resemble an old-fashioned keyhole. It is also the fundamental rhythm in salsa music, which was undoubtably playing as this species was being described. It is a noun in apposition.

Diagnosis: WL: 10.5-12.4 mm; Flag: 52-60; ATC of propodeum U-shaped above ASu; stemmaticum raised; occipital carina rounded; face coarsely densely punctate (most punctures separated by less than their diameter), and punctures connected by strong microreticulation.

Description: Head: Eyes slightly convergent to nearly parallel in frontal view; stemmaticum raised, sulci surrounding stemmaticum complete or nearly so; IOD/OL: 0.53-0.92; OOD/OL: 0.13-0.29; occipital carina rounded, OC/OL: 0.67-0.92; temple receding, approximately equal to width of eye in lateral view; clypeus weakly convex in lateral view and very weakly separated from face, coriaceous with irregularly scattered coarse punctures, often interspersed with at least a few minute punctures, smaller and denser towards lateral and dorsal margins, less dense than on face (see variation), CH/CAW: 0.50-0.64 (♀), 0.49-0.67 (♂); face evenly coarsely punctate, most punctures separated by less than their diameter and connected by strong microreticulation, smaller on sides than in centre (see variation); FW/FH: 1.18-1.26; antennae with 52-60 flagellomeres, F1: 3.00-4.17, F20: 1.36-1.83; MS/MW: 0.43-0.63; GI/MW: 0.36-0.67; Mesosoma: Mesoscutum polished to subpolished (see variation), evenly, shallowly punctate with small to minute punctures; mesopleuron coriaceous with strong punctures separated by approximately their diameter, polished with sparse minute punctures above mesopleural fovea; epicnemial carina of females with pleurosternal angle

approximately 90°, and more or less sharp, varying from slightly acute to slightly obtuse, in males rounded and slightly obtuse; pleural angle extremely obtuse so that carina curves evenly towards anterior margin of mesopleuron, angling slightly upwards at anterior end; SL/SW: 1.23-1.58 (♀), 1.43-1.67 (♂); lateral carina strong along anterior third to two-thirds of scutellum; metapleuron coriaceous with strong punctures separated by approximately their diameter; Propodeum: Females with ATC strong, strongly arched above ASu (so anterior margin of ASu is highly convex), occasionally weak to obsolete along AE; PTC present (occasionally obsolete) along ASu, usually produced as small crests where intersects with MLC, obsolete to absent between AD and APE, strong along APL; MLC weak and slightly convergent along ASu, strong (occasionally weak) and distinctly convergent (but not meeting) along AP; LLC weak to strong along APL, otherwise absent; PC strong, not connected to spiracle; spiracular area slightly to strongly sloping, closely punctate, subpolished to coriaceous; posterior area strongly sloping to apex, rugulopunctate basally, strongly wrinkled apically; Males with same pattern but all carinae tending to be weaker; Wings: WL: 10.5-12.4 mm; veins dark brown to black, stigma light brown; forewing with ramellus absent or present, discocubital cell entirely trichiose except for well-defined fenestra under stigma (not extending below prestigma); Legs: CL/CW: 1.58-1.93; FL/FW: 7.00-8.59 (♀), 7.00-7.18 (♂), MT1/MT2: 2.01-2.26 (♀), 1.69-2.02 (♂); MTS: 0.72-0.88; Metasoma: sides of petiole gently divergent from spiracles to apex in females, more abruptly expanded at spiracles in males

Colour: Reddish-orange; palps, mandibles and/or scutellum sometimes slightly paler; orbits narrowly yellowish; tegula and mesepimeron yellowish; ovipositor sheath concolourous with gaster

Variation: 2 ♀♀ with clypeal punctures more densely and regularly spaced than in remaining specimens; 2 ♀♀ have facial punctures slightly smaller and facial microreticulation slightly weaker that in the remaining specimens; 1 ♂, 1 ♀ (from same locality) with mesoscutum coriaceous

Remarks: Most similar to *O. brevipunctatus*, this species can be distinguished by the sculpture of the face, the more extensive yellow orbits and the smaller size. This species is also similar to *O. idoneus* and *O. importunus*, but can be most easily distinguished by the strongly convex ATC. There is some geographic variation in this species, with less morphological variation in specimens from within each locality than there is between localities.

Ophion aureus sp. n.
Figure 4-7

Type Material: Holotype ♀ (MS7632, DNA3970, GenBank KF615968) CAN: AB: Machesis Lk Forest Prov. Rec. Area, 32 km W Fort Vermilion; 318m; Jack pine forest; 58.325 -116.578; 10 vi 2008; UV trap; D.&S. Macaulay. Paratypes 9 ♂♂ (MS2318, DNA3961, GenBank KF594535, KF645966; MS2324, DNA3911, GenBank KF594487, KF615965; MS2310, DNA3975, GenBank KF615969; MS2311, MS2313-16, MS2320) nr. Tangent Park Campgrnd, 23km S of Peace River, meadow in spruce/aspen, 56.092 -117.542; 7 v 2008, UV trap, D.Macaulay.

Etymology: The name *aureus* is the Latin word for golden, referring to the golden-orange colour of this species.

Diagnosis: WL: 14.3-17.1 mm, Flag: 65-69. Largest species in subgroup B; stemmaticum raised with sulci complete; reduced propodeal carinae with posterior area strongly wrinkled; paler orange (less reddish) than the other species in this species group

Description: Head: eyes weakly convergent in frontal view; stemmaticum distinctly raised, sulci surrounding stemmaticum complete and deeply impressed; IOD/OL: 0.65-0.80, OOD/OL 0.26-0.33; occipital carina rounded, OC/OL: 0.73-

1.00; temple receding, approximately equal to width of eye in lateral view; clypeus moderately convex in lateral view and weakly separated from face, slightly more convex in males, coriaceous, sparsely punctate in males, very sparsely punctate in female, with irregularly sized (minute to coarse) punctures, punctures denser basally and especially laterally, CH/CAW: 0.0.53-0.64; face coriaceous with small punctures separated by slightly more than their diameter, smaller and denser on orbits than in centre, FW/FH: 1.25-1.41; antennae with 65-69 flagellomeres, F1: 3.25-4.00; F20: 1.47-2.38; MS/MW: 0.52-0.76; GI/MW: 0.32-0.66. Mesosoma: mesoscutum densely evenly punctate, subpolished with minute punctures in males, weakly coriaceous with slightly larger punctures in female; mesopleuron coriaceous with strong punctures separated by approximately their diameter in female, punctures smaller and separated by approximately 2x their diameter in males, subpolished with smaller sparser punctures above mesopleural fovea, epicnemial carina with pleurosternal angle 90º to slightly obtuse, rounded (occasionally somewhat sharp); pleural angle extremely obtuse so that carina curves sinuously towards anterior margin of mesopleuron, angling slightly upwards and becoming weak at extreme anterior end; SW/SL: 1.13-1.38, lateral carina strong at base, present along basal third to half of scutellum; metapleuron coriaceous (strongly coriaceous in female), punctures smaller and sparser than on mesopleuron; Propodeum: ♀: ATC strong along ASu, strongly arched (so anterior margin of ASu is highly convex), otherwise absent; PTC present along ASu, raised into crests where intersects with MLC, absent along APE, extremely strong (raised as a flange) along APL; MLC very weak and slightly convergent along ASu, stronger and nearly parallel along AP; LLC absent; two strong longitudinal wrinkles in APL, one of which probably represents remnant of LLC; PC strong, not connected to spiracle; propodeum short, spiracular area sloping, punctate, strongly coriaceous, posterior area abruptly sloping, nearly vertical at apex, rugose; ♂ similar, but with all carinae much less developed: ATC present as vestige in centre; PTC present only as slight crests where intersects with MLC and as a flange along APL, but much less raised than in female; MLC convergent, weak to obsolete, often reduced to longitudinal

wrinkles; punctures on spiracular area very shallow; Wings: WL: 14.3-17.1 mm; veins brown to dark brown, stigma light brown with an apical white spot, fenestra mostly confined to area below stigma, in several specimens indistinctly extending below prestigma; trichiae more or less sparse along basal vein; ramellus long; Legs: CL/CW: 1.67 (♀), 1.69-2.08 (♂), FL/FW: 7.95 (♀),8.11-9.63 (♂); MT1/MT2: 1.22 (♀),1.22-2.19 (♂); MTS: 0.70-0.90; Metasoma: sides of petiole gently divergent from spiracles to apex, some males with more abrupt expansion at spiracles

Colour: Uniformly golden-orange, males less reddish than other members of the species group, female distinctly less reddish. Orbits narrowly and indistinctly yellow, more yellow posterior to eye; tegula and extreme dorsal part of mesepimeron yellowish

Ophion brevipunctatus sp. n.
Figure 4-8

Type material: <u>Holotype</u> ♀ (MS7990, DNA3939, GenBank KF594513, KF615967, KF616314) CAN: ON: Carleton Co., Carp Ridge, nr. Carp; 45.385 - 76.008; 13 v 2008; UV light; B.C. Schmidt.

Etymology: The name is derived from the Latin words *brevis* and *punctatus*, referring to the unusually shallow punctures of the face.

Diagnosis: WL: 14.0 mm; Flag: 67; ATC strongly arched above ASu; stemmaticum raised with sulci complete; facial punctures small, very shallow, widely separated but connected with strong microreticulation; temple strongly receding, 0.6x eye width (other species in this group with temple approx. equal to eye width); stemmaticum dark, no yellow on orbits

Description: Eyes convergent in frontal view; stemmaticum raised, sulci complete; IOD/OL: 0.69, OOD/OL: 0.13; occipital carina rounded, OC/OL: 0.78; temple strongly receding, 0.6x as long as eye width in lateral view; CH/CAW 0.58x apical width, only slightly convex in lateral view, weakly separated from face; clypeal punctures irregularly sized, sparsely, irregularly distributed across coriaceous clypeus; punctures of face small, very shallow, separated by 2-3x their diameter, connected by strong microreticulation; FW/FH: 1.27; antennae with 67 flagellomeres; F1: 3.6; F20: 1.8; MS/MW: 0.5; GI/MW: 0.5; mesoscutum subpolished, evenly punctate with minute punctures; mesopleuron coriaceous with strong punctures separated by approximately their diameter, varying to subpolished with smaller punctures anteriodorsally; subpolished with minute punctures above mesopleural fovea; epicnemial carina with pleurosternal angle slightly obtuse, pleural angle obtuse, slightly angled dorsally at intersection with anterior margin of mesopleuron; scutellum 1.5, strongly carinate along the anterior third; metapleuron coriaceous with shallow medium-sized punctures, more sparsely distributed than on mesopleuron; Propodeum: ATC strong, strongly arched along ASu (so anterior margin of ASu strongly convex); PTC obsolete in centre, strong at intersection with MLC and for a short distance along AD, otherwise obsolete along AD, very strong along APL; MLC obsolete and slightly convergent along ASu, obsolete (represented by wrinkles) and strongly convergent along AP; LLC present along APL, very weakly represented at intersection with ATC, otherwise absent; PC strong, not connected to spiracle; spiracular area sloping, coriaceous with small shallow punctures; posterior area abruptly sloping, nearly vertical at apex, weakly rugopunctate, becoming wrinkled apically; Wing L: 14.0 mm, wing veins dark brown, stigma reddish-brown, fenestra restricted to area below stigma, trichiae slightly sparser below prestigma, ramellus long; CL/CW: 2.0; FL/FW: 9.5, MT1/MT2: 2.1; MTS: 0.78; Sides of petiole gradually expanding at spiracles.

Colour: Uniformly reddish-orange; stemmaticum distinctly darker, dark reddish-brown; palps and scutellum very slightly paler than base colour, tegula and

extreme dorsal part of mesepimeron dark yellowish, mesopleural fovea slightly darker than base colour; ovipositor sheath concolourous with abdomen

Ophion dombroskii sp. nov.
Figure 4-9

Type material: Holotype ♀ (MS13975, DNA6548, GenBank KF594760, KF615971, KF616341) CAN: SK: nr. Newton L., 49.301 -107.764, 20 v 2011, J. Dombroskie

Etymology: This species is named for Jason Dombroskie, who was kind enough to collect the only known specimen of this species on an otherwise rainy and utterly unsuccessful moth-collecting trip.

Diagnosis: WL: 10.8 mm, Flag: 51; The head and thorax of this species are almost entirely black, making this species easily recognizable. It also has unusually short antennal segments, widely separated ocelli and a long, narrow scutellum.

Description: Eyes slightly convergent in frontal view; stemmaticum slightly raised, sulci surrounding stemmaticum complete; IOD/OL: 1.20, OOD/OL: 0.45; occipital carina rounded, OC/OL: 1.20; temple receding, approximately equal to width of eye in lateral view; clypeus 0.5x as high as apical width, coriaceious, punctures irregularly sized (coarse to very small) and sparsely, irregularly distributed, denser on sides, CH/CAW: 0.50; face with medium-sized punctures, approximately separated by their diameter and connected with strong microreticulation, smaller on sides of face; FW/FH: 1.34; antennae short, 51 flagellomeres, F1: 2.7, F20: 1.1; MS/MW: 0.6; GI/MW: 0.8; mesoscutum coriaceous, evenly punctate with minute punctures, separated by several times their diameter; mesopleuron and metapleuron strongly coriaceous, evenly, coarsely punctate with punctures separated by their diameter or less; mesopleuron

above mesopleural fovea subpolished, punctures minute, separated by 2-3x their diameter; epicnemial carina pleurosternal angle obtuse, pleural angle very obtuse; scutellum with lateral carina strong along anterior half, SL/SW: 1.8; WL=10.8 mm; wings with veins dark brown, stigma light brown, fenestra not extending below prestigma, ramellus short; propodeum: ATC strong and strongly arched along ASu (so anterior margin of ASu strongly convex), weak to obsolete along AD; PTC mostly obsolete, represented by wrinkles, forming small crests where intersects with MLC, strong along APL, especially strong at propodeal apophysis; MLC obsolete, faintly represented by wrinkles along ASu and even more indistinctly along AP; LLC present as a wrinkle along APL, otherwise absent; PC strong, not connected to spiracle; spiracular area sloping, coriaceous with numerous small punctures; posterior area abruptly sloping, nearly vertical at apex, weakly wrinkled and punctate, more wrinkled apically; CL/CW: 1.5, FL/FW: 7.2, MT1/MT2: 3; MTS: 0.85.

Colour: Head: black; orbits, temple, vertex, mandibles, palps and clypeus except for extreme base reddish-orange; Mesosoma: black; mesocutum (except margins and base of notauli), scutellum, anterior margin and an indistinct area in the centre of mesopleuron, apical margin of propodeum, apical half of coxae and legs reddish-orange; mesepimeron reddish-orange ventrally and yellowish dorsally; metasoma reddish-orange; ovipositor sheath same colour as metasoma

Remarks: This species is unusual because of its extensive black markings. The unusually short antennae and black markings indicate that this species may be diurnally active (ref). Gauld (1985) mentions a few undescribed deserticolous species with short antennae and quadrate central flagellomeres; we have not seen these specimens, so it is unknown whether this species should be considered among them.

Ophion keala sp. n.

Figure 4-10

Type material: <u>Holotype</u> ♀ (MS2249, DNA3965, GenBank KF594539, KF615948) CAN: AB: Porcupine Hills, Skyline Rd; 49.972 -114.087; 29 v 2008; UV trap; J.Dombroskie, J.Walker. <u>Paratypes</u> 7 ♀♀, 1 ♂. CAN: AB: 2 ♀♀ (MS2244, DNA3980, GenBank KF594552, KF615947; MS2238, DNA3960, GenBank KF594534, KF615950) Same data as holotype; 1 ♂, 1 ♀ (MS2235, DNA3904, GenBank KF594480, 615945; MS2237) Same data as holotype except date 28 v 2008; 1 ♀ (MS8647, DNA6515, GenBank KF594730, KF615943, KF616332) Porcupine Hills, Skyline Rd, 49.972 -114.087, 15 vi 2009, UV trap J.Dombroskie, B.Brunet; 1 ♀ (MS7801, DNA7327, GenBank KF594917, KF615946) 62 km WNW of Dixonville, Mixedwood retention patch in clearcut, Site 7, 56.685 -118.641, 26 v 2008, UV trap, B.Bodeux; 1 ♀ (MS7912, DNA7324, GenBank KF594914, KF615944) 62 km WNW of Dixonville, Mixedwood retention patch in clearcut, Site 4, 56.684 -118.644, 7 vi 2008, UV trap, B.Bodeux; 1 ♀ (MS7746) 54 km NW of Dixonville, Mixedwood forest, 56.96 -118.31, 11 vi 2007, UV trap, B.Bodeux

Etymology: The name for this species is derived from *keala*, the Hawaiian word for path, as this large and distinctive species presents a rare clear path within the morphologically homogeneous jungle that is *Ophion*. It is a noun in apposition - and is also my daughter's name.

Diagnosis: ♀: WL: 18.6-19.7 mm, Flag: 69-73; ♂: WL: 16.2 mm, Flag: 65; Very large species, uniformly dark reddish-orange with interocellar area often darker; hind femur long and slender (8.4-11.6), scutellum strongly carinate.

Description: Head: female eyes convergent in frontal view, male eyes weakly convergent; stemmaticum weakly raised, sulci surrounding stemmaticum complete; IOD/OL: 0.27-0.44; OOD/OL: 0.11-0.22x (♀) 0.44 (♂); occipital carina rounded, often very slightly wavy, with a very small peak in the centre;

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OC/OL: 0.42-0.68 (♀), 0.81 (♂); temple receding, approximately equal to width of eye in lateral view; CH/CAW: 0.52-0.63, coriaceous with evenly distributed medium-sized punctures, separated by approximately 1-2x their diameter, punctures smaller basally; face weakly coriaceous, with small punctures separated by 1-2x their diameter, closer on sides than in centre; FW/FH: 1.24-1.49; antennae with 69-73 flagellomeres (♀), 65 (♂); F1: 3.56-4.50; F20: 1.54-2.24; MS/MW: 0.33-0.47; GI/MW: 0.46-0.69; mesoscutum polished, evenly punctate with minute punctures separated 1-2x their diameter; mesopleuron coriaceous, densely punctate with small to medium-sized relatively shallow punctures, separated by approximately their diameter; subpolished with minute punctures separated by 2-3x their diameter above mesopleural fovea; epicnemial carina with pleurosternal angle obtuse, distinctly so in male, rounded (see variation); SW/SL: 1.47-1.76, lateral carinae strong along almost entire length of scutellum, slightly weaker in male; metapleuron strongly coriaceous, punctures approximately equal to those of posterior corner of mesopleuron, equally or slightly less dense; WL: 18.6-19.7 mm (♀), 162 mm (♂); wings slightly brownish with black veins, stigma reddish-brown, ramellus very short to somewhat long, fenestra restricted to area under stigma; propodeum: ATC strong, very slightly arched above ASu; PTC weak to absent along ASu, represented by small crests at MLC, obsolete to absent along AD, very strong along APL where it is expanded as a flange; MLC obsolete, absent to very faintly represented by parallel or slightly convergent wrinkles along ASu, present as a series of stronger convergent wrinkles along AP; LLC weak to obsolete along APL, otherwise absent; PC strong, connected to spiracle by a very weak to obsolete carina; spiracular area sloping, subpolished with minute shallow punctures separated by 1-2x their diameter; posterior area abruptly sloping, nearly vertical at apex, weakly wavy-wrinkled with minute punctures to PTC, then more distinctly wrinkled to apex; CL/CW: 1.94-2.37 (♀), 1.83 (♂); FL/FW: 8.41-11.63, MT1/MT2: 2.06-2.33; MTS: 0.78-0.88 (male 0.91); Metasoma: first tergite often abruptly (sometimes gradually) expanded from petiole to postpetiole, spiracles sometimes raised on tubercles

Colour: Reddish-orange; stemmaticum often slightly darker, mandibles usually slightly paler; orbit (especially posterior to eye), tegula and dorsal part of mesepimeron yellowish; notauli and margins of mesocutum slightly darker; ovipositor sheath concolourous with metasoma

Variation: One female with all punctures slightly more sparsely distributed than in the remaining specimens; One female with pleurosternal angle of epicnemial carina approximately 90° and somewhat sharp

Ophion importunus sp. n.
Figure 4-11

Type material: <u>Holotype</u> ♀ (MS12343, DNA6907, GenBank KF594814, KF615963, KF616346) CAN: AB: 8 km NW of Winfield, Bird East Poplar Creek quarter, mixed woods, 900m; 53.01 -114.5; 15 v 2010; UV; C.D.Bird. <u>Paratypes</u> 2 ♀♀. CAN: AB: 1 ♀ (MS13904) 8 km NW of Winfield, Bird East Poplar Creek quarter, mixed woods, 900m, 53.01 -114.5, 21 v 2011, UV, C.D.Bird. ON: 1 ♀ (MS12153) Bells Corners, Stony Swamp, 45.295 -75.83, 3 v 2010, MV light, J.Dombroskie, B.C.Schmidt

Etymology: This species is most similar to *O. idoneus*. Since *idoneus* is the Latin word for "suitable" or "proper", the name for this species is derived from the Latin word *importunus*, meaning "unsuitable".

Diagnosis: WL: 12.6-13.3 mm, Flag: 54-57; Similar to *O. idoneus*, but can be recognized by the larger size, LMC convergent but not meeting (thus propodeum lacks Y-shaped carinae), and the long ramellus.

Description: Eyes weakly convergent in frontal view; stemmaticum weakly raised, sulci not complete; IOD/OL: 0.53-0.85, OOD: 0.15-0.23; occipital carina rounded, very slightly rippled or wavy, OC/OL: 0.80-1.00; temple receding,

approximately equal to eye width in lateral view; clypeus convex, weakly coriaceous, with small regular punctures only slightly larger than on face, separated by approximately their diameter, CH/CAW: 50-0.60; face subpolished with very small punctures separated by slightly more than their diameter in the centre and slightly less on the sides; FW/FH: 1.25-1.29; antennae with 54-57 flagellomeres, F1: 3.43-3.69; F20: 1.54-1.83; MS/MS: 0.46-0.58; GI/MW: 0.46-0.54; mesoscutum subpolished with very small regular punctures separated by 1-2x their diameter; mesopleuron and metapleuron coriaceous, strongly closely punctate, punctures separated by less than their diameter; area of mesopleuron above mesopleural fovea subpolished with minute punctures separated by 1-3x their diameter; epicnemial carina: pleurosternal angle obtuse, rounded; scutellum with lateral carina strong along most of length, SW/SL: 1.55-1.60; Wing L: 12.6-13.3 mm; wings with veins black, stigma reddish-brown with apex whitish-gray ramellus long, fenestra not extending below prestigma; Propodeum: ATC strong, moderately arched above ASu; PTC present along APL, otherwise absent; MLC very weak and weakly convergent along ASu, stronger at apex of ASu, obsolete and represented by strongly convergent wrinkles along AP; LLC represented by indistinct wrinkles along APL, otherwise absent; PC strong, sometimes weakly connected to spiracle by an obsolete carina; spiracular area sloping, subpolished with small punctures separated by less than their diameter; posterior area abruptly sloping, nearly vertical at apex, wavy-wrinkled with very shallow punctures basally, more wrinkled apically; CL/CW: 1.61-2.00, FL/FW: 8.25-8.87, MT1/MT2: 2.19-2.26; MTS: 0.79-0.87; expansion from petiole to postpetiole slightly abrupt.

*Colour***:** Reddish-orange, orbits and sometimes mandibles yellow, tegula and dorsal part of mesepimeron yellowish, scutellum slightly paler than base colour; ovipositor sheath concolourous with apex of metasoma

Ophion idoneus Viereck, 1905

Figure 4-12

Type material: <u>Holotype</u> ♀ USA: Kansas: Douglas Co. ft. 900; May; U. of K., Lot 878, 8ub (SEMC, examined); *Other material examined*: 108 ♀♀, 83 ♂♂, 2 unknown: CAN: AB: 2 ♀♀ (MS12403, MS12405) 11 km NE of Lacombe, J.J. Collett Natural Area, N-facing slope, P. glauca, B. papyrifera, 835m, 52.553 -113.641, 18 vi 2009, UV, C.D. Bird; 4 ♀♀, 1 ♂ (MS11824-28) 5km NEE of Dunstable, George Lake Research Station, aspen forest, MT-7, 53.957 -114.130, 25-29 v 2007, Malaise, M. Schwarzfeld; 11 ♀♀, 6 ♂♂ (MS13317, MS13319-20, MS13322-26, MS13328-36) same data except date is 29 v-6vi 2007; 2 ♀♀, 6 ♂♂ (MS12920, MS12922-28) 5km NEE of Dunstable, George Lake Research Station; Black spruce forest; MT-8, 53.957 -114.128, 29 v-6 vi 2007, Malaise, M. Schwarzfeld; 4 ♀♀ (MS12433-35, MS12444) 8 km NW of Winfield, Bird East Poplar Creek quarter, mixed woods, 900m, 53.01 -114.50, 12 vi 2010, UV, C.D. Bird; 1 ♂ (MS12335) same data except date is 15 v 2010; 1 ♂ (MS11457) same data except date is 16 v 2009; 1 ♀ (MS13925) same data except date is 21 v 2011; 1 ♀ (MS2261) 8 km SE Sherwood Park, aspen forest, 53.478 -113.229, 12-15 v 2008, MV light, G.R. Pohl; 1 ♀ (MS7373) Bragg Creek, 50.917 -114.533, 15 viii 2007, at light, F. Sperling; 1 ♀ (MS4026), Calgary, Edgemont, 51.115 -114.142, 18 v 2010, light, T. Pike; 3 ♀♀ (MS2275, DNA3910, GenBank KF594486, KF615953; MS2280; MS2274) Edmonton, nr. Fulton Ravine, 53.545 -113.439, 15-16 v 2008, light, G. Anweiler; 1 ♂ (MS2284) same data except date is 17 v 2008; 1 ♀ (MS9688, DNA3974, GenBank KF594547, KF615956) same data except date is 23 v 2009; 2 ♀♀ (MS2286, MS2290) same data except date is 24 v 2008; 1 ♀, 1 ♂ (MS5632, DNA3933, GenBank KF594507, KF615951; MS4301) same data except date is 26 v 2011; 9 ♀♀ (MS13809, MS13811, MS133815, MS13820-22, MS13826-28) same data exept date is 27 v 2011; 3 ♀♀ (MS12169, MS12171-72) same data except date is 31 v 2009; 7 ♀♀

(MS13834-35, MS13842-43, MS13858, MS13862) same data except date is 6-8 vi 2011; 5 ♀♀, 2 ♂♂ (MS13763-65, MS13767, MS13757, MS13774, MS13782) same data except date is 9-11 vi 2011; 2 ♂♂ (MS78, MS80) same data except date is v 2007; 2 ♀♀ (MS2879, MS2886) Edmonton, Edith Ravine, 53.510 -113.622, 12 v 2010, UV light, J. Acorn; 1 ♀ (MS2885) same data except date is 27 v 2010; 1 ♀ (MS2234, DNA5552, GenBank KF594576, KF615958) Edmonton, inside building, 53.521 -113.521, J. Dombroskie; 3 ♂♂ (MS100, MS103, MS107) Edmonton, River Valley at U.Alberta, 53.529 -113.519, 28 v 2007, Sweep, M. Schwarzfeld; 1 ♀ (MS2770, DNA6551, GenBank KF594763, KF615949) EMEND site, 48 km NW of Dixonville; Decid. forest, uncut, 56.7525 -118.3282, 28 v-10 vi 2008, Malaise, 852-2, M. Schwarzfeld; 2 ♀♀ (MS12420, MS12423) Erskine, 5 Maple Close, backyard with aspen, 830m, 52.322 -112.883, 19 v 2010, UV, C.D. Bird; 1 ♀, 1 ♂ (MS9763-64) George Lake Research Site, 53.953 -114.120, 29 v 2007, Sweep, M. Schwarzfeld; 1 ♂ (MS39) George Lake Research Site, Aspen forest, 53.957 -114.125, 25 v 2007, Sweep, M. Schwarzfeld; 1 ♀ (MS3613) N. Wyndham-Carseland Provincial Park, 50.8366 -113.4347, 31 v 2008, light, T. Pike; 2 ♀♀ (MS5650, DNA3977, GenBank KF594549, KF615957; MS5651, DNA3950, GenBank KF594524, KF615952) Pigeon Lake, Itasca, 53.072 – 114.072, 3 vi 2008, UV light, F. Sperling; 9 ♀♀ 14 ♂♂ (MS12460, MS12463, MS12472, MS12474-83, MS12485-93, MS12495) Rochon Sands Prov. Park, 15 km N Erskine, 720m, aspen, chokecherry, aspen, 52.46 -112.88, 2 vi 2010, UV, C.D. Bird; 1 ♂ (MS12197) Spruce Grove, 13 km South, 53.4 -113.9, 28 v-2 vi 1989, Malaise, A.T. Finnamore; 1 ♀ (MS12265) Summer Village of Gull Lake, 52.460 -113.947, 31 v 2009, UV trap, J.H. Acorn; 1 ♂ (MS13925) Wintering Hills West, 51.2552 – 112.6261, 29 v 2011, net, J. Dupuis; BC: 1 ♂ (RBCM ENT008-002270) Robson, "?" v 1951, H.R. Foxlee, Ex. H.R. Foxlee Collection U.B.C. 1971; 1 ♂ (RBCM ENT008-005274) same data except date is 14 v 1954; 1 ♀ (RBCM ENT008-00250) same data except

date is 15 v 1951; 1 ♂ (RBCM ENT008-002274) same data except date is 15 v 1954; 1 ♀, 1 ♂ (RBCM ENT008-002275) same data except date is 17 v 1954; 1 ♂ (RBCM ENT008-002230) same data except date is 29 v 53; ; 1 ♂ (RBCM ENT008-002239) same data except date is 3 v 51; ; 1 ♂ Robson, 8 v 1954, H.R. Foxlee (UBC); MB: 2 ♀♀ 10 mi. S. of Winnipeg, 1 vi 1973, coll. C. Starr, at UV light (SEMC); 2 ♀♀ same data except date is 30 v 73; 1 ♀ Brandon, 29 v 49, Light Trap (NoFC); ON: 4 ♀♀, 2 ♂♂ (MS2927, MS2932-34, MS2940, MS2943) Bells Corners, Monaghan Forest, 45.272 -75.808, 18 v 2010, Light, B.C. Schmidt; 1 ♀, 1 ♂ (MS8020, MS8039) Bells Corners, Stony Swamp, 45.295 -75.830, 25 v 2008, B.C. Schmidt; 1 ♂ (MS12164) Bells Corners, Stony Swamp, 45.295 -75.830, 3 v 2010, MV light, J. Dombroskie, B.C. Schmidt; 1 ♀, 1 unknown (MS10755, DNA5554, GenBank KF594578, KF615955; MS10754) Grenadier Is., St. Lawrence Islands Nat. Pk., *Carya* grove, 44.4 -75.9 10-21 vi 1994, YPT, Coll. CNC Hym Team; 1 ♀ (MS10710) same data except date is 24-9 vi 1994; 7 ♂♂ (MS10713, DNA6552, GenBank KF594764, KF615960; MS10711; MS10716-17; MS10719-21) same data except trap is Malaise, date is 24-9 vi 1994; 1 ♀ (MS10751) same data except trap is Malaise, date is 3-13 v 1994; 1 ♀ (MS8007, DNA3976, GenBank KF594548, KF615961) Leeds Grenville Co., Long Mtn. 44.487 -76.008, 7 vi 2008, B.D. Schmidt; 2 ♀♀ (MS10807, MS10813) same data except date is 21 v 2009; 5 ♀♀, 22 ♂♂ (MS10793, DNA5717, GenBank KF594635, KF615959; MS10794, DNA5552, GenBank KF594576, KF615958; MS10779, DNA5551, GenBank KF594575, KF615954; MS10768-78, MS10780-92) Ottawa, city garden, 45.356 -75.707, 5 v-5 vi 2008; Malaise, Coll. H. Goulet; 1 ♂ Waterloo Co., Cambridge, malaise, Skevington & Cannings (RBCM); SK: 1 ♀ S'toon, May 22, 1940, D.R. Foskett (RBCM); USA: MI: 1 ♀ Ag. Coll. Mich 5 – 23 93 22 (CUIC); 1 ♂ Ag. Coll. Mich 5-23 95 22 (CUIC); NY: 1 ♀, 1 ♂ Ithaca, v 23 1936 (CUIC); 1 unknown Orient, L.I. June 2, 1932, Roy Latham/ Roy Latham Collection (CUIC).

Diagnosis: WL: 8.4-11.4 mm, Flag: 51-57; Smallest species within the species group; MLC fused immediately apically of ASu, therefore appears Y-shaped; ramellus usually absent or extremely short

Description: Eyes weakly convergent in frontal view; stemmaticum very weakly raised, sulci not complete; IOD/OL: 0.61-0.86, OOD/OL: 25-0.43; occipital carina rounded, often very slightly rippled or wavy, usually with a small peak at centre, OC/OL: 0.58-0.95; temple receding, approximately equal to eye width in lateral view; clypeus convex in lateral view and distinctly separated from face, coriaceous, with small regular shallow punctures only slightly larger than on face, separated by approximately their diameter, CH/CAW: 0.57-0.70; face subpolished to very weakly coriaceous, with small shallow punctures separated by approximately their diameter, slightly more dense on sides; FW/FH: 1.28-1.45; antennae with 51-57 flagellomeres, F1: 2.93-4.00; F20: 1.33-2.00; MS/MW: 0.54-0.70; GI/MW: 0.46-0.73; mesoscutum subpolished with very small to minute regular punctures separated by approximately 2x their diameter; mesopleuron weakly coriaceous, strongly closely punctate, punctures separated by their diameter ore less, above mesopleural fovea subpolished with small punctures separated by 1-3x their diameter; metapleuron slightly more coriaceous, punctures similar in size to those of mesopleuron, only slightly less dense; epicnemial carina with pleurosternal angle obtuse; scutellum with lateral carina strong along most of length, SW/SL: 1.37-1.69; Wing L: 8.4-11.4 mm; wings with veins dark brown, stigma light brown with apex whitish-gray, ramellus completely absent or represented by a minute vestigial stub, rarely somewhat long, fenestra not extending below prestigma; Propodeum: ATC strong, weakly arched above ASu; PTC weak to obsolete along ASu, raised into small crests where intersects with MLC, absent along AD, strong along APL; MLC obsolete and weakly convergent along ASu, strongly convergent just apical to ASu, so that the carinae fuse into one stronger carina for most of AP; LLC almost absent, short carina in APL may be remnant of this carina; PC strong, not connected to spiracle; spiracular area slightly sloping, nearly horizontal, subpolished with shallow minute indistinct

punctures, separated by 2-3x their diameter ; posterior area abruptly sloping, wavy-wrinkled with very shallow indistinct punctures basally, more wrinkled apically; CL/CW: 1.41-1.85, FL/FW: 6.45-7.50, MT1/MT2: 2.09-2.50; MTS: 0.71-0.89; expansion from petiole to postpetiole gradual.

Colour: Reddish-orange, mandibles, palps and legs slightly paler; orbits, tegula and dorsal part of mesepimeron yellowish, ovipositor sheath concolourous with apex of metasoma

Remarks: Common and wide-spread early season species. Most small dark reddish individuals, lacking yellow notauli, collected in May will be this species. The original description (Viereck 1905) for this species is quite detailed, and is sufficient to identify this species; we have re-described it here so that the description is consistent with the other members of the species group. We excluded characters that apply to the species group as a whole, and focused on characters that distinguish it from other species within the group.

Key to the known Canadian species of the *Ophion scutellaris* species group

1a. Face and most of thorax black, central flagellomeres approximately quadrate….. *O. dombroskii* sp. n.
1b. Body entirely orange or reddish, central flagellomeres distinctly longer than wide 2

2a. Anterior transverse carina strongly convex in centre (U-shaped).....................3
2b. Anterior transverse carina weakly convex in centre..5

3a. Yellow-orange base colour, wing length greater than 14 mm, propodeal carinae generally reduced, greatly reduced in male, but apical part of LLC expanded as a flange .... *O. aureus* sp. n.

3b. Reddish-orange base colour, wing length 14 mm or less, propodeal carinae more developed, but apical part of LLC strong but not expanded into a flange 4

4a. Facial punctures strong and separated by less than their diameter; orbits yellowish, stemmaticum and mesopleural fovea concolourous with base colour ....*O. clave* sp. n.

4b. Facial punctures very shallow and separated by 2 – 3 X their diameter; orbits lacking yellow, concolourous with reddish head, stemmaticum and mesopleural fovea darker than base colour .... *O. brevipunctatus* sp. n.

5a. Forewing length greater than 16 mm; antennae with at least 65 flagellomeres, more in female .... *O. keala* sp. n.

5b. Forewing length less than 14 mm; antennae with less than 58 flagellomeres ...6

6a. Median longitudinal carinae of propodeum strongly convergent posterior to areola, usually arriving at apex of propodeum as a single carina; ramellus almost always extremely short to absent (rarely longer) ......*O. idoneus* Viereck

6b. Median longitudinal carinae of propodeum represented by wrinkles posterior to posterior transverse carina, convergent but not fusing into a single carina; ramellus long...*O. importunus* sp. n.

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Table 4-1. Summary of specimens used for morphometric analyses, arranged by species. GM = Geometric morphometrics; CM = Classical morphometrics; A-E refer to unplaced specimens (see text and Figures 4-3, 4-4).

Species	Provenance	Lat/Long	GM ♂/♀	CM ♂/♀	Both ♂/♀
clave	AB: Jenner	50.842 -111.151	-	-	1/3
	AB: Jenner	50.844 -111.154	-	-	1/3
	AB: Spruce Grove	53.4 -113.9	-	-	-/3
	AB: Stettler Co.	52.154 -112.713	-	-	1/1
	MB: Brandon	49.83 -99.96	-	-/1	-
	ON: Leeds	44.487 -76.008	-	-	-/2
	ON: Waterloo	43.46 -80.52	-	-/1	-
	TOTAL		**-**	**-/2**	**3/12**
aureus	AB: Machesis Lake PRA	58.325 -116.578	-	-	-/1
	AB: nr. Tangent Park	56.092 -117.542	-	-	9/-
	TOTAL		**-**	**-**	**9/1**
brevipunctatus	ON: Carleton Co.	45.385 -76.008	-	-	-/1
	TOTAL		**-**	**-**	**-/1**
dombroskii	SK: near Newton Lake	49.301 -107.764	-	-	-/1
	TOTAL		**-**	**-**	**-/1**
keala	AB: Porcupine Hills	49.972 -114.087	-	-	1/5
	AB: 62 km NW Dixonville	56.691 -118.641	-	-	-/1
	AB: 62 km NW Dixonville	56.684 -118.644	-	-	-/1
	AB: 54 km NW Dixonville	56.96 -118.31	-	-	-/1
	TOTAL		**-**	**-**	**1/8**
importunus	AB: Winfield	53.01 -114.50	-	-	-/2
	ON: Bells Corners	45.295 -75.830	-	-	-/1
	TOTAL		**-**	**-**	**-/3**
idoneus	AB: 48 km NW Dixonville	56.753 -118.328	-	-	-/1
	AB: Bragg Creek	50.917 -114.533	-	-	-/1
	AB: Calgary	51.115 -114.142	-	-	-/1
	AB: Edmonton	53.510 -113.622	-/3	-	-
	AB: Edmonton	53.521 -113.521	-	-	-/1
	AB: Edmonton	53.527 -113.519	1/-	-	2/-
	AB: Edmonton	53.545 -113.439	2/7	-	2/2

Species	Provenance	Lat/Long	GM ♂/♀	CM ♂/♀	Both ♂/♀
idoneus (cont'd)	AB: Erskine	52.322 -112.883		-	-/1
	AB: George Lake	53.953 -114.120	1/1	-	-
	AB: George Lake	53.957 -114.125	11/14	-	2/1
	AB: George Lake	53.957 -114.128	-/2	-	-
	AB: Pigeon Lake	53.072 -114.072	-	-	1/2
	AB: Rochon Sands PP	52.46 -112.88	-	-	1/1
	AB: Sherwood Park	53.478 -113.229	-/1	-	-
	AB: Spruce Grove	53.4 -113.9	-	-	1/-
	AB: Winfield	53.01 -114.50	1/3	-	1/1
	BC: Robson	49.34 -117.70	-	2/2	-
	MB: Brandon	49.83 -99.96	-	-/1	-
	MB: Winnipeg	49.74 -97.13	-	-/1	-
	ON: Bells Corners	45.272 -75.808	-	-	2/1
	ON: Grenadier Island	44.4 -75.9	-	-	1/-
	ON: Leeds	44.487 -76.008	-	-	-/1
	ON: Ottawa	45.356 -75.707	-	-	2/2
	SK: Saskatoon	52.1 -106.6	-	-/1	-
	TOTAL		**16/31**	**2/5**	**15/16**
A	AB: Winfield	53.01 -114.50	-	-	-/1
B	AB: Calgary	51.115 -114.142	-	-	-/1
C	AB: Beaver Mines Lake	49.371 -114.295	-	-	-/1
D	AB: nr. Tangent Park	56.092 -117.542	-	-	1/-
E	AB: George Lake	53.957 -114.125	-	-	1/-

Table 4-2. Morphometric variables included in the classical morphometric analysis of the *O. scutellaris* species-group.

Variable	Description	Details
FW/FM	Face width / face maximum	Face width: in frontal view between the inner eye margins at the level of the clypeal foveae; Face maximum: widest part of the face between the maximum indentation of the eyes
FW/HW	Face width / head width	Head width: across the widest part of the eyes in frontal view
FW/FH	Face width / face height	Face height: from the apex of the clypeus to the bottom of the facial tubercle in frontal view
MS/MW	Malar space / mandible width	Malar space: shortest distance between the eye and the base of the mandible; Mandible width: measured at the base
IOD/OL	Interocellar distance / ocellus length	Interocellar distance: shortest distance between the posterior ocelli in dorsal view; Ocellus length: maximum length of the posterior ocellus
OOD/OL	Ocellar-ocular distance / ocellus length	Ocellar-ocular distance is the shortest distance from the eye margin to the deepest part of the sulcus adjacent to the posterior ocellus
SL/SW	Scutellum length / scutellum width	Scutellum length: from the base of the scutellum to the apical scutellar carina; Scutellum width: across the base of the scutellum between the inner margins of the lateral scutellar carinae
TB/TS	Tergite 1 basal width / tergite 1 spiracle width	The width of the first tergite measured in dorsal view at the base and at the level of the spiracles
TS/TA	Tergite 1 spiracle width / tergite 1 apical width	The width of the first tergite measured in dorsal view at the level of the spiracles and at the apex
FL/FW	Hind femur length / hind femur width	Femur length: midpoint of the base of the hind femur to midpoint of the apex, in lateral view; Femur width: maximum lateral width of the hind femur
cu-a	cu-a above Cu1 / cu-a below Cu1	For most specimens this was measured in ImageJ 1 46r (Rasband 1997-2012) from the photographs taken for the wing geometric morphometric analysis. For those specimens not photographed (11 specimens), this was measured using an ocular micrometer
Wing L	Length of the forewing	For most specimens, this was measured in ImageJ from the photographs used in the wing geometric morphometric analysis; photographs were calibrated for size using the known size of the coverslip on the slide-mount; for those specimens not photographed for the wing analysis, wing length was measured using a calibrated ocular micrometer
Flag	Number of flagellomeres	3 specimens of *O. idoneus* (1 male and 2 females) had broken antennae. The number of flagellomeres for these specimens was estimated by averaging the number of flagellomeres for all remaining O. idoneus of the respective sex. One female specimen of an unknown species had broken antennae; the number of flagellomeres was thus estimated by averaging the number of flagellomeres of all female specimens of a similar size (i.e. excluding *O. aureus* and *O. keala*).

Table 4-3. Summary of statistics from maximum likelihood (ML) and maximum parsimony (MP) analyses of COI, ITS2 and 28S genetic markers.

Analysis	Statistics	COI	ITS2	28S
ML	Log likelihood	-3545.81	-4076.72	-1429.26
MP	Tree Length	579	467	75
	No. MP trees	8	10	10
	CI/RI	0.55/0.80	0.90/0.93	0.88/0.78

Table 4-4. K2P sequence divergence of COI and ITS2 between and within species. COI divergences are below the diagonal, ITS2 divergences are above the diagonal, and intraspecific distances are along the diagonal (COI/ITS2). *O. scutellaris* B was only sequenced for COI.

	idoneus	*clave*	*aureus*	*brevipunctatus*	*dombroskii*	*keala*	*importunus*	*scutellaris* A
idoneus	**0.046/0**	1.77	2.05	2.29	1.87	0.09	0.92	0.46
clave	9.43	**0.237/0**	0.28	0.51	0.10	1.68	2.08	1.59
aureus	9.85	3.28	**0.450/0**	0.61	0.31	1.96	2.37	1.85
brevipunctatus	8.46	3.20	2.20	**n/a**	0.30	2.19	2.6	2.09
dombroskii	9.63	3.34	2.28	1.05	**n/a**	1.78	2.19	1.67
keala	4.95	9.57	9.72	8.55	9.28	**0.396/0.026**	0.83	0.37
importunus	2.40	9.12	9.36	7.65	8.80	4.94	**n/a**	0.77
scutellaris A	7.12	9.36	9.50	9.11	9.63	5.44	7.24	**0/0.103**
scutellaris B	6.86	8.86	8.86	8.51	8.82	5.62	7.01	3.61

Table 4-5. Summary of measurements of 13 morphometric variables for females of the *O. scutellaris* species group. Morphometric variables are defined in the text. The mean ± standard deviation for each variable is given, followed by the range. A, B, C refer to unplaced specimens.

Variable	*clave* n = 13	*aureus* n = 1	*brevipunctatus* n = 1	*dombroskii* n = 1	*keala* n = 8	*importunus* n = 3	*idoneus* n = 21	**A** n = 1	**B** n =1	**C** n = 1
FW/FM	0.83±0.02 *0.81-0.85*	0.83	0.79	0.84	0.81±0.02 *0.78-0.84*	0.82±0.01 *0.82-0.83*	0.86±0.01 *0.84-0.89*	0.88	0.87	0.83
FW/FH	1.23±0.08 *1.19-1.26*	1.37	1.27	1.34	1.33±0.09 *1.24-1.49*	1.26±0.02 *1.25-1.29*	1.37±0.05 *1.28-1.45*	1.30	1.29	1.29
FW/HW	0.52±0.02 *0.50-0.54*	0.51	0.47	0.53	0.48±0.01 *0.46-0.50*	0.50±0.00 *0.49-0.50*	0.53±0.01 *0.51-0.55*	0.54	0.54	0.51
MS/MW	0.51±0.10 *0.43-0.63*	0.60	0.50	0.58	0.40±0.04 *0.33+0.47*	0.51±0.06 *0.46-0.58*	0.59±0.04 *0.54-0.70*	0.60	0.58	0.54
IOD/OL	0.77±0.08 *0.53-1.00*	0.71	0.69	1.20	0.36±0.06 *0.27-0.44*	0.72±0.16 *0.53-0.85*	0.74±0.08 *0.61-0.86*	1.00	0.73	0.71
OOD/OL	0.22±0.05 *0.13-0.29*	0.29	0.13	0.45	0.16±0.04 *0.11-0.22*	0.19±0.04 *0.15-0.23*	0.33±0.04 *0.25-0.43*	0.32	0.20	0.21
SL/SW	1.43±0.17 *1.23-1.58*	1.18	1.48	1.76	1.63±0.10 *1.47-1.76*	1.58±0.03 *1.55-1.60*	1.52±0.09 *1.37-1.69*	1.39	1.52	1.45
TB/TS	0.80±0.11 *0.71-0.92*	0.70	0.80	0.78	0.63±0.06 *0.57-0.74*	0.78±0.04 *0.75-0.82*	0.80±0.06 *0.71-0.92*	0.82	0.88	0.63
TS/TA	0.61±0.07 *0.58-0.67*	0.69	0.60	0.64	0.76±0.06 *0.57-0.74*	0.66±0.02 *0.64-0.68*	0.67±0.06 *0.59-0.79*	0.64	0.53	0.70
FL/FW	7.82±0.95 *7.00-8.59*	7.95	9.50	7.24	10.26±1.09 *8.41-11.63*	8.58±0.31 *8.25-8.87*	6.87±0.26 *6.41-7.50*	7.56	7.89	7.79
Flag	55.08±5.82 *52-60*	65	67	51	70.63±1.30 *69-73*	55.00±1.73 *54-57*	54.05±1.93 *51-57*	52	59	59
cu-a	0.69±0.11 *0.60-0.84*	0.88	0.66	0.83	1.08±0.13 *0.89-1.26*	0.72±0.09 *0.66-0.83*	0.83±0.12 *0.68-1.13*	0.62	0.68	0.64
Wing L	11.83±2.76 *10.49-12.44*	16.69	14.04	10.80	19.06±0.45 *18.64-19.73*	12.92±0.38 *12.57-13.33*	10.13±0.64 *8.98-11.48*	10.70	12.86	12.70

Table 4-6. Summary of measurements of 13 morphometric variables for males of the *O. scutellaris* species group. Morphometric variables are defined in the text. The mean ± standard deviation for each variable is given, followed by the range. D, E refer to unplaced specimens.

Variable	*clave* n = 3	*aureus* n = 9	*keala* n = 1	*idoneus* n = 17	D n =1	E n = 1
FW/FM	0.85±0.02 *0.83-0.87*	0.84±0.01 *0.82-0.89*	0.86	0.89±0.02 *0.85-0.91*	0.83	0.91
FW/FH	1.22±0.04 *1.18-1.26*	1.33±0.05 *1.25-1.41*	1.41	1.40±0.03 *1.32-1.45*	1.23	1.39
FW/HW	0.50±0.02 *0.49-0.52*	0.52±0.01 *0.50-0.54*	0.50	0.53±0.01 *0.51-0.56*	0.50	0.55
MS/MW	0.48±0.06 *0.43-0.54*	0.74±0.05 *0.65-0.80*	0.39	0.65±0.08 *0.50-0.82*	0.58	0.70
IOD/OL	0.79±0.13 *0.67-0.92*	0.74±0.05 *0.65-0.80*	0.44	0.87±0.16 *0.58-1.25*	1.04	0.90
OOD/OL	0.23±0.03 *0.20-0.27*	0.30±0.02 *0.26-0.33*	0.44	0.48±0.14 *0.35-0.92*	0.33	0.40
SL/SW	1.54±0.12 *1.43-1.67*	1.23±0.08 *1.13-1.38*	1.74	1.44±0.12 *1.21-1.68*	1.68	1.44
TB/TS	0.79±0.04 *0.76-0.84*	0.64±0.08 *0.54-0.78*	0.63	0.77±0.07 *0.61-0.87*	0.79	0.75
TS/TA	0.65±0.06 *0.61-0.71*	0.72±0.04 *0.67-0.78*	0.79	0.73±0.07 *0.62-0.90*	0.67	0.80
FL/FW	7.08±0.09 *7.00-7.18*	8.72±0.54 *8.11-9.63*	9.03	6.95±0.43 *7.85±6.31*	7.13	7.14
Flag	54.67±1.53 *53-56*	66.78±1.56 *65-69*	65	54.69±1.74 *51-57*	54	52
cu-a	0.69±0.09 *0.59-0.74*	0.69±0.10 *0.51-0.83*	1.43	0.97±0.22 *0.72-1.53*	0.76	0.96
Wing L	10.78±0.11 *10.65-10.85*	15.55±0.94 *14.37-17.07*	16.23	9.45±0.56 *8.42-10.74*	9.91	8.91
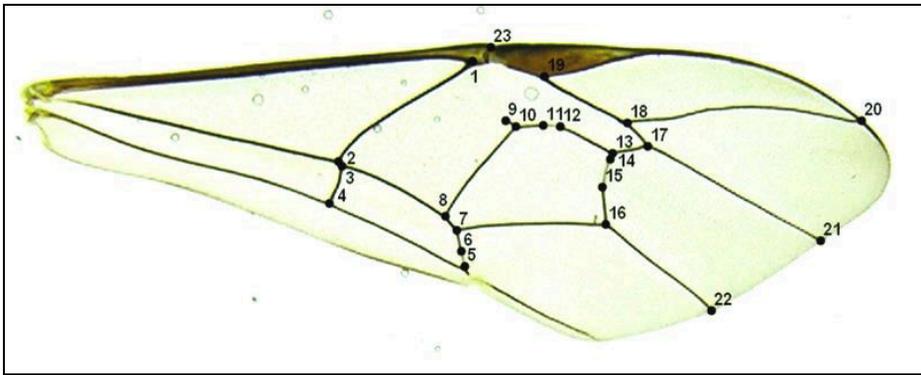
Figure 4-1. Locations of 23 landmarks used for geometric morphometrics analysis of *Ophion scutellaris* species-group forewings
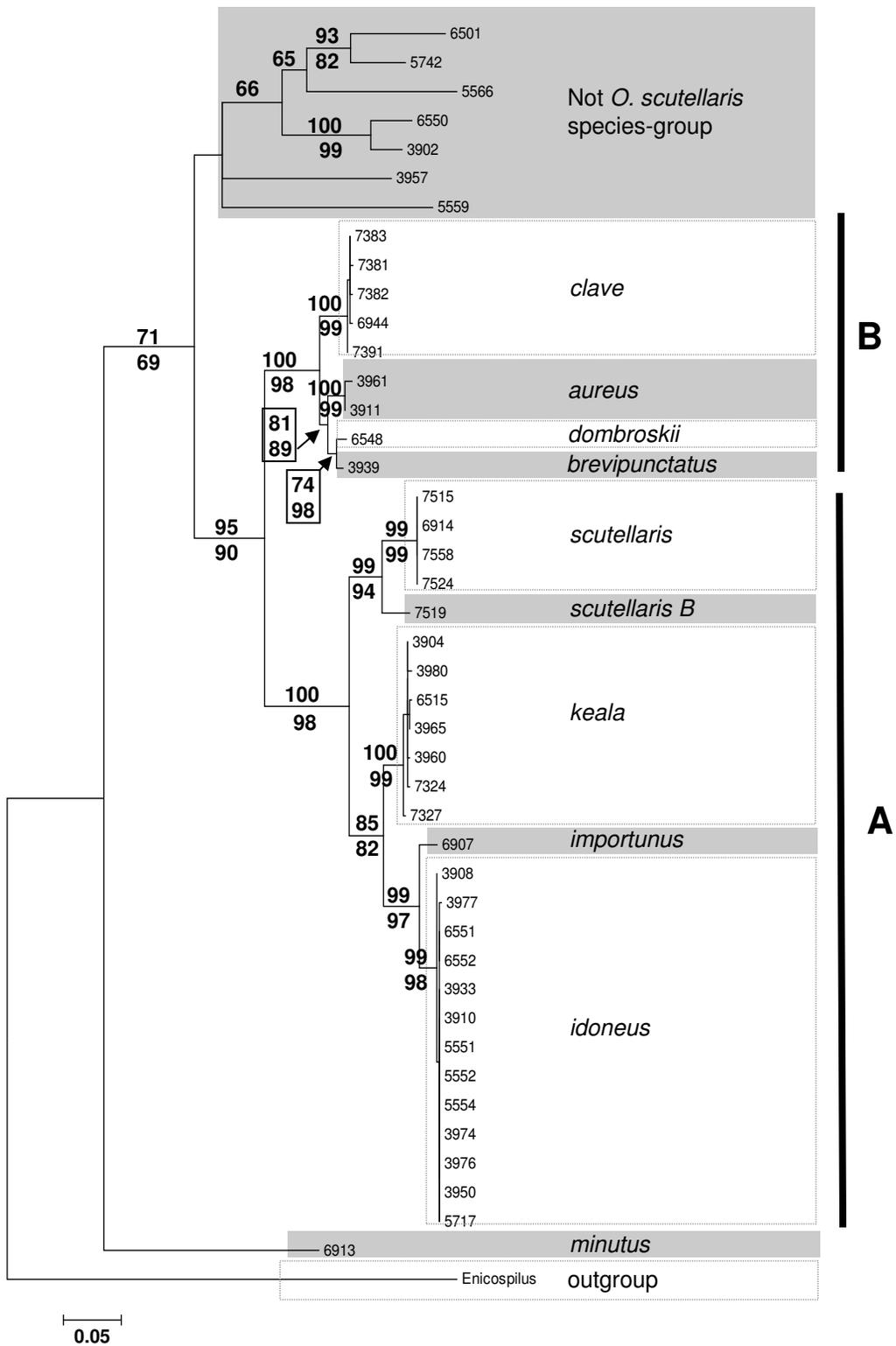
Figure 4-2a. Maximum likelihood tree of COI sequences. Maximum likelihood bootstrap support values are above branches and maximum parsimony bootstrap values are below branches.

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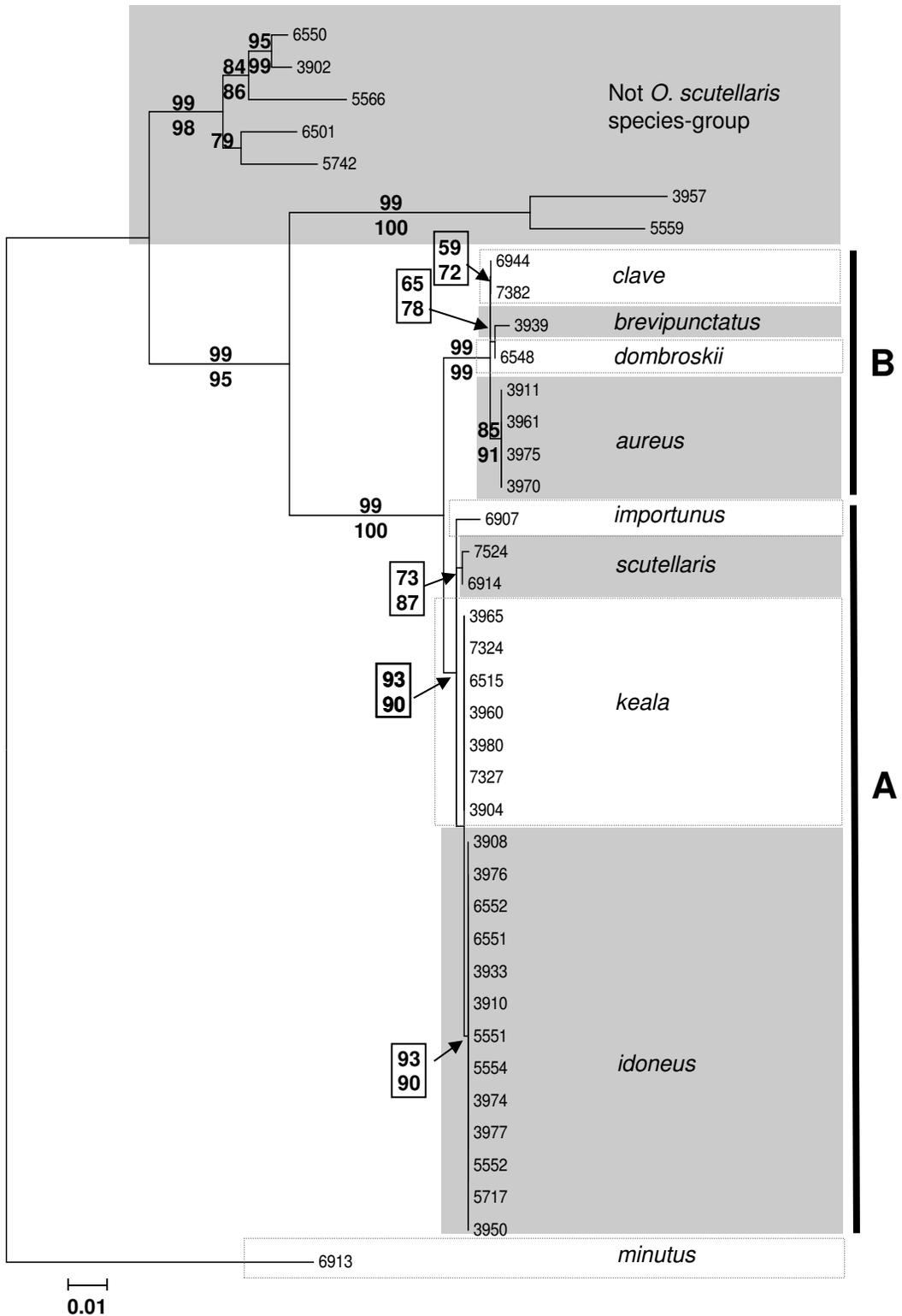
Figure 4-2b. Maximum likelihood tree of ITS2 sequences. Maximum likelihood bootstrap support values are above branches and maximum parsimony bootstrap values are below branches.
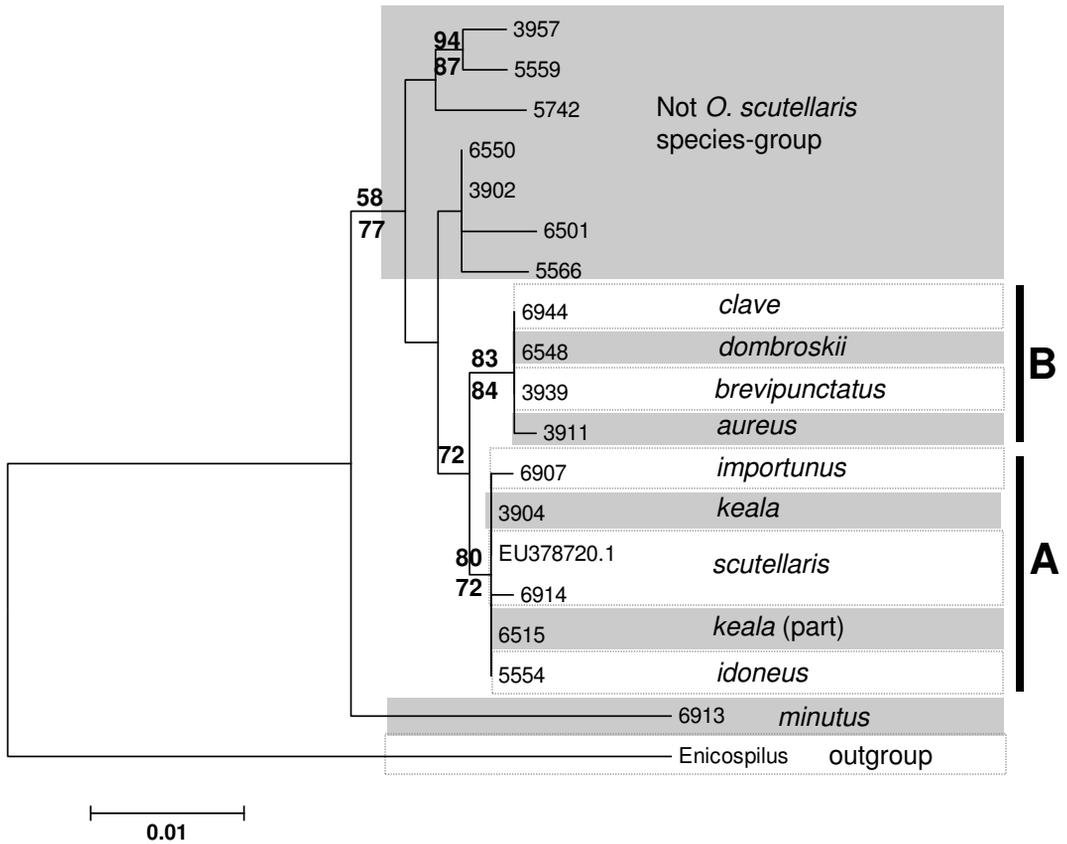
Figure 4-2c. Maximum likelihood tree of 28S sequences. Maximum likelihood bootstrap support values are above branches and maximum parsimony bootstrap values are below branches.
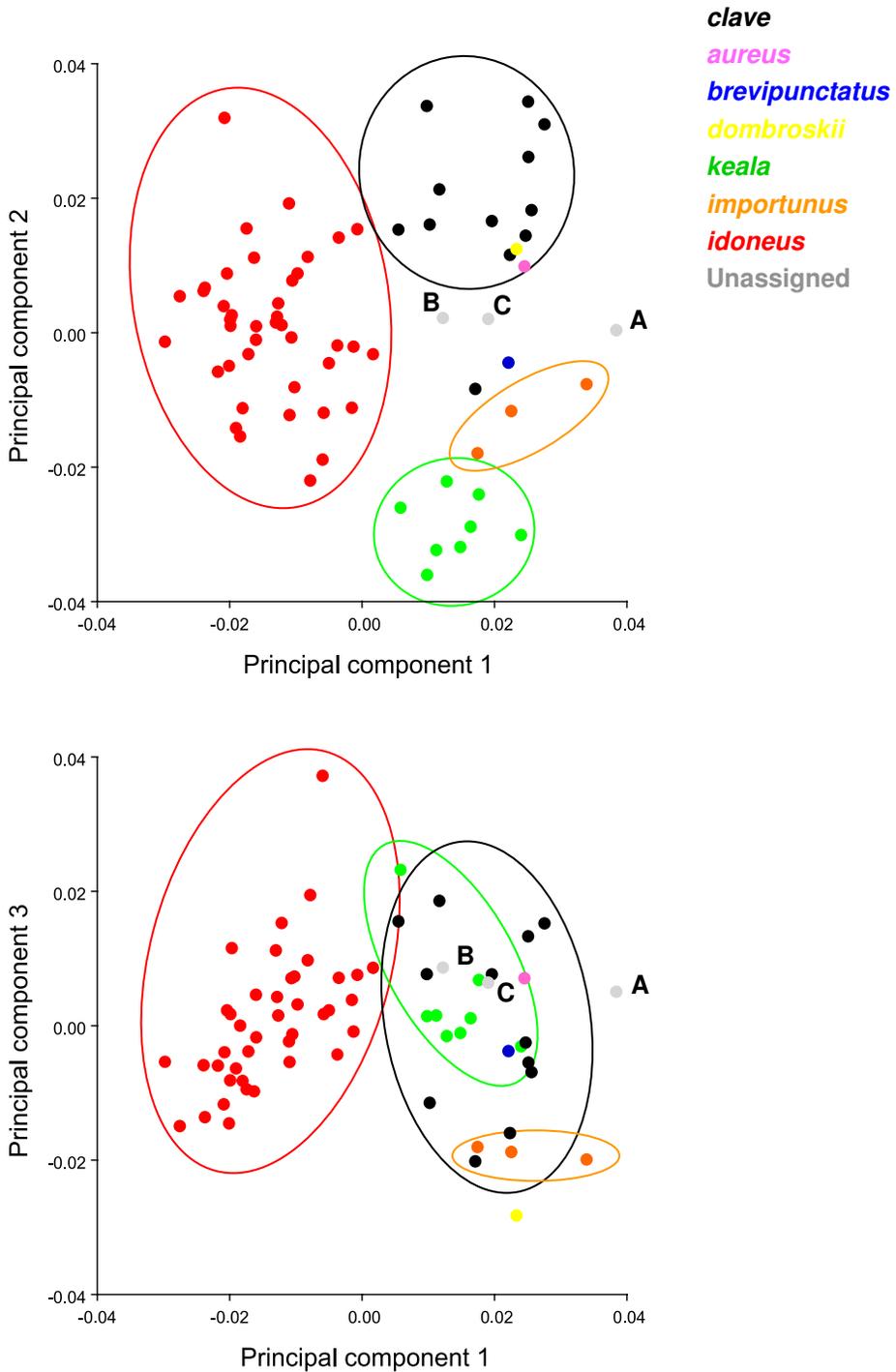
Figure 4-3a. Principal component analysis of 23 forewing landmarks for females of the *O. scutellaris* species-group. A, B, and C refer to unassigned specimens (see text).

Figure 4-3b. Principal component analysis of 23 forewing landmarks for males of the *O. scutellaris* species-group. D and E refer to unassigned specimens (see text).
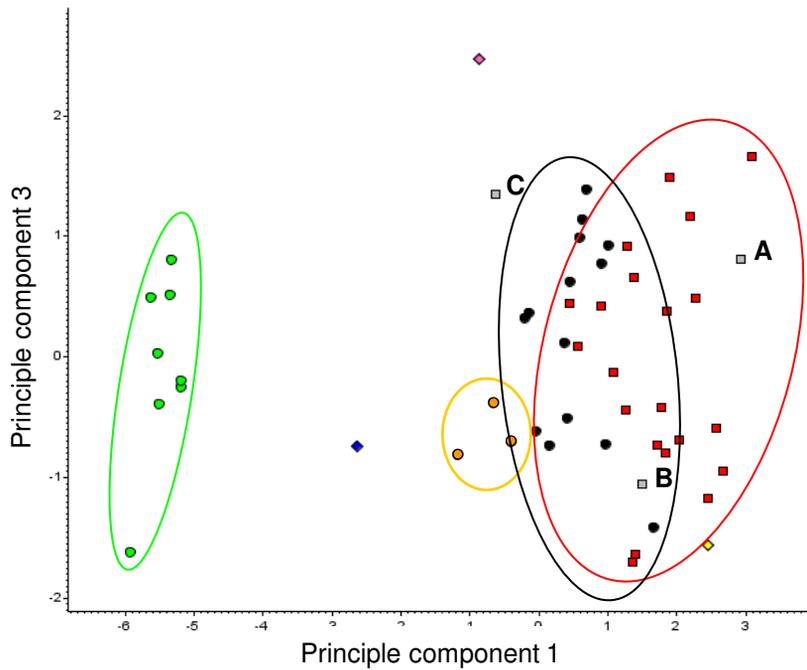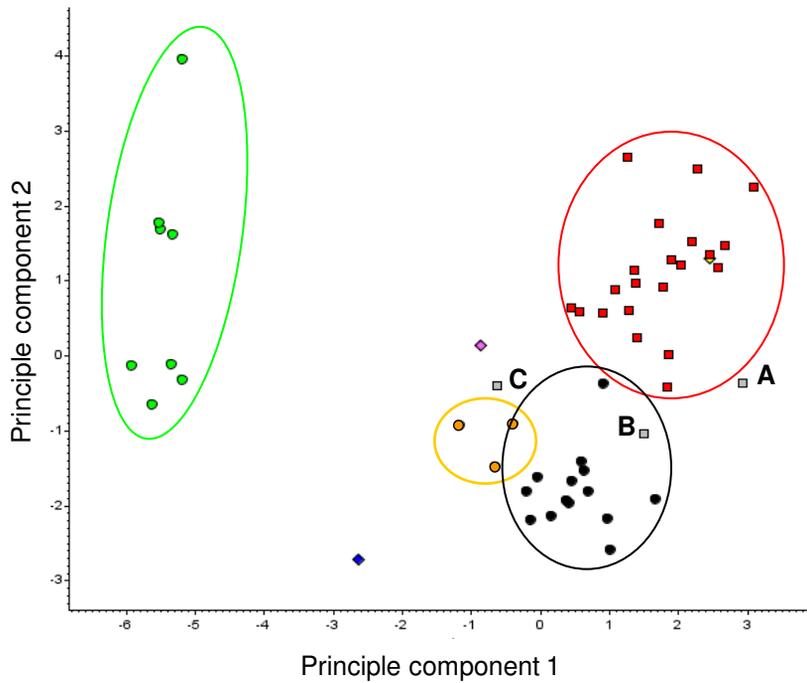
Figure 4-4a. Principal component analysis of 13 morphometric characters for females of the *O. scutellaris* species-group. A, B, and C refer to unassigned specimens (see text).
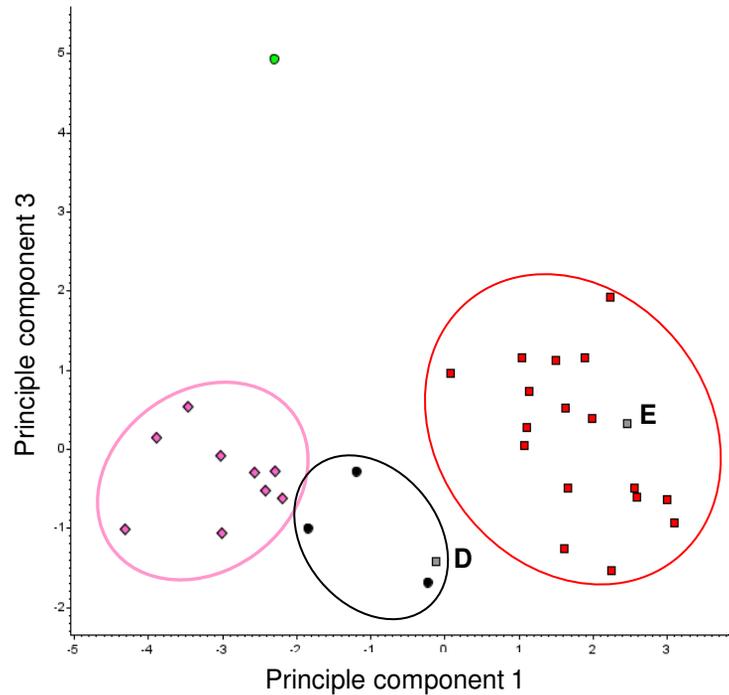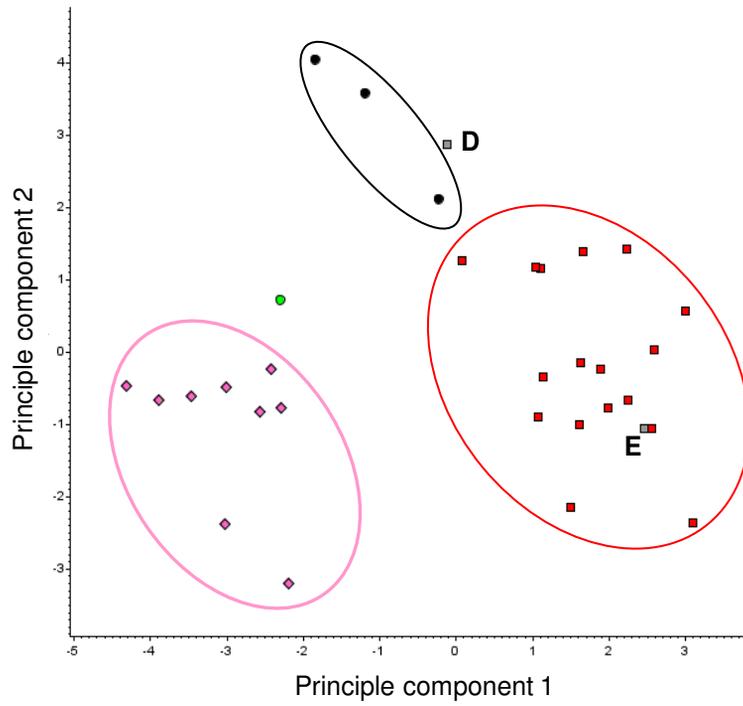
Figure 4-4b. Principal component analysis of 13 morphometric characters for males of the *O. scutellaris* species-group. D and E refer to unassigned specimens (see text).

Figure 4-5. Propodeum of *Ophion* demonstrating propodeal carinae and areas. Carinae: ATC = anterior transverse carina; PTC = posterior transverse carina; MLC = median longitudinal carinae; LLC = lateral longitudinal carina; PC = pleural carina; Areas: ASu = area superomedia; AP = area petiolaris; AD = area dentiparis; APE = area postero-externa; APL = area postero-lateralis
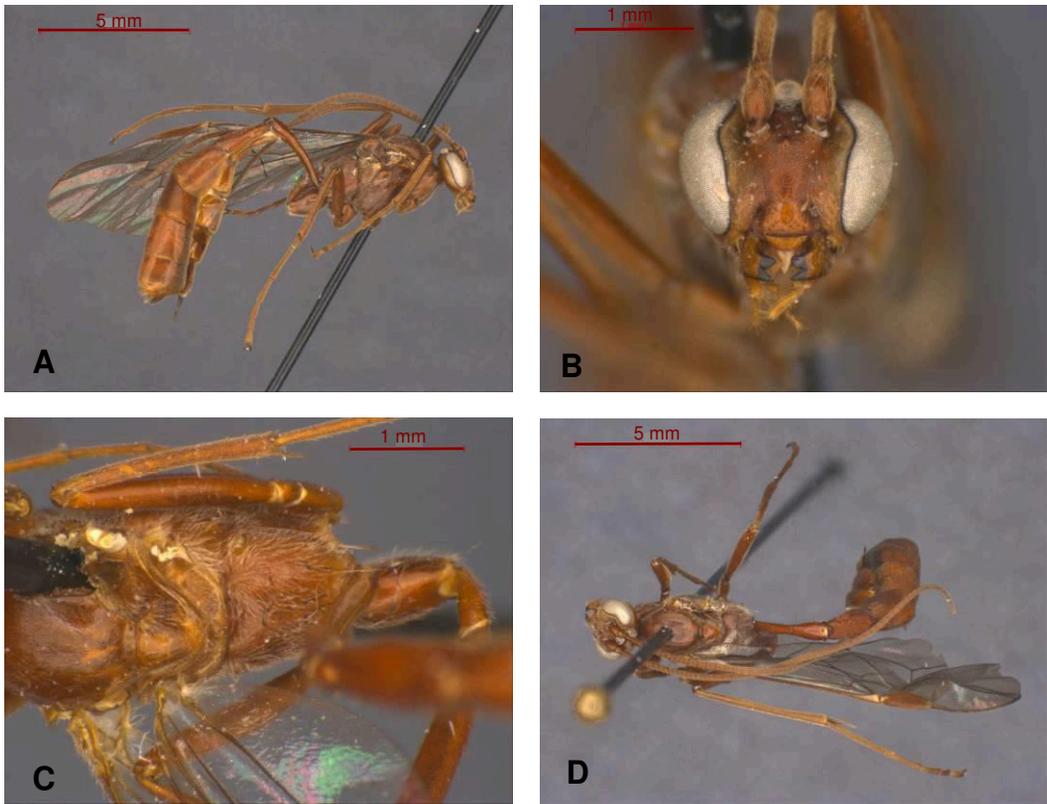
Figure 4-6. *Ophion clave* sp.n. **A:** lateral **B:** face and clypeus **C:** propodeum and scutellum **D:** dorsal
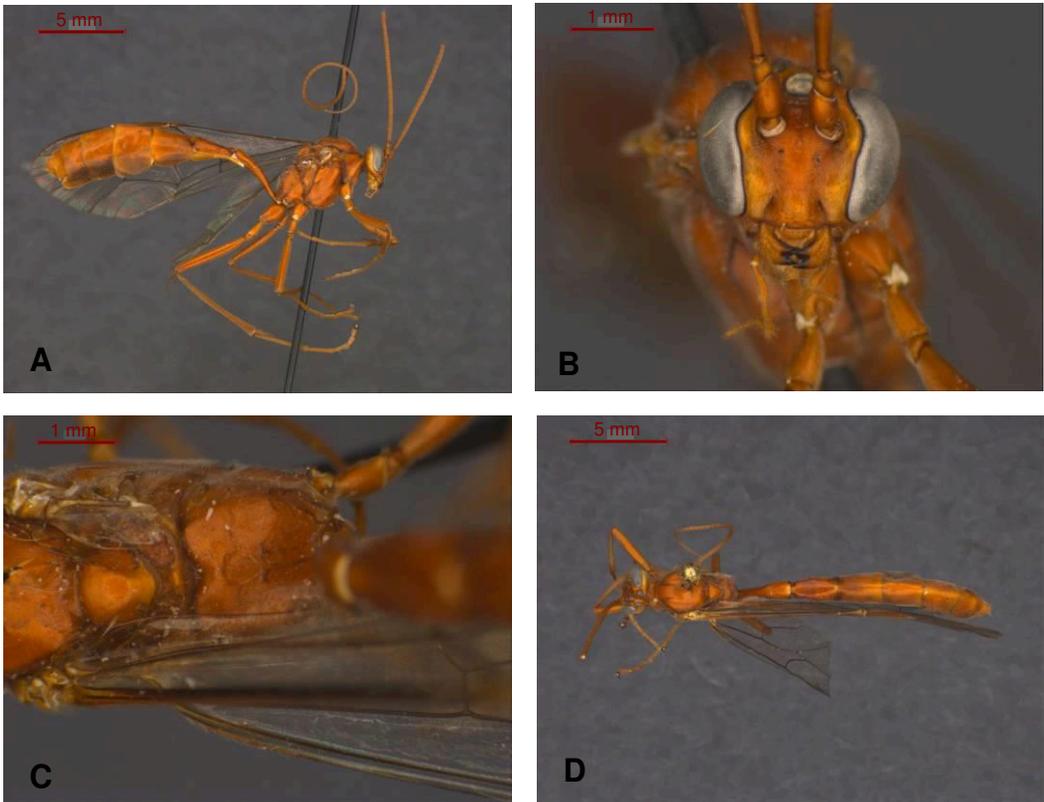
Figure 4-7. *Ophion aureus* sp.n. **A:** lateral **B:** face and clypeus **C:** propodeum and scutellum **D:** dorsal
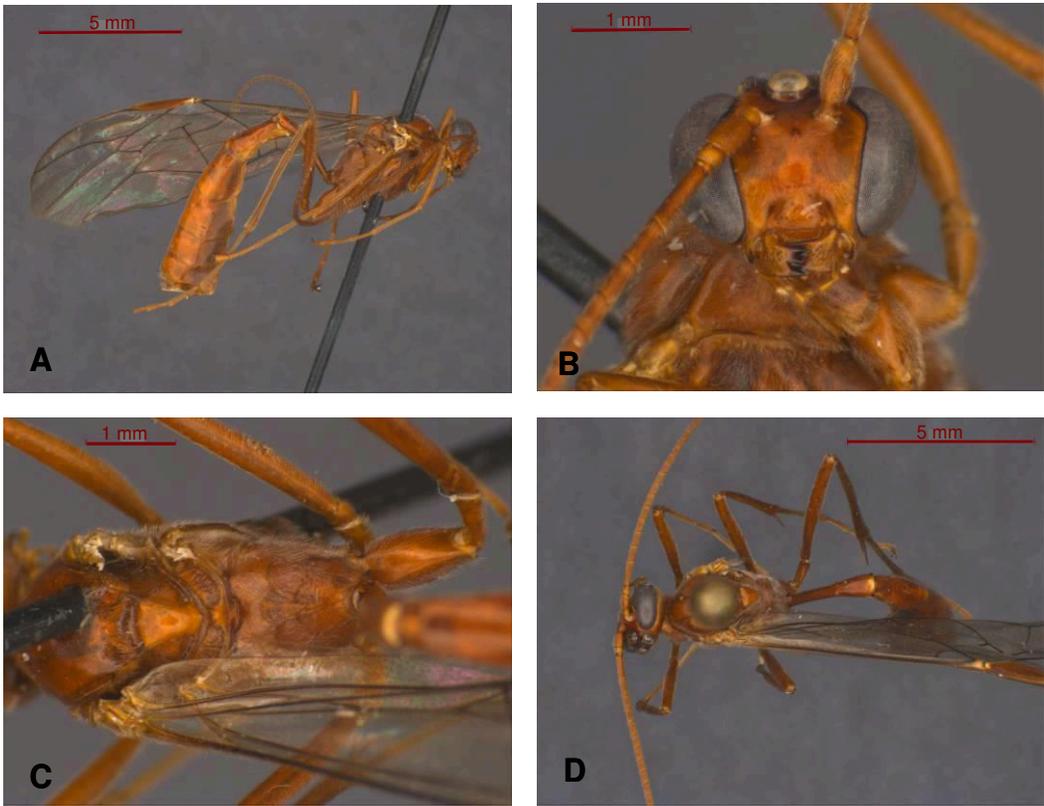
Figure 4-8. *Ophion brevipunctatus* sp.n. **A:** lateral **B:** face and clypeus
C: propodeum and scutellum **D:** dorsal
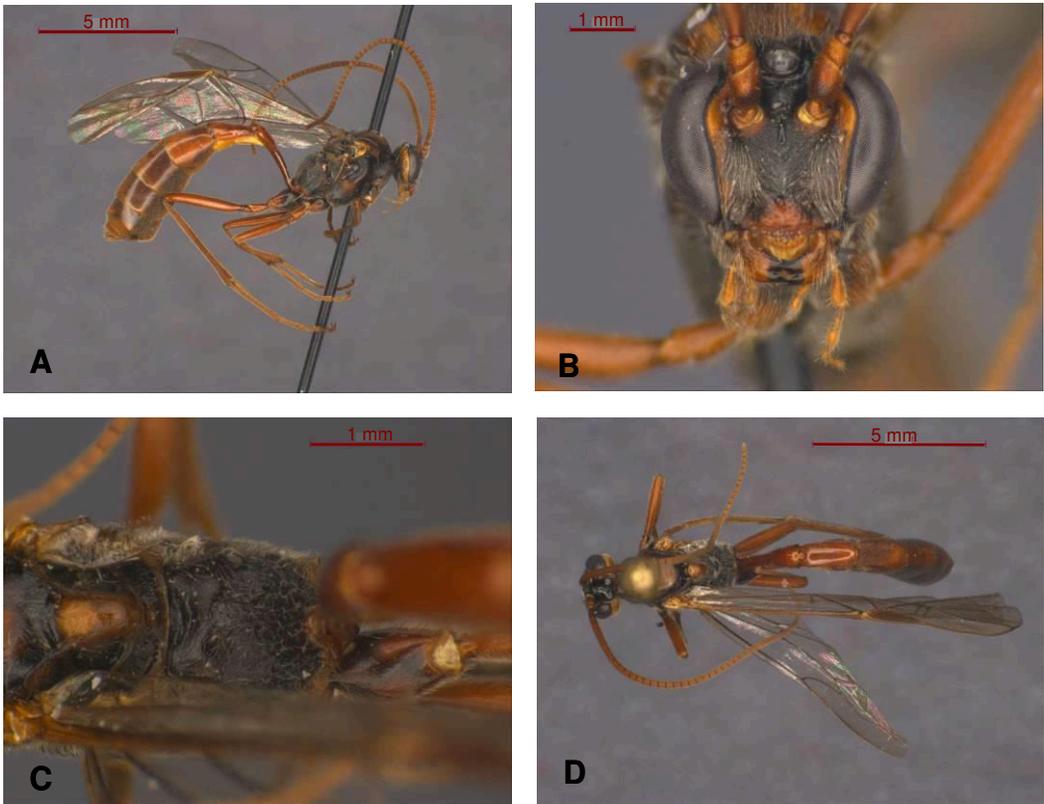
Figure 4-9. *Ophion dombroskii* sp.n. **A:** lateral **B:** face and clypeus
C: propodeum and scutellum **D:** dorsal
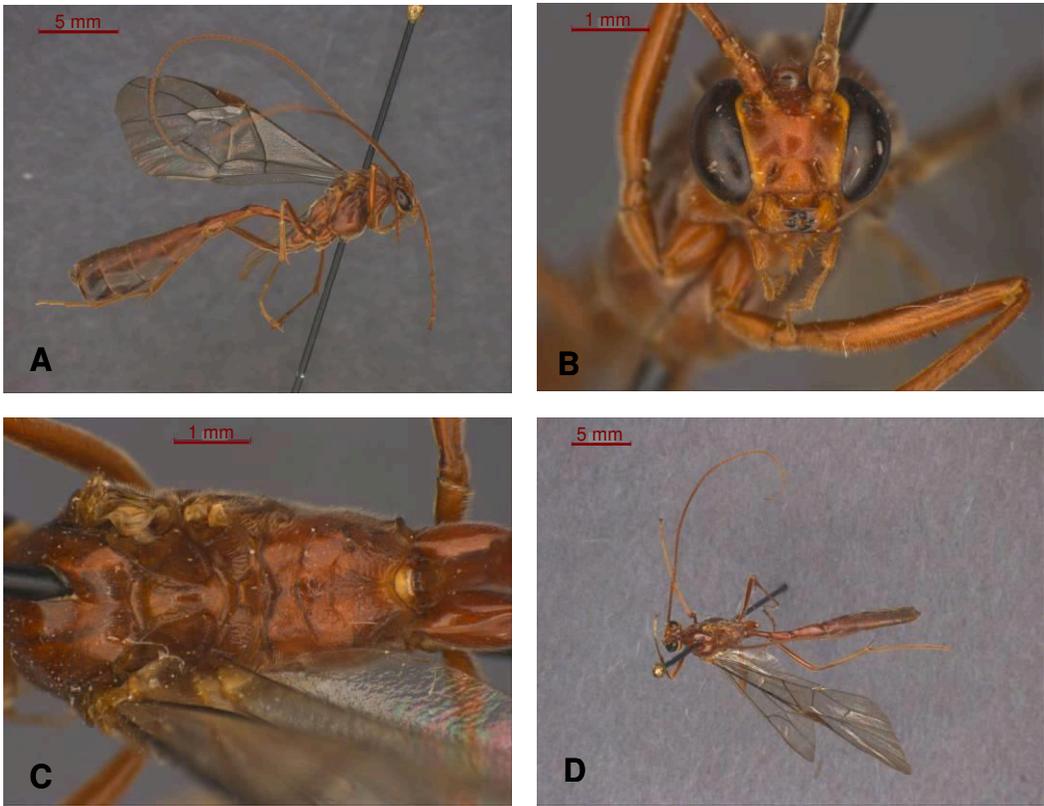
Figure 4-10. *Ophion keala* sp.n. **A:** lateral **B:** face and clypeus
C: propodeum and scutellum **D:** dorsal
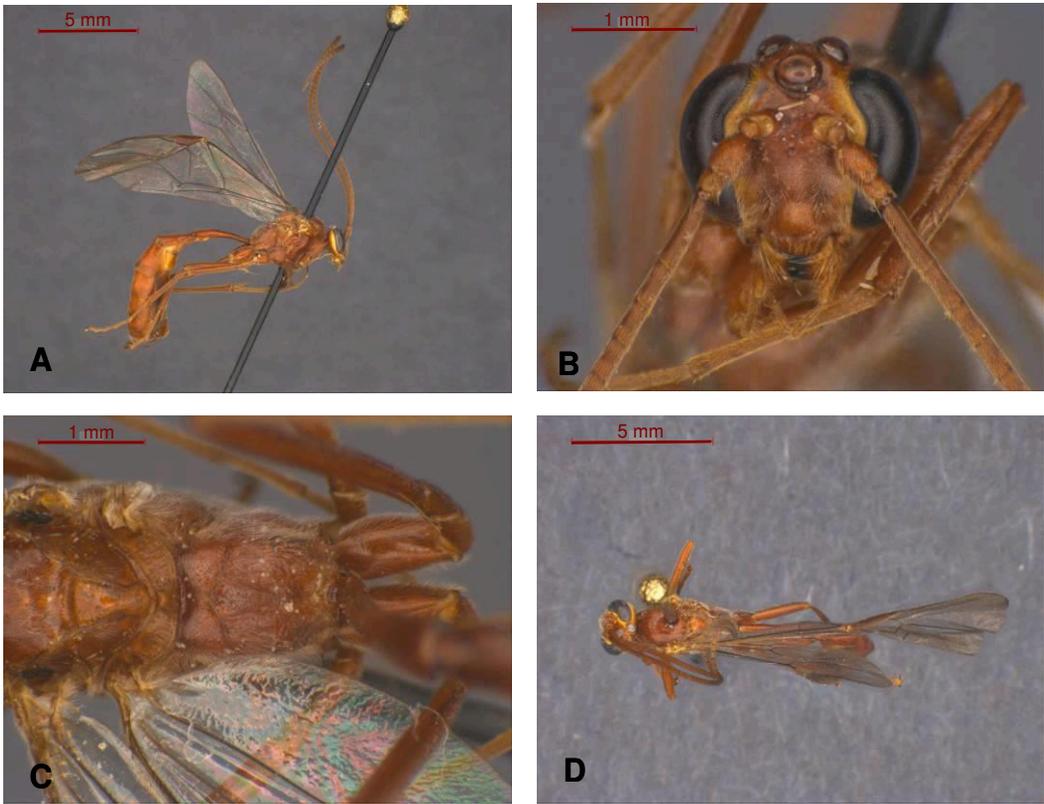
Figure 4-11. *Ophion importunus* sp. n. **A:** lateral **B:** face and clypeus **C:** propodeum and scutellum **D:** dorsal
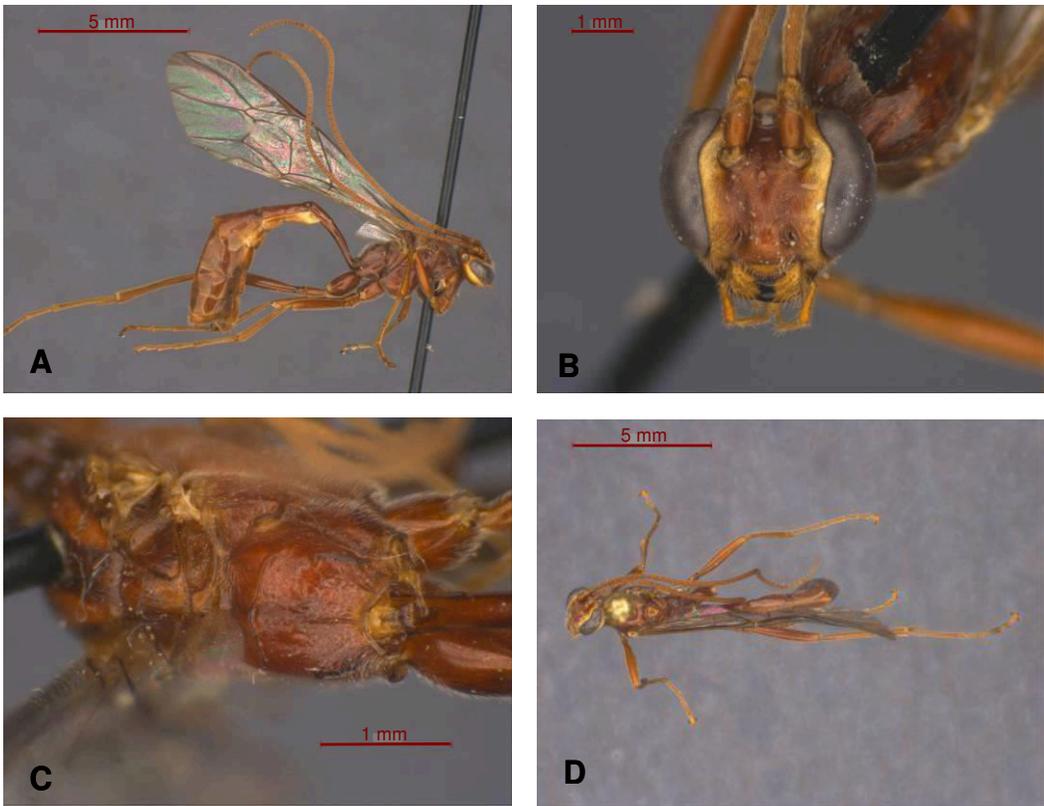
Figure 4-12. *Ophion idoneus* Vireck **A:** lateral **B:** face and clypeus **C:** propodeum and scutellum **D:** dorsal

Chapter 5

Pimplinae, Poemeniinae, and Rhyssinae (Hymenoptera, Ichneumonidae) of a boreal deciduous forest and the impact of variable retention harvesting

Introduction

        Arthropods are an essential part of any biodiversity assessment, due to their abundance, diversity and sensitivity to fine-grained changes in habitat quality (Kremen *et al.* 1993; Spence *et al.* 2008; McGeoch *et al.* 2011). However, these same characteristics make assessing arthropod communities one of the most challenging ways to study biodiversity (Langor and Spence 2006; Cardoso *et al.* 2011). Arthropod studies have therefore tended to focus on groups that can be relatively easily sampled and identified. For example, a disproportionate number of studies have focused on ground beetles (Niemelä *et al.* 1993; 2007; Work *et al.* 2008, 2010), saproxylic beetles (Jacobs *et al.* 2007; Johansson *et al.* 2007; McGeoch 2007; Langor *et al.* 2008), Lepidoptera (Summerville and Crist 2008) and spiders (Buddle *et al.* 2000; Buddle and Shorthouse 2008; Pinzon *et al.* 2012). Meanwhile some of the most speciose groups of arthropods have been largely ignored, due to a lack of identification resources (Nilsson *et al.* 2001; Langor and Spence 2006). These groups nevertheless have essential ecosystem functions that must be understood if we are to properly assess the impacts of land-use strategies.

        Arthropod surveys have frequently been used to inform forest management practices (e.g. Langor and Spence 2006; Maleque *et al.* 2006). Canada is no exception, with forest harvesting in Canada's boreal forests increasing at a rapid pace over the last three decades (Cumming *et al.* 1994; Burton *et al.* 2006). The primary method of harvesting has traditionally been clearcutting; however this has been criticized for its large-scale forest disturbance and impact on biodiversity and ecological processes (Schindler 1998; Kouki *et al.* 2001; Spence 2001; Burton *et al.* 2006). In response to this criticism, there has been a shift in management goals for boreal forests; instead of simply considering them a source of timber, there is an increasing emphasis on intrinsic forest values

such as biodiversity (Simberloff 1999; Spence 2001; Wang 2004; Klenner *et al*. 2009). Less intensive and more heterogeneous harvesting has been proposed as a method for maintaining these values (Vanha-Majamaa and Jalonen 2001; Rosenvald and Lõhmus 2008); however, studies are needed to determine whether they are in fact capable of preserving biodiversity and ecosystem processes (Spence 2001; Spence *et al*. 2008).

Parasitic Hymenoptera (parasitoids) are a particularly important component of forest ecosystems. All parasitoids complete their development by feeding internally or externally on a host arthropod, eventually killing it (Gauld and Bolton 1988). They play a large role in the regulation of potential pest species, and may be an important driver of diversity patterns in other organisms (Shaw and Hochberg 2001; LaSalle and Gauld 1993). Because of their highly specialized life histories and high trophic level, they may also be particularly vulnerable to ecological disturbances (Hochberg *et al*. 1998; LaSalle and Gauld 1991; Shaw and Hochberg 2001; Thies *et al*. 2003). As well, parasitoids do not always respond to habitat fragmentation in the same manner as their hosts (Price 1991; Roland and Taylor 1997; Jonsell *et al*. 1999; Anton *et al*. 2007), which can potentially increase outbreaks of herbivorous insects (Roland and Taylor 1997). The importance of parasitoids of saproxylic insects in forest ecosystems has recently been recognized (Hilszczański *et al*. 2005; Stenbacka *et al*. 2010; Ulyshen *et al*. 2011); however, they constitute just a small proportion of the total parasitoid fauna in forests.

Ichneumonidae are the largest parasitic hymenopteran family, with an estimated world fauna of over 100,000 species (Gauld 2002). Some estimates suggest that it may in fact be the most species-rich insect family on the planet (Owen *et al*. 1981; Gauld 1991). There are currently 38 recognized subfamilies of Ichneumonidae (Quicke *et al*. 2009) and life-history strategies vary widely both within and between subfamilies, although the most common hosts are Lepidoptera and Symphyta (Wahl 1993).

Within Ichneumonidae, Pimplinae are of particular interest, as they may be the subfamily with the largest range in hosts and life-histories. Pimplinae

include koinobionts and idiobionts, endoparasitoids and ectoparasitoids, and solitary and gregarious species (Gauld *et al*. 2002). The majority of species attack concealed hosts (in plant tissue, silk cocoons or wood), although some free-living hosts are also used. Orders parasitized include Lepidoptera, Hymenoptera, Coleoptera, and Araneae (Gauld *et al*. 2002). Two additional subfamilies (Poemeniinae and Rhyssinae) are primarily parasitoids of wood-boring insects and were historically included within the Pimplinae. Together, these three subfamilies are among the best known and most easily identified Ichneumonidae, and they are the group that has most commonly been used in ichneumonid biodiversity research (e.g. Fraser *et al*. 2007, 2008; Gaston and Gauld, 1993; Sääksjärvi *et al*. 2004). Nevertheless, even though keys to most species exist, this group has rarely been surveyed in Nearctic boreal habitats (but see Finnamore 1994).

One obstacle to including parasitoids in biodiversity studies is the difficulty in identifying parasitic wasps to the species level. Where parasitoids have been included in biodiversity surveys in North America, this has generally been overcome by assessing them at higher taxonomic levels, such as to family or subfamily (e.g. Deans *et al*. 2005; Vance *et al*. 2007; Rohr *et al*. 2009). However analyses of species may show significantly different results from analyses at higher taxonomic levels, and the value of doing biodiversity assessments above the species level is controversial (Spence *et al*. 2008; Timms *et al*. 2012).

The goal of this study is to a) provide a baseline survey of Ichneumonidae (Pimplinae, Poemeniinae, and Rhyssinae) in a boreal deciduous forest; b) investigate the impact of forest harvesting at various intensities (10 years post-harvest) on the Ichneumonidae community; and c) determine whether species-level community patterns of parasitoids are correlated with patterns at a higher taxonomic level.

Methods

Study Area and Sampling Design

This study was conducted in 2008 at the EMEND (Ecosystem Management Emulating Natural Disturbance) research site, approximately 90 km northwest of Peace River, Alberta (56°46'13"N, 118°22'28"W). EMEND is a large-scale research project, located within Alberta's boreal mixedwood forest, designed to examine the effects of variable-retention harvesting on forest biology and dynamics. The dominant tree species are balsam poplar (*Populus balsamifera* L.), trembling aspen (*Populus tremuloides* Michx), and white spruce (*Picea glauca* (Moench) Voss). While succession is variable depending on many factors, the general pattern is for deciduous-dominated forests to be gradually replaced by spruce-dominated stands in the absence of disturbance (Work *et al.* 2004).

All sampling for this study was conducted in deciduous-dominated stands (i.e. greater than 70% of the pre-harvest canopy was deciduous). Four harvest treatments were included: 0-2% retention (clearcut), 20% retention, 50% retention and unharvested controls. All harvesting was completed in the winter of 1998; complete details of the harvesting treatments can be found in Spence *et al.* (1999) and Sidders and Luchkow (1998) and on the EMEND website (www.emendproject.org).

Four 10 ha experimental compartments, representing each of the harvesting levels, were selected within each of two independent stands (Stand 66 and 77), with one replicate of each treatment in each stand (Figure 5-1). Since field logistics precluded the addition of additional replicates, the comparison between treatments is qualitative and preliminary; it nevertheless provides the first baseline data to guide further studies into the influence of boreal forest harvesting on Ichneumonidae communities.

Samples were collected using black and white Townes-style Malaise traps (Sante Traps, Lexington, KY). Two traps were used in each compartment for a total of 16 traps. Traps were located near two of the six permanent randomly-selected stations that are part of the larger EMEND experiment. Selected stations were at least 150 m from the edge of the compartment, at least 50 m from any

retention patches and at least 100 m from each other. Coordinates for all traps are found in Table 5-1. Traps were run continuously from late May until late August 2008, and were emptied at 10-14 day intervals. There were some instances of trap disturbance, primarily due to bears, and a total of 8 samples were lost entirely (Table 5-1). In several other instances, there were varying amounts of trap damage, yet the traps still collected large numbers of specimens and were thus included in all analyses.

Identification and Curation

Arthropod specimens were collected into 95% ethanol in the Malaise traps, and transported to the lab in the same collecting liquid. In the lab, samples were transferred to containers filled with fresh 95% ethanol and sorted.

Ichneumonidae were identified to subfamily, primarily using Wahl (1993) and Broad (2006), and each subfamily was retained in a separate vial of 95% ethanol. During the peak of insect abundance, some of the larger samples were subsampled prior to sorting, due to time constraints. Subsamples were obtained by straining bulk specimens through 0.5 mm mesh. They were then weighed, and a certain percentage by weight (either 25%, 33%, or 50%, depending on the size of the sample) was placed in a separate container for later sorting (Table 5-1). A trial examination of both portions of several samples indicated that the subsamples were representative in terms of number of individuals in each ichneumonid subfamily.

In order to not miss rare species, all Pimplinae, Poemeniinae, and Rhyssinae (from both the sorted and unsorted portions of each sample) were included in the species-level analyses. All individuals were pinned and identified to species or morphospecies using Townes and Townes (1960), with the exception of members of the genus *Delomerista*, which were identified using Gupta (1982). There is currently no key to male *Delomerista*. Since males could not be associated with females, male *Delomerista* were excluded from rarefaction curves and all *Delomerista* spp. were treated as a single taxon in multivariate analyses. A single male specimen of *Scambus* represents either *S. deceptor* or *S. granulosus*,

but could not be confidently assigned to either; it was thus excluded from all analyses. Identifications were confirmed by examination of specimens at the Canadian National Collection, Ottawa, ON. Taxonomic names follow Yu *et al*. (2012), with the exception of the "Polysphinctini" where the names follow Gauld and Dubois (2006). Representative voucher specimens have been deposited in the E. H. Strickland Entomological Museum, University of Alberta, Edmonton, AB. For the remainder of the paper, Pimplinae, Poemeniinae, and Rhyssinae will be collectively referred to as "pimplines", whereas "Pimplinae" will be used for the specific subfamily.

Data analysis

Pimpline species richness was compared between the four harvesting treatments and between the two stands using individual-based rarefaction in EcoSim Version 7 (Gotelli and Entsminger 2009), with the abundance of each species pooled over the entire collecting period. Rarefaction is a method used to standardize expected species richness by sampling effort (Buddle *et al*. 2005; Magurran 2004). Since the efficiency of Malaise traps is highly dependent on many factors, including sun direction, insect flight paths, and wind direction (Darling and Packer 1988), and some samples were incomplete due to damage by bears, this method was used to compare relative species richness at an equal sampling effort in terms of number of individuals. Significant differences in species richness were approximated by visually determining the overlap in the 95% confidence intervals of the rarefaction curves (Buddle *et al*. 2005). Rarefaction curves are also used to assess whether a community has been thoroughly sampled (in which case the curve will reach an asymptote), or whether there are still many species remaining to be discovered (the curve continues to rise steeply) (Gotelli and Colwell 2001; Magurran 2004; Buddle *et al*. 2005). A rarefaction curve was therefore calculated for all samples combined.

For both the species-level and subfamily data, I examined the abundance (standardized by the number of trap-days) and the relative proportion of each taxon. The advantage of using abundance data is that the abundance of one taxon

in the sample does not affect that of other taxa. However proportional data prevents the analysis from being unduly influenced by the overall differences in abundances between samples (whether due to trap efficiency, trap damage, or true ecological differences between sites). For the subfamily dataset, the standardized abundance was extrapolated based on the proportion of each sample that was identified (25%, 33%, 50%, or 100%). To qualitatively compare the community composition at the subfamily and species level, the 17 most abundant subfamilies and 10 most abundant species were plotted by treatment. For the remainder of the analyses, the data were square-root transformed to decrease the influence of highly abundant species on the analysis (McCune and Grace 2002).

To explore community patterns in relation to harvesting, I performed non-metric multidimensional scaling (NMS) of the subfamily and pimpline datasets, using the program PC-ORD (McCune and Mefford 2006). NMS is a multivariate ordination technique that arranges samples in ordination space such that the rank-order correlation between the ordination distance and the distance measure is maximized. The amount of correlation is determined by calculating "stress", which is a measure of the discrepancy between the two distances (McCune and Grace 2002). NMS is well-suited to community data in that it makes few assumptions about the underlying relationships within the data (McCune and Grace 2002). I used a step-down approach to calculate the number of dimensions beyond which stress does not improve significantly (distance measure: Bray-Curtis; maximum 500 iterations, 250 runs with real data, 250 runs with randomized data, instability criterion: 0.0001). I then reran the analysis using the number of dimensions recommended by the preliminary analysis (McCune and Grace 2002).

I conducted NMS on both the standardized abundance and relative proportion of each taxon, with data pooled over the season. To test the hypothesis of no difference between treatments, I used multi-response permutation procedure (MRPP) for the pimpline and subfamily datasets. This non-parametric technique uses randomization to compare variation within and between groups (Mielke and Berry 2001). Since there were only two replicates per treatment (with two traps

within each replicate), these analyses are a preliminary assessment of the relationship between harvesting intensity and community composition; nevertheless, they provide a useful way to explore patterns in the data.

To test whether there was a significant correlation between the subfamily and species-level community composition of each trap I conducted Mantel tests in PC-ORD on both the relativized and standardized abundance data (Bray-Curtis distance index, Monte Carlo randomization method with 10000 iterations).

In forest ecosystems, Malaise traps primarily collect specimens that are moving through the shrub layer (Fraser et el. 2008). To assess the influence of habitat on the ichneumonid community at a more fine-grained scale, I compared the Ichneumonidae data with the shrub layer species composition at each trap site. Shrub data were retrieved from the core EMEND database (J. Volney, unpub. data). All shrubs within two 20 m^2 plots (both located within an 80 m^2 plot centered near the trap sites) were counted, identified, and measured. I determined whether there was a correlation between each ichneumonid dataset and the vegetation data (number of live individuals of each species, square-root transformed) at each trap site by conducting Mantel tests in PC-ORD (Bray-Curtis distance, Monte Carlo test, 10000 iterations).

Results

Overall abundance and species richness

A total of 47,755 ichneumonids from 23 subfamilies were counted; the extrapolated total was 91,548 specimens or 68.2 specimens/trap/day (Table 5-2). The samples were dominated by two subfamilies, Cryptinae and Orthocentrinae, which together made up 66.7% of the total. Pimplinae was the fifth most abundant subfamily, making up 4.2% of the total. The ten least abundant subfamilies collectively made up 3.8% of the total (Table 5-2).

In the three target subfamilies (Pimplinae, Poemeniinae, and Rhyssinae, or "pimplines"), 3851 specimens representing 72 species and morphospecies were identified (Table 5-3), with a total of 2.87 specimens/trap/day. In comparison, the extrapolated total number of pimplines from the subfamily dataset was 3915

specimens (2.91 specimens/trap/day). This reasonably close estimate provides additional confidence that the subsampling method was representative of the subfamily composition of each sample. The estimation was more accurate for the relatively abundant subfamily Pimplinae (3904 estimated specimens compared to 3794 actual specimens) than for the rare subfamilies, Poemeniinae and Rhyssinae (32 and 15 estimated specimens compared to 46 and 9 actual specimens, respectively).

The species-level data had a highly skewed distribution, with few very common species and many rare species (Table 5-3). The samples were strongly dominated by two species of *Pimpla* (*P. aquilonia* Cresson and *P. pedalis* Cresson) that together made up 72.5% of the total number of individuals/trap/day. Both of these species are generalist parasitoids that have been recorded from a wide range of Lepidoptera (Yu *et al*. 2012). Thirty species were represented by singletons or doubletons, and the 50 least abundant species together made up only 4.7% of the total. The majority of species and individuals belonged to the subfamily Pimplinae. Poemeniinae was represented by seven species, making up 1.2% of the total, while Rhyssinae was even more rare, with three species making up 0.2% of the total number of specimens. Eleven species are newly recorded for Alberta, including one (*Piogaster* cf. *maculata*) that is a new record for Canada (Finnamore 1994; Yu *et al*. 2012). An estimated fourteen species were identified to morphospecies, but could not be assigned to any species. The total rarefaction curve approached but did not reach an asymptote (Figure 5-2a), indicating there are still unsampled species at these sites.

Influence of forest harvesting

The rarefaction-based estimates of pimpline species richness did not follow a consistent pattern with regard to treatment, with broad overlap in the 95% confidence intervals of each curve (Figure 5-2b), though there is an indication that the clearcut sites may have higher richness than any of the others. None of the curves approached an asymptote.

Overall, there was a weak trend toward increasing numbers of Ichneumonidae collected as retention level increased, with the traps in the control stands collecting an average of 84.7 ± 24.3 specimens per trap-day, compared to 62.0 ± 13.2 specimens per trap-day in the clearcut treatments. However, the trap catches were highly stochastic, with broad overlap in the numbers collected between each treatment and high variability within them (Figure 5-3). The 20% retention traps were particularly variable, ranging from a low of 15.1 specimens/day to a high of 86.5 specimens/day.

Few subfamilies showed a strong association with any treatment (Figure 5-4 a,b), though several subfamilies (e.g. Cryptinae, Ctenopelmatinae, Pimplinae, Ichneumoninae, Tersilochinae, Anomaloninae, Adelognathinae, Cylloceriinae) showed a weak trend toward increasing abundance with increasing retention levels. Orthocentrinae did not show a similar increase, with a wide range in capture rates between traps and no consistent pattern with regard to treatment.

While there was a weak trend towards increasing numbers of several Pimplinae species as green-tree retention increased (*Pimpla aquilonia*, *Pimpla pedalis*, *Zaglyptus varipes incompletus* (Cresson), *Acrodactyla* nr. *ocellata*), only a single species, *Dreisbachia slossonae* (Davis) appeared to be strongly associated with the control stands (Figure 5-5 a,b). No species showed a strong affiliation to any other treatment.

The analyses of standardized abundance and proportional data were highly congruent within each dataset (subfamily or species). NMS produced a 2-dimensional solution for each subfamily dataset (standardized abundance and proportions). The analysis of the proportional data explained 89.8% of the variance in the data (axis 1 = 0.407, axis 2 = 0.491, final stress = 10.7), while the abundance analysis explained 95.6% of the variance (axis 1 = 0.831 , axis 2 = 0.125, final stress = 8.2; Figure 5-6 a,b). Both pimpline datasets were best explained by 3-dimensional solutions, explaining 88.4% of the variance in the proportional data (axis 1 = 0.065, axis 2 = 0.525, axis 3 = 0.294, final stress = 8.2) and 91.9% of the variance of the abundance data (axis 1 = 0.511, axis 2 = 0.051, axis 3 = 0.357, final stress = 8.0). Since in both cases one dimension contained

very little of the overall variance (5.1 % and 6.5 %), I have only shown the plots of the other two axes (Figure 5-6 c,d).

The four harvesting treatments were not distinctly separated in any of the analyses, though in most cases there was little overlap between the control and clearcut treatments (Figure 5-6). In general, the control and 50% retention treatments clustered more closely together, whereas there was a wider spread of points for the clearcut and 20% retention treatments. Despite the weak patterns within the NMS ordinations, MRPP found a significant difference between groups for all datasets (Table 5-4). Post-hoc tests of the subfamily data found significant differences between the clearcut and control compartments, while species-level analyses found significant differences between the control and each of the three other treatments. To test the effect of the single species that was strongly associated with uncut forest, I ran the species-level analyses again with *Dreisbachia slossonae* excluded. In these analyses the control and clearcut stand were still significantly different, however there was no longer any significant difference between the control and partially cut stands.

Based on Mantel tests, there was significant correlation between the subfamily and pimpline community composition collected by each trap, for both proportional (p = 0.0003) and abundance data (p = 0.0001).

Influence of shrub layer composition

The composition of the shrub layer was distinctly different between the control and clearcut treatments (Figure 5-7a). The control stands were all dominated by green alder (*Alnus crispa* (Ait) Turrill), with a stem count of 21 – 53 shrubs per 40 m^2 sampling area. In comparison, the clearcut stands were dominated by regenerating balsam poplar (*Populus balsamifera*), as well as varying amounts of mountain alder (*Alnus tenuifolia* Nutt.) and regenerating trembling aspen *(Populus tremuloides*). There were over 100 shrubs per 40 m^2 at most trap sites, however one site had only 22 shrubs, almost all of which were balsam poplar. The partially cut stands were highly variable (Figure 5-7b). The two stands having the 20% retention treatment differed strongly in shrub

composition, with Stand 66 having large amounts of green alder, with a smaller amount of trembling aspen, and an overall count of 191 – 207 shrubs per 40 m^2. In comparison, the 20% plots in Stand 77 had many fewer shrubs (78 – 88) and were dominated by trembling aspen and balsam poplar. The four traps in the 50% retention treatments were each surrounded by a different shrub composition, with the number of stems varying from 21 – 112 per 40 m^2.

According to the Mantel tests, both pimpline datasets were significantly correlated with the shrub community composition. The association was particularly strong with the proportional dataset (p = 0.0002), but was also significant using the abundance data (p = 0.027). In contrast, neither subfamily dataset was correlated with the shrub composition (proportions: p = 0.182; abundance: p = 0.374).

Discussion

Ichneumonidae are extremely abundant in northern boreal habitats (Deans *et al.* 2005; Stahlhut *et al.* 2013). This study supports this finding, with a total of 68.2 specimens collected per trap-day. Both at the subfamily and species-level, Ichneumonidae followed the typical right-skewed distribution of insect biodiversity, with many rare species and a few common species (Novotný and Basset 2000; Summerville and Crist 2002; Scharff *et al.* 2003; Pinzon and Spence 2010). There are three main hypotheses for the observed rarity. The first is that the species may be relatively common in the habitat being assessed, but trap biases limit the number that are collected. The second possibility is that rare species are transients from adjacent habitats where they are more common. Finally, it is possible that the species are genuinely rare on the landcape (Novotný and Basset 2000; Lim *et al.* 2012).

All traps have biases and sample arthropods differentially depending on their activity patterns (Missa *et al.* 2009). Malaise traps are the standard method to sample Ichneumonidae, as they are specifically designed to collect flying arthropods (Townes 1972; Gauld 1991; Gaston and Gauld 1993; Skillen *et al.* 2000; Sääksjärvi *et al.* 2004; Fraser *et al.* 2007, 2008). However, at least for some

species, yellow pan traps may be equally or more effective (Missa *et al*. 2009; Aguiar and Santos 2010). Since only Malaise traps were used, I am unable to assess sampling biases in the current study. However in another survey of boreal Ichneumonidae based on one Malaise trap and multiple pan traps, only Cryptinae and Orthocentrinae had multiple species that were best collected using pan traps (Finnamore 1994). Pimplinae were well-represented in the Malaise trap, with only one out of 36 species restricted to the pan traps.

Very little is known about dispersal distances of ichneumonids. In a recent review of studies examining the movement rates of woodland-associated invertebrates, none of the studies were of parasitoids (Brouwers *et al*. 2009). Even within agricultural systems, where most research into parasitoids has occurred, there is a lack of knowledge about the distance traveled by parasitoids (Lavandero 2004). Many ichneumonids, particularly among the larger species, are likely strong fliers; it is therefore likely that several species were transients in the habitat. Preliminary studies of the surrounding forest types (e.g. white spruce-dominated stands) indicate that the ichneumonid community differs significantly between habitats (MDS, unpublished data). More extensive surveys in a wider range of habitats would help to distinguish transient from genuinely rare species.

Finally, many species are likely to be truly rare. While rare species are difficult to study, they are very important biologically (Novotný and Basset 2000). Parasitoids in particular present challenges, since by necessity they are rarer than their prey. This emphasizes the importance of baseline surveys, even if we are only able to record presence/absence. Without intensive and extensive surveys, it would be easy for these rare species to disappear from the landscape without attracting any notice (Fraser *et al*. 2008). Such baseline data are also essential in a world shaped by climate change. Some researchers predict that climate change could have a disproportionate effect on parasitoids, by disrupting the thermal, phenological, or geographic adaptations of parasitoids to their hosts, and potentially by eliminating parasitoid endosymbionts (Shaw 2006; Hance *et al*. 2007).

Pimplinae is a relatively small subfamily in terms of numbers of species, and was only the 5th most abundant subfamily in this study. Nevertheless, a total of 72 species and morphospecies were collected within this subfamily, and the species accumulation curve indicates that sampling is not complete. The number of newly recorded species for Alberta and Canada and the presence of several morphospecies that cannot be assigned to species demonstrate that even in this relatively well-known group, there is much unexplored diversity. In particular, the genera *Delomerista*, *Dolichomitus*, *Scambus*, and *Zatypota* are in need of revisionary work.

Harvesting and habitat associations

There was a weak trend toward increasing numbers of Ichneumonidae as retention levels increased, and several subfamilies or pimpline species mirrored the trend. However the results were highly stochastic between traps. The efficiency of Malaise trap samples is highly influenced by environmental variables, such as wind direction and insect flight paths, that are difficult to control for in structurally complex forest ecosystems (Darling and Packer 1988). It is thus difficult to determine whether trap catches are the result of the immediate environment and placement of the trap or else due to true differences between sites. Since the trend was not equivalent for all taxa, likely a combination of these factors was at play.

Dreisbachia slossonae was the only species that appeared to be highly associated with unharvested forest. This species is a spider parasitoid; however, its specific host or host range is not known (Yu *et al*. 2012). This strong pattern was not observed with spider parasitoids in general; therefore, the explanation must lie with the particular host/habitat association of this species, as opposed to being an indication of increased spider fauna in the control treatments. More research into the biology of this species would be of great interest for understanding this pattern.

Clearcutting is often thought to homogenize faunas in comparison to unharvested forests (Buddle and Shorthouse 2008; Work *et al*. 2010). However, in

this study, the samples from the clearcuts were more dispersed in the NMS ordinations than were those of the unharvested controls, indicating greater heterogeneity in the harvested forest. MRPP found a significant difference between clearcut and control treatments for both subfamilies and species, despite the weak separation between treatments in the NMS ordinations. This is likely due to the greater variability in the clearcut samples, since MRPP is sensitive to the spread of samples as well as the location (Mielke and Berry 2001).

It is expected that clearcut and uncut forests would have different species assemblages, with open habitat-associated or forest-associated species, respectively. The goal of variable retention harvesting is to determine if partial-cuts maintain elements of uncut forests, to facilitate the recovery of forest ecosystems following harvesting. In this study the results of partial-cutting on the Ichneumonidae community were inconclusive. No differences were found between the partial-cut treatments and either control or clearcut treatments in the subfamily data. For the species level data, the partially cut treatments differed from the control treatment, but this was almost entirely due to a single, apparently forest-associated, spider parasitoid species. Greater replication and more traps are needed to increase the power of this study to assess the fine-grained differences between retention levels.

Despite the weak association with treatments, there was a strong correlation between the pimpline community and the shrub composition at each site. The composition and structure of the vegetation community has similarly been correlated with ichneumonid community composition in both tropical and temperate habitats (Sääksjärvi *et al*. 2004, 2006; Shaw 2006; Steinbauer *et al*. 2006; Fraser *et al*. 2007, 2008). This indicates that Malaise traps are effectively sampling the resident ichneumonid community, rather than being dominated by transient species. In comparison, the Ichneumonidae community at the subfamily level shows no association with the shrub community composition. This is unexpected since there is a strong correlation between the ichneumonid subfamily composition and pimpline species composition in each trap. It indicates that the two measures of ichneumonid community structure may not be responding to the

same environmental variables. One possibility is that subfamily composition is more influenced by abiotic factors affecting trap efficiency with the specific habitat requirements of different species masked by the pooling of species into broad taxonomic categories. In comparison, it is possible to associate species-level community structure with specific habitat elements, thus contributing to our ability to assess the habitat requirements for parasitoid species.

Conclusion

This study provides a baseline survey of the Pimplinae, Poemeniinae, and Rhyssinae in a deciduous boreal forest ecosystem. While there is evidence that the community composition of both pimpline species and ichneumonid subfamilies is influenced by harvesting, a great deal of additional sampling is needed to determine if variable retention harvesting is an effective method at maintaining intact parasitoid communities. There is an overall correlation between the species-level community in the three target subfamilies and the ichneumonid community at a higher taxonomic level; however, the lack of association between the specific habitat and the subfamily data lends support to the importance of assessing community patterns at a lower taxonomic level.

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Table 5-1. Summary of trapping sessions, subsamples, and trap damage of Malaise traps. All collections were from 2008. Collecting sessions: 1 = May 27/28 (Stand B/A, respectively) – June 10; 2 = June 10 – 24; 3: June 24 – July 8; 4: July 8 – 18; 5: July 18 – 29; 6: July 29 – August 12; 7: August 12 – 26. Numbers indicate the proportion of each sample that was sorted to subfamily; X = no sample.

| Treatment | Stand | Trap | Coordinates | Collecting session (no. days) | | | | | | |
				1 (13/14)	2 (14)	3 (14)	4 (10)	5 (11)	6 (14)	7 (14)
Clearcut	A	850-1	56.7488 -118.3209	1	X	0.25	0.5	0.33	0.5	1
		850-2	56.7491 -118.3236	1	1	0.25	0.5	0.33	0.5	1
	B	864-1	56.7489 -118.3627	X	1	0.5	X	0.33	0.33	0.5
		864-2	56.7502 -118.3627	1	0.33	0.25	0.33	X	0.5	1
20% retention	A	854-1	56.7529 -118.3333	1	X	0.25	1	0.33	0.5	1
		854-2	56.7541 -118.3336	1	1	0.5	1	1	1	1
	B	860-1	56.7492 -118.3544	1	1	0.25	0.5	0.5	0.5	1
		860-2	56.7486 -118.3557	X	0.5	0.33	1	1	1	1
50% retention	A	853-1	56.7499 -118.3258	1	1	0.25	1	0.25	0.5	1
		853-2	56.7506 -118.3304	1	X	0.25	0.33	0.33	0.5	0.5
	B	863-1	56.7485 -118.3598	1	0.25	1	1	0.5	1	1
		863-2	56.7503 -118.3592	1	1	0.25	1	0.5	0.5	X
Control	A	852-1	56.7520 -118.3246	1	0.33	0.25	0.5	0.33	0.5	1
		852-2	56.7525 -118.3282	1	0.25	0.25	0.5	0.33	0.5	1
	B	862-1	56.7458 -118.3615	1	0.25	0.25	1	0.25	0.5	1
		862-2	56.7475 -118.3631	1	1	1	0.25	1	0.5	1

Table 5-2. List of subfamilies collected at the EMEND experimental site in northwestern Alberta in sixteen Malaise traps with a total of 1346 trap-days. No. counted = total number of individuals identified in samples and subsamples; extrapolated total = estimated total number of individuals extrapolated from percentage of each sample that was sorted.

Subfamily	No. counted	Extrapolated total	Extrapolated no. per trap-day
Acaenitinae	3	5	0.004
Adelognathinae	562	1158	0.862
Anomaloninae	738	1205	0.897
Banchinae	564	1180	0.879
Campopleginae	2284	4565	3.399
Cryptinae	15730	31382	23.367
Ctenopelmatinae	1413	2868	2.136
Cyllocerinae	299	783	0.583
Diacritinae	617	944	0.703
Diplazontinae	1041	2054	1.529
Eucerotinae	20	40	0.03
Ichneumoninae	2197	4457	3.319
Mesochorinae	1563	3184	2.371
Metopiinae	521	936	0.697
Ophioninae	288	505	0.376
Orthocentrinae	15958	29681	22.101
Orthopelmatinae	60	145	0.108
Pimplinae	2312	3904	2.907
Poemeniinae	15	32	0.024
Rhyssinae	5	15	0.011
Tersilochinae	547	969	0.722
Tryphoninae	783	1460	1.087
Xoridinae	35	76	0.057
Total	**47555**	**91548**	**68.167**

Table 5-3. List of Pimplinae, Poemeniinae, and Rhyssinae collected at the
EMEND experimental site in northwestern Alberta in sixteen Malaise traps with a
total of 1346 trap-days. * = newly recorded from Alberta; ** = newly recorded
from Canada.

Species	No. specimens	No. per trap-day
Pimplinae		
Acrodactyla nr. *ocellata*	74	0.055
Acrodactyla quadrisculpta (Gravenhorst, 1820)	5	0.004
Acrodactyla sp. 1	8	0.006
Acrodactyla sp. 2	2	0.001
Acrodactyla sp. 3	1	0.001
Acropimpla alboricta (Cresson, 1870)	4	0.003
Apechthis ontario (Cresson, 1870)	14	0.010
Apechthis picticornis (Cresson, 1870)	18	0.013
Clistopyga maculifrons Cushman, 1921	5	0.004
Delomerista mandibularis (Gravenhorst, 1829)	22	0.016
Delomerista masoni Gupta, 1982*	16	0.012
Delomerista novita novita (Cresson, 1870)	6	0.004
Delomerista townesorum Gupta, 1982	8	0.006
Delomerista walkleyi Gupta, 1982*	1	0.001
Delomerista sp. 1	2	0.001
Delomerista sp. 2	1	0.001
Delomerista sp. 3	1	0.001
Delomerista sp. 4	1	0.001
Delomerista spp. (unassociated males)	18	0.013
Dolichomitus imperator (Kriechbaumer, 1854)	10	0.007
Dolichomitus pterelas (Say, 1829)	9	0.007
Dolichomitus pygmaeus (Walsh, 1873)*	5	0.004
Dolichomitus terebrans (Ratzeburg, 1844)	1	0.001
Dolichomitus sp. 1	1	0.001
Dolichomitus sp. 2	1	0.001
Dreisbachia frigida (Cresson, 1870)	76	0.057
Dreisbachia slossonae (Davis, 1898)	151	0.112
Endromopoda producta (Walley, 1960)	2	0.001
Ephialtes macer Cresson, 1868	9	0.007
Ephialtes duplicauda Heinrich, 1949	11	0.008
Eruga lineata Townes, 1960*	4	0.003
Iseropus stercorator orgyiae (Ashmead, 1896)	3	0.002
Itoplectis fustiger Townes, 1960*	25	0.019
Itoplectis quadricingulata (Provancher, 1880)	37	0.028
Liotryphon dentatus (Townes, 1960	2	0.001
Oxyrrhexis carbonator texana (Cresson, 1870)	12	0.009
Perithous scurra (Panzer, 1804)	1	0.001
Pimpla aquilonia Cresson, 1870	2003	1.491

Species	No. specimens	No. per trap-day
Pimpla pedalis Cresson, 1865	790	0.588
Pimpla stricklandi (Townes, 1960)	22	0.016
Pimpla tenuicornis Cresson, 1865	72	0.054
Pimpla sp. 1	1	0.001
Piogaster cf. maculata Townes, 1960**	1	0.001
Polysphincta burgessii Cresson, 1870	1	0.001
Polysphincta koebeli Howard, 1892*	1	0.001
Scambus atrocoxalis (Ashmead, 1902)	14	0.010
Scambus brevicornis (Gravenhorst, 1829)	1	0.001
Scambus deceptor Walley, 1960	2	0.001
Scambus decorus Walley, 1960	47	0.035
Scambus granulosus Walley, 1960	10	0.007
Scambus hispae (Harris, 1835)	70	0.052
Scambus nucum (Ratzeburg, 1844)*	1	0.001
Scambus pterophori (Ashmead, 1890)	1	0.001
Scambus vesicarius euurae (Ashmead, 1890)	5	0.004
Scambus sp. 1	1	0.001
Scambus deceptor/granulosus (unassoc. male*)*	1	0.001
Sinarachna pallipes (Holmgren, 1860)	31	0.023
Theronia atalantae fulvescens (Cresson, 1865)	2	0.001
Tromatobia ovivora (Boheman, 1821)	8	0.006
Zabrachypus primus Cushman, 1920	1	0.001
Zaglyptus varipes incompletus (Cresson, 1870)	88	0.066
Zatypota anomala (Holmgren, 1860)	13	0.010
Zatypota percontatoria (Müller, 1776)	2	0.001
Zatypota sp. 1	40	0.030
Poemeniinae		
Neoxorides borealis (Cresson, 1870)	1	0.001
Neoxorides pilulus Townes, 1960	2	0.001
Podoschistus vittifrons (Cresson, 1868)*	7	0.005
Poemenia albipes (Cresson, 1870)	6	0.004
Poemenia americana (Cresson, 1870)	15	0.011
Poemenia thoracica (Cresson, 1879)	13	0.010
Pseudorhyssa ruficoxa (Kriechbaumer, 1887)	2	0.001
Rhyssinae		
Rhyssa alaskensis (Cresson, 1902)	1	0.001
Rhyssa persuasoria (Linnaeus, 1758)	7	0.005
Rhyssella humida (Say, 1835)*	1	0.001
TOTAL	**3851**	**2.867**

Table 5-4. Results of MRPP analyses of proportional data and abundance data of Ichneumonidae subfamilies and Pimplinae, Poemeniinae, and Rhyssinae species (including and excluding *Dreisbachia slossonae*). Abundances are standardized as specimens per trap-day (species) or extrapolated specimens per trap-day (subfamily).

Comparison	Subfamily		Species		Excluding *D. slossonae*	
	Proportion	Abundance	Proportion	Abundance	Proportion	Abundance
All	0.043*	0.034*	0.015*	0.012*	0.038*	0.041*
Clearcut vs. 20 % retention	0.260	0.373	0.167	0.477	0.185	0.534
Clearcut vs. 50 % retention	0.065	0.141	0.502	0.410	0.480	0.401
Clearcut vs. control	0.010*	0.008*	0.024*	0.013*	0.042*	0.026*
20 % retention vs. 50 % retention	0.420	0.149	0.365	0.227	0.355	0.230
20 % retention vs. control	0.317	0.079	0.029*	0.037*	0.062	0.087
50% retention vs. control	0.602	0.358	0.035*	0.018*	0.089	0.075
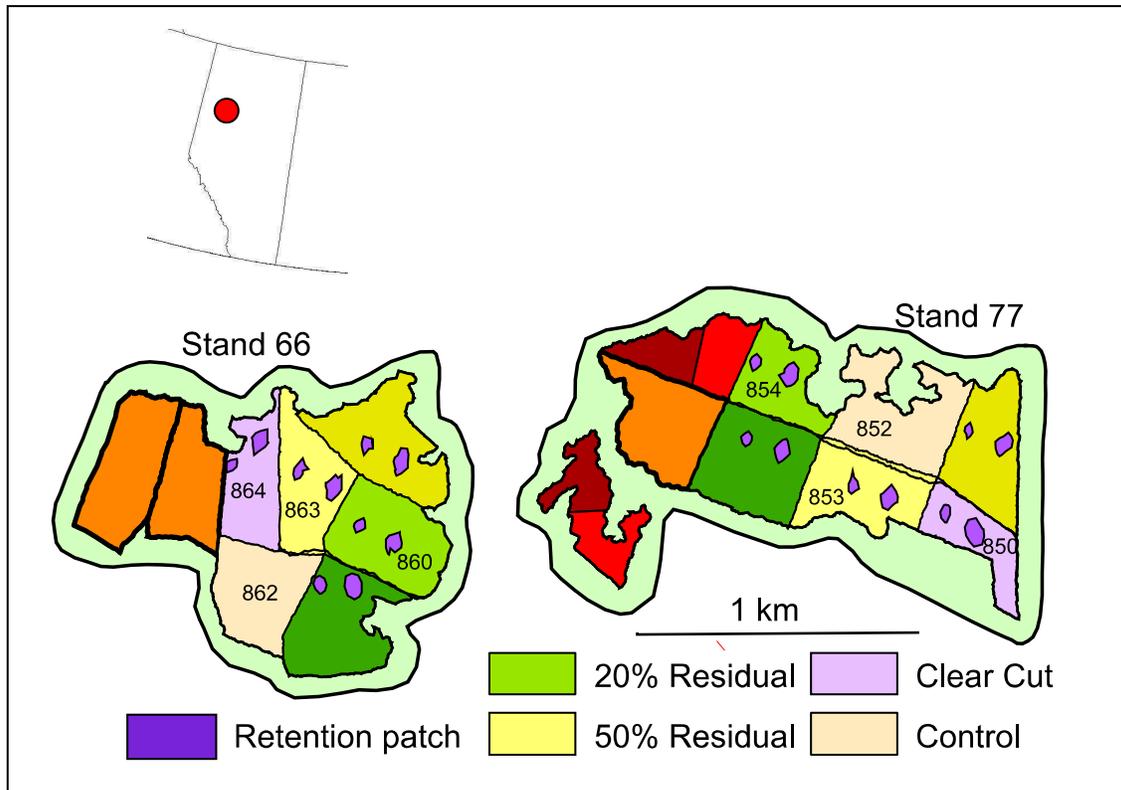
Figure 5-1. Map of study sites, located with the EMEND experimental area in northwestern Alberta. Two Malaise traps were used within each of four treatments in two stands. Compartment numbers: 850 and 864 = clearcut; 854 and 860 = 20% retention; 853 and 863 = 50% retention; 852 and 862 = uncut control.
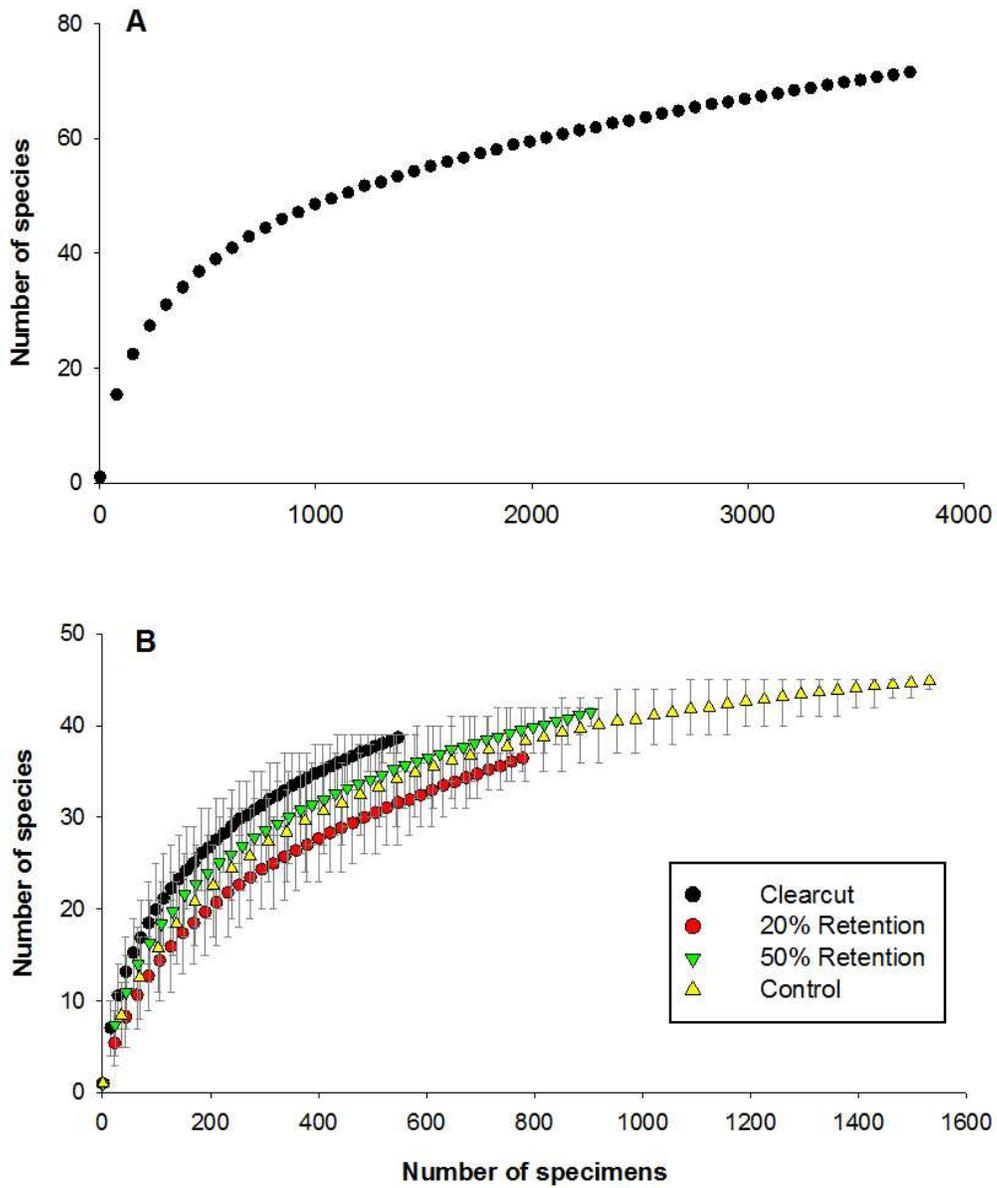
Figure 5-2. Rarefaction curves of species diversity of Pimplinae, Poemeniinae, and Rhyssinae. **A:** All traps combined; **B:** Each treatment calculated separately. Error bars represent 95% confidence intervals.
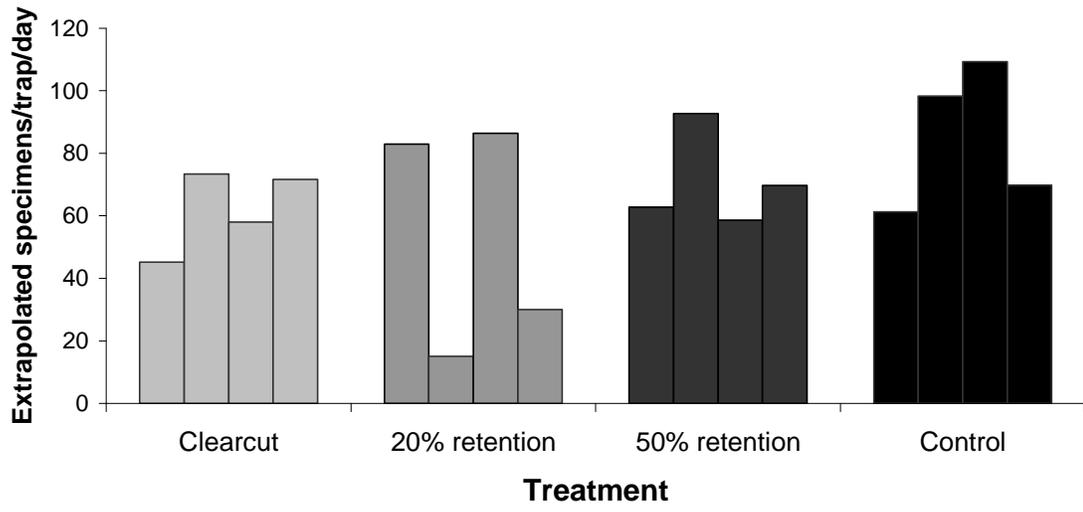
Figure 5-3. Abundance of Ichneumonidae in each of sixteen Malaise traps in four harvesting treatments in northwestern Alberta.
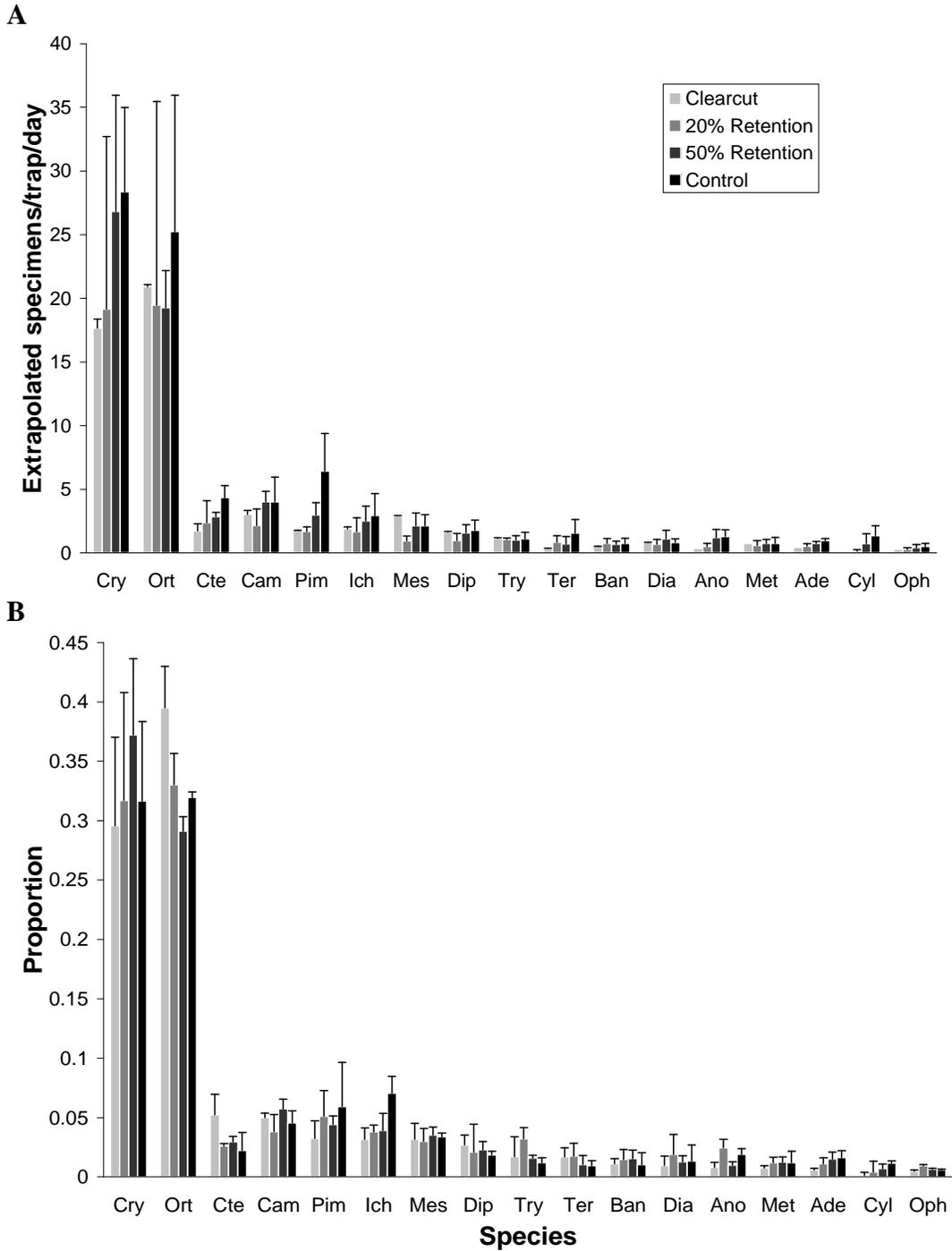
Figure 5-4. Summary of seventeen most abundant Ichneumonidae subfamilies collected in four harvesting treatments in northwestern Alberta. Cry = Cryptinae; Ort = Orthocentrinae; Cte = Ctenopelmatinae; Cam = Campopleginae; Pim = Pimplinae; Ich = Ichneumoninae; Mes = Mesochorinae; Dip = Diplazontinae; Try = Tryphoninae; Ter = Tersilochinae; Ban = Banchinae; Dia = Diacritinae; Ano = Anomaloninae; Met = Metopiinae; Ade = Adelognathinae; Cyl = Cylloceriinae; Oph = Ophioninae. Error bars represent 95% confidence intervals.

A
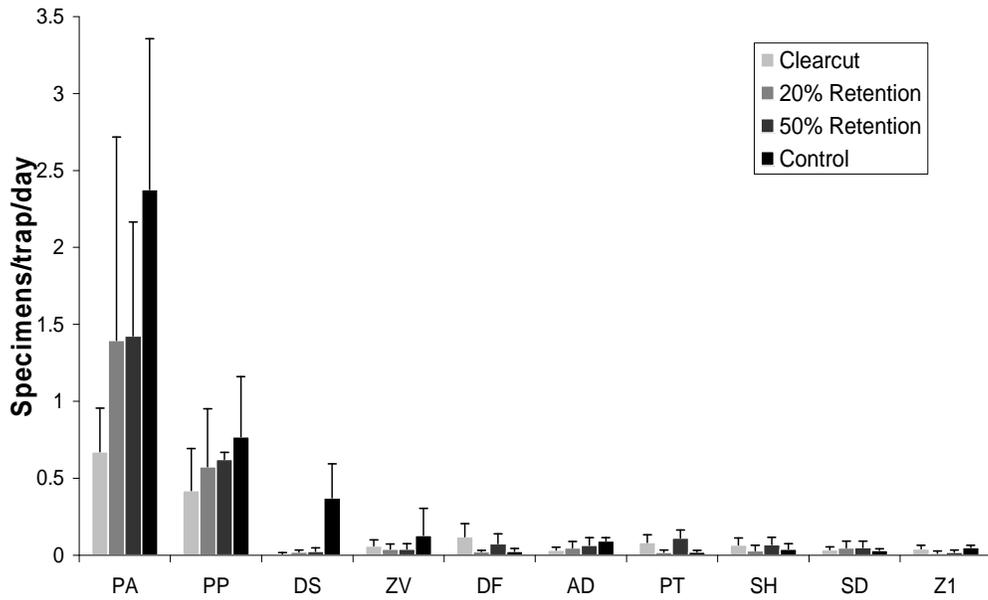
B
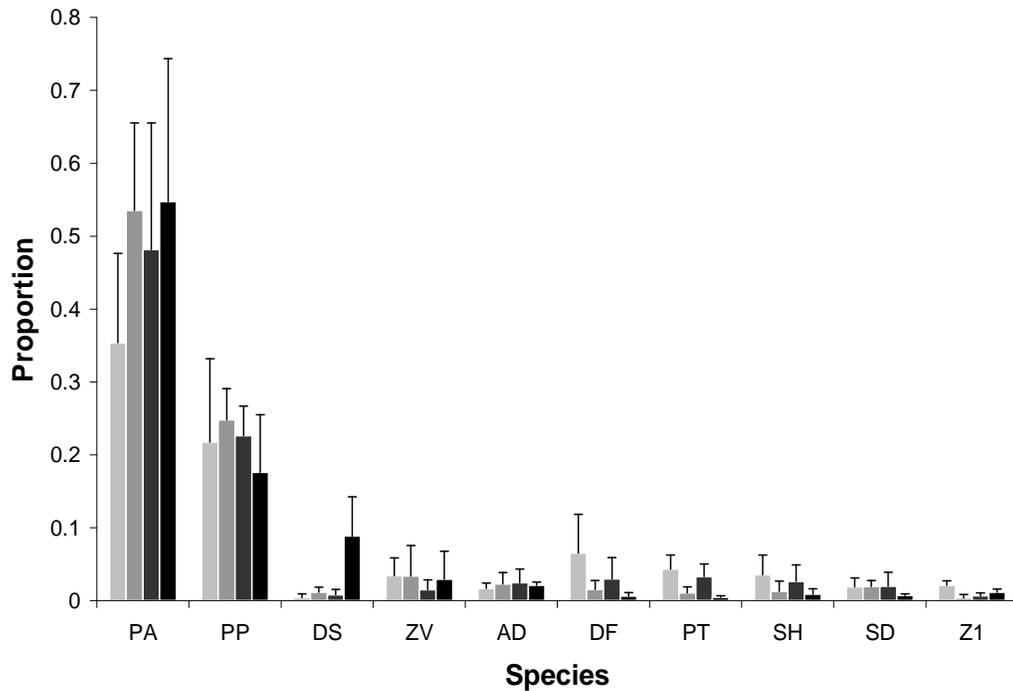
Figure 5-5. Summary of ten most abundant species of Pimplinae collected in four harvesting treatments in northwestern Alberta. PA = *Pimpla aquilonia*; PP = *Pimpla pedalis*; DS = *Dreisbachia slossonae*; ZV = *Zaglyptus varipes incompletus*; AD = *Acrodactyla* nr. *ocellata*; DF = *Dreisbachia frigida*; PT = *Pimpla tenuicornis*; SH = *Scambus hispae*; SD = *Scambus decorus*; Z1 = *Zatypota* sp. 1. Error bars represent 95% confidence intervals.
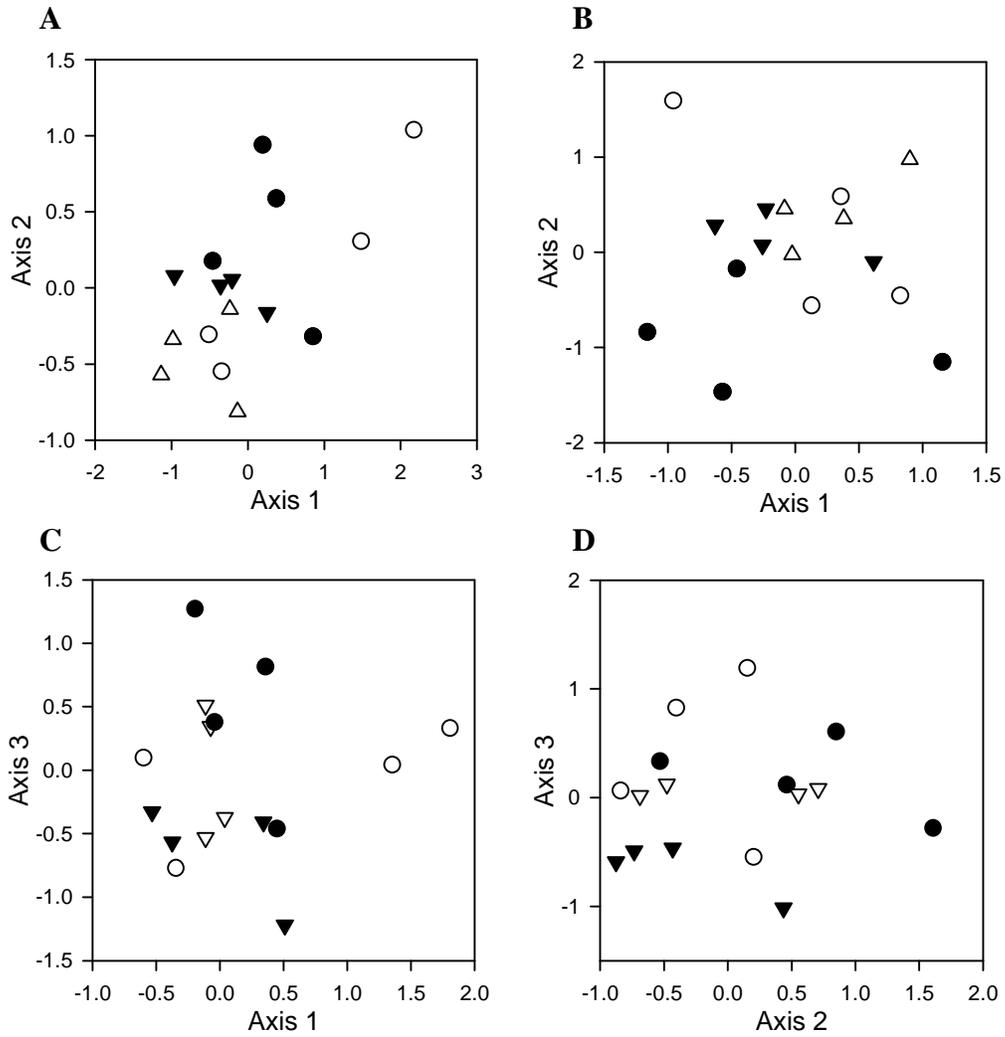
Figure 5-6. Non-metric multidimensional scaling of Ichneumonidae subfamilies (a,b) and Pimplinae, Poemeniinae, and Rhyssinae species (c,d) in four harvesting treatments. a) subfamily abundance per trap-day; b) subfamily relative proportions; c) species abundance per trap-day; d) species relative proportions; Filled circles = clearcut; open circles = 20% retention; open triangles = 50% retention; closed triangles = uncut control
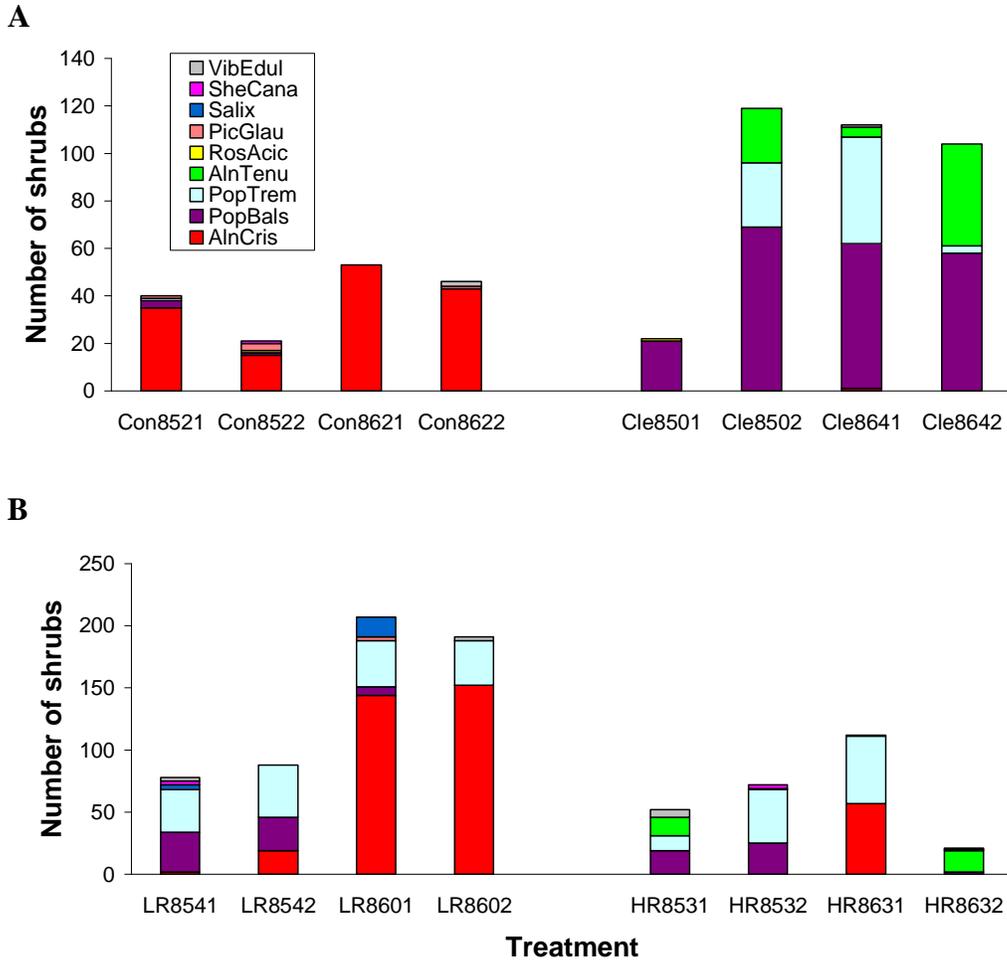
Figure 5-7. Comparison of shrub composition surrounding each Malaise trap. **A:** Control (Con) and clearcut (Cle) treatments; **B:** Partial-cut treatments: LR = 20% retention; HR = 50% retention; Shrubs: VibEdul = *Viburnum edule*; SheCana = *Shepherdia canadensis*; Salix = *Salix* spp.; PicGlau = *Picea glauca*; RosAcic = *Rosa acicularis*; AlnTenu = *Alnus tenuifolia*; PopTrem = *Populus tremuloides*; PopBals = *Populus balsamifera*; AlnCris = *Alnus crispa.*

Chapter 6

General Conclusions

Thesis summary

Ichneumonidae are the most species-rich family of parasitoid Hymenoptera and exhibit a wide range of life-history strategies. They have great potential as a source for theoretical studies into species distributions (e.g. Janzen 1981; Gauld *et al.* 1992; Sime and Brower 1998) or host-parasitoid dynamics (e.g. Várkonyi *et al.* 2002; Anton *et al.* 2007); as biological control in agricultural and forest ecosystems (Langor *et al.* 2000; Dosdall *et al.* 2010); and to contribute to our understanding of the ecology of managed ecosystems (Hilszczański *et al.* 2005; Stenbacka *et al.* 2010; Ulyshen *et al.* 2011). However, we are extremely limited in terms of what we can learn from ichneumonids since at the most fundamental levels of species identity, species distributions, and habitat requirements we lack basic information for the vast majority of species (Shaw 2006; Quicke 2012; Schwarzfeld 2013).

Species identity is the essential building block for studies of biodiversity, ecology, biogeography and evolution (Claridge *et al.* 1997; Sites and Marshall 2004). However, it is estimated that only approximately 25% of Ichneumonidae are currently described (Gauld 2002; Yu *et al.* 2012). In Chapters 2 – 4, I addressed this issue by examining the taxonomically neglected ichneumonid genus *Ophion* at three different scales.

In Chapter 2, I used molecular data to construct a preliminary phylogeny of the genus and define several new species groups. This chapter thus provides the necessary structure for all further studies of *Ophion* systematics.

In Chapter 3, I investigated the diversity of *Ophion* at the species level by comparing three quantitative species delimitation methods with each other and with several morphologically-defined species. This chapter also addressed the question of whether "DNA taxonomy" (Tautz *et al.* 2003; Blaxter 2004; Pons *et al.* 2006) is an effective tool for investigating diverse, morphologically

challenging taxa. While each of the methods was generally successful at delimiting well-differentiated species, they differed in their ability to discriminate closely related species. As well, within each method the results were highly dependent on the parameters used. This emphasizes the importance of the approach by Puillandre *et al*. (2012), wherein automated species delimitation algorithms (in their case automatic barcode gap discovery) provide "primary species hypotheses", that then need to be addressed with other sources of data in an integrative framework (Dayrat 2005; Roe and Sperling 2007; Schlick-Steiner *et al*. 2010). However while these methods differed in the details of species delimitation, they all agreed with the qualitative assessment that *Ophion* is highly diverse in the Nearctic region. The eleven described Nearctic species (Yu *et al*. 2012) thus represent a minute fraction of the total diversity, and even the prediction of 50 Nearctic species (Gauld 1985) is a significant underestimate. Perhaps more unexpectedly, the well-studied British fauna (Gauld 1978; Brock 1982) also seems to contain several previously unrecognized species.

In Chapter 4, I focused in detail on the newly defined *O. scutellaris* Thomson species group, and described six new species based on an integrative analysis of molecular data, morphology, geometric morphometrics of wing venation and classical morphometrics. *Ophion* is often considered to be a particularly challenging group due to high intraspecific and low interspecific morphological variability (Gauld 1980; Brock 1982). The methods used in this chapter provide multiple additional, semi-independent datasets that can be used to inform species hypotheses. All methods were broadly congruent, but each provided essential information aiding the discrimination of these species.

The above three chapters focused on the essential task of identifying and putting names on organisms. This is a crucial step before any further meaningful studies can take place. In Chapter 5, I addressed some of the other fundamental questions that can be asked and answered once species names are available, namely the species distributions and habitat requirements of Ichneumonidae.

I collected Ichneumonidae with Malaise traps in a boreal deciduous ecosystem in northwestern Alberta. I identified three ichneumonid subfamilies

(Pimplinae, Poemeniinae, and Rhyssinae) to species, and all Ichneumonidae to subfamily. Community composition and species diversity were weakly affected by harvesting treatments; however, this may be due to insufficient replication of treatments. There was stronger evidence that the community composition at the species level was associated with the immediate vegetation surrounding each trap. This correlation was not observed at the subfamily level, however, thus supporting the need for lower-level identifications for ecological monitoring. Overall, this study found 72 species and morphospecies, eleven of which were previously unrecorded in Alberta (Finnamore 1994; Yu *et al*. 2012). Rarefaction curves indicated that not all species present in the plots were sampled. This study provided a baseline survey of these subfamilies in Alberta's boreal forest, and demonstrated that even within this relatively well-known group, there is a great deal of unexplored diversity.

Future research

The scope for discovery, whether within *Ophion* in particular or Ichneumonidae in general, is virtually limitless. In this thesis, I laid the groundwork for taxonomic study of Nearctic *Ophion*; however, the real work to revise the genus has only just begun. While Chapter 2 provides an essential structure within which *Ophion* can be examined, it would be of great interest to expand the phylogeny to include additional described and undescribed species worldwide. In particular, I would like to include species from the remaining species groups defined by Gauld (1985) that were not included in this study, both to test their monophyly and to determine their relationship to the rest of *Ophion.* In addition, more work is needed to define the species groups morphologically. While some groups appear to have useful diagnostic characters, many are morphologically weakly characterized at best. More research is needed to confirm those characters that appear to be consistent and to find additional characters, particularly for morphologically variable groups such as the *O. slossonae* species group.

The species delimitation methods used in Chapter 3 provided many testable species hypotheses that now need to be further assessed in an integrative framework. I would therefore like to use the same approach as in Chapter 4 and apply it sequentially to each of the other species groups. Several putative species of Nearctic *Ophion* are quite morphologically homogeneous within the species, and distinct from closely-related species. However, more research is needed to put them into the context of their respective species groups and to distinguish them from distantly-related but morphologically similar species. Other species are well-supported by molecular data, but thus far lack obvious morphological characters.

The *O. obscuratus* species group in particular would benefit from the integrative methods developed in Chapter 4. While some species within this group are morphologically quite recognizable, there appear to be multiple species-complexes in this group, with weak molecular and/or morphological gaps between taxa. This group is of particular interest, as it contains the most common Nearctic *Ophion* species, at least in most Canadian localities. This undescribed species is a distinctly yellow-patterned, early-season species. However preliminary morphological, molecular and geometric morphometric studies (MDS, unpublished) have found several specimens that appear to be weakly differentiated, and that may represent additional species. Along with quantitative morphometrics, a population genetics approach (e.g. Shaffer and Thomson 2007; Lumley and Sperling 2011) may be necessary to resolve this species group.

Finally, studies are needed into the biology and ecology of *Ophion* species. For example, little is known about the habitat and host requirements for *Ophion* species. Rearing of hosts, in particular, would contribute extremely valuable information into both the life history and species identities of *Ophion.*

The biodiversity survey of Ichneumonidae in a boreal ecosystem also laid the foundation for more comprehensive studies. The experimental forest where this study was conducted (Ecosystem Management Emulating Natural Disturbances, or EMEND) has perhaps the most extensively studied terrestrial arthropod fauna in Canada (e.g. Pinzon *et al*. 2013; Kamunya 2012; Diaz-Aguilar 2010; Pengelly and Cartar 2010; Work *et al*. 2010). However, with the exception

of four species that were reared from bark beetles (Wesley *et al*. 2006), this is the first list of parasitoid Hymenoptera from this study system. Parasitoids may not respond to habitat fragmentation in the same manner as their hosts, potentially increasing outbreaks of herbivorous insects (Roland and Taylor 1997; Anton *et al*. 2007). The neglect of parasitic Hymenoptera therefore represents an important gap in our knowledge of, and ability to manage, forest ecosystems.

While the need to study parasitoids has often been expressed (e.g. Shaw and Hochberg 2001), conducting biodiversity surveys of Ichneumonidae in boreal ecosystems is not a trivial task. Aside from the challenges of dealing with bears who have developed a taste for the alcohol in Malaise traps, the abundance, diversity, and poor state of taxonomy of many subfamilies and genera all contribute to the difficulties of working with this group. In this study, I collected an average of nearly 70 specimens per trap per day, and I estimate that the total number of species present is well over 1000. Since many species of Pimplinae, Poemeniinae, and Rhyssinae are generalist parasitoids (Yu *et al*. 2012), these subfamilies may not be the best indicators of habitat change; however, they are among the only ichneumonid subfamilies that can be readily identified to species. While it would take several lifetimes to accomplish all of these goals, I believe a three-pronged approach is needed to promote our understanding of the diversity and ecology of Ichneumonidae in forest ecosystems.

One priority is to conduct additional species-level surveys in order to better understand the distributions and habitat requirements of known species, to identify the gaps in our taxonomic knowledge, and to provide the raw material for systematic and taxonomic research. The second priority is to continue the fundamental taxonomic work of species discovery, delimitation, and description. Rearing of host species is an important component of both of these priorities, as knowledge of host-parasitoid relationships is fundamental to elucidating the species identity and ecology of ichneumonid species. Finally, one of the major obstacles to the use of Ichneumonidae in biodiversity studies is the lack of user-friendly keys, or for many groups the lack of any keys at all. For non-taxonomists, including both amateur entomologists and ecologists, ease of identification plays

a significant role in the likelihood of pursuing an interest in a particular group (Walter and Winterton 2007; Gerlach *et al*. 2013). A particularly high priority is therefore to make Ichneumonidae identifications more accessible to non-specialists, for example through extensively-illustrated online keys (e.g. Gauld and Wahl 2013), matrix-based keys (e.g. Clutts *et al*. 2011; Dombroskie 2011) or online species pages (e.g. Strickland Virtual Museum, University of Alberta). These would in turn hopefully encourage further studies into this ecologically important and fascinating family of insects.

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Appendix 1. List of all *Ophion* and *Enicospilus* sequences included in Chapters 2 - 4.

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
O. areolaris species-group											
6910	UK: Aldbury	51.799 -0.601	3 vi 2011	GRB	*ocellaris*	KF594817	KF616299	KF616347	a,b,c,d	a,b	
7512	UK: Bucks., Burnham Beeches	51.547 -0.646	24 v 2007	MA	*ocellaris*	KF594991			a	a	
7550	UK: Radnage	51.659 -0.858	7 vi 2008	AMG	*ocellaris*	KF595023			a	a	
	UK	not listed	not listed		*ocellaris*			EU378719	c		
O. flavidus species-group											
5542	FL: Sarasota Co.	27.246 -82.302	17 iii 2008	WH	*flavidus*	KF594566	KF616301	KF616388	a,b,c,d	a,b	
	CR: Guanacaste	10.904 -85.309	25 iii 2007	EA	*flavidus*	HCWC788-07			a	a	
	CR: Guanacaste	10.877 -85.586	2 vii 2005	FQ	*flavidus*	JF793278			a	a	
5550	ON: Woodlawn	45.45 -76.09	1-6 viii 2008	LM		KF594574		KF616390	a,c	a	
5556	ON: 8 km E Almonte	45.25 -76.15	7-11 vi 1999	JD,SN		KF594580		KF616389	a,c	a	
	CR: Guanacaste	10.973 -85.315	30 ix 2008	BioLep		JQ576869			a		
	CR: Guanacaste	10.843 -85.78	23 v 2009	BioLep		JQ576880			a		
	CR: Guanacaste	10.843 -85.78	23 v 2009	BioLep		JQ576882			a		
	CR: Guanacaste	10.843 -85.78	23 v 2009	BioLep		JQ576883			a		
	CR: Guanacaste	10.843 -85.78	23 v 2009	BioLep		JQ576884			a		
	CR: Guanacaste	10.843 -85.78	23 v 2009	BioLep		JQ576885			a		
O. luteus species-group											
6915	UK: Suffolk, East Bridge	52.2 -1.6	29 v 2011	PAB	*luteus*	KF594820			a	a	
6916	UK: Suffolk, East Bridge	52.2 -1.6	29 v 2011	PAB	*luteus*	KF594821	KF616257	KF616364	a,b,c,d	a,b	
7508	UK: Aldbury	51.799 -0.601	1 ix 2010	GRB	*luteus*	KF594987			a	a	
7509	UK: London, Barnes	51.477 -0.235	3 ix 2010	MRH	*luteus*	KF594988			a	a	
7551	UK: Aldbury	51.799 -0.601	11 viii 2008	GRB	*luteus*	KF595024			a	a	
7553	UK: Aldbury	51.799 -0.601	2 viii 2011	GRB	*luteus*	KF595026			a	a	
7557	UK: London, Barnes	51.477 -0.235	5 viii 2011	MRH	*luteus*	KF595029			a	a	
7560	UK: London, Nat. Hist. Mus.	51.496 -0.176	7 viii 2008	CR	*luteus*	KF595032			a	a	
7562	UK: Radnage	51.659 -0.858	29 viii 2008	AMG	*luteus*	KF595034			a	a	
7563	UK: Radnage	51.659 -0.858	5 viii 2007	AMG	*luteus*	KF595035			a	a	
7564	UK: Aldbury	51.799 -0.601	11 viii 2008	GRB	*luteus*	KF595036			a	a	
7565	UK: Aldbury	51.799 -0.601	3 ix 2008	GRB	*luteus*	KF595043			a	a	
7567	UK: Aldbury	51.799 -0.601	8 viii 2008	GRB	*luteus*	KF595038			a	a	
7568	UK: Aldbury	51.799 -0.601	27 vii 2011	GRB	*luteus*	KF595039			a	a	
3921	AB: Notikewan PP	57.28 -117.138	9 vi 2008	DM		KF594496	KF616247	KF616359	a,b,c,d	a,b	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
						GenBank/BOLD*			Dataset[5]		
3934	ON: Bells Corner	45.295 -75.83	25 v 2008	BCS		KF594508	KF616264		a,b	a,b	
3937	ON: Bells Corner	45.295 -75.83	25 v 2008	BCS		KF594511	KF616265		a,b	a,b	
3978	AB: Notikewan PP	57.28 -117.138	9 vi 2008	DM		KF594550			a	a	
5540	BC: Boss Lake	49.875 -120.742	17 vii 2009	DGH		KF594564	KF616261	KF616363	a,b,c,d	a,b	
5561	NM: 14 mi N Silver City	32.913 -108.227	2 viii 2003	JEO		KF594584	KF616266		a,b	a,b	
5568	AZ: Cochise Co.	31.39 -110.24	15 iii-30 iv 1994	NM		KF594591			a	a	
5702	NL: Wiltondale	49.37 -57.738	20 vii 2008	DM		KF594622	KF616248		a,b	a,b	
5758	AB: Jenner	50.842 -111.151	07 vi 2007	GGA		KF594675			a	a	
5760	AB: 8 km NW Winfield	53.01 -114.5	22 viii 2009	CDB		KF594677			a	a	
5764	AB: Jenner	50.842 -111.151	07 vi 2007	GGA		KF594681			a	a	
5771	NL: Deer Lake	49.179 -57.442	23 vi 2009	DWL		KF594688	KF616254	KF616361	a,b,c,d	a,b	
5772	AB: Waterton Lakes NP	49.1 -113.953	17 vii 2010	MDS		KF594689	KF615941		a,b	a,b	
6501	AB: 6 km N Guy	55.607 -117.161	22 vii-16 viii	BAM		KF594717	KF616256	KF616362	a,b,c,d	a,b	a,b,c
6502	AB: 6 km N Guy	55.607 -117.161	22 vii-16 viii	BAM		KF594718	KF616260		a,b	a,b	
6504	AB: Erskine	52.322 -112.883		CDB		KF594719			a	a	
6507	AB: Porcupine Hills	49.972 -114.087	14 vi 2009	JJD,BMTB		KF594722		KF616358	a,c	a	
6509	AB: Edgemont	51.115 -114.142	20 viii 2009	TP		KF594724	KF616258		a,b	a,b	
6525	BC: Sicamous	50.819 -118.869	3 viii 2009	JdW		KF594740	KF616249		a,b	a,b	
6541	BC: Okanagan Falls	49.318 -119.507	24 v 2009	JdW		KF594753	KF616262		a,b	a,b	
6554	NL: nr. Marble Mountain	48.988 -57.724	21 vi 2010	DWL		KF594765	KF616253		a,b	a,b	
6556	AB: 11 km NE Lacombe	52.553 -113.641	18 vi 2009	CDB		KF594767	KF616267		a,b	a,b	
6558	AB: Jenner	50.842 -111.151	07 vi 2007	GGA		KF594769	KF616250		a,b	a,b	
6571	AB: 8 km NW Winfield	53.01 -114.5	15 viii 2010	CDB		KF594780	KF616263		a,b	a,b	
6572	AB: 8 km NW Winfield	53.01 -114.5	22 viii 2009	CDB		KF594781			a	a	
6573	AB: 8 km NW Winfield	53.01 -114.5	22 viii 2009	CDB		KF594782			a	a	
6574	AB: 8 km NW Winfield	53.01 -114.5	15 viii 2010	CDB		KF594783	KF616259		a,b	a,b	
6906	AB: 8 km NW Winfield	53.01 -114.5	12 vi 2010	CDB		KF594813			a	a	
6941	AB: Cooking Lake-Blackfoot PRA	53.505 -112.945	20 viii 2009	GGA		KF594830			a	a	
7360	AB: Pakowki sand dunes	49.397 -110.875	12-13 vii 2009	GGA		KF594947			a	a	
7361	AB: Pakowki sand dunes	49.397 -110.875	8 vii 2008	JJD		KF594948			a	a	
7364	AB: Pakowki sand dunes	49.397 -110.875	28 vi 2008	LML		KF594949	KF616251		a,b	a,b	
7377	AB: Jenner	50.844 -111.154	2 vi 2010	GGA		KF594957			a	a	
7378	AB: Jenner	50.842 -111.151	07 vi 2007	GGA		KF594958			a	a	
7379	AB: Jenner	50.842 -111.151	26 v 2007	GGA, JJD		KF594959			a	a	
7384	AB: Jenner	50.842 -111.151	26 v 2007	GGA, JJD		KF594964			a	a	
7389	AB: Pakowki sand dunes	49.397 -110.875	10 v 2007	MDS		KF594969	KF616252		a,b	a,b	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	GenBank/BOLD*			Dataset[5]		
						COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
7390	AB: Pakowki sand dunes	49.397 -110.875	10 v 2007	MDS			KF616255		b	b	
	BC: Houston	54.633 -126.424	7 viii 2008	JdW		ICHBC009			a	a	
	BC: Sicamous Creek	50.819 -118.869	10 vii 2008	JdW		ICHBC012			a	a	
	BC: Winfield	50.022 -119.325	5 vi 2008	JdW		ICHBC015			a	a	
	BC: Winfield	50.022 -119.325	5 vi 2008	JdW		ICHBC018			a	a	
	BC: Winfield	50.022 -119.325	5 vi 2008	JdW		ICHBC019			a	a	
	BC: nr Kamloops	50.643 -120.485	12 vi 2008	JdW		ICHBC058			a	a	
	BC: nr Kamloops	50.643 -120.485	12 vi 2008	JdW		ICHBC059			a	a	
	BC: nr Kamloops	50.643 -120.485	12 vi 2008	JdW		ICHBC061			a	a	
	BC: Kamloops	49.312 -119.502	8 vi 2009	JdW		ICHBC096			a	a	
	BC: Kamloops	49.312 -119.502	8 vi 2009	JdW		ICHBC098			a	a	
	BC: Kamloops	49.318 -119.507	8 vi 2009	JdW		ICHBC103			a	a	
	BC: Kamloops	49.318 -119.507	8 vi 2009	JdW		ICHBC104			a	a	
	BC: Kamloops	49.318 -119.507	8 vi 2009	JdW		ICHBC105			a	a	
	BC: Kamloops	49.318 -119.507	8 vi 2009	JdW		ICHBC106			a	a	
	BC: Kamloops	49.318 -119.507	8 vi 2009	JdW		ICHBC107			a	a	
	BC: Kamloops	49.318 -119.507	8 vi 2009	JdW		ICHBC108			a	a	
	BC: Kamloops	49.318 -119.507	8 vi 2009	JdW		ICHBC109			a	a	
	BC: Kamloops	49.318 -119.507	8 vi 2009	JdW		ICHBC110			a	a	
	BC: Mahoney Lake	49.285 -119.586	19 vi 2009	JdW		ICHBC137			a	a	
	BC: Kamloops	49.318 -119.507	19 vi 2009	JdW		ICHBC140			a	a	
	BC: Kamloops	49.318 -119.507	19 vi 2009	JdW		ICHBC141			a	a	
	BC: Kamloops	49.318 -119.507	19 vi 2009	JdW		ICHBC142			a	a	
	BC: Sicamous Creek	50.82 -118.86	3 viii 2009	JdW		ICHBC154			a	a	
	MB: Churchill	58.634 -93.786	25 viii 2006	PDNH		JX829722			a		

O. minutus **species-group**

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
6912	UK: Aldbury	51.799 -0.601	21 iv 2011	GRB	*minutus*	KF594849			a	a	
6913	UK: Aldbury	51.799 -0.601	9 v 2011	GRB	*minutus*	KF594818	KF616300	KF616360	a,b,c,d	a,b	
7525	UK			GRB	*minutus*	KF595000			a	a	
7534	UK: London, Barnes	51.477 -0.235	10 v 2012	MRH	*minutus*	KF595008			a	a	
7535	UK: London, Barnes	51.477 -0.235	10 v 2012	MRH	*minutus*	KF595009			a	a	
7536	UK: London, Barnes	51.477 -0.235	10 v 2012	MRH	*minutus*	KF595010			a	a	
7539	UK: Bucks., Steps Hill		23 v 2012	PH	*minutus*	KF595013			a	a	
7540	UK: Bucks., Steps Hill		23 v 2012	PH	*minutus*	KF595014			a	a	
	UK	not listed	not listed		*minutus*			EU378717	c		

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DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	GenBank/BOLD*			Dataset[5]		
						COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
	UK	not listed	not listed		*minutus*	JF963665			a		
7510	UK: Aldbury	51.799 -0.601	6 vi 2010	GRB	*ventricosus*	KF594989			a	a	
7511	UK: S Lyndhurst, Denny Wood	50.851 -1.523	2 v 2008	GRE	*ventricosus*	KF594990			a	a	
7544	UK: Bucks., Barley End		24 v 2012	GRB	*ventricosus*	KF595018			a	a	
7566	UK: Ruislip, Copse Wood	51.59 -0.44	22 iv 2011	AMG	*ventricosus*	KF595037			a	a	
	Not listed				*ventricosus*			z97888	c		
O. obscuratus species-group											
	Taiwan	not listed	not listed		*bicarinatus*			EU378715	c		
7503	UK: Ascot	51.4 -0.6	1 vi 1999	GRB	*brevicornis*	KF594983			a	a	
7504	UK: Ascot	51.4 -0.6	1 vi 1999	GRB	*brevicornis*	KF594998			a	a	
7548	UK: New Haw	5.4 -0.5	23 vi 2012	AJB	*brevicornis*	KF595022			a	a	
6922	UK: Aldbury	51.799 -0.601	5 v 2011	GRB	*costatus*	KF594822	KF616235		a,b	a,b	
6923	UK: Aldbury	51.799 -0.601	3 vi 2011	GRB	*costatus*		KF616238		b	b	
6925	UK: Aldbury	51.799 -0.601	25 v 2011	GRB	*costatus*	KF594824	KF616237	KF616351	a,b,c,d	a,b	
6926	UK: Aldbury	51.799 -0.601	5 v 2011	GRB	*costatus*	KF594825	KF616236		a,b	a,b	
7521	UK				*costatus*	KF594997			a	a	
7526	UK: Aldbury	51.799 -0.601	10 vi 2012	GRB	*costatus*	KF595001			a	a	
7533	UK: London, Barnes	51.477 -0.235	10 v 2012	MRH	*costatus*	KF595007			a	a	
7538	UK: London, Barnes	51.477 -0.235	28 v 2012	MRH	*costatus*	KF595012			a	a	
7541	UK: Bucks., Barley End		24 v 2012	GRB	*costatus*	KF595015			a	a	
7542	UK: Bucks., Barley End		24 v 2012	GRB	*costatus*	KF595016			a	a	
7543	UK: Bucks., Barley End		24 v 2012	GRB	*costatus*	KF595017			a	a	
7545	UK: Bucks., Barley End		24 v 2012	GRB	*costatus*	KF595019			a	a	
7555	UK: Radnage	51.659 -0.858	11 vi 2007	AMG	*costatus*	KF595027			a	a	
	UK	not listed	not listed		*costatus*			EU378716	c		
	UK	not listed	not listed		*costatus*	JF963664			a		
6921	UK: Aldbury	51.799 -0.601	6 v 2011	GRB	*crassicornis*	KF594886	KF616241	KF616350	a,b,c,d	a,b	
7505	UK: Scotland: Kinnaird	56.448 -3.233	14-20 vi 2007	JATW	*crassicornis*	KF594984			a	a	
7506	UK: Scotland: Kinnaird	56.448 -3.233	2-3 vi 2007	JATW	*crassicornis*	KF594985			a	a	
7507	UK: Westcott	51.848 -0.962	21 v 2009	DW	*crassicornis*	KF594986			a	a	
7570	FR: Aude, Pouzols-Minervois	43.3 2.8	26-31 v 2012	MRS	*forticornis*	KF595041			a	a	
6919	UK: Aldbury	51.799 -0.601	9 vi 2011	GRB	*mocsaryi*	KF594839		KF616349	a,c	a	
6920	UK: Aldbury	51.799 -0.601	9 vi 2011	GRB	*mocsaryi*		KF616239		b	b	
7520	UK: Aldbury	51.799 -0.601	3 vii 2010	GRB	*mocsaryi*	KF594996			a	a	
7528	UK: Aldbury	51.799 -0.601	18 vi 2012	GRB	*mocsaryi*	KF595003			a	a	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
						GenBank/BOLD*			Dataset[5]		
7530	UK: Aldbury	51.799 -0.601	6 vii 2012	GRB	*mocsaryi*	KF595004				a	a
7531	UK: Aldbury	51.799 -0.601	14 vii 2012	GRB	*mocsaryi*	KF595005				a	a
7532	UK: Aldbury	51.799 -0.601	12 vii 2012	GRB	*mocsaryi*	KF595006				a	a
7537	UK: London, Barnes	51.477 -0.235	23 v 2012	MRH	*mocsaryi*	KF595011				a	a
7559	UK: Radnage	51.659 -0.858	22 v 2008	AMG	*mocsaryi*	KF595031				a	a
	UK	not listed	not listed		*mocsaryi*			EU378718	c		
	Not listed	not listed	not listed		*obscuratus*			z97889	c		
7523	UK: Aldbury	51.799 -0.601	9 v 2012	GRB	*obscuratus A*	KF594999				a	a
7549	UK: Aldbury	51.799 -0.601	24 ii 2012	GRB	*obscuratus A*	KF595044				a	a
	UK	not listed	not listed		*obscuratus A*	FN662468				a	a
	UK	not listed	not listed		*obscuratus A*	JF963666				a	a
6917	UK: Aldbury	51.799 -0.601	21 iv 2011	GRB	*obscuratus A*	KF594838	KF616246	KF616348	a,b,c,d	a,b	
7561	ESP: Parque Nacional Mondrago	39.4 3.2	27 ii 2007	MRH	*obscuratus B*	KF595033				a	a
7527	UK	?	?	GRB	*obscuratus C*	KF595002				a	a
7546	UK: Bucks., Barley End		24 v 2012	GRB	*obscuratus C*	KF595020				a	a
7569	UK: Aldbury	51.799 -0.601	23 vi 2012	GRB	*"parvulus"*	KF595040				a	a
3902	AB: Kootenay Plains PRA	52.064 -116.422	14 vii 2007	MDS		KF594478	KF615984	KF616308	a,b,c,d	a,b	a,b,c
3903	AB: Cooking Lake-Blackfoot PRA	53.465 -112.981	17 v 2008	MDS		KF594479	KF616050		a,b	a,b	
3905	AB: 18 km SE Deadwood	56.659 -117.200	3 v 2008	DM		KF594481	KF616081		a,b	a,b	
3906	AB: Kootenay Plains PRA	52.064 -116.422	14 vii 2007	MDS		KF594482	KF615993		a,b	a,b	
3907	BC: Tranquille ER	50.755 -120.589	05 v 2008	JJD		KF594483	KF616148	KF616309	a,b,c,d	a,b	
3909	AB: Sherwood Park	53.478 -113.229	12-15 v 2008	GRP		KF594485	KF616076		a,b	a,b	
3912	AB: Jasper NP	53.205 -117.927	3 vii 2008	WH		KF594488	KF616008	KF616312	a,b,c,d	a,b	
3916	AB: Erskine	52.322 -112.883	11 vi 2008	CDB		KF594491	KF616089	KF616310	a,b,c,d	a,b	
3917	AB: nr. Arrowwood	50.757 -113.128	17 v 2008	CDB		KF594492	KF616078		a,b	a,b	
3920	AB: nr. Tangent Park	56.092 -117.542	22 vi 2008	DM		KF594495	KF616114		a,b	a,b	
3922	AB: Portage Lake	54.966 -112.033	18 vi 2008	DM		KF594497	KF616079		a,b	a,b	
3923	AB: Machesis Lake PRA	58.325 -116.578	10 vi 2008	DM			KF616080		b	b	
3924	AB: Peace River	56.342 -117.318	3 v 2007	DM		KF594498	KF616083		a,b	a,b	
3925	NL: Wiltondale	49.37 -57.738	20 vii 2008	DM		KF594499				a	a
3927	AB: Pembina River PP	53.607 -115.003	4 vi 2007	WH		KF594501	KF615990		a,b	a,b	
3928	AB: Porcupine Hills	49.972 -114.087	29 v 2008	JJD		KF594502				a	a
3929	AB: Porcupine Hills	49.972 -114.087	8 vii 2008	JJD		KF594503	KF615985		a,b	a,b	
3930	AB: Clifford E. Lee Nature Sanctuary	53.523 -113.774	5 vii 2008	JJD		KF594504	KF615992		a,b	a,b	
3931	BC: Tranquille ER	50.755 -120.589	15 vii 2008	JJD		KF594505	KF616224		a,b	a,b	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	GenBank/BOLD*			Dataset[5]		
						COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
3932	BC: Valemount	52.835 -119.31	14 vii 2008	JJD		KF594506	KF616026		a,b	a,b	
3935	ON: Bells Corner	45.295 -75.83	25 v 2008	BCS		KF594509	KF616099		a,b	a,b	
3936	ON: Bells Corner	45.295 -75.83	25 v 2008	BCS		KF594510	KF616169	KF616313	a,b,c,d	a,b	
3940	ON: nr. Carp	45.385 -76.008	13 v 2008	BCS		KF594514		KF616334	a,c	a	
3941	AB: nr. Tangent Park	56.092 -117.542	22 vi 2008	DM		KF594515	KF616192		a,b	a,b	
3942	AB: Kootenay Plains PRA	52.064 -116.422	14 vii 2007	MDS		KF594516	KF616003	KF616318	a,b,c,d	a,b	
3943	AB: Pakowki sand dunes	49.397 -110.875	8 vii 2008	JJD		KF594517	KF616161		a,b	a,b	
3944	ON: nr. Carp	45.385 -76.008	13 v 2008	BCS		KF594518	KF616094		a,b	a,b	
3946	AB: nr. Tangent Park	56.092 -117.542	22 vi 2008	DM		KF594520	KF616115		a,b	a,b	
3947	AB: Pakowki sand dunes	49.397 -110.875	24 vii 2008	JJD		KF594521	KF616037		a,b	a,b	
3948	AB: Pembina River PP	53.607 -115.003	4 vi 2007	WH		KF594522	KF616086		a,b	a,b	
3949	AB: Buffalo	50.808 -110.675	7 vii 2008	GGA		KF594523	KF616162		a,b	a,b	
3951	ON: Leeds Grenville Co.	44.487 -76.008	7 vi 2008	BCS		KF594525	KF616004	KF616315	a,b,c,d	a,b	
3952	AB: Bindloss	50.878 -110.259	9 vii 2008	GGA		KF594526	KF616163		a,b	a,b	
3953	AB: nr. Tangent Park	56.092 -117.542	22 vi 2008	DM		KF594527	KF616170		a,b	a,b	
3955	AB: nr. Arrowwood	50.757 -113.128	17 v 2008	CDB		KF594529	KF616082		a,b	a,b	
3958	AB: 21 km S Whitelaw	55.928 -117.995	9 viii 2008	DM		KF594532	KF616000		a,b	a,b	
3959	BC: Tranquille ER	50.755 -120.589	31 v 2007	JJD		KF594533	KF616194		a,b	a,b	
3962	AB: 21 km S Whitelaw	55.928 -117.995	9 vi 2008	DM		KF594536	KF616092		a,b	a,b	
3963	AB: 21 km S Whitelaw	55.928 -117.995	29 vi 2008	DM		KF594537	KF616130		a,b	a,b	
3964	AB: Tangent Park	56.092 -117.542	8 viii 2008	DM		KF594538	KF616001		a,b	a,b	
3967	AB: 48 km NW Dixonville	56.753 -118.333	24 vi-8 vii 2008	MDS		KF594541	KF616085		a,b	a,b	
3968	AB: 48 km NW Dixonville	56.753 -118.328	8-18 vii 2008	MDS		KF594542	KF616193		a,b	a,b	
3969	AB: 48 km NW Dixonville	56.749 -118.324	28 v-10 vi 2008	MDS		KF594543	KF616090		a,b	a,b	
3972	AB: Edmonton	53.545 -113.439	4 vi 2008	GGA		KF594545			a	a	
3973	AB: Edmonton	53.545 -113.439	23 v 2009	GGA		KF594546	KF616088		a,b	a,b	
3981	AB: Buffalo Lake CA	52.499 -112.738	19 vi 2008	CDB		KF594553	KF616197		a,b	a,b	
3982	NL: Wiltondale	49.37 -57.738	20 vii 2008	DM		KF594554	KF616186		a,b	a,b	
3983	AB: Porcupine Hills	49.972 -114.087	8 vii 2008	JJD		KF594555	KF615987		a,b	a,b	
3984	AB: Notikewan PP	57.28 -117.138	9 vi 2008	DM		KF594556	KF616097		a,b	a,b	
3987	AB: 48 km NW Dixonville	56.750 -118.326	28 v-10 vi 2008	MDS		KF594559	KF616061		a,b	a,b	
5538	BC: Murray Lake	49.802 -121.005	18 vii 2009	DGH		KF594562	KF616208		a,b	a,b	
5543	BC: Salt Spring Island	48.89 -123.53	14-21 viii 2008	JE		KF594567	KF616038	KF616316	a,b,c,d	a,b	
5545	AB: Pigeon Lake	53.072 -114.072	16-29 vi 2007	FAHS		KF594569	KF616182		a,b	a,b	
5553	ON: Leeds Grenville Co.	44.487 -76.008	21 v 2009	BCS		KF594577	KF616051		a,b	a,b	
5555	ON: Grenadier Is.	44.4 -75.9	10-21 vi 1994	CNC		KF594579	KF616064		a,b	a,b	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	GenBank/BOLD*			Dataset[5]		
						COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
5557	AZ: Cochise Co.	31.39 -110.24	15 vii -14 viii 1994	NM		KF594581	KF616120	KF616335	a,b,c,d	a,b	
5562	PE: Tryon	46.2 -63.5	26 vi 1991	MEMS		KF594585	KF616102		a,b	a,b	
5569	AZ: Animas Mountains	31.592 -108.775	13-14 viii 1999	JEO		KF594592	KF616233	KF616322	a,b,c,d	a,b	
5574	AB: 48 km NW Dixonville	56.749 -118.354	18-29 vii 2008	MDS		KF594596	KF616202		a,b	a,b	
5575	AB: 48 km NW Dixonville	56.749 -118.321	28 v-10 vi 2008	MDS		KF594597	KF616142		a,b	a,b	
5576	AB: 48 km NW Dixonville	56.746 -118.362	27 v-10 vi 2008	MDS		KF594598	KF616124		a,b	a,b	
5577	AB: 48 km NW Dixonville	56.751 -118.330	28 v-10 vi 2008	MDS		KF594599	KF616101		a,b	a,b	
5578	AB: 48 km NW Dixonville	56.753 -118.328	8-18 vii 2008	MDS		KF594600	KF616211		a,b	a,b	
5579	ON: Leeds Grenville Co.	44.487 -76.008	21 v 2009	BCS		KF594601			a	a	
5580	ON: Leeds Grenville Co.	44.487 -76.008	21 v 2009	BCS		KF594602	KF616103		a,b	a,b	
5585	BC: Pender Island	48.771 -123.300	20 vii 2009	MDS		KF594605	KF616035		a,b	a,b	
5586	AB: Banff NP	51.194 -115.52	03 viii 2009	MDS		KF594606	KF616009		a,b	a,b	
5587	AB: Edmonton	53.545 -113.439	17 v 2008	GGA		KF594607	KF616104		a,b	a,b	
5588	AB: Sherwood Park	53.478 -113.229	12-15 v 2008	GRP		KF594608	KF616093		a,b	a,b	
5589	AB: Cooking Lake-Blackfoot PRA	53.465 -112.981	17 v 2008	MDS		KF594609	KF616087		a,b	a,b	
5590	AB: Kootenay Plains PRA	52.064 -116.422	14 vii 2007	MDS		KF594610	KF616075		a,b	a,b	
5591	AB: Jasper NP	53.205 -117.927	3 vii 2008	WH		KF594611	KF616131	KF616324	a,b,c,d	a,b	
5592	AB: Jasper NP	53.205 -117.927	3 vii 2008	WH		KF594612	KF616014		a,b	a,b	
5593	AB: Pakowki sand dunes	49.397 -110.875	8 vii 2008	JJD		KF594613			a	a	
5594	AB: Pakowki sand dunes	49.397 -110.875	8 vii 2008	JJD		KF594614	KF615989		a,b	a,b	
5595	AB: Pakowki sand dunes	49.397 -110.875	24 vii 2008	JJD		KF594615	KF616218		a,b	a,b	
5596	AB: Buffalo Lake CA	52.499 -112.738	19 vi 2008	CDB		KF594616	KF616117		a,b	a,b	
5597	AB: nr. Arrowwood	52.499 -112.738	19 vi 2008	CDB		KF594617	KF616055		a,b	a,b	
5599	AB: Portage Lake	54.966 -112.033	18 vi 2008	DM		KF594619	KF615991		a,b	a,b	
5600	AB: 18 km SE Deadwood	56.659 -117.2	4 vii 2008	DM		KF594620	KF615924		a,b	a,b	
5701	NL: Wiltondale	49.37 -57.738	20 vii 2008	DM		KF594621	KF616187		a,b	a,b	
5703	AB: Pembina River PP	53.607 -115.003	4 vi 2007	WH		KF594623	KF616116		a,b	a,b	
5704	BC: Sheridan Lake	51.535 -120.952	27 vii 2009	DGH		KF594624	KF616006		a,b	a,b	
5710	AB: 48 km NW Dixonville	56.750 -118.326	8-18 vii 2008	MDS		KF594628	KF616220		a,b	a,b	
5711	AB: 33 km NW Dixonville	56.727 -118.267	12-19 vi 2007	MDS		KF594629	KF616189	KF616326	a,b,c,d	a,b	
5713	AB: 31 km NW Dixonville	56.703 -118.216	7-16 vii 2007	MDS		KF594631	KF616215		a,b	a,b	
5714	AB: 48 km NW Dixonville	56.746 -118.362	8-18 vii 2008	MDS		KF594632	KF616183	KF616327	a,b,c,d	a,b	
5716	AB: Porcupine Hills	49.972 -114.087	14 vi 2009	JJD		KF594634			a	a	
5718	BC: Burnaby	49.28 -122.939	29 vii 2010	MDS		KF594636	KF616227		a,b	a,b	
5719	BC: Green Lake	51.431 -121.198	26 vii 2010	MDS		KF594637	KF615988		a,b	a,b	
5720	BC: Cinnamon Forest Rec Site	50.614 -122.105	27 vii 2010	MDS		KF594638	KF616028		a,b	a,b	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
									GenBank/BOLD*		Dataset[5]
5721	BC: 21 km NE Pemberton	50.37 -122.498	27 vii 2010	MDS		KF594639	KF616185		a,b	a,b	
5722	BC: Metchosin	48.394 -123.512	30 vii 2010	MDS		KF594640	KF616234	KF616336	a,b,c,d	a,b	
5727	BC: Mt. Begbie, nr. 100 Mile House	51.477 -121.369	26 vii 2010	MDS		KF594645	KF616027		a,b	a,b	
5729	BC: Mt. Begbie, nr. 100 Mile House	51.477 -121.369	26 vii 2010	MDS		KF594647	KF616217		a,b	a,b	
5731	BC: 6 km S Squamish	49.655 -123.193	28 vii 2010	MDS		KF594649	KF616230		a,b	a,b	
5732	BC: Glacier NP	51.243 -117.649	25 vii 2010	MDS		KF594650	KF616039		a,b	a,b	
5733	BC: Hope	49.381 -121.428	1 viii 2010	MDS		KF594651	KF616240	KF616325	a,b,c,d	a,b	
5734	BC: 7 km NW Bridesville	49.05 -119.059	2 viii 2010	MDS		KF594652	KF616159		a,b	a,b	
5737	BC: Kootenay NP	50.675 -115.888	9 vii 2010	MDS		KF594655	KF616040		a,b	a,b	
5738	BC: Glacier NP	51.427 -117.477	8 vii 2010	MDS		KF594656	KF616201		a,b	a,b	
5739	BC: 9 km NW Pt. Alberni	49.297 -124.925	31 vii 2010	MDS		KF594657	KF616036		a,b	a,b	
5741	BC: Kamloops	50.663 -120.433	8 vi 2010	MDS		KF594659	KF616138		a,b	a,b	
5744	WA: Wenatchee NF	47.584 -120.367	9 vii 2010	JA		KF594662			a	a	
5745	BC: Kootenay NP	50.621 -116.061	9 vii 2010	MDS		KF594663	KF616226		a,b	a,b	
5747	BC: Kootenay NP	50.621 -116.061	9 vii 2010	MDS		KF594665	KF616232		a,b	a,b	
5748	AB: 5 km NEE Dunstable	53.957 -114.128	15-22 vi 2007	MDS		KF594666	KF616184		a,b	a,b	
5750	BC: New Denver	49.998 -117.372	9 vi 2010	MDS		KF594668	KF616198		a,b	a,b	
5753	BC: Pink Mountain	57.024 -122.849	18 vi 2010	JRD		KF594670	KF616118		a,b	a,b	
5757	AB: Jasper NP	52.963 -118.058	2 vi 2007	JJD		KF594674	KF616175		a,b	a,b	
5759	AB: 62 km WNW Dixonville	56.692 -118.625	26 v 2008	BBB		KF594676	KF616100		a,b	a,b	
5768	NL: Labrador: Muskrat Falls	60.777 -53.254	30 vi 2009	DWL		KF594685	KF616171		a,b	a,b	
5769	NL: Labrador: Muskrat Falls	60.777 -53.254	30 vi 2009	DWL		KF594686	KF616005	KF616339	a,b,c,d	a,b	
5774	AB: Waterton Lakes NP	49.067 -114	18 vii 2010	MDS		KF594691	KF615939		a,b	a,b	
5775	AB: Waterton Lakes NP	49.021 -114.047	18 vii 2010	MDS		KF594692	KF615923		a,b	a,b	
5776	AB: Waterton Lakes NP	49.081 -113.878	18 vii 2010	MDS		KF594693		KF616340	a,c		
5777	AB: Barrier Lake	51.03 -115.03	17 vii 2010	BMTB		KF594694	KF615995		a,b	a,b	
5778	AB: Cypress Hills PP	49.657 -110.036	6 viii 2008	JJD		KF594695	KF616002		a,b	a,b	
5779	AB: Cypress Hills PP	49.672 -110.147	6 viii 2008	JJD		KF594696	KF615994		a,b	a,b	
5780	AB: Porcupine Hills	49.972 -114.087	19 vi 2010	HB		KF594697	KF616047		a,b	a,b	
5781	BC: 6 km S Squamish	49.655 -123.193	28 vii 2010	MDS		KF594698	KF616032		a,b	a,b	
5783	BC: Oliver's Landing	49.593 -123.22	28 vii 2010	MDS		KF594700	KF616228		a,b	a,b	
5784	BC: West Vancouver	49.351 -123.224	28 vii 2010	MDS		KF594701	KF616231	KF616329	a,b,c,d	a,b	
5785	BC: Hope	49.343 -121.45	1 viii 2010	MDS		KF594702	KF616229		a,b	a,b	
5786	BC: 10.5 km SW Lillooet	50.64 -122.073	27 vii 2010	MDS		KF594703	KF616166		a,b	a,b	
5788	BC: 16 km SW 100 Mile House	51.497 -121.389	26 vii 2010	MDS		KF594705	KF616021		a,b	a,b	
5789	BC: 100 Mile House	51.563 -121.361	26 vii 2010	MDS		KF594706	KF615998		a,b	a,b	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	GenBank/BOLD*			Dataset[5]		
						COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
5790	BC: Green Lake	51.431 -121.198	26 vii 2010	MDS		KF594707	KF615996		a,b	a,b	
5791	BC: Cinnamon Forest Rec Site	50.614 -122.105	27 vii 2010	MDS		KF594708	KF616010		a,b	a,b	
5792	BC: Cinnamon Forest Rec Site	50.614 -122.105	27 vii 2010	MDS		KF594709	KF616013		a,b	a,b	
5793	BC: Mt. Begbie, nr. 100 Mile House	51.477 -121.369	26 vii 2010	MDS		KF594710	KF616022		a,b	a,b	
5794	BC: 9 km NW Pt. Alberni	49.297 -124.925	31 vii 2010	MDS		KF594711	KF616033		a,b	a,b	
5800	BC: Kootenay NP	50.627 -116.053	9 vii 2010	MDS		KF594716	KF616225		a,b	a,b	
6505	AB: Waterton Lakes NP	49.092 -113.886	7 vi 2010	DWL		KF594720	KF616143		a,b	a,b	
6506	AB: Waterton Lakes NP	49.092 -113.886	7 vi 2010	DWL		KF594721	KF616077		a,b	a,b	
6511	WA: Wenatchee NF	47.584 -120.367	9 vii 2010	JA		KF594726			a	a	
6512	WA: Wenatchee NF	47.584 -120.367	9 vii 2010	JA		KF594727	KF616034		a,b	a,b	
6513	BC: Kootenay NP	50.621 -116.061	9 vii 2010	MDS		KF594728	KF616024		a,b	a,b	
6514	AB: 24 km NW Dixonville	56.667 -118.062	8-12 vi 2007	MDS		KF594729	KF616113		a,b	a,b	
6516	WA: Wenatchee NF	47.584 -120.367	9 vii 2010	JA		KF594731	KF616164		a,b	a,b	
6517	AB: George Lake	53.957 -114.128	27 vi-4 vii 2007	MDS		KF594732	KF616178		a,b	a,b	
6518	BC: Mt. Begbie, nr. 100 Mile House	51.477 -121.369	26 vii 2010	MDS		KF594733			a	a	
6519	AB: Porcupine Hills	49.972 -114.087	15 vi 2009	JJD,BMTB		KF594734	KF616098		a,b	a,b	
6520	BC: nr. Fort Steele	49.636 -115.609	16 vi 2009	JJD		KF594735	KF616112		a,b	a,b	
6521	AB: Porcupine Hills	49.972 -114.087	15 vi 2009	JJD,BMTB		KF594736	KF616107		a,b	a,b	
6522	BC: Cinnamon Forest Rec Site	50.614 -122.105	27 vii 2010	MDS		KF594737	KF616023		a,b	a,b	
6523	AB: George Lake	53.957 -114.125	29 v-6 vi 2007	MDS		KF594738	KF616145	KF616333	a,b,c,d	a,b	
6532	BC: 40 km NE Houston	54.588 -126.408	13 vii 2009	JdW		KF594746			a	a	
6533	BC: 40 km NE Houston	54.633 -126.424	13 vii 2009	JdW		KF594747			a	a	
6536	BC: Haida Gwaii, Graham Is.	54.024 -131.994	26 vii 2009	JdW		KF594750	KF616133		a,b	a,b	
6539	BC: 40 km NE Houston	54.6167 -126.411	22 viii 2009	JdW		KF594751	KF616030		a,b	a,b	
6540	BC: Okanagan Falls	49.318 -119.507	24 v 2009	JdW		KF594752	KF616084		a,b	a,b	
6542	BC: Okanagan Falls	49.32 -119.51		JdW		KF594754	KF616160		a,b	a,b	
6549	SK: nr. Newton Lake	49.301 -107.764	20 v 2011	JJD		KF594761	KF616149	KF616342	a,b,c,d	a,b	
6550	SK: nr. Newton Lake	49.301 -107.764	20 v 2011	JJD		KF594762	KF616150	KF616343	a,b,c,d	a,b	a,b,c
6553	AB: Kootenay Plains PRA	52.064 -116.422	14 vii 2007	MDS			KF615997		b	b	
6555	ON: nr. Carp	45.385 -76.008	13 v 2008	BCS		KF594766	KF616156		a,b	a,b	
6559	SK: Manitou Beach	51.713 -105.449	13 vii 2008	MDS		KF594770	KF615986		a,b	a,b	
6560	AB: Jenner	50.842 -111.151	09 v 2007	MDS			KF616152		b	b	
6575	AB: 8 km NW Winfield	53.01 -114.5	18 vii 2009	CDB		KF594816	KF616199		a,b	a,b	
6576	AB: 8 km NW Winfield	53.01 -114.5	1 viii 2009	CDB		KF594784	KF615999		a,b	a,b	
6577	AB: 8 km NW Winfield	53.01 -114.5	18 vii 2009	CDB		KF594785	KF616221		a,b	a,b	
6578	AB: 8 km NW Winfield	53.01 -114.5	24 vii 2010	CDB		KF594786	KF616167		a,b	a,b	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	GenBank/BOLD* COI	ITS2	28S	Dataset[5] Ch. 2	Ch. 3	Ch. 4
6579	AB: 8 km NW Winfield	53.01 -114.5	10 vii 2010	CDB		KF594787	KF616031		a,b	a,b	
6580	AB: 8 km NW Winfield	53.01 -114.5	1 viii 2009	CDB		KF594788	KF616020		a,b	a,b	
6581	AB: 8 km NW Winfield	53.01 -114.5	2 v 2009	CDB		KF594789	KF616095		a,b	a,b	
6582	AB: 8 km NW Winfield	53.01 -114.5	15 v 2010	CDB		KF594790	KF616147		a,b	a,b	
6583	AB: 8 km NW Winfield	53.01 -114.5	16 v 2009	CDB		KF594791	KF616158		a,b	a,b	
6584	AB: 8 km NW Winfield	53.01 -114.5	12 vi 2010	CDB		KF594792	KF616057		a,b	a,b	
6585	AB: 8 km NW Winfield	53.01 -114.5	12 vi 2010	CDB		KF594793	KF616066		a,b	a,b	
6586	AB: 8 km NW Winfield	53.01 -114.5	31 v 2008	CDB		KF594794	KF616119		a,b	a,b	
6587	AB: 8 km NW Winfield	53.01 -114.5	31 v 2008	CDB		KF594795	KF616139		a,b	a,b	
6588	AB: 8 km NW Winfield	53.01 -114.5	16 v 2009	CDB		KF594796	KF616121		a,b	a,b	
6589	AB: 8 km NW Winfield	53.01 -114.5	12 vi 2010	CDB		KF594797	KF616072		a,b	a,b	
6590	AB: 8 km NW Winfield	53.01 -114.5	4 vi 2010	CDB		KF594798	KF616058		a,b	a,b	
6591	AB: 8 km NW Winfield	53.01 -114.5	20 vi 2008	CDB		KF594799	KF616210		a,b	a,b	
6592	AB: 8 km NW Winfield	53.01 -114.5	20 vi 2008	CDB		KF594800	KF616195		a,b	a,b	
6593	AB: 8 km NW Winfield	53.01 -114.5	20 vi 2008	CDB		KF594801	KF616044		a,b	a,b	
6594	AB: 8 km NW Winfield	53.01 -114.5	31 v 2008	CDB		KF594979	KF616177	KF616345	a,b,c,d	a,b	
6595	AB: 8 km NW Winfield	53.01 -114.5	31 v 2008	CDB		KF594802	KF616109		a,b	a,b	
6596	AB: 8 km NW Winfield	53.01 -114.5	16 v 2009	CDB		KF594803	KF616071		a,b	a,b	
6597	AB: 8 km NW Winfield	53.01 -114.5	16 v 2009	CDB		KF594804	KF616048		a,b	a,b	
6598	AB: 8 km NW Winfield	53.01 -114.5	2 v 2009	CDB		KF594805	KF616062		a,b	a,b	
6599	AB: 8 km NW Winfield	53.01 -114.5	12 vi 2010	CDB		KF594806	KF616096		a,b	a,b	
6600	AB: 8 km NW Winfield	53.01 -114.5	12 vi 2010	CDB		KF594807	KF616059		a,b	a,b	
6901	AB: 8 km NW Winfield	53.01 -114.5	16 v 2009	CDB		KF594808	KF616069		a,b	a,b	
6902	AB: 8 km NW Winfield	53.01 -114.5	16 v 2009	CDB		KF594809	KF616073		a,b	a,b	
6903	AB: 8 km NW Winfield	53.01 -114.5	15 v 2010	CDB		KF594810	KF616052		a,b	a,b	
6904	AB: 8 km NW Winfield	53.01 -114.5	16 v 2009	CDB		KF594811	KF616056		a,b	a,b	
6905	AB: 8 km NW Winfield	53.01 -114.5	16 v 2009	CDB		KF594812	KF616045		a,b	a,b	
6927	AB: 8 km NW Winfield	53.01 -114.5	15 v 2010	CDB		KF594826	KF616146		a,b	a,b	
6928	AB: 8 km NW Winfield	53.01 -114.5	15 v 2010	CDB		KF594827	KF616111		a,b	a,b	
6929	AB: 8 km NW Winfield	53.01 -114.5	15 v 2010	CDB		KF594887	KF616054		a,b	a,b	
6930	AB: Edmonton	53.528 -113.523	4 ix 2010	JJD			KF616273		b	b	
6932	AB: Edmonton	53.545 -113.439	19 vi 2008	GGA			KF616041		b	b	
6934	AB: Edmonton	53.510 -113.622	8 vi 2010	JHA		KF594889			a	a	
6942	AB: Spruce Grove	53.4 -113.9	28 v-2 vi 1989	ATF		KF594891			a	a	
6943	AB: Spruce Grove	53.4 -113.9	28 v-2 vi 1989	ATF		KF594892			a	a	
6945	AB: Spruce Grove	53.4 -113.9	28 v-2 vi 1989	ATF		KF594832			a	a	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	GenBank/BOLD*			Dataset[5]		
						COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
6946	AB: George Lake	53.957 -114.125	6-15 vi 2007	MDS		KF594893	KF616060		a,b	a,b	
6947	AB: George Lake	53.957 -114.128	15-22 vi 2007	MDS		KF594894			a	a	
6948	AB: George Lake	53.957 -114.128	22-27 vi 2007	MDS		KF594833			a	a	
6949	AB: George Lake	53.957 -114.125	15 vi 2007	MDS		KF594834			a	a	
6957	AB: 48 km NW Dixonville	56.746 -118.362	8-18 vii 2008	MDS		KF594840	KF616029		a,b	a,b	
6958	AB: 48 km NW Dixonville	56.751 -118.330	8-18 vii 2008	MDS		KF594841	KF616019		a,b	a,b	
6964	AB: 48 km NW Dixonville	56.751 -118.330	24 vi-8 vii 2008	MDS		KF594846			a	a	
6965	AB: 48 km NW Dixonville	56.750 -118.326	24 vi-8 vii 2008	MDS		KF594847	KF616018		a,b	a,b	
6966	AB: 48 km NW Dixonville	56.750 -118.359	24 vi-8 vii 2008	MDS		KF594848			a	a	
6967	AB: 48 km NW Dixonville	56.749 -118.354	24 vi-8 vii 2008	MDS		KF594850			a	a	
6968	AB: 48 km NW Dixonville	56.748 -118.363	8-18 vii 2008	MDS		KF594851	KF616179		a,b	a,b	
6970	AB: 48 km NW Dixonville	56.753 -118.333	8-18 vii 2008	MDS		KF594853	KF616188		a,b	a,b	
6972	AB: 48 km NW Dixonville	56.749 -118.321	8-18 vii 2008	MDS		KF594855	KF616209		a,b	a,b	
6974	AB: 48 km NW Dixonville	56.749 -118.354	18-29 vii 2008	MDS		KF594857			a	a	
6981	AB: 48 km NW Dixonville	56.754 -118.334	24 vi-8 vii 2008	MDS		KF594864			a	a	
6982	AB: 48 km NW Dixonville	56.752 -118.325	28 v-10 vi 2008	MDS		KF594865	KF616068		a,b	a,b	
6983	AB: 48 km NW Dixonville	56.752 -118.325	28 v-10 vi 2008	MDS		KF594866	KF616070		a,b	a,b	
6984	AB: 48 km NW Dixonville	56.746 -118.362	24 vi-8 vii 2008	MDS		KF594867	KF616141		a,b	a,b	
6985	AB: 48 km NW Dixonville	56.749 -118.360	27 v-10 vi 2008	MDS		KF594868	KF616043		a,b	a,b	
6986	AB: 48 km NW Dixonville	56.749 -118.321	28 v-10 vi 2008	MDS		KF594869	KF616065		a,b	a,b	
6987	AB: 48 km NW Dixonville	56.749 -118.360	10-24 vi 2008	MDS		KF594870	KF616123		a,b	a,b	
6988	AB: 48 km NW Dixonville	56.749 -118.360	10-24 vi 2008	MDS		KF594871	KF616046		a,b	a,b	
6989	AB: 48 km NW Dixonville	56.749 -118.324	10-24 vi 2008	MDS		KF594872	KF616049		a,b	a,b	
6990	AB: 48 km NW Dixonville	56.749 -118.324	10-24 vi 2008	MDS		KF594873	KF616074		a,b	a,b	
6992	AB: 48 km NW Dixonville	56.753 -118.328	28 v-10 vi 2008	MDS		KF594874	KF616190		a,b	a,b	
6993	AB: 48 km NW Dixonville	56.753 -118.328	10-24 vi 2008	MDS		KF594875			a	a	
6994	AB: 48 km NW Dixonville	56.746 -118.362	10-24 vi 2008	MDS		KF594876			a	a	
6995	AB: 48 km NW Dixonville	56.751 -118.330	8-18 vii 2008	MDS		KF594877	KF616135		a,b	a,b	
6997	AB: 48 km NW Dixonville	56.753 -118.328	18-29 vii 2008	MDS		KF594878	KF616129	KF616354	a,b,c,d	a,b	
6998	AB: 48 km NW Dixonville	56.754 -118.334	10-24 vi 2008	MDS		KF594879	KF616180		a,b	a,b	
6999	AB: 48 km NW Dixonville	56.752 -118.325	10-24 vi 2008	MDS		KF594880	KF616203		a,b	a,b	
7000	AB: 48 km NW Dixonville	56.749 -118.324	10-24 vi 2008	MDS		KF594881	KF616214		a,b	a,b	
7301	AB: 48 km NW Dixonville	56.751 -118.330	24 vi-8 vii 2008	MDS		KF594882			a	a	
7302	AB: 48 km NW Dixonville	56.750 -118.363	24 vi-8 vii 2008	MDS		KF594883	KF616017		a,b	a,b	
7303	AB: 48 km NW Dixonville	56.753 -118.328	24 vi-8 vii 2008	MDS		KF594884			a	a	
7304	AB: 48 km NW Dixonville	56.750 -118.326	24 vi-8 vii 2008	MDS		KF594885			a	a	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	GenBank/BOLD*			Dataset[5]		
						COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
7305	AB: 62 km WNW Dixonville	56.691 -118.675	8 vii 2008	BBB		KF594895	KF616204		a,b	a,b	
7306	AB: 54 km NW Dixonville	56.86 -118.31	11 vi 2007	BBB		KF594896	KF616219		a,b	a,b	
7307	AB: 62 km WNW Dixonville	56.698 -118.648	26 v 2008	BBB		KF594897	KF616105		a,b	a,b	
7308	AB: 62 km WNW Dixonville	56.694 -118.648	7 vi 2008	BBB		KF594898	KF616106		a,b	a,b	
7309	AB: 62 km WNW Dixonville	56.683 -118.648	7 vi 2008	BBB		KF594899			a	a	
7310	AB: 62 km WNW Dixonville	56.684 -118.644	18 vi 2008	BBB		KF594900	KF616053		a,b	a,b	
7311	AB: 62 km WNW Dixonville	56.682 -118.636	18 vi 2008	BBB		KF594901	KF616155		a,b	a,b	
7313	AB: 62 km WNW Dixonville	56.686 -118.645	18 vi 2008	BBB		KF594903	KF616108		a,b	a,b	
7314	AB: 62 km WNW Dixonville	56.697 -118.653	7 vi 2008	BBB		KF594904	KF616063		a,b	a,b	
7315	AB: 62 km WNW Dixonville	56.683 -118.648	8 vii 2008	BBB		KF594905	KF616222		a,b	a,b	
7316	AB: 62 km WNW Dixonville	56.682 -118.636	21 vi 2008	BBB		KF594906	KF616223		a,b	a,b	
7317	AB: 62 km WNW Dixonville	56.689 -118.644	21 vi 2008	BBB		KF594907	KF616200		a,b	a,b	
7318	AB: 62 km WNW Dixonville	56.682 -118.647	21 vi 2008	BBB		KF594908	KF616212		a,b	a,b	
7319	AB: 62 km WNW Dixonville	56.689 -118.644	7 vi 2008	BBB		KF594909	KF616173		a,b	a,b	
7320	AB: 62 km WNW Dixonville	56.690 -118.647	7 vi 2008	BBB		KF594910	KF616174		a,b	a,b	
7321	AB: 62 km WNW Dixonville	56.691 -118.672	7 vi 2008	BBB		KF594911	KF616176		a,b	a,b	
7322	AB: 62 km WNW Dixonville	56.682 -118.647	21 vi 2008	BBB		KF594912	KF616206		a,b	a,b	
7323	AB: 62 km WNW Dixonville	56.682 -118.636	21 vi 2008	BBB		KF594913	KF616196		a,b	a,b	
7326	AB: 62 km WNW Dixonville	56.691 -118.54	8 vii 2008	BBB		KF594916	KF616172		a,b	a,b	
7328	AB: 62 km WNW Dixonville	56.682 -118.647	21 vi 2008	BBB		KF594918	KF616011		a,b	a,b	
7329	AB: 62 km WNW Dixonville	56.690 -118.638	8 vii 2008	BBB		KF594919	KF616007		a,b	a,b	
7330	AB: 62 km WNW Dixonville	56.683 -118.648	18 vi 2008	BBB		KF594920	KF616016		a,b	a,b	
7331	AB: 62 km WNW Dixonville	56.684 -118.644	8 vii 2008	BBB		KF594921	KF616015		a,b	a,b	
7332	AB: 62 km WNW Dixonville	56.691 -118.675	8 vii 2008	BBB		KF594922	KF616213		a,b	a,b	
7333	AB: 62 km WNW Dixonville	56.694 -118.648	21 vi 2008	BBB		KF594923	KF616136		a,b	a,b	
7334	AB: 62 km WNW Dixonville	56.692 -118.666	8 vii 2008	BBB		KF594924	KF616205		a,b	a,b	
7335	AB: 62 km WNW Dixonville	56.691 -118.675	8 vii 2008	BBB		KF594925	KF616207		a,b	a,b	
7336	AB: 62 km WNW Dixonville	56.682 -118.636	26 v 2008	BBB		KF594926	KF616140		a,b	a,b	
7337	AB: 62 km WNW Dixonville	56.691 -118.672	26 v 2008	BBB		KF594927	KF616122		a,b	a,b	
7338	AB: 62 km WNW Dixonville	56.698 -118.648	26 v 2008	BBB		KF594928	KF616067		a,b	a,b	
7339	AB: 48 km NW Dixonville	56.753 -118.328	24 vi-8 vii 2008	MDS		KF594929	KF616125		a,b	a,b	
7340	AB: 48 km NW Dixonville	56.746 -118.362	24 vi-8 vii 2008	MDS		KF594930	KF616128		a,b	a,b	
7341	AB: 48 km NW Dixonville	56.753 -118.328	24 vi-8 vii 2008	MDS		KF594931			a	a	
7342	AB: 48 km NW Dixonville	56.750 -118.363	24 vi-8 vii 2008	MDS		KF594932	KF616168		a,b	a,b	
7343	AB: 48 km NW Dixonville	56.749 -118.354	18-29 vii 2008	MDS		KF594933	KF616181		a,b	a,b	
7344	AB: 33 km NW Dixonville	56.727 -118.267	29 vi-7 vii 2007	MDS			KF616132	KF616355	b,c	b	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
						GenBank/BOLD*			**Dataset[5]**		
7345	AB: 33 km NW Dixonville	56.727 -118.267	21-29 vi 2007	MDS		KF594934	KF616134		a,b	a,b	
7346	AB: 33 km NW Dixonville	56.727 -118.267	12-19 vi 2007	MDS		KF594935	KF616191		a,b	a,b	
7349	SK: nr. Newton Lake	49.301 -107.764	20 v 2011	JJD		KF594938	KF616153		a,b	a,b	
7350	SK: nr. Newton Lake	49.301 -107.764	20 v 2011	JJD		KF594939	KF616151		a,b	a,b	
7351	AB: 31 km NW Dixonville	56.703 -118.216	21-29 vi 2007	MDS		KF594940	KF616025		a,b	a,b	
7352	AB: 31 km NW Dixonville	56.703 -118.216	24 vii-2 viii 2007	MDS		KF594941			a	a	
7353	AB: Jenner	50.842 -111.151	26 v 2007	GGA, JJD			KF616110		b,c	b	
7355	AB: Pakowki sand dunes	49.397 -110.875	8 vii 2008	JJD		KF594943	KF616127		a,b	a,b	
7357	AB: Pakowki sand dunes	49.397 -110.875	8 vii 2008	JJD		KF594945	KF616126		a,b	a,b	
7358	AB: Jenner	50.842 -111.151	07 vi 2007	GGA		KF594946			a	a	
7365	AB: Jenner	50.842 -111.151	07 vi 2007	GGA		KF594950			a	a	
7366	AB: Jenner	50.842 -111.151	26 vi 2009	TP		KF594951	KF615922		a,b	a,b	
7372	AB: Pakowki sand dunes	49.397 -110.875	8 vii 2008	JJD		KF594952			a	a	
7373	AB: Pakowki sand dunes	49.397 -110.875	12-13 vii 2009	GGA		KF594953			a	a	
7385	AB: Pakowki sand dunes	49.397 -110.875	8 vii 2008	JJD		KF594965	KF616091		a,b	a,b	
7386	AB: Pakowki sand dunes	49.397 -110.875	8 vii 2008	JJD		KF594966	KF616144		a,b	a,b	
7387	AB: Pakowki sand dunes	49.397 -110.875	8 vii 2008	JJD		KF594967	KF616157		a,b	a,b	
7388	AB: Pakowki sand dunes	49.397 -110.875	8 vii 2008	JJD		KF594968	KF616012		a,b	a,b	
7392	AB: Jenner	50.844 -111.154	2 vi 2010	GGA		KF594971	KF616137		a,b	a,b	
7393	AB: Jenner	50.844 -111.154	2 vi 2010	GGA		KF594972	KF616042		a,b	a,b	
7394	AB: Pakowki sand dunes	49.397 -110.875	8 vii 2008	JJD		KF594973			a	a	
7396	AB: Jenner	50.842 -111.151	26 v 2007	GGA, JJD			KF616154		b	b	
7397	AB: Pakowki sand dunes	49.397 -110.875	8 vii 2008	JJD		KF594975	KF616216		a,b	a,b	
7398	AB: Pakowki sand dunes	49.397 -110.875	12-13 vii 2009	GGA		KF594976	KF616165		a,b	a,b	
7399	AB: Pakowki sand dunes	49.397 -110.875	8 vii 2008	JJD		KF594977			a	a	
7400	AB: Pakowki sand dunes	49.397 -110.875	13-14 vii 2009	GGA		KF594978			a	a	
	BC: Sicamous Creek	50.814 -118.881	10 vii 2008	JdW		ICHBC006			a	a	
	BC: Winfield	50.022 -119.325	5 vi 2008	JdW		ICHBC014			a	a	
	BC: Winfield	50.022 -119.325	5 vi 2008	JdW		ICHBC016			a	a	
	BC: Winfield	50.022 -119.325	5 vi 2008	JdW		ICHBC017			a	a	
	BC: Winfield	50.022 -119.325	5 vi 2008	JdW		ICHBC020			a	a	
	BC: Winfield	50.022 -119.325	5 vi 2008	JdW		ICHBC021			a	a	
	BC: nr Kamloops: Sugarloaf	50.638 -120.451	9 vii 2008	JdW		ICHBC023			a	a	
	BC: nr Kamloops: Sugarloaf	50.638 -120.451	9 vii 2008	JdW		ICHBC027			a	a	
	BC: Kamloops	50.67 -120.408	12 vi 2008	JdW		ICHBC038			a	a	
	BC: Kamloops	50.67 -120.408	12 vi 2008	JdW		ICHBC042			a	a	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	GenBank/BOLD*			Dataset[5]		
						COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
	BC: Date Creek	55.464 -127.81	22 v 2008	JdW		ICHBC056				a	a
	BC: 2 km N Otter L	49.6 -120.8	5 vii 2008	JdW		ICHBC070				a	a
	BC: nr Kamloops: Sugarloaf	50.638 -120.451	4 vii 2008	JdW		ICHBC074				a	a
	BC: nr Kamloops: Sugarloaf	50.638 -120.451	4 v 2009	JdW		ICHBC077				a	a
	BC: Okanagan Falls	49.312 -119.502	8 vi 2009	JdW		ICHBC078				a	a
	BC: nr Kamloops: Dewdrop	50.747 -120.555	4 v 2008	JdW		ICHBC080				a	a
	BC: nr Kamloops: Dewdrop	50.747 -120.555	4 v 2008	JdW		ICHBC082				a	a
	BC: nr Kamloops: Dewdrop	50.747 -120.555	4 v 2008	JdW		ICHBC083				a	a
	BC: nr Kamloops: Dewdrop	50.747 -120.555	4 v 2008	JdW		ICHBC084				a	a
	BC: nr Kamloops: Dewdrop	50.747 -120.555	4 v 2008	JdW		ICHBC085				a	a
	BC: Kamloops	50.739 -120.727	4 v 2008	JdW		ICHBC088				a	a
	BC: Date Creek	55.439 -127.814	29 v 2009	JdW		ICHBC089				a	a
	BC: Date Creek	55.464 -127.81	22 v 2008	JdW		ICHBC090				a	a
	BC: Date Creek	55.464 -127.81	29 v 2008	JdW		ICHBC091				a	a
	BC: Date Creek	55.464 -127.81	29 v 2008	JdW		ICHBC092				a	a
	BC: Date Creek	55.464 -127.81	29 v 2008	JdW		ICHBC093				a	a
	BC: Date Creek	55.465 -127.806	29 v 2009	JdW		ICHBC094				a	a
	BC: Okanagan Falls	49.318 -119.507	8 vi 2009	JdW		ICHBC095				a	a
	BC: Okanagan Falls	49.312 -119.502	8 vi 2009	JdW		ICHBC100				a	a
	BC: Okanagan Falls	49.312 -119.502	8 vi 2009	JdW		ICHBC101				a	a
	BC: Mahoney Lake	49.285 -119.586	8 vii 2009	JdW		ICHBC112				a	a
	BC: Mahoney Lake	49.285 -119.586	8 vii 2009	JdW		ICHBC113				a	a
	IBC: Mahoney Lake	49.285 -119.586	8 vi 2009	JdW		ICHBC115				a	a
	BC: Mahoney Lake	49.291 -119.591	8 vi 2009	JdW		ICHBC118				a	a
	BC: Mahoney Lake	49.291 -119.591	8 vi 2009	JdW		ICHBC119				a	a
	BC: Date Creek	55.459 -127.812	22 vi 2009	JdW		ICHBC122				a	a
	BC: Date Creek	55.431 -127.81	22 vi 2009	JdW		ICHBC123				a	a
	BC: Houston	54.6 -126.4	23 vi 2009	JdW		ICHBC124				a	a
	BC: Houston	54.6 -126.4	23 vi 2009	JdW		ICHBC125				a	a
	BC: Houston	54.617 -126.411	23 vi 2009	JdW		ICHBC126				a	a
	BC: Houston	54.704 -126.282	23 vi 2009	JdW		ICHBC127				a	a
	BC: Houston	54.704 -126.282	23 vi 2009	JdW		ICHBC128				a	a
	BC: Mahoney Lake	49.285 -119.586	19 vi 2009	JdW		ICHBC132				a	a
	BC: Mahoney Lake	49.285 -119.586	19 vi 2009	JdW		ICHBC133				a	a
	BC: Mahoney Lake	49.285 -119.586	19 vi 2009	JdW		ICHBC134				a	a
	BC: Mahoney Lake	49.285 -119.586	19 vi 2009	JdW		ICHBC135				a	a

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
	BC: Mahoney Lake	49.285 -119.586	19 vi 2009	JdW		ICHBC138			a	a	
	BC: Kamloops	49.318 -119.507	19 vi 2009	JdW		ICHBC139			a	a	
	BC: nr Kamloops	50.638 -120.451	9 vi 2009	JdW		ICHBC146			a	a	
	BC: Mahoney Lake	49.291 -119.591	10 vii 2009	JdW		ICHBC155			a	a	
	BC: Mahoney Lake	49.291 -119.591	10 vii 2009	JdW		ICHBC156			a	a	
	BC: Kamloops	49.318 -119.507	8 vi 2009	JdW		ICHBC162			a	a	
	MB: Churchill	58.626 -94.23	14 vi 2007			JX832644			a	a	
	MB: Churchill	58.626 -94.23	14 vii 2007			JX832902			a	a	

O. parvulus species-group

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
7514	UK: Cornwall, Ding Dong	50.158 -5.58	viii 2010	JH	*parvulus A*	KF594992			a	a	
6911	UK: Aldbury	51.799 -0.601	25 v 2011	GRB	*parvulus B*		KF615925		b	b	
6924	UK: Aldbury	51.799 -0.601	25 v 2011	GRB	*parvulus B*	KF594823	KF615917	KF616377	a,b,c,d	a,b	
5558	BC: Vancouver	49.2 -123.2	3-17 ix 1997	IK		KF594582	KF615980		a,b	a,b	
5559	BC: Vancouver	49.2 -123.2	3-17 ix 1997	IK		KF594583	KF615978	KF616371	a,b,c,d	a,b	a,b,c
5563	BC: Vancouver	49.2 -123.2	24 ix 1997	IK		KF594586	KF615926		a,b	a,b	
6547	NH: Hubbard Brook Forest	43.93 -71.75	6 ix 2010	NL		KF594759			a	a	
6566	AB: 8 km NW Winfield	53.01 -114.5	5 ix 2009	CDB		KF594776			a	a	
6567	AB: 8 km NW Winfield	53.01 -114.5	5 ix 2009	CDB		KF594777	KF615979		a,b	a,b	
6569	AB: 8 km NW Winfield	53.01 -114.5	22 viii 2009	CDB		KF594778	KF615914		a,b	a,b	
6570	AB: 8 km NW Winfield	53.01 -114.5	5 ix 2009	CDB		KF594779	KF615981		a,b	a,b	
6908	AB: 8 km NW Winfield	53.01 -114.5	15 viii 2010	CDB		KF594980		KF616368	a,c	a	
6978	AB: 48 km NW Dixonville	56.749 -118.363	29 vii-12 viii 2008	MDS		KF594861	KF615982		a,b	a,b	
6979	AB: 48 km NW Dixonville	56.753 -118.333	12-26 viii 2008	MDS		KF594862	KF615983	KF616379	a,b,c,d	a,b	

O. pteridis species-group

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
7501	UK: Cornwall, Ding Dong	50.158 -5.58	viii 2010	JH	*pteridis*	KF594981			a	a	
7552	UK: Radnage	51.659 -0.858	30 vii 2008	AMG	*pteridis*	KF595025			a	a	
7556	UK: Saundersfoot	51.7 -4.7	4 viii 2007	JS	*pteridis*	KF595028			a	a	
3919	AB: Buffalo Lake CA	52.494 -112.696	10 viii 2008	CDB		KF594494	KF615913		a,b	a,b	
3926	NL: Wiltondale	49.37 -57.738	20 vii 2008	DM		KF594500	KF616281		a,b	a,b	
3945	AB: Buffalo Lake CA	52.494 -112.696	10 viii 2008	CDB		KF594519		KF616357	a,c	a	
3971	AB: 21 km S Whitelaw	55.928 -117.995	24 viii 2008	DM		KF594544			a	a	
3979	AB: Buffalo Lake CA	52.494 -112.696	10 viii 2008	CDB		KF594551			a	a	
3985	AB: 48 km NW Dixonville	56.749 -118.354	12-26 viii 2008	MDS		KF594557	KF616283		a,b	a,b	
3986	AB: 48 km NW Dixonville	56.7486 -118.356	18-29 vii 2008	MDS		KF594558		KF616317	a,c	a	
5536	BC: Sheridan Lake	51.535 -120.952	27 vii 2009	DGH		KF594560	KF616282		a,b	a,b	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	GenBank/BOLD* COI	ITS2	28S	Dataset[5] Ch. 2	Ch. 3	Ch. 4
5537	BC: Sheridan Lake	51.535 -120.952	27 vii 2009	DGH		KF594561	KF616279		a,b	a,b	
5546	AB: Pigeon Lake	53.072 -114.072	12-26 viii 2007	FAHS		KF594570	KF616274		a,b	a,b	
5547	12.6 km NWW of Dixonville	56.566 -117.865	24-28 vii 2007	MDS		KF594571	KF616293		a,b	a,b	
5548	AB: Pigeon Lake	53.072 -114.072	12-26 viii 2007	FAHS		KF594572	KF616289	KF616319	a,b,c,d	a,b	
5572	AB: 48 km NW Dixonville	56.750 -118.363	12-26 viii 2008	MDS		KF594594	KF616291	KF616323	a,b,c,d	a,b	
5573	AB: 48 km NW Dixonville	56.749 -118.354	18-29 vii 2008	MDS		KF594595	KF616287		a,b	a,b	
5705	BC: Sheridan Lake	51.535 -120.952	27 vii 2009	DGH		KF594625			a	a	
5712	AB: 38 km NW Dixonville	56.742 -118.332	29 vi-16 vii 2007	MDS		KF594630			a	a	
5723	BC: Revelstoke NP	51.108 -117.906	25 vii 2010	MDS		KF594641	KF616286		a,b	a,b	
5725	BC: Mt. Begbie, nr. 100 Mile House	51.477 -121.369	26 vii 2010	MDS		KF594643	KF615916		a,b	a,b	
5728	BC: Mt. Begbie, nr. 100 Mile House	51.477 -121.369	26 vii 2010	MDS		KF594646	KF616280		a,b	a,b	
5742	BC: Glacier NP	51.26 -117.57	8 vii 2010	MDS		KF594660	KF615911	KF616337	a,b,c,d	a,b	a,b,c
5743	BC: Glacier NP	51.242 -117.649	8 vii 2010	MDS		KF594661			a	a	
5754	AB: 48 km NW Dixonville	56.749 -118.356	12-26 viii 2008	MDS		KF594671	KF616285	KF616328	a,b,c,d	a,b	
5755	AB: 48 km NW Dixonville	56.750 -118.418	25-30 vii 2007	MDS		KF594672	KF615909		a,b	a,b	
5756	AB: Kootenay Plains PRA	52.064 -116.422	14 vii 2007	MDS		KF594673		KF616338	a,c	a	
5762	AB: Rochon Sands PP	52.461 -112.887	26 vii 2009	CDB		KF594679	KF616278		a,b	a,b	
5763	AB: 48 km NW Dixonville	56.560 -117.865	8-22 viii 2007	MDS		KF594680	KF616294		a,b	a,b	
5766	AB: Porcupine Hills	49.972 -114.087	31 vii 2007	JJD		KF594683		KF616330	a,c	a	
5782	BC: 6 km S Squamish	49.655 -123.193	28 vii 2010	MDS		KF594699	KF616290		a,b	a,b	
6510	AB: George Lake	53.957 -114.128	27 vi-4 vii 2007	MDS		KF594725			a	a	
6526	BC: Sicamous	50.82 -118.86	?	JdW		KF594741			a	a	
6535	BC: Date Creek	55.427 -127.803	11 ix 2009	JdW		KF594749			a	a	
6561	AB: 8 km NW Winfield	53.01 -114.5	1 viii 2009	CDB		KF594771	KF616277	KF616344	a,b,c,d	a,b	
6562	AB: 8 km NW Winfield	53.01 -114.5	15 viii 2010	CDB		KF594772	KF616276		a,b	a,b	
6563	AB: 8 km NW Winfield	53.01 -114.5	5 ix 2009	CDB		KF594773	KF616271	KF616356	a,b,c,d	a,b	
6564	AB: 8 km NW Winfield	53.01 -114.5	22 viii 2009	CDB		KF594774			a	a	
6565	AB: 8 km NW Winfield	53.01 -114.5	5 ix 2009	CDB		KF594775	KF615910		a,b	a,b	
6568	AB: 8 km NW Winfield	53.01 -114.5	24 vii 2010	CDB		KF594815	KF616275		a,b	a,b	
6931	AB: Edmonton	53.506 -113.613	9 ix 2009	GGA		KF594888	KF616272		a,b	a,b	
6940	AB: Cooking Lake-Blackfoot PRA	53.505 -112.945	20 viii 2009	GGA		KF594890			a	a	
6956	AB: George Lake	53.957 -114.128	13 ix 2009	MDS		KF594837	KF616270		a,b	a,b	
6959	AB: 48 km NW Dixonville	56.751 -118.330	8-18 vii 2008	MDS		KF594842	KF615908		a,b	a,b	
6960	AB: 48 km NW Dixonville	56.749 -118.360	8-18 vii 2008	MDS		KF594843	KF616292	KF616353	a,b,c,d	a,b	
6962	AB: 48 km NW Dixonville	56.751 -118.330	24 vi-8 vii 2008	MDS		KF594844			a	a	
6963	AB: 48 km NW Dixonville	56.749 -118.321	12-26 viii 2008	MDS		KF594845			a	a	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	GenBank/BOLD* COI	ITS2	28S	Dataset[5] Ch. 2	Ch. 3	Ch. 4
6969	AB: 48 km NW Dixonville	56.742 -118.332	16-24 vii 2007	MDS		KF594852	KF616295		a,b	a,b	
6971	AB: 48 km NW Dixonville	56.749 -118.360	29 vii-12 viii 2008	MDS		KF594854	KF616284		a,b	a,b	
6973	AB: 48 km NW Dixonville	56.749 -118.360	18-29 vii 2008	MDS		KF594856			a	a	
6975	AB: 48 km NW Dixonville	56.749 -118.360	8-18 vii 2008	MDS		KF594858			a	a	
6976	AB: 48 km NW Dixonville	56.754 -118.334	29 vii-12 viii 2008	MDS		KF594859	KF616297		a,b	a,b	
6977	AB: 48 km NW Dixonville	56.749 -118.363	18-29 vii 2008	MDS		KF594860	KF616298		a,b	a,b	
6980	AB: 48 km NW Dixonville	56.750 -118.363	12-26 viii 2008	MDS		KF594863			a	a	
7325	AB: 62 km WNW Dixonville	56.695 -118.656	19 viii 2008	BBB		KF594915			a	a	
7347	AB: 33 km NW Dixonville	56.727 -118.267	24 vii-2 viii 2007	MDS		KF594936	KF616296		a,b	a,b	
7348	AB: 33 km NW Dixonville	56.727 -118.267	16-24 vii 2007	MDS		KF594937	KF616288		a,b	a,b	
7354	AB: Pakowki sand dunes	49.397 -110.875	12-13 vii 2009	GGA		KF594942			a	a	
	MB: Churchill	58.619 -93.828	23 vi 2007			JX832540			a		
	MB: Churchill	58.755 -93.998	8 viii 2007			JX832888			a		
***O. scutellaris* species-group**											
3911	AB: nr. Tangent Park	56.092 -117.542	7 v 2008	DM	*aureus*	KF594487	KF615965	KF616311	a,b,c,d	a,b	a,b,c
3961	AB: nr. Tangent Park	56.092 -117.542	7 v 2008	DM	*aureus*	KF594535	KF615966		a,b	a,b	a,b
3970	AB: Machesis Lake PRA	58.325 -116.578	10 vi 2008	DM	*aureus*		KF615968		b	b	b
3975	AB: nr. Tangent Park	56.092 -117.542	7 v 2008	DM	*aureus*		KF615969		b	b	b
3939	ON: nr. Carp	45.385 -76.008	13 v 2008	BCS	*brevipunctatus*	KF594513	KF615967	KF616314	a,b,c,d	a,b	a,b,c
6944	AB: Spruce Grove	53.4 -113.9	28 v-2 vi 1989	ATF	*clave*	KF594831	KF615972	KF616352	a,b,c,d	a,b	a,b,c
7381	AB: Jenner	50.844 -111.154	2 vi 2010	GGA	*clave*	KF594961			a	a	a
7382	AB: Jenner	50.842 -111.151	09 v 2007	MDS	*clave*	KF594962	KF615970		a,b	a,b	a,b
7383	AB: Jenner	50.844 -111.154	2 vi 2010	GGA	*clave*	KF594963			a	a	a
7391	AB: Jenner	50.842 -111.151	07 vi 2007	GGA	*clave*	KF594970			a	a	a
6548	SK: nr. Newton Lake	49.301 -107.764	20 v 2011	JJD	*dombroskii*	KF594760	KF615971	KF616341	a,b,c,d	a,b	a,b,c
3908	AB: Edmonton	53.521 -113.521	20 v 2008	JJD	*idoneus*	KF594484	KF615962		a,b	a,b	a,b
3910	AB: Edmonton	53.545 -113.439	15-16 v 2008	GGA	*idoneus*	KF594486	KF615953		a,b	a,b	a,b
3933	AB: Edmonton	53.545 -113.439	15 vi 2008	GGA	*idoneus*	KF594507	KF615951		a,b	a,b	a,b
3950	AB: Pigeon Lake	53.072 -114.072	3 vi 2008	FAHS	*idoneus*	KF594524	KF615952		a,b	a,b	a,b
3974	AB: Edmonton	53.545 -113.439	23 v 2009	GGA	*idoneus*	KF594547	KF615956		a,b	a,b	a,b
3976	ON: Leeds Grenville Co.	44.487 -76.008	7 vi 2008	BCS	*idoneus*	KF594548	KF615961		a,b	a,b	a,b
3977	AB: Pigeon Lake	53.072 -114.072	3 vi 2008	FAHS	*idoneus*	KF594549	KF615957		a,b	a,b	a,b
5551	ON: Ottawa	45.356 -75.707	5 v-5 vi 2008	HG	*idoneus*	KF594575	KF615954		a,b	a,b	a,b
5552	ON: Ottawa	45.356 -75.707	5 v-5 vi 2008	HG	*idoneus*	KF594576	KF615958		a,b	a,b	a,b
5554	ON: Grenadier Is.	44.4 -75.9	10-21 vi 1994	CNC	*idoneus*	KF594578	KF615955	KF616320	a,b,c,d	a,b	a,b,c

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	GenBank/BOLD*			Dataset[5]		
						COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
5717	ON: Ottawa	45.356 -75.707	5 v-5 vi 2008	HG	*idoneus*	KF594635	KF615959		a,b	a,b	a,b
6551	AB: 48 km NW Dixonville	56.753 -118.328	28 v-10 vi 2008	MDS	*idoneus*	KF594763	KF615949		a,b	a,b	a,b
6552	ON: Grenadier Is.	44.4 -75.9	24 v - 9 vi 1994	CNC	*idoneus*	KF594764	KF615960		a,b	a,b	a,b
6907	AB: 8 km NW Winfield	53.01 -114.5	15 v 2010	CDB	*importunus*	KF594814	KF615963	KF616346	a,b,c,d	a,b	a,b,c
3904	AB: Porcupine Hills	49.972 -114.087	28 v 2008	JJD	*keala*	KF594480	KF615945	KF616307	a,b,c,d	a,b	a,b,c
3960	AB: Porcupine Hills	49.972 -114.087	28 v 2008	JJD	*keala*	KF594534	KF615950		a,b	a,b	a,b
3965	AB: Porcupine Hills	49.972 -114.087	29 v 2008	JJD	*keala*	KF594539	KF615948		a,b	a,b	a,b
3980	AB: Porcupine Hills	49.972 -114.087	29 v 2008	JJD	*keala*	KF594552	KF615947		a,b	a,b	a,b
6515	AB: Porcupine Hills	49.972 -114.087	15 vi 2009	MDS	*keala*	KF594730	KF615943	KF616332	a,b,c,d	a,b	a,b,c
7324	AB: 62 km WNW Dixonville	56.684 -118.644	7 vi 2008	BBB	*keala*	KF594914	KF615944		a,b	a,b	a,b
7327	AB: 62 km WNW Dixonville	56.685 -118.641	26 v 2008	BBB	*keala*	KF594917	KF615946		a,b	a,b	a,b
	UK	not listed	not listed		*scutellaris*			EU378720	c		c
6914	UK: Pitstone Commons	51.81 -0.593	22 iv-7 v 2011	GRB	*scutellaris A*	KF594819	KF615964	KF616305	a,b,c,d	a,b	a,b,c
7515	UK: Radnage	51.659 -0.858	3 v 2008	AMG	*scutellaris A*	KF594993			a	a	a
7524	UK	?	?	GRB	*scutellaris A*	KF595042			a	a	a
7558	UK: Radnage	51.659 -0.858	21 vii 2011	AMG	*scutellaris A*	KF595030			a	a	a
7519	UK: Westcott	51.848 -0.962	20 iii 2009	DW	*scutellaris B*	KF594995			a	a	a
	UK	not listed	not listed		*scutellaris B*	JF963667			a		
***O. slossonae* species-group**											
6543	AZ: Franklin Co.	35.659 -93.748	17 vi 2008	JJD,DL	*slossonae*	KF594755			a	a	
6544	NH: Hubbard Brook Forest	43.93 -71.75	6 ix 2010	NL	*slossonae*	KF594756			a	a	
6545	NH: Hubbard Brook Forest	43.93 -71.75	13 ix 2010	NL	*slossonae*	KF594757			a	a	
3914	AB: Pakowki sand dunes	49.397 -110.875	24 vii 2008	JJD		KF594490	KF615921	KF616384	a,b,c,d	a,b	
3938	ON: Leeds Grenville Co.	44.487 -76.008	7 vi 2008	BCS		KF594512		KF616302	a,c	a	
3954	AB: 18 km SE Deadwood	56.659 -117.2	2 viii 2008	DM		KF594528	KF615976		a,b	a,b	
3956	AB: 18 km SE Deadwood	56.659 -117.2	2 viii 2008	DM		KF594530	KF615927	KF616380	a,b,c,d	a,b	
3957	AB: 21 km S Whitelaw	55.928 -117.995	29 vi 2008	DM		KF594531	KF615930	KF616381	a,b,c,d	a,b	a,b,c
3966	AB: 21 km S Whitelaw	55.928 -117.995	29 vi 2008	DM		KF594540			a	a	
5539	BC: Murray Lake	49.802 -121.005	18 vii 2009	DGH		KF594563	KF615974		a,b	a,b	
5541	FL: Highlands Co.	27.26 -81.36	19 iii 2008	WH		KF594565		KF616366	a,c	a	
5544	AB: Pakowki sand dunes	49.397 -110.875	18-19 vii 2007	MDS		KF594568	KF615973	KF616369	a,b,c,d	a,b	
5549	BC: Pender Island	48.771 -123.300	20 vii 2009	MDS		KF594573	KF615935	KF616370	a,b,c,d	a,b	
5570	AZ: Animas Mountains	31.592 -108.775	13-14 viii 1999	JEO		KF594593	KF615929	KF616385	a,b,c,d	a,b	
5584	BC: Pender Island	48.771 -123.3	20 vii 2009	MDS		KF594604	KF615912		a,b	a,b	
5598	AB: Lowden Springs CA	52.152 -112.706	28 vi 2008	CDB		KF594618	KF615932		a,b	a,b	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
						GenBank/BOLD*			Dataset[5]		
5715	BC: nr. Fort Steele	49.636 -115.609	16 vi 2009	JJD		KF594633		KF616372	a,b,c,d	a,b	
5726	BC: Mt. Begbie, nr. 100 Mile House	51.477 -121.369	26 vii 2010	MDS		KF594644	KF615919	KF616382	a,b,c,d	a,b	
5730	BC: 6 km S Squamish	49.655 -123.193	28 vii 2010	MDS		KF594648		KF616373	a,c	a	
5735	BC: 7 km NW Bridesville	49.05 -119.059	2 viii 2010	MDS		KF594653	KF615938	KF616383	a,b,c,d	a,b	
5736	AB: Jasper NP	52.963 -118.058	11 ix 2008	DL		KF594654		KF616374	a,c	a	
5740	BC: 7 km E Osoyoos	49.012 -119.354	2 viii 2010	MDS		KF594658	KF615920		a,b	a,b	
5746	WA: Wenatchee NF	47.584 -120.367	9 vii 2010	JA		KF594664			a	a	
5749	BC: New Denver	49.996 -117.352	9 vi 2010	MDS		KF594667	KF615942	KF616387	a,b,c,d	a,b	
5752	BC: Metchosin	48.395 -123.513	7 vi 2010	MDS		KF594669	KF615933	KF616375	a,b,c,d	a,b	
5761	AB: Big Knife PP	52.487 -112.217	30 viii 2009	CDB		KF594678	KF615934		a,b	a,b	
5765	AB: Writing-on-Stone Prov. Park	49.084 -111.615	19 vii 2007	MDS		KF594682			a	a	
5770	NL: Deer Lake	49.179 -57.442	23 vi 2009	DWL		KF594687	KF615940	KF616367	a,b,c,d	a,b	
5773	AB: Waterton Lakes NP	49.1 -113.953	17 vii 2010	MDS		KF594690			a	a	
5787	BC: 10.5 km SW Lillooet	50.64 -122.073	27 vii 2010	MDS		KF594704	KF615918		a,b	a,b	
5796	AB: Big Knife PP	52.487 -112.217	30 viii 2009	CDB		KF594712			a	a	
5797	AB: 62 km WNW Dixonville	56.686 -118.645	1 viii 2008	BBB		KF594713		KF616376	a,c	a	
5798	AB: 21 km S Whitelaw	55.928 -117.995	9 viii 2008	DM		KF594714			a	a	
5799	AB: Edmonton	53.506 -113.613	30 viii 2009	JHA		KF594715			a	a	
6524	BC: Date Creek	55.466 -127.815	23 viii 2009	JdW		KF594739	KF615936		a,b	a,b	
6527	BC: Haida Gwaii, Moresby Is.	53.126 -131.707	28 vii 2009	JdW		KF594742	KF615937		a,b	a,b	
6528	BC: Haida Gwaii, Graham Is.	53.579 -131.922		JdW		KF594743	KF615975		a,b	a,b	
6530	BC: Date Creek	55.464 -127.810	23 viii 2009	JdW		KF594744	KF615915		a,b	a,b	
6531	BC: Haida Gwaii, Graham Is.	53.579 -131.922	23 vii 2009	JdW		KF594745			a	a	
6534	BC: Haida Gwaii	?	?	JdW		KF594748			a	a	
6546	NH: Hubbard Brook Forest	43.93 -71.75	12 vii 2010	NL		KF594758			a	a	
6557	NV: Elko Co.	41.026 -115.085	13-14 vii 2010	JA		KF594768			a	a	
6937	AB: Edmonton	53.510 -113.622	8 vi 2010	JHA		KF594829	KF615931	KF616378	a,b,c,d	a,b	
6951	AB: George Lake	53.957 -114.128	15-22 vi 2007	MDS		KF594835			a	a	
6952	AB: George Lake	53.957 -114.124	4 vii 2007	MDS		KF594836	KF615928		a,b	a,b	
7312	AB: 62 km WNW Dixonville	56.691 -118.672	8 vii 2008	BBB		KF594902	KF615977		a,b	a,b	
7356	AB: Pakowki sand dunes	49.397 -110.875	28 vi 2008	LML		KF594944			a	a	
7374	AB: Pakowki sand dunes	49.397 -110.875	13-14 vii 2009	GGA		KF594954			a	a	
7375	AB: Pakowki sand dunes	49.397 -110.875	13-14 vii 2009	GGA		KF594955			a	a	
7376	AB: Pakowki sand dunes	49.397 -110.875	10 viii 2008	GGA		KF594956			a	a	
7395	AB: Pakowki sand dunes	49.397 -110.875	28 vi 2008	LML		KF594974			a	a	
	BC: Kelowna	49.937 -119.398	14 vi 2008	JdW		ICHBC001			a	a	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	GenBank/BOLD*			Dataset[5]		
						COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
	BC: Kelowna	49.937 -119.398	14 vi 2008	JdW		ICHBC002				a	a
	BC: nr Kamloops: Sugarloaf	50.638 -120.451	4 vii 2008	JdW		ICHBC025				a	a
	BC: nr Kamloops: Sugarloaf	50.638 -120.451	4 vii 2008	JdW		ICHBC028				a	a
	BC: Kamloops	50.666 -120.376	12 vi 2008	JdW		ICHBC044				a	a
	BC: Kamloops	50.666 -120.376	12 vi 2008	JdW		ICHBC045				a	a
	BC: Kamloops	50.666 -120.376	12 vi 2008	JdW		ICHBC046				a	a
	BC: nr Kamloops: Dewdrop	50.747 -120.555	31 vii 2008	JdW		ICHBC049				a	a
	BC: Kamloops	50.675 -120.424	12 vi 2008	JdW		ICHBC055				a	a
	BC: Mahoney Lake	49.291 -119.591	8 vi 2008	JdW		ICHBC117				a	a
	BC: Mahoney Lake	49.291 -119.591	8 vi 2008	JdW		ICHBC120				a	a
	BC: Mahoney Lake	49.285 -119.586	19 vi 2009	JdW		ICHBC136				a	a
Species-group 1											
3913	AB: Pakowki sand dunes	49.397 -110.875	8 vii 2008	JJD		KF594489				a	
5566	MB: Winnipegosis	51.651 -99.945	16 vi 2007	HG		KF594589	KF616242	KF616321	a,b,c,d	a,b	a,b,c
5581	MB: Winnipegosis	51.651 -99.945	16 vi 2007	HG		KF594603	KF616243		a,b	a,b	
5708	QC: Gatineau Park	45.5 -75.9	7-10 ix 1986	HG		KF594626	KF616245		a,b	a,b	
5709	KY: 8 km W Hopkinsville	36.9 -87.6	v 1986	C		KF594627				a	a
5724	BC: Revelstoke NP	51.108 -117.906	25 vii 2010	MDS		KF594642		KF616386	a,c	a	
5767	AB: Jasper NP	52.912 -117.989	9-12 vii 2007	MDS		KF594684				a	a
6508	AB: Medecine Lake Rec Area	52.747 -114.739	11 vii 2009	CDB		KF594723	KF616244	KF616331	a,b,c,d	a,b	
6936	AB: Edmonton	53.545 -113.439	14 vi 2008	GGA		KF594828				a	a
	BC: 2 km N Otter L	49.6 -120.8	5 vii 2008	JdW		ICHBC071				a	a
	BC: 2 km N Otter L	49.6 -120.8	5 vii 2008	JdW		ICHBC073				a	a
	BC: Kamloops	50.739 -120.727	20 vi 2009	JdW		ICHBC121				a	a
Unplaced *Ophion*											
7518	UK: Eaton Bray	51.876 -0.597	13 v-31 xii 2004	PG	*longigena*	KF594994				a	a
3918	AB: Lowden Springs CA	52.152 -112.706	28 vi 2008	CDB	*nigrovarius*	KF594493	KF616268	KF616304	a,b,c,d	a,b	
5564	QC: nr. Frelighsburg	45 -72.8	28 v-3 vi 1991	LD	*nigrovarius*	KF594587				a	a
5565	QC: nr. Frelighsburg	45 -72.8	28 v-3 vi 1991	LD	*nigrovarius*	KF594588				a	a
5567	MB: Winnipegosis	51.651 -99.945	16 vi 2007	HG	*nigrovarius*	KF594590	KF616269	KF616306	a,b,c,d	a,b	
7380	AB: Jenner	50.842 -111.151	07 vi 2007	GGA	*nigrovarius*	KF594960				a	a
7502	UK: Aldbury	51.799 -0.601	1 vii 2010	GRB	*perkinsi*	KF594982				a	a
7547	UK: Aldbury	51.799 -0.601	23 vii 2012	GRB	*perkinsi*	KF595021				a	a
	Australia	not listed	not listed		*zerus*			EU378721	c		
	Madagascar: Ranamafana			HH		JF963662				a	

DNA[1]	Provenance[2]	Lat-long	Date	Coll.[3]	Species[4]	GenBank/BOLD*			Dataset[5]		
						COI	ITS2	28S	Ch. 2	Ch. 3	Ch. 4
	Madagascar: Ranamafana			HH		JF963663			a		
Outgroup											
	Malaysia	not listed	not listed		*Enicospilus*			EU378708	c		
3901	AB: Kootenay Plains PRA	52.064 -116.422	14 vii 2007	MDS	*Enicospilus*	KF594477		KF594477	a,c	a	a,c
5795	BC: Tranquille ER	50.755 -120.589	29 vi 2009	JJD	*Enicospilus*			KF616303	c		
	BC: Kamloops	50.638 -120.451	9 vii 2008	JdW	*Enicospilus*	ICHBC022			a	a	
	BC: Kamloops	50.638 -120.451	9 vii 2008	JdW	*Enicospilus*	ICHBC026			a	a	

1. DNA: Sperling lab (University of Alberta) DNA voucher number for newly sequenced specimens

2. Abbreviations: CA = Conservation Area; ER - Ecological Reserve; NP = National Park; PP = Provincial Park; PRA = Provincial Recreation Area;

3. Collectors: AJB = A.J. Beasley; AMG = A.M. George; ATF = A.T. Finnamore; BAM = B.A. Mori; BBB = B.B. Bodeux; BCS = B.C. Schmidt; BMTB = B.M.T.Brunet; C = Campbell; CDB = C.D. Bird; CNC = Canadian National Collection Hym team; CR = C.Rowe; DGH = D.G. Holden; DL = D. Lawrie; DM = D. Macaulay; DW = D. Wilton; DWL = D.W. Langor; EA = E. Araya; FAHS = F.A.H. Sperling; F.Q. = F. Quesada; GGA = G.G. Anweiler; GRP = G.R. Pohl; HB = H. Bird; HG = H. Goulet; HH = H. Hala; IK = I. Klimeszewski; JA = J. Adams; JATW = J.A.T. Woodford; JD = J. Denis; JdW = J.R. deWaard; JE = J. Edwards; JEO = J.E. O'Hara; JH = J. Herbert; JHA = J.H. Acorn; JJD = J.J. Dombroskie; JRD = J.R. Dupuis; JS = J. Sirrett; LD = L. Dumouchel; LM = L. Masner; LML = L.M. Lumley; MA = M. Albertini; MDS = M.D. Schwarzfeld; MEMS = M.E.M. Smith; MRH = M.R. Honey; MRS = M.R. Shaw; NL = N. Lany; NM = N. McFarland; PAB = P.A. Brown; PDNH = P.D.N. Hebert; PG = P. Gould; PH = P. Hall; SN = S. Nargundkar; TP = T. Pike; WH = W. Hunting

4. Species: All newly sequenced Nearctic species were determined by MDS; all newly sequenced Palearctic species were determined by G.R. Broad, except for *O. forticornis*, which was determined by M.R. Shaw.

5. Datasets: Chapter 2: a = COI, b = ITS2, c = 28S, d = combined; Chapter 3: a = COI_{all}, b = $ITS2_{all}$, a,b = COI_{common} and $ITS2_{common}$; Chapter 4: a = COI; b = ITS2; c = 28S

Biography

I was born on March 20, 1978, in Vancouver, B.C. to Rita Dahlie and Earl Schwarzfeld. I grew up as a city-girl in Vancouver, but always had a fascination for birds, bugs and flowers. Weekends and summers spent on Pender Island, one of B.C.'s Gulf Islands, further spurred my interest, with lots of bird-watching and catching insects, tadpoles and newts in the lake.

In 1996, I began my undergraduate studies in Biology at the University of Victoria, with the intention of becoming an ornithologist. By the end of my first year, I was sure my destiny was to be a botanist. Then in my third year, I took my first entomology class, and never looked back. Co-op positions at the Victoria Bug Zoo and at the Royal British Columbia Museum gave me opportunities to play with bugs (living and dead), and inspired me to want to lean more and more about terrestrial arthropods. While sorting Malaise traps for the Royal BC Museum, I was first introduced to parasitoid Hymenoptera, as I became fascinated by the tiny jewel-like chalcidoids in the traps. Upon learning that they were no keys to identify them, I knew I wanted to work towards filling that gap – and a (future) taxonomist was born.

 First, however, I spent two summers working on the breeding biology of Chestnut-backed Chickadees in northern British Columbia. While working on this project, I conducted an honours project studying bark-dwelling and foliage-dwelling arthropods, with an emphasis on spiders. This was my first introduction to independent research, and to the challenges of arthropod biodiversity surveys.

Upon graduating from UVic in 2001, I spent eight months working in a freshwater ecology lab, where I learned to sort arthropods from mud samples, and identify chironomid larvae. I then took time off to travel in Central America for six months, where I learned Spanish, rekindled my love of birds, and got to see some really large insects. Returning to Victoria, I spent a further eight months in the same lab, this time focusing on zooplankton and fish.

The bugs were calling, however, and in January 2004, I left Victoria to work on an entomology project on the Big Island of Hawai'i. This position included work on a wide variety of insects, including identifying moths, rearing

caterpillars, surveying for ants, and collecting foliage-dwelling arthropods. However, a major component of my work was identifying ichneumonids from Malaise trap samples. I was thrilled to finally be working on parasitoid Hymenoptera, and my interest in this group continued to grow. After almost three years in Hawai'i, I decided it was time to pursue graduate studies, and I knew that I wanted to continue learning about parasitoids.

I was drawn to the University of Alberta based on its reputation of strongly supporting its graduate students, as well as its strong tradition of entomological research. I began an M.Sc. in Felix Sperling's lab in 2006 and switched to a Ph.D. in 2008. I feel extremely fortunate to have spent these years surrounded by passionate entomologists, and to have learned so many new skills and concepts. Despite having completed my doctorate, I have just begun my entomological journey; I look forward to spending my life learning about ichneumonids in particular and insects in general.