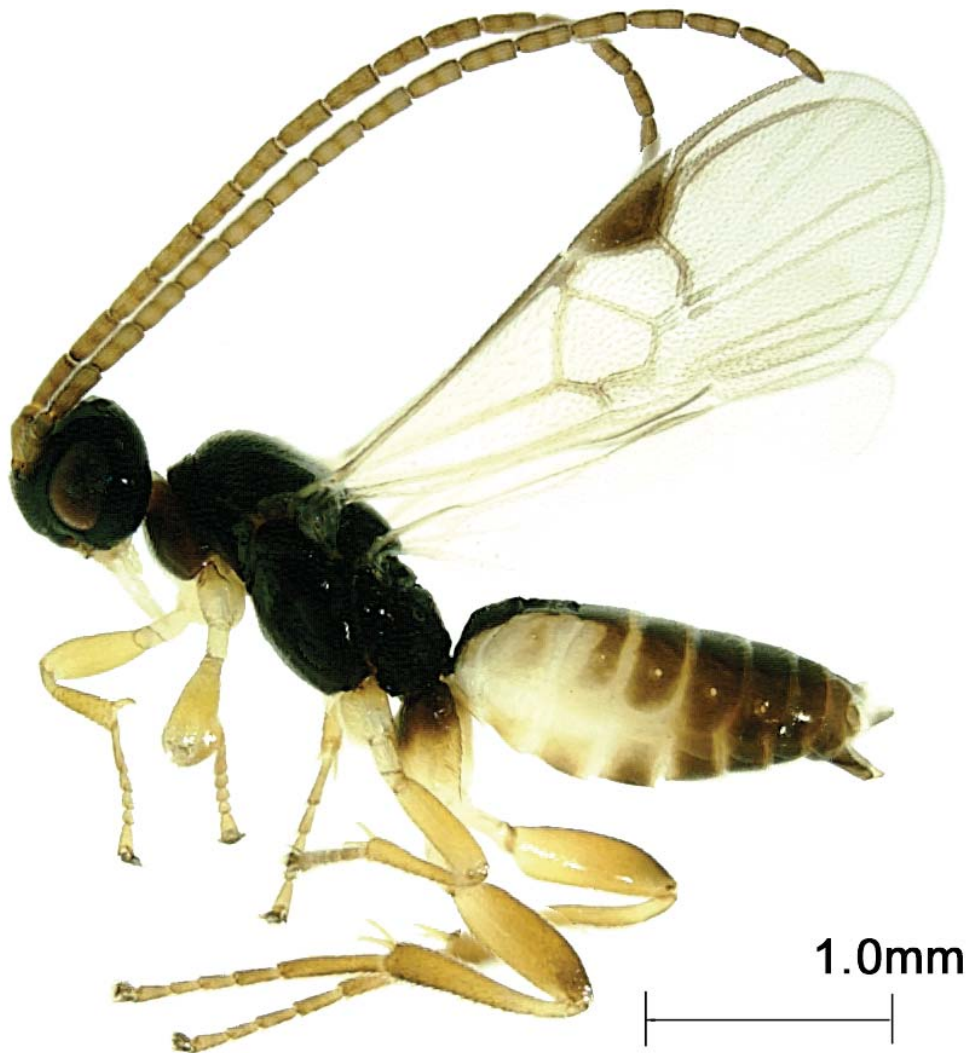


**PHYLOGENETICS OF THE *COTESIA FLAVIPES* SPECIES
COMPLEX: TOWARDS THE EFFECTIVE CONTROL OF
STEMBORER PESTS IN AUSTRALIA**



KATE MUIRHEAD

Presented for the degree of Doctor of Philosophy
School of Earth & Environmental Sciences
Ecology & Evolutionary Biology
The University of Adelaide

December 2009

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in text.

I give consent to this copy of my thesis when deposited in the University Library being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holders of those works.

This study was funded by an Australian Research Council industry-linkage postgraduate scholarship (project LP0348703) awarded to Professor Andrew Austin, Professor Steve Donnellan and Dr Nader Sallam.

Kate Muirhead

1 December, 2009

DEDICATION

To my dearly loved parents,
Valentina and Robb Muirhead

ACKNOWLEDGEMENTS

I would like to thank my principal supervisor, Andy Austin for opening my eyes as an undergraduate to the world of parasitoid wasps, and providing me with countless work and travel opportunities. I am grateful for his relaxed approach, ‘open door policy’ and all the support and guidance throughout the course of my PhD. I would also like to thank Nader Sallam for initiating this project, collecting most of the overseas specimens and for invaluable advice and assistance. Sallam’s passion and enthusiasm for this work was inspiration and made long hours of fieldwork truly enjoyable. I am indebted to Steve Donnellan, Nick Murphy and Michelle Guzik for training and direction in the molecular lab and help with the analyses.

I’ve had the pleasure of interacting with a wonderful group of faculty, postdocs and students in the Austin lab at the University of Adelaide. I would like to thank, John Jennings, Lars Krogman, Tessa Bradford and Kym Abrams, with special thanks to my wonderful friends Michelle Guzik, Tim Moulds, Nick Murphy, Deb O’Keefe, Steven Delean, Alice Shields, Nick Stevens and Trude Hallaraker, for all their help, humour and for simply being amazing individuals.

I am grateful to the endless support of my family in Australia and America. I thank my parents for their encouragement and unparalleled generosity; my brother for his wit and excellent sense of humour - long distance phone calls are always a source of amusement and cheer. Massive thanks are also due to my Aunt Olga for her wonderful good nature and for taking us in for almost a year while I was writing up. Last but definitely not least, I thank my husband Clay: your support, love and pride in my achievements is humbling.

This work was funded by an Australian Research Council industry-linkage postgraduate scholarship (project LP0348703), with BSES limited and the South Australian Museum. Additional funding was provided by the Sugar Research and Development Corporation, The University of Adelaide Research Abroad Grant and the Early Career Researcher Travel Grant from the ARC Environmental Futures Network.

The *Cotesia flavipes* species complex of parasitoid wasps are economically important worldwide for the biological control of lepidopteran stemborers. The complex currently comprises three species: *Cotesia flavipes* Cameron, *C. sesamiae* (Cameron) and *C. chilonis* (Matsumura), which appear morphologically similar. Despite their economic importance, considerable confusion surrounds the identity of species and host-associated biotypes. Differences in the biology and variation in host range of geographic populations have generally been interpreted as genetic divergence among strains, but direct genetic evidence is lacking. In Australia, several stemborer pests in neighbouring countries have been identified as significant threats to the sugar industry. However, the status of *C. flavipes* in Australia is unknown. To examine the genetic variation among worldwide populations of the *C. flavipes* complex and investigate the status of the Australian *C. flavipes*-like species, a pilot study based on 21 geographic populations of the complex and four outgroups was carried out using partial sequence data generated for mitochondrial gene regions, *16S rRNA* and *COI*. Phylogenetic analyses supported the monophyly of the complex and the existence of genetically divergent populations of *C. flavipes* and *C. sesamiae*. The geographically isolated Australian haplotypes formed a distinct lineage within the complex and were ~3.0% divergent from the other species.

Based on molecular, morphological and preliminary investigations into biological differences, the Australian species *Cotesia nonagriæ* Olliff stat. rev. was redescribed and formally removed from synonymy with *C. flavipes*. Investigations of biology and behaviour of *C. nonagriæ* on the native stem borer host, *Bathytricha truncata* (Walker) (Noctuidae) revealed that duration of the larval stages and adult longevity of *C. nonagriæ* were longer than previously recorded for other members of the species complex. In addition, *C. nonagriæ* oviposited an average of over 100 eggs into each host, almost three times more than for other species in the *C. flavipes* complex (30-40). During microhabitat location, both naïve and experienced females demonstrated a strong response towards the plant host complex, with experienced wasps benefiting by having a more rapid response time to infested than noninfested plants.

Genetic variation and relationships among the complex were further studied by generating nucleotide sequence data for two partial mtDNA gene regions (*COI*, *16S*) and three anonymous nuclear loci (*CfBN*, *CfCN*, *CfEN*) among 42 worldwide populations within the *C. flavipes* complex and three outgroups. Phylogenetic reconstructions provided strong support for the monophyly of the complex and the presence of at least four species, *C. chilonis* (from China and Japan), *C. sesamiae* (from Africa), *C. flavipes* (originating from the Indo-Asia region but introduced into Africa and the New World), and *C. nonagriae* (from Australia and PNG). Although there was geographic variation within species, the analyses did not support the overall separation and monophyly of clades associated with different host species.

Members of the complex harbour polydnavirus (PDV) symbionts, which play a key role in determining host range by host immune suppression during the course of parasitoid development. A worldwide phylogeny of the *C. flavipes* complex PDV *CrVI* locus was determined to investigate cophylogeny between wasps and their PDV symbionts. The results showed that there were numerous PDV *CrVI* haplotypes within worldwide populations. However, not all coevolutionary analyses supported the cophylogeny between wasp and PDV trees. Phylogenetic incongruence was most likely a result of the ability of PDVs to coevolve with host resistance through a process of natural selection, whereas the wasp genes were not under selection. The most important result of this study was the implication for the use of the *CrVI* locus as a virulence marker in biological control.

TABLE OF CONTENTS

DECLARATION	II
DEDICATION	III
ACKNOWLEDGMENTS	IV
ABSTRACT	V
TABLE OF CONTENTS	VII
CHAPTER I	
General introduction	1
CHAPTER II	
Mitochondrial DNA phylogeography of the <i>Cotesia flavipes</i> complex of parasitoid wasps (Hymenoptera: Braconidae)	17
CHAPTER III	
The systematics and biology of <i>Cotesia nonagriæ</i> (Olliff) stat. rev. (Hymenoptera: Braconidae: Microgastrinae), a newly recognised member of the <i>Cotesia flavipes</i> species complex	37
CHAPTER IV	
Life history traits and foraging behaviour of <i>Cotesia nonagriæ</i> (Olliff) (Hymenoptera: Braconidae), a newly recognised member of the <i>Cotesia flavipes</i> species complex of stemborer parasitoids	53
CHAPTER V	
Molecular phylogeography and haplotype diversity of the <i>Cotesia flavipes</i> complex of parasitoid wasps (Hymenoptera: Braconidae)	74
CHAPTER VI	
Polydnavirus haplotype diversity in the <i>Cotesia flavipes</i> complex and phylogenetic incongruence between wasp and PDV phylogenies	100
CHAPTER VII	
General Discussion	116
REFERENCES	125
APPENDIX I	
Published Papers	150